Acta Biomed. - Vol. 90 - N. 2 - June 2019 | ISSN 0392 - 4203

ACTA BIOMEDICA

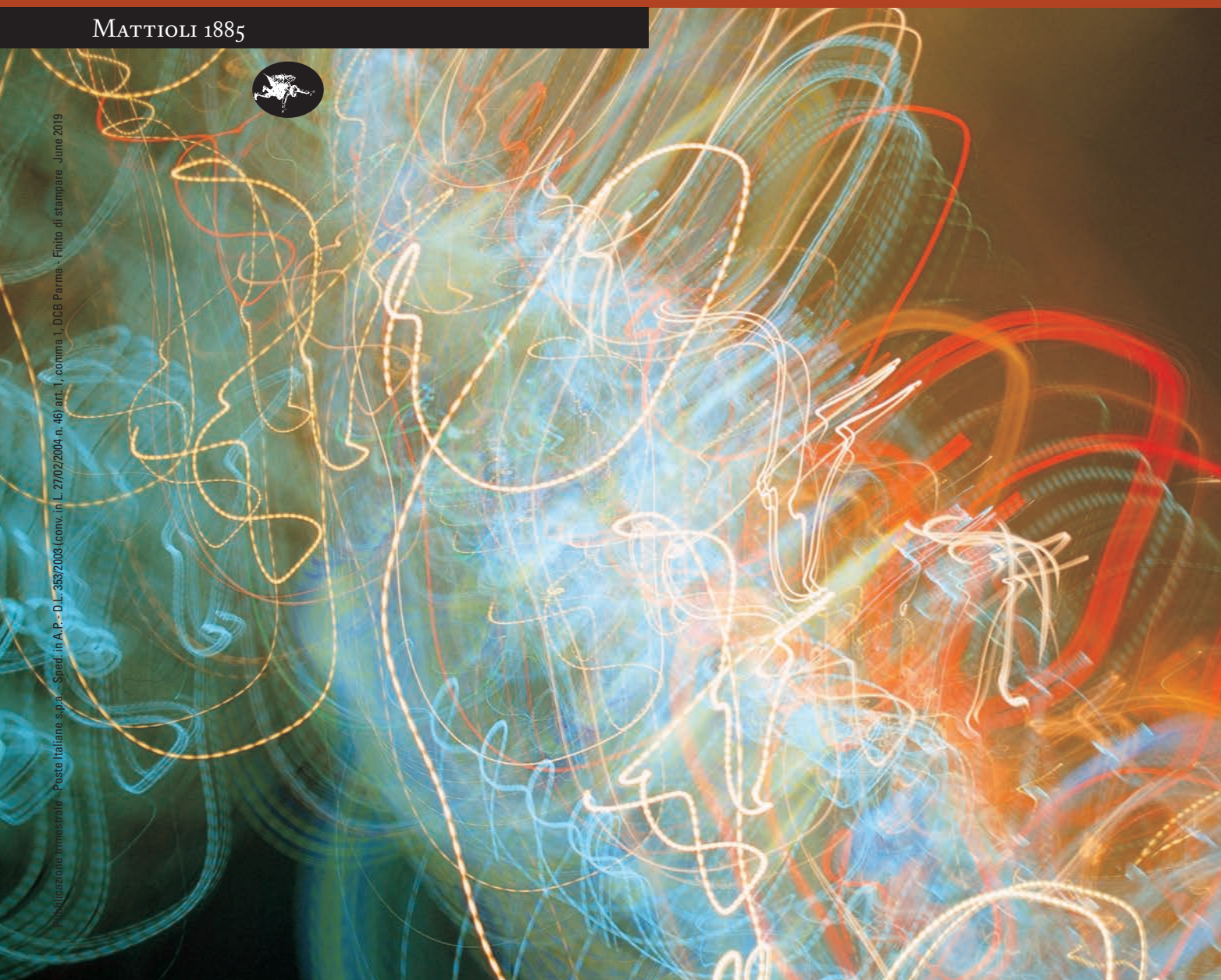
ATENEI PARMENSIS | FOUNDED 1887

Official Journal of the Society of Medicine and Natural Sciences of Parma and Centre on health systems' organization, quality and sustainability, Parma, Italy

The Acta Biomedica is indexed by Index Medicus / Medline Excerpta Medica (EMBASE), the Elsevier BioBASE, Scopus (Elsevier) and Bibliovigilance

Free on-line www.actabiomedica.it

Mattioli 1885



Pubblicazione trimestrale - Poste Italiane s.p.a. - Sped. in A.P. - D.L. 353/2003 (conv. in L. 27/02/2004 n. 46) art. 1, comma 1, DCB Parma - Finito di stampare June 2019

Acta Bio Medica

Atenei parmensis

founded 1887

Official journal of the Society of Medicine and Natural Sciences of Parma and Centre on Health System's Organization, Quality and Sustainability, Parma, Italy

free on-line: www.actabiomedica.it

EDITORIAL OFFICE MANAGER
Anna Scotti
Mattioli 1885 srl - Casa Editrice
Strada di Lodesana 649/sx, Loc. Vaio
43036 Fidenza (PR), Italy
Tel. ++39 0524 530383
Fax ++39 0524 82537
contact@actabiomedica.it

Francesco Covino
Società di Medicina e Scienze Naturali
Azienda Ospedaliero-Universitaria
di Parma - Cattani Building, 2nd floor
Via Gramsci, 14 - Parma, Italy
Tel./Fax ++39 0521 033730
francesco.covino@unipr.it

PUBLISHER
Mattioli 1885 srl Casa Editrice
Strada di Lodesana, 649/sx, Loc. Vaio
43036 Fidenza (PR), Italy
Tel. ++39 0524 530383
Fax ++39 0524 82537
E-mail: edit@mattioli1885.com

Acta BioMedica is the official Journal of the Society of Medicine and Natural Sciences of Parma. The Journal publishes Original Articles, Commentaries, Review Articles, Case Reports of experimental and general medicine. The manuscript must be submitted using the journal web site: **http://www.actabiomedica.it**
The Editorial Office will forward the text to the Editor-in-Chief, Prof. Maurizio Vanelli (University of Parma).
For any information please refer to:
Acta BioMedica – Editorial Office
Dr. Anna Scotti
Mattioli 1885 srl
Strada di Lodesana 649/sx, Loc. Vaio - 43036 Fidenza (PR) - Italy
E-mail: contact@actabiomedica.it - Fax: 0039-(0)524-82537
The Journal does not hold itself responsible for statements made by contributors or for loss or damage of mailed manuscripts. They should be ac-companied by an undertaking that they are submitted to this Journal only. Papers must be submitted in English. Papers are accepted on the understanding that they may be subject to editorial revision.
All Original Articles are subject to review and authors are urged to be brief. Long papers with many tables and figures may require shortening if they are to be accepted for publication. All manuscripts should include a total text word count and an abstract word count on the cover page. Total text word count does not include title page, figure legends, references, or tables. Only under exceptional circumstances will Original Articles longer than 5500 words be considered, and under no circumstances will abstracts greater than 250 words be published. Editorials and Reviews are normally invited contributions but suitable papers may be submitted to the Editor for consideration for this purpose. The presentation of Case Reports should be as short as possible. Reports of co-existence of two diseases or conditions without proof of causal relationship are discouraged. Letters to the Editor should not exceed 600 words of text, one figure or table and up to six references. Because space limitation, publication of submitted Letters will depend on priority rating.

TITLE PAGE must contain:
- a concise informative title
- author(s) names
- department or institution where work was done
- name and address of author to whom correspondence about the manuscript and request for reprints should be referred, as well as fax, E-mail and telephone number
- a running title of no more than 40 characters.

Be certain to list the FAX number and E-mail of the corresponding author on the title page. **All correspondence will be by E-mail and web site only.**

MANUSCRIPT should be typed in 12-point type and double spacing should be used throughout. It should carry an abstract of not more than 250 words including 4 paragraphs labeled: Background and aim of the work, Methods, Results, and Conclusions. Below the abstract provide 3-10 key words that will assist indexers in cross-indexing the article. Paragraphs to be set in a smaller type should be marked with an "s" (small) in the left hand margin. Avoid footnotes; when essential they are numbered consecutively and typed at the foot of the appropriate page.

ILLUSTRATIONS. It is the authors' responsibility to obtain permission (from the author and copyright holder) to reproduce illustrations, tables, etc. from other publications. Photographs and graphics should be sent as high resolution files: not less than 300 d.p.i. and with a base of the same size as a column of the Journal (8 cm). A letter of permission must accompany all photographs when there is a possibility of identification. Authors will pay for colour illustrations. Present rate for a full page colour illustration is about $ 600-1200. Final quotation will be given by the publisher. Legends should be typed on a separate "word" document.

TABLES should be numbered consecutively with Arabic numerals. Type each table on a separate document, together with a brief caption. We do not welcome large tables of unanalysed data.

REFERENCES should be numbered consecutively in the order in which they appear in the text. References cited only in tables or in legends to figures should be numbered in accordance with the sequence established by the first identification in the text. The list of references should be typed in numerical order and indicate: authors' names (all authors when six or less; when seven or more list only the first three and add "et al."); article title, name of the Journal (abbreviated as in Index Medicus), publication year, volume and first and last page numbers. Example:
Rizzato G, Marazzini L. Thoracoabdominal mechanics in elderly men. J Appl Physiol 1970; 28: 457-60.
If the reference is concerning a book, give authors' names, full title, name and address of publisher and publication year. Personal communications should not be included in the references, but may be cited in the text in parentheses.

COPYRIGHT. Please include a signed release of copyright to Acta Bio Medica Society of Medicine and Natural Sciences of Parma with your text. Include the title of the article being submitted, as well as the date. Include the signature of coauthors.
The corresponding author must certify that the submitted manuscript is an original article and that he is able to prove this originality if required from the Referees. Without this declaration the manuscript will not be considered.

GALLEY PROOF. Unless indicated otherwise, galley proofs are sent to the first author and should be returned without delay. Alterations to galley proofs, other than those due to printer's error, are charged to the author. Accepted and rejected manuscripts are retained for six months after publication or rejection, then destroyed.

REPRINTS. Reprints are available at cost if they are ordered when the proof is returned. Order form and a price list are sent with the galley proofs; payment must be made with the order.

NOTICE TO SUBSCRIBERS

Acta Bio Medica Society of Medicine and Natural Sciences of Parma is published four-monthly. Individual annual subscription for 2018 is 35,00 Euro in Italy, 45,00 Euro outside Italy. Institutional subscription is 45,00 Euro in Italy, 45,00 Euro outside Italy. The publisher accepts no responsibility for replacing Journal issues unless notified of non-receipt within 5 months of issue date. Payment should be made to the publisher: Mattioli 1885 srl, Strada di Lodesana 649/sx, Loc. Vaio, 43036 Fidenza (PR), Italy, Tel. 0039-(0)524-530383, Fax 0039-(0)524-82537, E-mail: subscribe@mattioli1885.com

COPYRIGHT

Editor-in-Chief: M. Vanelli
Printed in: June 2019
Registrazione del Tribunale di Parma n° 253 del 21/7/1955

Mattioli 1885
srl- Strada di Lodesana 649/sx
43036 Fidenza (Parma)
tel 0524/530383
fax 0524/82537
www.mattioli1885.com

Index

Volume 90 / n. 2 June 2019

Acta Biomed 2019; Vol. 90, N. 2: 181-183 DOI: 10.23750/abm.v90i2.8354

EDITORIAL

Why urbanisation and health?

Andrea Lenzi

President Health City Institute; President National Committee for Biosafety, Biotechnology and Life Sciences of Prime Minister and President Health City Institute

In 2007 more than 50% of the world's population were living in metropolitan cities, which was the first time in history that the urban population level had risen above 50%.The urban population have grown ever since and will continue to do so according WHO estimates. By 2030it has been estimated that 6 people out of 10 will live in urban agglomerates and an estimate of 70% of the inhabitants will be living in urban environments by 2050.

During the last 50 years there has been a change in the population with people are moving from rural to urban areas (Fig. 1).

According to the WHO there will be a steady increase in the numbers of people concentrated in the big cities, attracted by the mirage of welfare, employment and a different quality of life. The small and medium sized income countries will experience a population growth of 1.84% per year between 2015 and 2020 (Fig. 2).

This is an unstoppable social phenomenon and an irreversible tendency to be administered by many different stakeholders. The phenomenon is studied from different points of views; such as urban planning, transport, the industrial context, employment, and health.

Cities and their development models are today in the forefront of the fight against the potential problems caused by an increasing urbanization. Public health is one of the core issues.

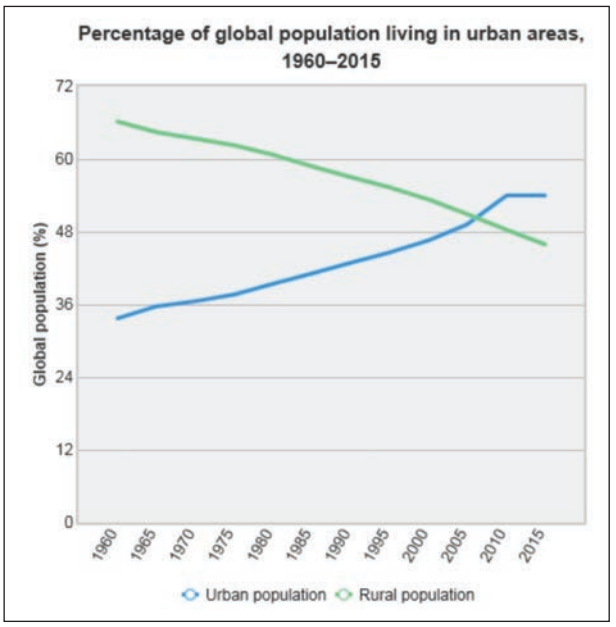


Figure 1. Source: WHO - World Urbanization Prospects 2014

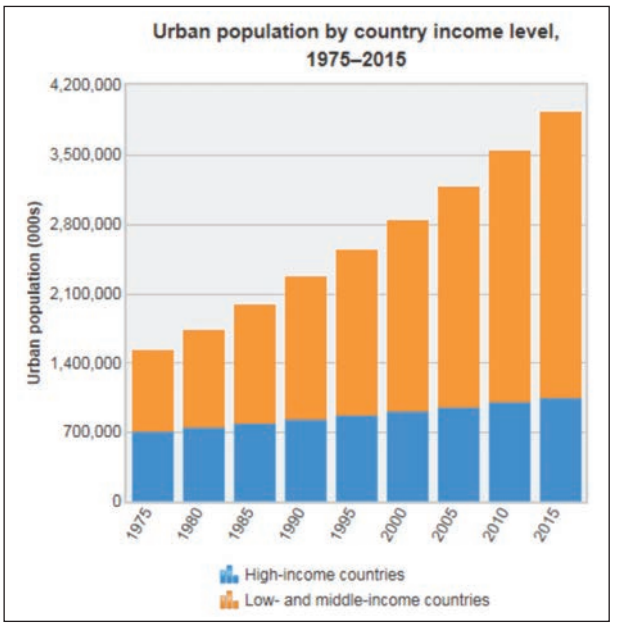


Figure 2. Source: WHO - World Urbanization Prospects 2014

In September 2015, 193 members of the United Nations met in New York with the aim of adopting a series of 17 targets in the Sustainable Development Goals (SDGs). The new SDGs cover a wide range of critical global issues, such as an end to poverty, the achievement of universal education and the fight against climate change. It is, however, important that a specific goal (SDG 11) has been added **in order to make cities inclusive, sustainable and able to deal with changes**. The key instruments in achieving goals, such as: housing development, air quality, good nutrition and transport, and the importance of the **determinants of health in the cities**.

The SDG 3 is part of the more general context of better health, as a global priority in the 2016-2030 planning. This goal is to focus on the improvement of mental and physical wellbeing for everyone at all age. The prevalence of health within in metropolitan cities with a high density of population consists of several risk factors affecting health, health inequalities, social and economic impact, are to be dealt with and discussed. Short-term interventions will not solve the basic problems and to understand the challenge ahead.

Today Cities are not only economic engines but also innovation centres which have to manage and face dramatic demographic and epidemiological transitions.

In 2014, a group of mayors and leaders of the world´s largest cities met and committed to tackle global climate change, thus reducing greenhouse gas emissions. This initiative has been launched and promoted by United Nations (UN) through a network made up by *Cities Climate Leadership Group* (*C40*), *United Cities and Local Governments Network* (*UCLG*) and *International Council for Local Environmental Initiatives* (*ICLEI*).

Through this agreement 206 cities with a total population of 270 million people, were able to commit to a specific programme in order to reduce the level of fine dust emissions in urban areas, thereby reducing the vulnerability of their territories.

On 15 October 2015 at EXPO, mayors from 115 metropolitan cities with a total of 400 million inhabitants, signed the *Milan Urban Food Policy Pact.* This commitment involves a series of local actions in order to face global emergencies as hunger, malnutrition and food waste. The cities signed the agreement and committed to develop sustainable food systems and healthy food at affordable prices, in order to reduce food waste.

These two examples highlight the role that mayors and cities play in order to face the development of the planet and its people through a proactive approach.

In the *Sustainable Development Goals, the* health goals relating to the achievement of urban development, such as SDG 3.3 and 3.4 are set. These are goals with a specific focus on the HIV epidemic and mortality reduction of **non-communicable diseases (NCDs).** It is known that HIV is mainly concentrated in cities, whereas urban lifestyle is a determining factor for the growth of NCDs. NCD's have only recently had a specific focus on city environment. The increasing of urbanization and non-communicable chronic diseases are two closely interconnected factors.

Policymakers and administrators, and mainly mayors, must deal with the increasing urbanization through a new approach. The new approach is to understand the burden of the disabilities related to chronic diseases, will affect the development and sustainability of their cities.

An entire new **urban welfare** model is to be understood, analysed and studied under different aspects. It is a welfare that, also referring to a national framework, must be assessed also locally through new questions and scenarios. It is necessary to ask whether the welfare systems in big cities and small cities differ, whether we are moving from a state of welfare to a local welfare and whether cities will be available to implement strategies enabling changes and innovations in planning and affecting socio-economic issues. Only the administrators and citizens will be more inclined to support the improvement of life quality and health through a gradual improvement of lifestyle.

An integrated approach to the SDGs will be useful in achieving health targets in cities, which are facing many challenges and will require multi-sectorial cooperation.

Clearly the increase in life expectancy and good life quality is related to the reduction of preventable deaths due to non-communicable diseases, will lead to the establishment of **coordination structures between different sectors of urban governance interacting with health.** A coordination through the involvement

of government- local, regional and national levels and they must be supported by global actions and as a primary factor from a dynamic view of the determinants of health in cities.

Another big challenge for the world´s health authorities is to fight the NCDs' evolution and among these diabetes has an important role.

Diabetes can be considered the biggest epidemiy in human history.

According to the lnternational Diabetes Federation (IDF), in 2013 more than 82 million people suffered from diabetes. By 2035 this number will rise to 595 million.

It is in the big cities that the majority of with type 2 diabetes lives in. The current estimates indicate that the disease will affect two people out of three. In fact, according to data provided by the lnternational Diabetes Federation (IDF), 246 million urban people (65%) in the world are diagnosed with type 2 diabetes compared to 136 million living in rural areas. This number is expected to grow**. It has been estimated that in 2035, 70% of people in the world will have, diabetes: 347 million urban compared to 147 million in rural areas.** It seems that cities are the "catalyst" for diabetes: those who move to the city does have higher chances of getting diabetes than people living outside big cities. This is of great concern, especially considering that for the first time in human history, the majority of the population live in urban areas. **From these propositions the Cities Changing Diabetes came about, an ambitious programme aiming to challenge the diabetes epidemic in big cities.** It started in Mexico City followed by Beijing, Beirut, Buenos Aires, Copenhagen, Hangzhou, Houston, Jakarta, Johannesburg, Koriyama, Leicester, Madrid, Mérida, Milan, Rome, Shanghai, Tianjin, Xiamen, Vancouver.

The project is intended to actively involve municipality authorities of the world´s largest metropolitan cities, including Italy, in analysing the underlying reasons for the increase of the disease and in identifying specific interventions in order to stop it.

There is an obvious need for a Health Observatory in cities which can identify the critical matters and facilitate the best practices to harmonise the benefits in order to avoid further discrimination.

In fact, health benefits in cities can be significantly different between people. It is known that in the same urban contexts there are different life expectations. In London, for example, people live longer if they are residents in Westminster, which is few metro stops from the *City*, whereas inhabitants of a decentralized suburb of Baltimore live shorter than people resident in other areas of the city.

The epigenetics have been studied for a long time and the correlation between circadian rhythm, environment and outbreak of certain diseases. Recent studies indicate that there is a strong correlation between genetic factors, individual lifestyle and the environment where we live. These are factors to be studied through a methodical approach and are relevant to identify in order to get the underlying reasons for the different situations between cities and its regions.

A roadmap on urbanization and health should be created. In order to raise awareness of health challenges related to urbanization and in order to increase the need to face these challenges through urban planning and inter-sector actions is it recommended that a *roadmap* I made to promote concrete actions and government strategies on health risks. The *roadmap* should take into account how urbanization increasingly exposes citizens to environmental factors (as air, water and noise pollution, problems related to public hygiene, sewage and waste disposal, etc.) and, therefore, to different health risk factors. **The *roadmap* should indicate actions to be promoted in the different cities, in order to avoid inequalities and to enable citizens to live a healthy life, as a common good and as an engine for everyone´s development and wealth.**

Italy could be at the forefront of the study on health issues related to urbanization if government, mayors, universities, health authorities and experts interact through a virtuous and multidisciplinary approach and not through a virtual, sectoral and individualistic one thus avoiding the logic of silos, which means the lack of cooperation between the different institutions involved.

Acta Biomed 2019; Vol. 90, N. 2: 184-186 DOI: 10.23750/abm.v90i2.8458

Editorial

New perspectives in Gerontology and Geriatrics

Jean Pierre Baeyens
AZ Alma, Eeklo, Belgium, University of Luxembourg, Former President IAGG-European Region

Introduction

In the development of Gerontology and Geriatrics in the next twenty years important questions have to be resolved, as following questions are suggesting:

- Is the cooperation between Gerontology and Geriatrics today optimal?
- How to detect and reverse pre-frailty?
- The increasing number of hip fractures: what is primary: osteoporosis or sarcopenia?
- All the Geriatric patients admitted in a general hospital have to be admitted in geriatric wards?
- Is the always earlier retirement age one of the important cause of the increase in incidence and prevalence of Alzheimer Dementia?
- How to stop the polypharmacy and inappropriate prescription for geriatric patients?
- How to start with clinical trials in very old frail patients?

What are the possible answers to these questions?

- Is the cooperation between Gerontology and Geriatrics today optimal?

It looks more and more difficult to take together Gerontology and Geriatrics. Geriatrics is really focusing on keeping the steady increasing number of people of very old age as autonomous as possible. As in the seventies Geriatric departments presented a mean age of 78 years, it is now in Western Europe 86 years or even higher.

The Gerontology is generally doing research on people in the sixties. It is indeed very important to prevent more and more incapacity in later age, and that begins earlier than the care of the very old people. Both should do efforts to keep more in touch. It was already one of the goals of the creation of the International Association of Gerontology in Liège (Belgium) in 1950.

- How to detect and reverse pre-frailty?

Both the Gerontology and the Geriatrics have to learn a lot from each other. The prevention of the frailty syndrome is an excellent example for cooperation between both disciplines. Prevention of frailty and reversing of pre-frailty in non-frailty is really a major challenge for mankind today (1-4). The General Practitioners have here a major role to play (1). They have to detect the Pre-Frailty situations in their patients. The Geriatric Day Hospitals can help them to change the situation when detected. This will be the only way to stop the massive inflow of geriatric patients in the emergency departments as it is today. Public health recommendations to eat an optimal diet with the right amount of energy and proteins should be moved to the public domain (2).

- The increasing number of hip fractures: what is primary: osteoporosis or sarcopenia?

The ortho-geriatric wards are starting in many hospitals. The number of hip fractures is increasing. Many geriatric patient have an extreme low vitamin D concentration, what could be easily corrected. Vitamin D shortage has a negative effect as well on the calcium resorption as on the incorporation of calcium in the muscle stem cells (3). The very expensive treatment of osteoporosis has till today no significant effect on the occurrence of hip fractures on population level. We see

indeed in the geriatric patients very frequently extreme situations of cachexia and sarcopenia. Undernutrition is a major problem in this population group. Not only their fat is burned, but also their muscles.

In many cases they have also inactivity: a typical aspect of geriatric patients; This also diminish the muscle strength and endurance. Treatment of these problems will also diminish the degree of osteoporosis.

The key question is "Osteoporosis and sarcopenia: two diseases or one?" (4).

- All the Geriatric patients admitted in a general hospital have to be admitted in geriatric wards?

In the General Hospitals we find today patients with geriatric profile in nearby all the departments. This was the situation 50 years ago with paediatrics. We found in the sixties children in all departments, between older and very old patients. Now this is no longer the case worldwide. Children are concentrated in the Paediatric departments, cared for by specialised paediatric nurses and paediatricians. Concentration of people with geriatric profile is necessary, because it is now proven by many studies that the results of this approach is much more rewarding than the conventional approach mixing all patients profile (not the age as such). The care by specialised geriatricians and the geriatric multidisciplinary team appears to be very efficient (5).

- Is the always earlier retirement age one of the important cause of the important increase in incidence and prevalence of Alzheimer Dementia?

The increase in prevalence and incidence of Dementia is a major problem in geriatric medicine today. as always in Medicine, prevention is more rewarding than cure (the major example is the high degree of efficiency of vaccination). Major questions are raising up: why this increase? What was modified in our society recently?

All papers are now proving that prevention of Alzheimer disease includes regular physical and mental activity.

In contrast we see more and more people retiring earlier, without any residual activity. The question is raising why we should not abolish the fixed mandatory retirement age and change it in a flexible age, with progressive adapted decrease in activity over the time. Recently some publications are making the possible link between later retirement and delaying of the onset of Alzheimer Dementia (6-8). Longer professional activity is in any case a major guarantee for physical and mental activity.

It has to be mentioned that some people appealed to the European Court of Justice in Luxemburg, with the result that the court concluded that the mandatory age related retirement is a real discrimination by age, but was not able to change this.

- How to stop the polypharmacy and inappropriate prescription for geriatric patients?

Polypharmacy is still an increasing major problem in the healthcare of geriatric patients. This was already mentioned in papers in the sixties, but the problem is increasing and is not solved. More and more "organ specialists" are treating geriatric patients and all of them likes to prescribe a lot of medicines. Each "organ specialist" is considering his speciality as the most important.

Good choices have to be made by the General Practitioner of the patient, and if too complex, the geriatrician's advice can be very useful. Some geriatric departments have started special out-patient clinics for this problem with success.

In many cases these prescriptions are not appropriate for the geriatric patient. The former Beers list (USA) and the recently published adapted to the European region STOPP-and START (9, 10) is now good disseminate but has to be introduced in the prescription software programmes to facilitate the generalisation.

-How to start with clinical trials in very old frail patients?

All geriatricians are now convinced that the principle of "start low, go slow" in geriatric patients is a good one, but that is not enough to avoid complications due to the medication side effects and medication interactions in these geriatric patients (11).

The situation is improving: we see more clinical trials with "fit older people". Clinical trials however with the "real frail geriatric patients" are still lacking. This is unacceptable and in contradiction with what has been decided in the European Parliament. The Eu-

ropean Parliament adopted a regulation that clinical trials have to be performed in "real patients".

Again what was started for the Paediatrics has also to be started up for the geriatrics: to start in the EMA (European Medicine Agency) with a Geriatric Committee as there is a Paediatric Committee (12).

References

1. Martin-Kleisch A, et al. Feasibility of assessing frailty in general medicine patients aged over 65. Rev. Epidemiol Sante Publique 2019 Mar 7, pii S0398-7620(19) 30171-3.
2. Cruz-Jentoft AJ, et al. Nutritional interventions to prevent frailty. Curr opin Clin Nutr Metab Care 2019 May; 22(3): 191-5. Doi 10.1097/MCO.0000000000000556.
3. Scimeca M, et al. Vitamine D receptor in muscle atrophy of elderly patients: a key element of osteoporosis-sarcopenia connection. Aging and Disease 2018 dec 9; (6): 952-064.
4. Reginster JY, et al. Open Clin Nutr Metab Care 2018; 19: 31-36.
5. Rubenstein L. The cklinical effectiveness of multidimensional geriatric assessment. J Am Ger Soc 1983 dec 31; (12): 578-62.
6. Lupton MK, et al. Int J Geriatric Psychiatry 2010 Jan; 25 (1): 30-6. doi.10.1002/gps.2294.
7. Grotz C, et al. Plos One DOI:10.1371/journal pone.0115056 february 25, 2015
8. Dufouil C, Pereira E, Chêne G, Glymour MM, Alpérovitch A, Saubusse E, Risse-Fleury M, Heuls B, Salord JC, Brieu MA, Forette F. Older age at retirement is associated with decreased risk of dementia. Eur J Epidemiol 2014 May; 29 (5): 353-61. doi: 10.1007/s10654-014-9906-3. Epub 2014 May 4.
9. Conejos M. et al. Potentially inappropriate drug prescription in older subjects across health care settings. European Geriatric Medicine 2010; 1: 9-14.
10. O'Mahony D, et al. STOPP/START criteria for potentially inappropriate prescribing in older people: version 2. Age and Ageing 2015; 44: 213-218.
11. Topinkova E, et al. Evidence-based strategies for the optimization of pharmacotherapy in older people. Drugs Aging 2012 Jun 1; 29 (6): 477-94.
12. Baeyens JP. The European Medicine Agency discovers the Geriatric Patient. Drugs Aging 2011; 28 (11): 849-851.

Acta Biomed 2019; Vol. 90, N. 2: 187-196 DOI: 10.23750/abm.v90i2.8374

Original article

Efficacy of 12-weeks velpatasvir plus sofosbuvir-based regimen in HCV-naive subjects with mild fibrosis: a meta-analysis

Mariantonietta Pisaturo[1], Antonio Russo[1], Lorenzo Onorato[1], Nicola Coppola[1,2]

[1] Department of Mental Health and Public Medicine - Infectious Diseases Unit, University of Campania Luigi Vanvitelli, Italy; [2] Infectious Diseases Unit, AORN Caserta, Italy

Summary. *Background and aims:* In literature systematic data on treatment with the fixed-dose combination of sofosbuvir and velpatasvir for 12 weeks in anti-HCV/HCV RNA positive subjects with mild fibrosis and naïve to previous Interferon free regimen are scanty. A meta-analysis has been performed to evaluate the efficacy of velpatasvir plus sofosbuvir combination in these patients. *Methods:* All randomized or non-randomized studies, investigating the sustained virological response rate to sofosbuvir plus velpatasvir without ribavirin for 12 weeks in subjects naïve to previous DAA therapy and with fibrosis F0-F2 or F0-F3, were included in the meta-analysis. *Results:* A total of 16 studies enrolling 4,907 subjects met the inclusion criteria and were included in this meta-analysis. The prevalence of SVR by sofosbuvir and velpatasvir was 98% (95% CI 96-99%) in the 4,907 subjects without cirrhosis. The prevalence of SVR was similar considering the 9 clinical studies and the 7 real-world studies (98%, CI 95%: 96-99% and 98%; CI 95%: 96-99%, respectively). Considering the 4 studies enrolling 1,371 subjects without advanced liver fibrosis the prevalence of SVR was also high [96% (95% CI: 94-98%)]. Data indicate a prevalence of SVR ranging to 95-100% according to the different HCV genotypes. *Conclusion:* Sofosbuvir plus velpatasvir therapeutic regimen was highly effective in HCV patients without advanced liver disease naïve to previous DAA regimen independently the different HCV genotypes. (www.actabiomedica.it)

Key words: velpatasvir, sofosbuvir, hepatitis C, mild fibrosis, HCV infection, initial fibrosis

Introducion

The World Health Organization has estimated that 71 million people are infected with hepatitis C virus (HCV) worldwide and that more than 399,000 people die each year of HCV-related liver diseases (1).

Since 2014 regimens without interferon, which combine several classes of directly acting antiviral agents (DAAs), have improved the response rate and tolerability. Nowadays, thanks to the high and rapid effect of the DAAs regimen, Interferon-free regimens yield a sustained virological response rate at week 12 (SVR12) of approximately 95%, even in patients with advanced liver diseases (2, 3).

Among DAAs, the NS5B nucleotide inhibitor (sofosbuvir) is effective agains all HCV genotypes with a favorable safety profile and a low risk for development of resistance; velpatasvir is an inhibitor of the HCV NS5A protein with a potent activity against all HCV genotypes. Several randomized controlled trails (RCTs) have evaluated the efficacy of this combination (sofosbuvir plus velpatasvir) with or without ribavirin in the treatment of different HCV genotypes showing a high efficacy. Thus, treatment-naïve and treatment-

experienced patients infected with different HCV genotypes, without cirrhosis or with compensated (Child-Pugh A) cirrhosis, could be treated with the fixed-dose combination of sofosbuvir and velpatasvir for 12 weeks (2, 3).

Few data are available in literature on the efficacy of this combination in subjects without advanced liver disease, when ribavirin is not indicated, especially in real-word scenario. Thus, a meta-analysis has been conducted to evaluate the efficacy of velpatasvir plus sofosbuvir combination without ribavirin for 12 weeks, assessed as sustained virological response at week 12 after the stop of therapy, in anti-HCV/HCV RNA positive subjects without advanced fibrosis and naïve to Interferon-free regimen.

Methods

Search strategy and selection criteria

The present systematic review and meta-analysis was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and of the Meta-Analysis of Observational Studies in Epidemiology (MOOSE).

Two researchers (LO and AR) conducted a comprehensive computerized literature search to identify original reports using MEDLINE and the Cochrane Library from January 2015 to March 2019, involving both medical subject heading (MeSH) terminology and relevant keywords for search strings to locate articles that analyzed the efficacy of velpatasvir plus sofosbuvir combination in anti-HCV/HCV RNA positive subjects without cirrhosis and advanced fibrosis and naïve to Interferon-free regimen.

The following items were used to search the studies: "Velpatasvir", "HCV infection", "HCV hepatitis". In addition, the reference lists of all studies meeting the inclusion criteria, of the studies excluded and of the published review articles were manually searched to identify any other study that might merit inclusion.

All studies included had to fulfill the following characteristics and inclusion criteria: (a) they presented original data from randomized or non-randomized trials; (b) they investigated the efficacy of sofosbuvir plus velpatasvir without ribavirin for 12 weeks in subjects without cirrhosis or advanced fibrosis, naïve to previous DAA therapy; (c) identified fibrosis by liver histology according to Metavir score (F0-F3 score for patients without cirrhosis and F0-F2 for those without advanced fibrosis) or Fibroscan (Transient Elastography-TE <12.5Pa for patients without cirrhosis and TE <9.5 for those without advanced fibrosis) or FIB-4 (score<3.25 for patients without cirrhosis and <1.45 for those without advanced fibrosis) or APRI (score <1 for patients without cirrhosis and <0.70 for those without advanced fibrosis) or Fibro-test (score <0.75 for patients without cirrhosis and <0.58 for those without advanced fibrosis); (d) report the primary outcomes clearly defined as Sustained Virological Response 12 (SVR), undetectable HCV RNA 12 weeks after therapy completion; (e) were available as a full text manuscript; (f) were written in the English language, and (g) were published online and indexed up to March 2019. The exclusion criteria of the meta-analysis were: (a) meta-analyses, letters, reviews, meeting abstracts, or editorial comments; (b) studies using ribavirin; (c) investigating patients with advanced liver fibrosis or cirrhosis did not reporting separate data for mild fibrosis. If more than one publication dealt with the same patient population and offered the same outcome messages, only the most recent or most complete article was included in the analysis.

Data extraction

Two reviewers (LO and AR) working independently extracted the data using a standard protocol and data-collection form according to the inclusion criteria. The following relevant information was collected from every article selected according to the inclusion criteria: last name of the first author, year of publication, country where the population was investigated, study design, sample size, participant characteristics (age range, sex), HCV genotype, type of methods used to stage liver disease, the achievement of SVR according to the stage of liver disease (patients without cirrhosis or with advanced liver disease). The discrepancies between these reviewers were resolved with discussion. The corresponding author was contacted via email if the data presentation was incomplete or if it

was necessary to resolve an apparent conflict or inconsistency in the article.

Statistical analysis

We estimated the SVR rate of velpatasvir plus sofosbuvir combination, in anti-HCV/HCV RNA positive subjects without cirrhosis and advanced fibrosis and naïve to Interferon-free regimen, based on data from all eligible studies together with 95% confidence intervals (CIs).

Statistical heterogeneity between studies included in the meta-analysis was assessed using the Cochran Q test, and the proportion of total variation in study estimates due to heterogeneity was quantified with the I^2 statistic. I^2values between 25% and 49% indicated low heterogeneity, between 50% and 75% indicated moderate heterogeneity and an I^2 value of 75% or above indicated high heterogeneity (4). For heterogeneity, a threshold p value less than 0.1 was considered statistically significant. The Mantel-Haenszel method for a fixed-effects model was applied in the absence of heterogeneity between the studies (Q-statistic: p>0.1 and I^2< 50%) (5), otherwise, the DerSimonian and Laird method for a random-effects model was used if substantial heterogeneity was detected (Q-statistic: p<0.1 or I^2>50%) (6). Subgroup analyses were additionally conducted based on the type of study enrolled (clinical studies vs. real-world studies) and HCV genotype (HCV genotype 1 or 2 or 3 r 6). Potential publication bias was assessed by visual inspections of the Begg funnel plots (6). A two-tailed p value of less than 0.05 was considered statistically significant. All statistical analyses were performed using Stata/IC, version 15.1 software (Stata Corporation, College Station, TX, USA).

Ethics Statement

Approval for the specific study was not required. However, all procedures used in the study were in accordance with the current international guidelines, with the standards on human experimentation of the Ethics Committee of the Azienda Ospedaliera of the University of Campania, Italy, and with the Helsinki Declaration of 1975, revised in 1983.

Results

Literature search

Figure 1 shows a flow diagram of the process of identification and selection of the articles included in the meta-analysis. A total of 1,103 potentially relevant articles were identified from the search of electronic databases. Of these, 1,050 articles were excluded after the first screening based on the title and abstracts, 53 were considered potentially valuable and full texts were retrieved for detailed evaluation. After further evaluation and manual search of the bibliography references of the relevant publications, a total of 16 articles met (7-22) the inclusion criteria and were included in this meta-analysis.

Study characteristics

The main characteristics of the 16 studies included in the meta-analysis are summarized in Table 1; 12 studies (8-12, 16-22) enrolled evaluated the SVR only in subjects without cirrhosis, 4 (7, 13, 14, 15) evaluated the SVR both in subjects without advanced liver disease and in those without cirrhosis. The number of patients per study ranged from 21 to 3,721 subjects, with a total of 6,453 subjects enrolled: 4,907 patients meet inclusion criteria for the definition of "patients without cirrhosis" and 1,371 patients meet the criteria for the definition of "patients without advanced fibrosis".

All the 6,453 patients enrolled were treated with sofosvuvir (400 mg/die) plus velpatasvir (100 mg/die)

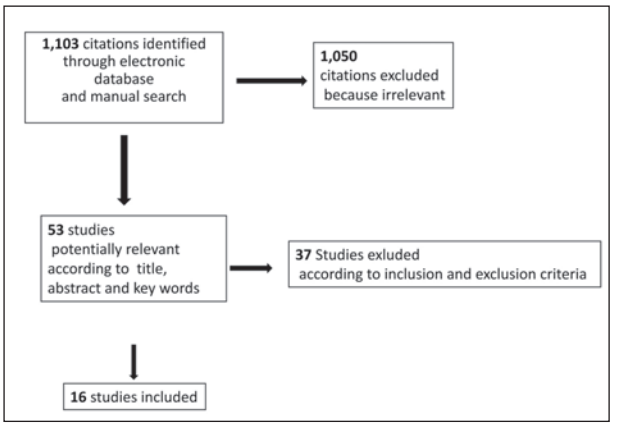


Figure 1. Flow-chart of article selection

Table 1. Characteristic of studies included in the meta analysis

First Author, year [Reference No.]	Country	Type of Study	No. of Patients	Age, mean (SD)	Males (%)	n° (%) with HCV genotype 1a, 1b, 2, 3, 4, 5, 6	Methods for liver fibrosis	N° SVR/pts without cirrhosis	N° SVR/pts without advanced fibrosis
Belpiero, 2019 (7)	USA	Real-word study	3,792	GT2: 62.9 (8.1) VEL/SOF; 63.4 (6.4) VEL/SOF+RIBA; GT3: 56.9 (10.9) VEL/SOF, 61 (6.7) VEL/SOF+RIBA	2273(96) GT2 VEL/SOF, 252 (98.4) GT2 VEL/SOF+RIBA;1360 (95.5) GT3 VEL/SOF, 442 (97.1) GT3 VEL/SOF+RIBA	2939 (51) HCV genotype 2; 2824 (49) HCV genotype 3	FIB-4	2,707/2,881^	1,071/1,134°
Von Felden, 2017 (8)	Germany	Real-word study	293	48(18-77)£	205(70)	293(100)	Liver histology, APRI, TE	158/163^^	Not reported
Wyles, 2017* (9)	USA	Open-label study	106	54(25-72)£	91(86)	66(63) HCV genotype 1a, 12(11) HCV genotype 1b, 11(10) HCV genotype 2, 12(11) HCV genotype 3, 5(5) HCV genotype 4	liver histology, TE, FibroTest, APRI.	82/87	Not reported
Hu, 2018 (10)	China	Real-word study	31	42.7(15.2)	12(39)	12(38.7) HCV genotype 1b, 6(19.4) HCV genotype 2a, 5(16.1) HCV genotype 3a, 5(16.1) HCV genotype 3b, 3(9.7) HCV genotype 6a	Not reported	21/21	Not reported
Gayan, 2018** (11)	USA	Real-word study	78	60.7(28-94)£	53(67.9)	60(76.9) HCV genotype 1a, 18(23.1) genotype 1b	Fibrosure score, liver histology	68/69	Not reported
Isakov, 2018 (12)	Russia, Sweden	Open-label study	119	44(18-71)£	50(50)	8 (7) HCV genotype 1a, 70 (59) HCV genotype 1b, 7 (6) HCV genotype 2, 34 (29) HCV genotype 3	Liver histology, TE, Fibro-test, APRI	96/97	Not reported
Grebely, 2018*** (13)	Australia, Canada, New Zeland, Norway, Switzerland, United Kingdom	Open-label study	103	48(41-43)#	74(72)	35 (34) HCV genotype 1a, 1 (1) HCV genotype 1b, 5 (5) HCV genotype 2, 60 (58) HCV genotype 3, 2 (2) HCV genotype 4	TE	82/86	57/59°°

Table 1. *(Continued)* Characteristic of studies included in the meta analysis

Liu, 2018**** (14)	Taiwan	Real-word study	228	60 (12) no HIV-subjects; 40(10) in HIV-subjects	70(44.0) no HIV-patients; 67(97.1) in HIV subjects	21 (9) HCV genotype 1a, 92 (40) HCV genotype 1b, 89 (39) HCV genotype 2, 7 (3) HCV genotype 3, 3 (1) HCV genotype 4, 16 (7) HCV genotype 6	TE	171/175	144/148°
Nguyen, 2019 (15)	USA	Real-word study	43	65.6(9.8)	19(44.2)	43(100) HCV genotype 6	Liver histology, Fibrotest, TE	38/38	30/30°
Wei, 2018 (16)	China, Thailand, Vietnam, Malaysia, Singapore	Open-label study	375	45(36-54) £	197(53)	22 (6)HCV genotype 1a, 107 (29) HCV genotype 1b, 64 (17) HCV genotype 2, 84 (22) HCV genotype 3, 98 (26) HCV genotype 6	Liver histology, Fibrotest, TE	302/308	Not reported
Wu, 2019 (17)	China	Open-label study	23	41(25-76) £	16(26.67)	23(100) HCV genotype 6	APRI, TE, FIB-4	23/23	Not reported
Sood, 2019 (18)	India	Open-label study	129	42(19-75) £	76(59)	6 (5) HCV genotype 1a, 22 (17) HCV genotype 1b, 90 (70) HCV genotype 3, 7 (5) HCV genotype 4, 1 (1) HCV genotype 6	Not specified	79/87^^^	Not reported
Tao, 2018 (19)	China	Cohort study	21	37 (33.10-41.67)	13(62)	21 (100) HCV genotype 3	TE	16/16	Not reported
Everson, 2015 (20)	USA	RCT	77	Genotype 1 49 (20-68) £, Genotype 3 50 (20-70) £, Genotype 4-6 54 (23-70) £	Genotype 1 17 (61), Genotype 3 17 (63), Genotype 4-6 15 (68)	28 (36) HCV genotype 1, 27 (35) HCV genotype 3, 22 (29) HCV genotype 4-6	Liver hisology, TE, APRI, FibroTest	74/77	Not reported
Feld, 2015 (21)	USA, Canada, Europe, Hong Kong	RCT	624	54 (18-82) £	374(60)	210 (34) HCV genotype 1a, 118 (19) HCV genotype 1b, 104 (17) HCV genotype 2, 116 (19) HCV genotype 4, 35 (6) HCV genotype 5, 41 (7) HCV genotype 6	Liver hostology, FibroTest, TE	496/501^^^^	Not reported
Foster 2015 (22)	USA, Canada, Europe	RCT	413	Genotype 2 57(26-81) £, Genotype 3 49(21-76) £	Genotype 2 86(64), Genotype 3 170(61)	134 (33) HCV genotype 2, 277 (67) HCV genotype 3	Liver histology, FibroTest, TE, APRI	274/278	Not reported

TE: transient elastography, RCT: randomized controlled study; £ mean (range), # median(IQR); *: in HIV-subjects; **: in Africa-American subjects; ***: in PWID; ****: 68 HIV-subjects; ^: 56 patients with a previous failure to Interferon-free regimen ; ^^: 5 patients with a previous failure to Interferon-free regimen ^^^: 1 patient with a failure to previous Interferon-free regimen; ^^^^: 23 patients with failure to previous Interferon-free regimen; °: patients with F0-F2 fibrosis score °°: patients with F0-F1 fibrosis score

for 12 weeks without ribavirin, except 54 patients in which ribavirin has been used (8).

All patients were naive to previous antiviral treatment, except 56 patients in the Belpiero study (7), 5 in Von Felden study (8), 1 in Sood study (18) and 23 in Feld study (21) who were previously treated with Interferon-free regimen. One study (9) enrolled only anti-HIV-positive patients, and one (11) only African-American subjects.

Considering the type of the studies, 7 were real-world studies (7, 8,10, 11, 14, 15, 19) and 9 clinical studies, specifically 6, were open-labelled trial (9, 12, 13, 16, 17, 18) and 3 randomized controlled trials (20-22) (RCTs).

Meta-analyses of the data

The results of the meta-analysis for the estimated prevalence of SVR are shown in Table 2. Considering all the 4,907 subjects without cirrhosis included in the 16 studies enrolled (7-22), the prevalence of SVR by a 12-week sofosbuvir plus velpatasvir-regimen was 98% (95% CI: 96-99%) (Table 2 and Figure 2). The prevalence of SVR was similar considering the 1,532 subjects from the 9 clinical studies (9, 12, 13, 16, 17, 18,

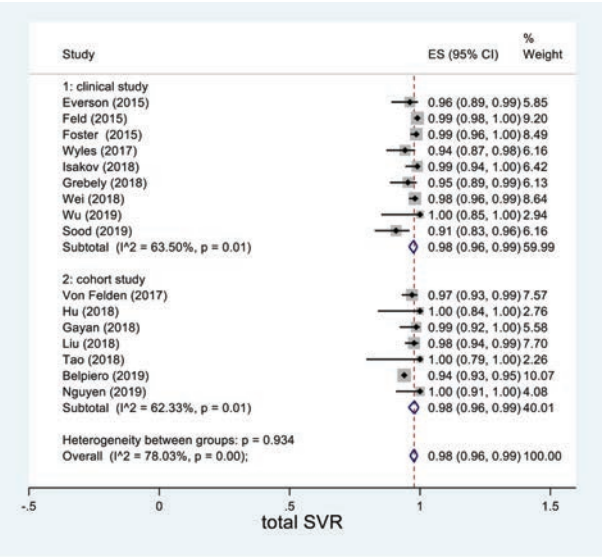


Figure 2. Meta-analysis of the prevalence of SVR in subjects without cirrhosis according to type of study

Table 2. Summary of meta-analysis results in the achievement of the sustained virological response by velpatasvir plus sofosbuvir in naïve patients with chronic hepatitis C and mild fibrosis

	N° of studies	N° of patients	N° of subjects with SVR	Summary of SVR prevalences (%)	95% CI (%)	Heterogeneity test (I^2%; p)
All subjects without cirrhosis	16 (7-21)	4,907	4,687	98	96-99	78; <0.0001
- In clinical studies	9 (9, 12, 13, 16, 17, 18, 20-22)	1,544	1508	98	97-99	63; 0.01
- In real-world studies	7 (7, 8, 10, 11, 14, 15, 19)	3,363	3179	98	96-99	62; 0.01
- with genotype 1	3 (11. 20, 21)	352	347	99	97-100	0; 0.9
- With genotype 2	2 (7, 21)	1,940	1,836	95	94-96	0; NR
- With genotype 3	6 (7, 8, 17, 19, 20, 22)	1,431	1,348	96	93-99	61.47; 0.02
- With genotype 6	3 (15, 17, 21)	96	96	100	98-100	0; 0.98
All subjects without advanced fibrosis	4 (7, 13, 14, 15)	1,371	1,302	96	94-98	35.81; 0.20

20-22) and the 3,363 subjects from the 7 real-world studies (7, 8, 10, 11, 14, 15, 19) (98%, CI 95%: 96-99% and 98%; CI 95%: 96-99%, respectively).

Similarly, considering the 4 studies (7, 13, 14, 15) enrolling 1,371 subjects without advanced liver fibrosis the prevalence of SVR was 96% (95% CI: 94-98%) (Table 2 and Figure 3).

Table 2 and Figures 3-6 show the prevalence of SVR considering HCV genotype stratification. Data indicate a prevalence of SVR of 99% (95% CI: 97-100%) in the 3 studies (11, 20, 21) enrolling 352 patients with HCV genotype 1 (Figure 4), of 95% (95% CI: 94-96%) in the 2 studies (7, 21) enrolling 1,940 patients with HCV genotype 2 (Figure 5), of 96% (95% CI: 93-99%) in the 6 studies (7, 8, 17, 19, 20, 22) enrolling 1,431 patients with HCV genotype 3 (Figure 6) and 100% (95% CI: 98-100%) in the 3 studies (15, 17, 21) enrolling 96 patients with HCV genotype 6 (Figure 7).

Heterogeneity was calculated among all studies using the I^2 test. As shown in Table 2, heterogeneity was found in all meta-analyses except for the meta-analyses in patients without cirrhosis and with genotype 1 or 2 or 6 and in those without advanced fibrosis (Table 2).

Visual inspection of the funnel plots and Egger's tests were performed to assess the potential publication bias of the studies included in this meta-analysis.

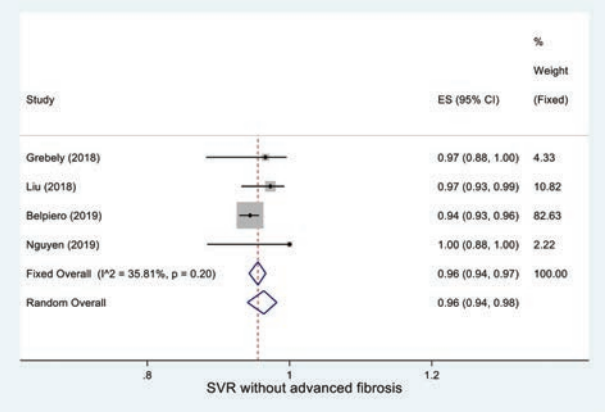


Figure 3. Meta-analysis of the prevalence of SVR in subjects without advanced fibrosis (F0-F2)

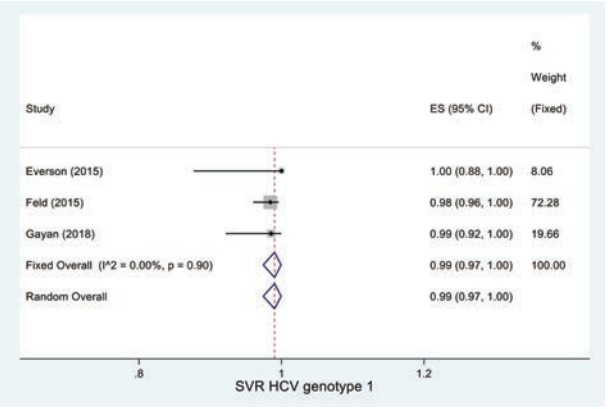


Figure 4. Meta-analysis of the prevalence of SVR in HCV genotype-1 subjects without cirrhosis

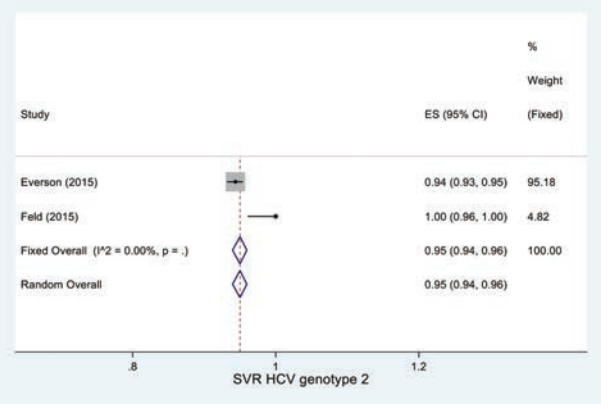


Figure 5. Meta-analysis of the prevalence of SVR in HCV genotype-2 subjects without cirrhosis

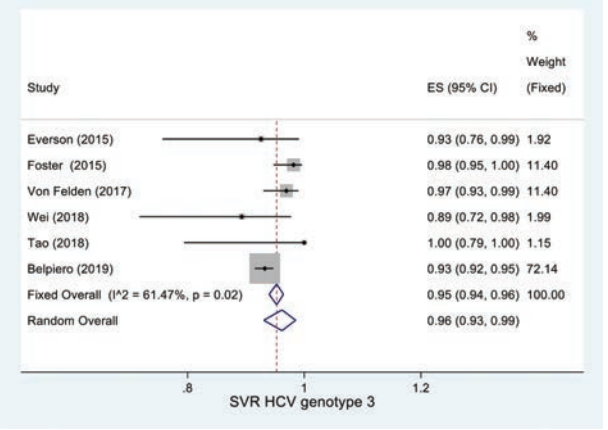


Figure 6. Meta-analysis of the prevalence of SVR in HCV genotype-3 subjects without cirrhosis

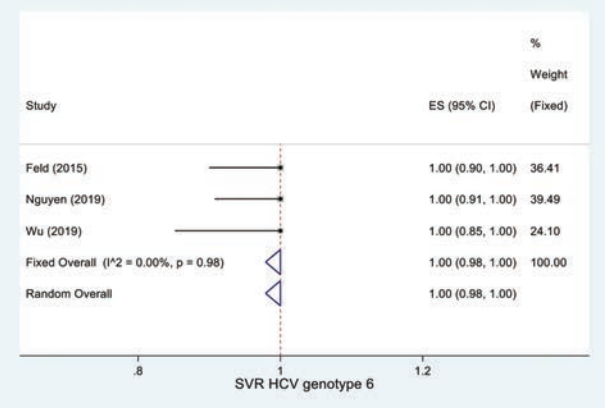


Figure 7. Meta-analysis of the prevalence of SVR in HCV genotype-6 subjects without cirrhosis

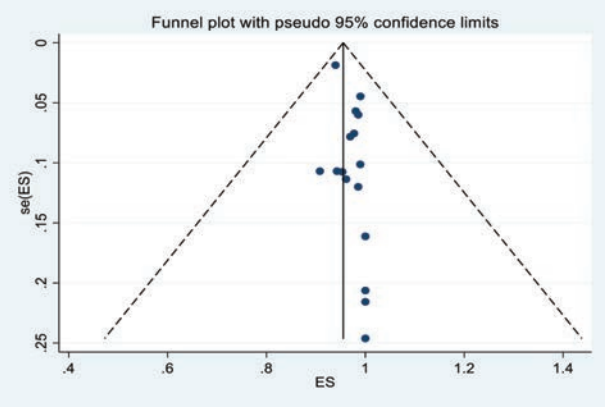


Figure 8. Funnel plot of the risk ratios vs. the reciprocal of their standard errors of all studies included in the meta-analysis

The shapes of the funnel plots did not reveal any clear evidence of obvious asymmetry in the analysis of the whole study (Figure 8). The Egger test results showed no significant statistical evidence of publication bias in the analysis of all studies included, which indicated a low risk of publication bias.

Discussion

The sofosbuvir plus velpatasvir combination is a powerful pan-genotypic regimen with a high genetic barrier against the emergence of resistance associated substitution (RAS) and consequently with high level of SVR regardless HCV genotypes. Moreover, this combination has an optimal safety profile, even for difficult-to-treat patients such as decompensated cirrhotic subjects (2, 3). However, few data are available in literature for patients with initial fibrosis, especially from real-word experiences.

Data of our meta-analysis analyzed in naïve patients with chronic HCV infection and mild fibrosis the efficacy of the single-tablet regimen of sofosbuvir plus velpatasvir without ribavirin, showing that it is highly effective in chronic HCV patients without cirrhosis (SVR12 rate = 98%) and in HCV patients without advanced liver fibrosis (SVR12 rate = 96%). Furthermore, it is of great interest to note that according to our study the prevalence of SVR was similar considering both clinical trials and real-world studies (98%, CI 95%: 96-99% and 98%; CI 95%: 96-99%, respectively). Therefore, a 12-week sofosbuvir plus velpatasvir-regimen is suitable for all stages of liver disease, as well demonstrated both by the data present in literature and by the correspondence between the results of clinical studies and real-life studies. The clarification that the rate of SVR was very high also in subjects with initial fibrosis and in real-word studies seems to be important, also considering that today most of HCV subjects starting DAA-regimen has not advanced liver fibrosis (24, 25).

Evaluating the stratification of the data according to the different HCV genotypes, the prevalence of SVR is high ranging to 95-100% also in HCV genotypes difficult-to-treat such as genotypes 1, 3 and 6 with a prevalence of SVR of 99%, 96% and 100% respectively, confirming international literature on this topics. Thus, Sofosbuvir plus velpatasvir regimen makes HCV treatment easier as the same therapy schedule are suitable for all the genotypes, irrespective of the fibrosis stage, making it a pangenotypic and panfibrotic regimen. Moreover, the single-pill, once-a-day posology improves the adherence to the therapy and the absence of lactose and gluten make it suitable to patients intolerant or allergic to these substances. Considering also the minimal drug-drug-interactions, this regimen may be consider a standard of care for the treatment of chronic HCV infection.

This meta-analysis has several strengths. First, a comprehensive literature search strategy was applied to minimize identification and selection bias and many

studies were identified as evaluating the prevalence of SVR in naïve subjects with chronic HCV infection without advanced fibrosis treated with sofosbuvir plus velpatasvir without ribavirin for 12-weeks. Second, the extensive amount of data reviewed. Third, in the present meta-analysis no between-study heterogeneity was observed. Heterogeneity is a potential problem when interpreting the results of all meta-analyses and finding the sources of heterogeneity is one of the most important goals.

However, there are some limitations which should be addressed when interpreting the findings of this meta-analysis. First, the findings are in part based on the results of observational studies and, therefore, as in observational studies themselves, recall and selection biases cannot be ruled out, and it is not possible to exclude potential confounding by various variables associated with exposure. Second, we did not search for unpublished studies, and this meta-analysis included only studies which were published in English and, as in any meta-analysis of published data, a publication bias may have occurred because small studies with null results tend not to be published, but there was no statistical evidence of a non-publication bias from the visualization of the funnel plot or from Egger's test.

Conclusion

Sofosbuvir plus velpatasvir therapeutic regimen was highly effective in HCV patients without advanced liver disease naïve to previous DAA regimen regardless the different HCV genotypes. Also considering that this combination is highly safe with a very low rate of severe adverse event such as identified both in clinical and real-word studies (7, 14, 19-22), it can therefore be considered a therapeutic regimen adaptable to all stages of liver disease and could be considered as well pan-genotypic as pan-fibrotic regime, confirmed not only by clinical trials but also by real life studies.

Author Contributions: MP was responsible for the conception and design of the study, assessed the quality of the studies, analysed the data and wrote the manuscript; AR: performed the literature search and data extraction; LO participated in the conception of the study, performed the literature search, data extraction, and assessed the quality; NC was responsible for the conception and design of the study, interpreted the data and wrote the manuscript. All authors read and approved the final manuscript

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Mohd Hanafiah K, Groeger J, Flaxman AD, et al. Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013 Apr; 57:1333-42.
2. EASL Recommendations on Treatment of Hepatitis C 2018. J Hepatol. 2018; 69: 461-511
3. AASLD-IDSA HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C. https://www.hcvguidelines.org
4. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959; 22: 719-48.
5. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7: 177-88.
6. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315: 629-34.
7. Belperio PS, Shahoumian TA, Loomis TP, Mole LA, Backus LI. Real-world effectiveness of daclatasvir plus sofosbuvir and velpatasvir/sofosbuvir in hepatitis C genotype 2 and 3. J Hepatol. 2019 Jan;70(1):15-23. doi: 10.1016/j.jhep.2018.09.018. Epub 2018 Sep 26.
8. von Felden J, Vermehren J, Ingiliz P, et al. High efficacy of sofosbuvir/velpatasvir and impact of baseline resistance-associated substitutions in hepatitis C genotype 3 infection. Aliment Pharmacol Ther. 2018 May;47(9):1288-1295. doi: 10.1111/apt.14592. Epub 2018 Mar 14.
9. Wyles D, Bräu N, Kottilil S, et al. Sofosbuvir and Velpatasvir for the Treatment of Hepatitis C Virus in Patients Coinfected With Human Immunodeficiency Virus Type 1: An Open-Label, Phase 3 Study. Clin Infect Dis. 2017 Jul 1;65(1):6-12. doi: 10.1093/cid/cix260.
10. Hu C, Yuan G, Liu J, Huang H, Ren Y, Li Y, Chen X, Li W, Wu T, Deng H, Peng Y, Zhang YY, Zhou Y. Sofosbuvir-Based Therapies for Patients with Hepatitis C Virus Infection: Real-World Experience in China. Can J Gastroenterol Hepatol. 2018 Nov 13;2018:3908767. doi: 10.1155/2018/3908767. eCollection 2018.
11. Gayam V, Tiongson B, Khalid M, et al. Sofosbuvir based regimens in the treatment of chronic hepatitis C genotype 1 infection in African-American patients: a community-based retrospective cohort study. Eur J Gastroenterol Hepatol. 2018 Oct;30(10):1200-1207. doi: 10.1097/MEG.0000000000001233.

12. Isakov V, Chulanov V, Abdurakhmanov D, et al. Sofosbuvir/velpatasvir for the treatment of HCV: excellent results from a phase-3, open-label study in Russia and Sweden. Infect Dis (Lond). 2018 Nov 30:1-9. doi: 10.1080/23744235.2018.1535186. [Epub ahead of print].
13. Grebely J, Dalgard O, Conway B, et al. Sofosbuvir and velpatasvir for hepatitis C virus infection in people with recent injection drug use (SIMPLIFY): an open-label, single-arm, phase 4, multicentre trial. Lancet Gastroenterol Hepatol. 2018 Mar;3(3):153-161. doi: 10.1016/S2468-1253(17)30404-1. Epub 2018 Jan 6.
14. Liu CH, Huang YJ, Yang SS, et al. Generic sofosbuvir-based interferon-free direct acting antiviral agents for patients with chronic hepatitis C virus infection: a real-world multicenter observational study. Sci Rep. 2018 Sep 12;8(1):13699. doi: 10.1038/s41598-018-32060-7.
15. Nguyen E, Trinh S, Trinh H, et al. Sustained virologic response rates in patients with chronic hepatitis C genotype 6 treated with ledipasvir+sofosbuvir or sofosbuvir+velpatasvir. Aliment Pharmacol Ther. 2019 Jan;49(1):99-106. doi: 10.1111/apt.15043. Epub 2018 Nov 22.
16. Wei L, Lim SG, Xie Q, et al. Sofosbuvir-velpatasvir for treatment of chronic hepatitis C virus infection in Asia: a single-arm, open-label, phase 3 trial. Lancet Gastroenterol Hepatol. 2019 Feb;4(2):127-134. doi: 10.1016/S2468-1253(18)30343-1. Epub 2018 Dec 14.
17. Wu DB, Jiang W, Wang YH, et al. Safety and efficacy of sofosbuvir-based direct-acting antiviral regimens for hepatitis C virus genotype 6 in Southwest China: Real-world experience of a retrospective study. J Viral Hepat. 2019 Mar;26(3):316-322. doi: 10.1111/jvh.13033. Epub 2018 Dec 3.
18. Sood A Duseja A, Kabrawala M, Amrose P, et al. Sofosbuvir-velpatasvir single-tablet regimen administered for 12 weeks in a phase 3 study with minimal monitoring in India. Hepatol Int. 2019 Feb 21. doi: 10.1007/s12072-019-09927-6. [Epub ahead of print].
19. Tao YC, Deng R, Wang ML, et al. Satisfactory virological response and fibrosis improvement of sofosbuvir-based regimens for Chinese patients with hepatitis C virus genotype 3 infection: results of a real-world cohort study. Virol J. 2018 Oct 1;15(1):150. doi: 10.1186/s12985-018-1066-8.
20. Everson GT, Towner WJ, Davis MN, et al. Sofosbuvir With Velpatasvir in Treatment-Naive NoncirrhoticPatients With Genotype 1 to 6 Hepatitis C Virus Infection: A Randomized Trial. Ann Intern Med. 2015 Dec 1;163(11):818-26. doi: 10.7326/M15-1000. Epub 2015 Nov 10.
21. Feld JJ, Jacobson IM, Hézode C, et al. Sofosbuvir and Velpatasvir for HCV Genotype 1, 2, 4, 5, and 6 Infection. N Engl J Med. 2015 Dec 31;373(27):2599-607. doi: 10.1056/NEJMoa1512610. Epub 2015 Nov 16.
22. Foster GR, Afdhal N, Roberts SK, et al. Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 Infection. N Engl J Med. 2015 Dec 31;373(27):2608-17. doi: 10.1056/NEJMoa1512612. Epub 2015 Nov 17.
23. Gentile I, Scotto R, Coppola C, et al. Treatment with direct-acting antivirals improves the clinical outcome in patients with HCV-related decompensated cirrhosis: results from an Italian real-life cohort (Liver Network Activity-LINA cohort). Hepatology International. 2019 Jan;13(1):66-74. doi: 10.1007/s12072-018-9914-6.
24. Viganò M, Andreoni M, Perno CF, et al. Real life experiences in HCV management in 2018. AL. Exp Rev Anti-Infect Ther. 2019;17(2):117-128.
25. Sagnelli E, Stroffolini T, Sagnelli C, et al. Characteristics of patients with hepatitis C virus-related chronic liver diseases just before the era of oral direct-acting antiviral therapy in Italy. Eur J Gastroenterol Hepatol 2018; 30(6):676-681

Received: 6 March 2019
Accepted: 16 April 2019
Correspondence:
Nicola Coppola
Department of Mental and Public Health,
Section of Infectious Diseases,
University of Campania
Via L. Armanni 5 - 80133 Naples, Italy
Tel. 081/5667719
Fax 081/5666207
E-mail: nicola.coppola@unicampania.it

Acta Biomed 2019; Vol. 90, N. 2: 197-208 DOI: 10.23750/abm.v90i2.6619

Review

From liposuction to adipose-derived stem cells: indications and technique

Francesco Simonacci, Nicolò Bertozzi, Michele Pio Grieco, Edoardo Raposio

Department of Medicine and Surgery, Plastic Surgery Division, University of Parma, Parma, Italy and the Cutaneous, Minin-vasive, Regenerative and Plastic Surgery Unit, Parma University Hospital, Parma, Italy

Summary. *Background and aim of the work:* Adipose tissue is an organ of energy storage, an endocrine organ, a soft tissue filler and a cosmetically unnecessary tissue discarded by liposuction. Liposuction was designed to correct unaesthetic deposits of subcutaneous fat; it produces satisfactory silhouette contouring when performed by appropriately trained operators using properly selected technologies. However, from lipoaspirate it is possible to obtain autologous fat graft and adipose-derived stem cells (ASCs) for reconstructive surgery and regenerative medicine. Autologous fat transplantation uses include the correction of body contour, malformations and post-surgical outcomes. The regenerative properties of ASCs allow treating damaged tissues such as wounds, burns, scars and radiodermatitis. The aim of this study was to perform a literature review highlighting the crucial role of adipose tissue in plastic and reconstructive surgery, from liposuction to lipofilling and ASCs, exposing the indications, procedures and complications of these surgical techniques. *Methods:* Literature review of publications concerning liposuction, lipofilling and adipose-derived stem cells (ASCS). *Results:* The introduction of liposuction allowed the use of adipose tissue for many clinical uses. The adipose tissue filling properties have been highlighted by the advent of lipofilling. The regenerative properties evidence of autologous fat transplantation encouraged the research on the clinical use of ASCs. *Conclusions:* Adipose tissue is not only the main energy storage of our body but also an important source of stem cells that can be used in various fields of regenerative medicine and tissue engineering with encouraging results for the future. (www.actabiomedica.it)

Key words: adipose tissue, liposuction, lipofilling, adipose-derived stem cells

Introduction

Adipose tissue has been considered an organ of energy storage, an endocrine organ, a soft tissue filler and a cosmetically unnecessary tissue discarded by liposuction. It is now also regarded as a promising source of adult stem cells, as adipose tissue has plenty of progenitor cells, some of which can differentiate into diverse lineages (1, 2). A component of fibroblast-like stromal cells obtained from liposuction aspirates can differentiate into various cell lineages, including adipogenic, osteogenic, chondrogenic, myogenic, cardiomyogenic and neurogenic. Thus, adipose tissue-derived stromal cells are now called adipose-derived stem cells (ASCs) and are expected to become a valuable tool for a wide range of cell-based therapies (1, 2). Liposuction is the surgical removal of subcutaneous fat by means of aspiration cannulas, introduced through small skin incisions, assisted by suction. Synonyms include liposuction surgery, suction- assisted lipectomy, suction lipoplasty, fat suction, blunt suction lipectomy, and liposculpture (3). Several variations of the technique have been described since. Its basic principles have been elaborated more recently by Illouz, who was

the first to introduce the modern, safe, and widespread method of liposuction with a blunt-tipped cannula as well as subcutaneous infiltration to facilitate adipose breakdown and aspiration (4-7). The procedure preserves neurovascular structures while maintaining fluid balance and minimizing patient discomfort (4, 5) Surprisingly, the basic principles remain unchanged despite the introduction of modern technologies enabling more efficient fat removal by enhancing liquefaction and disruption of the adipocyte membrane (4, 7) Despite the hard clear differentiation between aesthetic and therapeutic indications, liposuction is considered the main surgical technique in refinements of body contouring surgery in addition to other surgical procedures such as abdominoplasty, brachioplasty and thighplasty to name just a few (8-11). Furthermore, liposuction is the surgical technique by which it is possible to obtain the adipose tissue for autologous fat transplantation and the isolation of adipose-derived stem cells (ASCs). In 1893, Neuber performed the first autologous fat transplantation to fill in depressed scars. The liposuction technique, introduced by Fisher in 1974, followed by the tumescent technique, introduced by Klein in 1985, accelerated the development of the lipofilling technique. In 1987, Coleman introduced a new technique to decrease traumatic handling of fat during liposuction. His technique consisted of three steps: manual lipoaspiration under low pressure, centrifugation for 3 min at 3000 rpm, and reinjection in 3D. This technique remains the gold standard for liposuction and lipofilling, but has undergone some technical modifications (12). Autologous fat grafting is a technique shown to be beneficial as a reconstructive and cosmetic procedure for patients with volume loss due to disease, trauma, congenital defects, or the natural process of aging (12). By the early 1990s, more positive reports of fat grafting were published, including an improvement in skin quality, tissue quality, and scar revision, in addition to volume improvement (13). In fact, emerging evidence shows that fat tissue is a rich source of pluripotent stem cells named adipose-derived stem cells (ASCs) that have regenerative capacity in multiple tissues and diseases. ASCs are a plastic-adherent, multipotent stem cell population, which display a similar differentiation potential to other MSCs (mesenchymal stem cells), and the ability to differentiate into cells of several lineages from all three germinal layers (14, 15). The discovery that ASCs can readily be expanded and have the capacity to undergo adipogenic, osteogenic, chondrogenic, neurogenic and myogenic differentiation in vitro was a significant milestone in ASCs therapeutic applicability (15, 16).

Liposuction

Liposuction is the most commonly performed cosmetic surgical procedure worldwide. Originally designed to correct unaesthetic superficial and deep deposits of subcutaneous fat, it produces highly satisfactory silhouette contouring when performed by appropriately trained operators using properly selected technologies for well-selected patients and anatomical areas (5, 6, 17). Liposuction was initially was performed under general anaesthesia without any introduction of fluid, hence, called “dry liposuction”. Later, a small amount of fluid was introduced into the fat (the “wet technique”). These methods were associated with much blood loss, and patients frequently required blood transfusions. In 1985, Dr. Jeffrey A. Klein, revolutionised liposuction surgery when he developed the tumescent technique, which permits liposuction totally by local anaesthesia and with minimal surgical blood loss (18). Further modifications such as power-assisted liposuction (PAL), ultrasound assisted-liposuction (UAL) and laser assisted-liposuction (LAL) have been introduced with variable results. Despite these advances, the tumescent technique remains the worldwide standard of care for liposuction (3, 19). This technique involves subcutaneous infiltration of large volumes of crystalloid fluid called Klein’s solution, which contains low concentrations of lignocaine and epinephrine, followed by suction-assisted aspiration of fat by using small aspiration cannulae called micro-cannulae (20). Infiltration begins by creating a small stab incision, just enough to accommodate the infiltration needle. Blunt-tipped cannulas of varying lengths are used to infiltrate the fluid into the desired deep subcutaneous adipose layer, using either a hand piece or foot pedal to control administration (21). The suction cannula is introduced into the deep fat layer. The vacuum is activated and the cannula is pushed through the fat, creating a radial

pattern (21). Cross-hatching, or inserting the cannula from two different axes (usually perpendicularly), creates a smoother result (21). Connected to the aspirator (or sometimes a syringe), the liposuction cannula is placed through the insertion site while the nondominant hand (also known as 'the thinking hand') continually monitors the placement and trajectory of the cannula, enabling the surgeon to feel the progress in the area and to determine the end point of surgery (21) In general, blunt-tip cannulas are used to minimize perforation risk, and smaller diameter cannulas are used to minimize contour irregularities (22). Non–blunt-tip cannulas are typically used for breaking up scar or discontinuous undermining (22). Aspiration has been found to be directly proportional to cannula and suction-tubing diameter and inversely proportional to cannula and suction- tubing length (22). Specific depths of subcutaneous fat should be suctioned, which vary from different body locations and patient-specific goals (22). The syringe technique used blunt-tip suction cannulas connected to a syringe. In case of manual liposuction the drawing back the syringe plunger generates the negative pressures needed to remove fat during liposuction and replaces the electric vacuum pump and connecting tubing (22, 23). Power-assisted liposuction (PAL) is a commonly used technology that uses a variable-speed motor to provide reciprocating motion to the cannula which, in combination with the reciprocating action of the surgeon's arm, facilitates removal of adipose tissue (22,24) The principal advantages of power assisted liposuction is treatment speed, economy of motion, and reduced operator fatigue (22, 24). Ultrasound-assisted liposuction (UAL) uses ultrasound vibration of the cannula to break down connective tissue and emulsify fat (25). The thermal energy produced has been reported to help with skin tightening but also has been associated with higher rates of complications (25). Ultrasound is the process which turns electric energy into mechanical vibrations that cause thermal effects and micro-mechanical effects (acoustic) or cavitational effects in contracting and expanding circles. This causes microcavities in the fat tissue, which burst, resulting in cell destruction and fat liquefaction (26-28). The thermal effect is caused by acoustic waves, cannula friction, and the conversion of the ultrasonic waves into heat as they pass tissue (25) The heat must be dissipated by tissue infiltration (28). One of the most important aspects that distinguishes ultrasound-assisted liposuction from other methods of liposuction is the result on the postoperative haematocrit level (25). With ultrasound-assisted liposuction there is better vessel preservation and consequently, less haematocrit decrease (25). Another positive aspect of this technique is the possibility of greater skin retraction in the treated areas, as the increased local temperature stimulates collagen contraction. The disadvantages of ultrasound-assisted liposuction are the increased operative time and the training necessary for to efficiently use the technique and the equipment. In addition, swollen and fibrotic areas necessitate extended postoperative lymphatic drainage (25). Laser lipolysis is now a commonly used and accepted modality for removal of unwanted fatty tissue. Since its United States Food and Drug Administration (FDA) approval in October of 2006, studies have continued to corroborate early clinical observations of decreased adiposity, shorter recovery times, and improved skin tightening (29). Laser-assisted liposuction (LAL) can be used to treat defined areas in the body, with claims of producing skin tightening and thermal coagulation to minimize bleeding. Different kinds of LAL have recently been developed and some are still at the experimental stage. An initial type of LAL has been tested by Apfelberg (30, 31). The operator inserts the cannula (special design, single holed, 4-6 mm diameter), activates the suction, and then depresses the foot pedal to activate the laser. The negative suction draws the fat globule into the hole of the cannula where the laser beam (YAG laser 40W) shears it off bloodlessly. The well-established and reviewed skin-tightening effect is perhaps the most significant advantage of laser lipolysis (32, 33). Early reports regarding the lack of efficacy of skin tightening may be related to the steep learning curve of the procedure, inadequate energy application, or insufficient heat accumulation (29). The authors emphasize the goal internal temperature should range between 48 and 50°C and external temperature of treatment location approximately 38 to 40°C (29). Surgeons and patients should also remember that skin tightening continues to improve several months after laser irradiation due to the delayed nature of neocollagenesis. For large areas, laser lipolysis alone

may be inadequate for proper correction, and many surgeons still insist that laser lipolysis is an adjunctive treatment to liposuction rather than a liposuction replacement (29). The flexibility and thin calibre of the laser fiber cannula may inhibit the surgeon's ability to perceive the exact depth in the tissues. Since many surgeons perform suction aspiration in addition to laser lipolysis, the procedural time is increased. The cost of equipment certainly is an impediment to many physicians considering adding laser lipolysis to their menu of services (29). As reported in literature the main complications of liposuction are hypesthesia, paresthesias, edema, ecchymosis, hematoma, seroma and infection usually resolve quickly (21). The most common long-term complication is contour irregularity (21). It should be treated conservatively for at least 6 months (21). Autologous fat grafting, further liposuction or skin excision should be performed as needed (21). In January 2000, Grazer published an article in which he reported the fatal outcomes of liposuction using a census survey of cosmetic surgeons (21, 34, 35). Of those surveyed, 917 surgeons reported that from 1994-1997, 95 fatalities occurred after 496,245 lipoplasties (21,35). This yields a mortality rate of 1 in 5224 (<0.5%). This is similar to rates quoted elsewhere (35) Pulmonary thromboembolism was the major cause of death in 23.4 (±2.6%) of these deaths (35). The American Society of Plastic Surgeons recommends that outpatient lipoplasty be limited to 5000 ml of total aspirate, irrespective of the technique (21).

Lipofilling

Historically, the use of fat grafts to correct congenital deformities and complex traumatic wounds with soft-tissue loss after radical oncological surgery was proposed in 1893 by Neuber, by Hollander in 1912, by Neuhof in 1921, and by Josef in 1931 (36, 37). Fat is a filler with ideal properties: it naturally integrates into tissues, is autologous, and is 100% biocompatible. However, this is not the only function of lipofilling; fat is an active and dynamic tissue composed of several different cell types, including adipocytes, fibroblasts, smooth muscle cells, endothelial cells, and adipogenic progenitor cells called "preadipocytes" (38-40). ASCs (adipose-derived stem cells) in fat grafts allow the regeneration of damaged tissues through their paracrine, immunomodulatory, chemotactic, and differentiating effects (36). For this reason fat transplantation techniques have dramatically changed over the last two decades, from simple free transfers of intact adipose tissue, which had limited success in the consistent replacement of volume defects, to free composite fat-cell transplantation strategies that, if properly executed, could have a high regenerative potential for both simple volume replacement as well as functional enhancement of recipient tissues (36). It is widely accepted that less-traumatic methods of fat harvesting result in increased adipocyte viability and graft survival (41, 42). The most frequently used methods for fat harvesting are vacuum aspiration or syringe aspiration with or without the infiltration of tumescent fluid (43) (Fig. 1-2). No difference in cell viability, cell metabolic activity, or adipogenic response was found after harvesting fat by syringe liposuction compared with pump-assisted liposuction (44). The tumescent technique causes hydrodissection and enlarges the target fat layer, facilitating the subsequent aspiration and decreasing pain and ecchymosis (45). However, the "dry" technique may lead to a greater requirement for analgesics (45). Cannula size may also affect the viability of harvested fat (46). Campbell

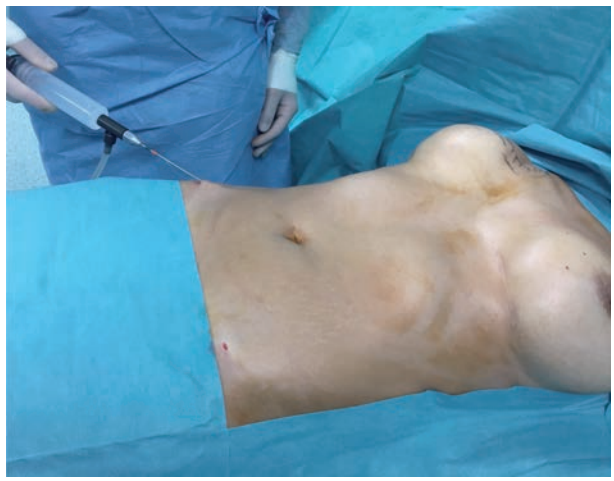


Figure 1. 40 year old patient subjected to mastectomy and reconstruction with implants. Klein's Solution introduced at the the donor site by means of a 50 ml syringe connected to a closed aspiration–injection system

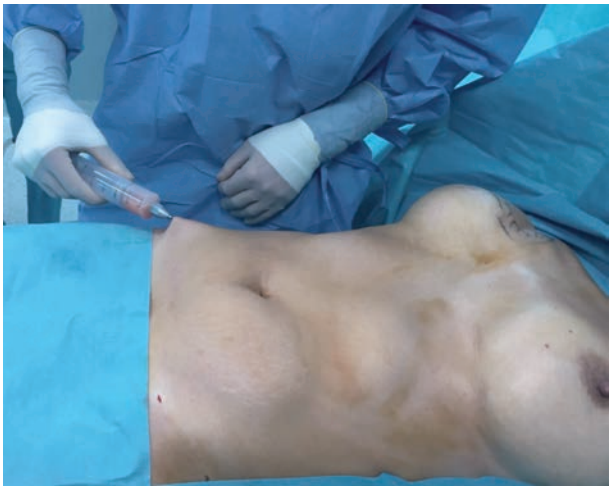


Figure 2. 40 year old patient subjected to mastectomy and reconstruction with implants. Lipoaspirate harvested using a 4 mm suction cannula and a 50 ml syringe connected to a closed aspiration–injection system, with a -650 mmHg vacuum

et al. found an inverse relationship between cellular damage and the diameter of the instrument used to extract fat (47). Erdim et al. (48) reported higher graft viability with lipoaspirates that were obtained using a 6-mm cannula rather than a 4-mm or 2-mm cannula. Coleman et al. (11) described a technique for fat harvesting that minimized trauma to the adipocytes. With a 3-mm, blunt-edged, 2-hole cannula connected to a 10-mL syringe, fat is suctioned manually by withdrawing the plunger. The cannula is pushed through the harvest site, as the surgeon uses digital manipulation to pull back on the plunger of the syringe and create a gentle negative pressure (11). A combination of slight negative pressure and the curetting action of the cannula through the tissues allows parcels of fat to move through the cannula and Luer-Lok aperture into the barrel of the syringe (11). When filled, the syringe is disconnected from the cannula, which is replaced with a plug that seals the Luer-Lok end of the syringe (11). The plunger is removed from the syringe before it is placed into a centrifuge (11). The most commonly used methods to process grafts are sedimentation, filtering, washing, and centrifugation. Fat processing is necessary because the lipoaspirate contains not only adipocytes but also collagen fibres, blood, and debris. These elements can cause inflammation at the recipient site, which can be detrimental for the fat graft (49). Blood must be extracted because blood accelerates the degradation of the transplanted fat (50). Centrifugation based processing resulted in higher ADSC numbers but decreased cell viability counts than decantation (51). Coleman suggested a processing method that has gained popularity and has been since integrated in many fat-transfer clinical protocols. Aspirated fat in syringes is spun at 3000 rpm for 3 min to isolate the fat (46) (Fig. 3). After the centrifugation, three layers are observed: the first layer includes lipids, which can be poured off using absorbent material; the second layer consists of fatty tissue; and the third layer contains blood, tissue fluid, and local anaesthetic and is ejected from the base of syringe (Fig. 4). The middle layer is routinely used for adipose tissue grafting (52-55). The identification of an optimal processing method will increase the number of viable cells and ultimately increase fat engraftment and retention over time. Through a skin incision of a size corresponding to the diameter of the cannula, the fat graft is inserted at the

Figure 3. 50 ml syringes with lipoaspirate placed inside the centrifuge

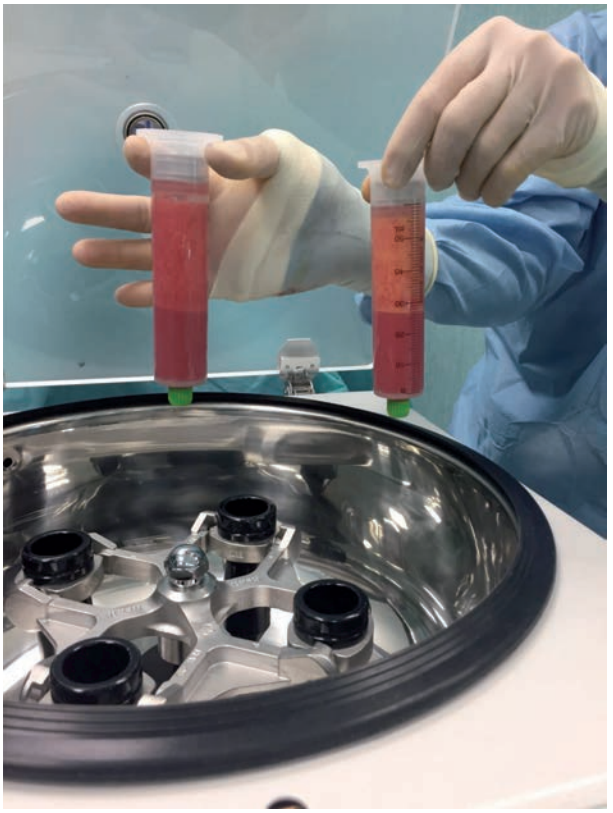


Figure 4. Lipoaspirate after centrifugation at 3000 rpm for 3 min. It is possible to observe three layers: the first consists of lipids; the second is composed of fatty tissue; and the third contains blood, tissue fluid, and local anaesthetic

level of the anatomical region affected (Fig. 5). Small-gauge cannulas are thought to reduce trauma to the recipient site, thus reducing the risks of bleeding, haematoma formation, and poor graft oxygen diffusion (41). Because revascularization starts at the periphery, ischaemic time is longer in the centre of the graft (49). Therefore, fat reinjection in multiple small-volume sessions is preferred over one single injection (49). Usually, through multiple access sites, multiple tunnels are created on insertion, but fat is injected only during withdrawal of the cannula in a "fanning-out" pattern (46). Ozsoy et al. (46) observed a greater vitality of adipose tissue if infiltrated with cannulas of at least 2.5 mm in diameter. However, Erdim et al. (48) found no significant differences in cell viability with differing needle gauge. Fat injection has been used for more than 20 years as a relatively low-risk and low-morbidity procedure to correct a variety of soft tissue defects

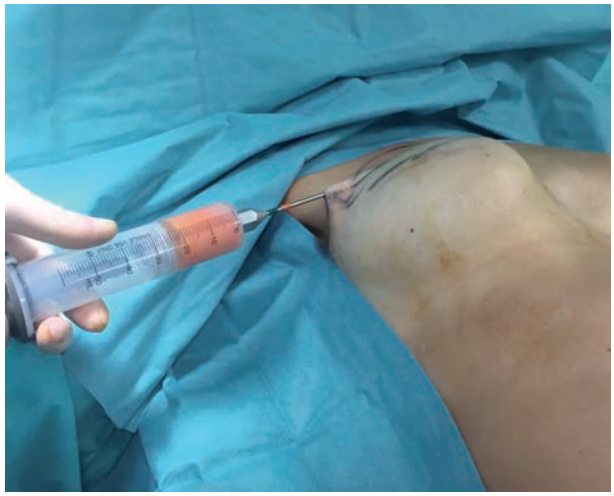


Figure 5. The injection of fat graft to restore the profile of the right breast

in the face, trunk, and extremities. Autologous fat transplantation represents a simple solution to restore the profile of the breast after reconstruction. In fact, in breast cancer surgery, lipofilling is usually used for the correction of defects and asymmetry following tumour excision (or breast conservative surgery), with/without radiotherapy (56). Acquired contour deformities of the reconstructed breast are relatively common and independent of the technique used, presenting a frequent therapeutic challenge to reconstructive surgeons (57). Primary breast reconstruction usually meets the goal of establishing a natural-appearing breast shape (57). However, in the immediate or late postoperative period, secondary contour defects of the reconstructed breast can develop (57, 58). There are important landmarks in the female breast, and the creation of a well-defined inframammary fold during breast reconstruction is a fundamental element in obtaining a good aesthetic result (59). Autologous fat transplantation represents a simple solution to restore the profile of the breast after reconstruction. Plastic surgeons and patients seeking breast reconstruction may have drastically different images in mind of what constitutes an attractive, natural, and ideal breast shape (60, 61).Autologous fat transplantation can be used to improve soft-tissue coverage following prosthesis or tissue expander implantation and the volume replacement of implants in unsatisfactory oncoplastic breast recon-

struction outcomes (62, 63). Other applications of autologous fat transplantation are volume augmentation and refinement after autologous flap or whole breast reconstruction with serial fat grafting or scar correction (56). Patients with retractile and painful scars compromising the normal daily activity/mobility of the joint involved can take advantage of lipofilling treatment (64). In fact, fat transplantation can be used not only to fill atrophic scars but also to reduce scar contracture as a regenerative alternative to other surgical techniques (65). This is made possible by the presence of ASCs in the fat tissue. Hypertrophic burn scars occur in approximately 75% of white patients with third-degree burns (66, 67). Burn outcomes still represent a problem because of aesthetic and functional concerns as well as concerns regarding the patient's social and psychological life (68). Subscar and intrascar fat grafting are relatively recent techniques that improve scar quality. Radiation dermatitis is caused by prolonged exposure of the skin to ionizing radiation (69). It can be seen in patients receiving radiation therapy, with or without adjuvant chemotherapy (70). Rigotti et al. (71) reported that the transplantation of lipoaspirates containing adult ASCs is a highly effective therapeutic approach for the treatment of degenerative, chronic lesions induced as late effects of oncologic radiation treatments. In fact, ultrastructural analysis of the radio-damaged tissue revealed a significant reduction of the capillary bed (71). Every step in fat transplantation, i.e., harvesting, processing, and transplantation, is important, but viability of the harvested fat cells is crucial (72). The chances of survival are higher the less the fat graft is manipulated and the more quickly it is reinjected (73). Early experience noted that graft re-absorption was the main drawback of autologous fat graft, with 50%-90% graft-loss rates being reported (74-77). Large grafts exhibit higher rates of liquefaction, necrosis, and cyst formation, while very small grafts tend to be reabsorbed (74). To ensure maximal take, many surgeons perform repeated transfers (74). Donor-site complications appear to be minimal and related to the liposuction technique. The possible complications include bruising, swelling, haematoma formation, paraesthesia or donor-site pain, infection, hypertrophic scarring, contour irregularities, and damage to the underlying structures for example due to the intraperitoneal or intramuscular penetration of the cannula (56). Fat tissue that is not perfused can die and result in necrotic cysts and even calcifications; however, these complications can occur after any type of breast surgery (78). It was thought that fat grafting to the breast could potentially interfere with breast cancer detection; however, no conclusive evidence of such interference has been found (79).

Adipose-derived stem cells

The emerging fields of regenerative medicine and stem cell-based therapies hold great promise for wound healing. Recently, many plastic surgeons have studied the potential clinical application of adipose-derived stem cells (ASCs), which represent a readily available adult stem cell population that has gathered a lot of attention in the field of regenerative medicine (13, 80, 81). Currently, ASCs are being investigated as a therapeutic strategy for a diverse group of pathological conditions, including hard-toheal wounds. Wound healing is not a series of individual and independent progressive steps, but a complex process involving inflammation, epithelialization, neoangiogenesis, proliferation, and collagen matrix formation (82-84). ASCs are part of the stromal vascular fraction (SVF) of adipose tissue, together with a heterogeneous population of many other cell types, including preadipocytes, endothelial cells, pericytes, haematopoietic-lineage cells, and fibroblasts (85). The regenerative features of the SVF are attributable to its paracrine effects: SVF cells secrete vascular endothelial growth factor, hepatocyte growth factor, and transforming growth factor-b in the presence of stimuli such as hypoxia and other growth factors (86, 87) and strongly influence the differentiation of stem cells, promoting angiogenesis and wound healing, and potentially aiding new tissue growth and development (88). Stem cells isolated from lipoaspirates have demonstrated a broad in vitro adipogenic, chondrogenic, osteogenic, and myogenic lineage commitment (89, 90) as well as differentiation into pancreatic cells, hepatocytes, and neurogenic cells (91-93). ASCs are similar to bone marrowe-derived stem cells in that they are capable of differentiating into multiple mesodermal tissue types and show similar surface pro-

tein marker expression (86, 94). Cytometric analysis of adipose-derived stem cells (ASCs) has shown that these cells do not express CD31 and CD45, but do express CD34, CD73, CD105, and the mesenchymal stem cell marker CD90 (95, 96). ASCs have a differentiation potential similar to that of other mesenchymal stem cells as well as a higher yield upon isolation and a greater proliferative rate in culture than bone marrow-derived stem cells (97-98). However, ASCs are different from bone marrowe-derived mesenchymal stem cells because they can be easily obtained using a standard wet liposuction procedure under local anaesthesia, without the need for expansion in culture (86). In 1964, Martin Rodbell (99) was the first to present a method for the in vitro isolation of mature adipocytes and adipogenic progenitors from rat fat tissue. In 2001, Zuk et al. (100) were the first to isolate ASCs from adipose tissue after a liposuction procedure by means of existing enzymatic strategies. Since then, interest in ASCs has grown dramatically and several groups working independently have developed procedures to isolate and characterize them (101). In 2016, Raposio et al (95, 96, 102) described a method that was specifically designed for clinical application, which appeared easy, safe, and fast (80 min), allowing collection of a ready-to-use ASC pellet. After a conventional liposuction, the harvested fat tissue (100 ml) was subjected to a first centrifugation (1600 RPM × 6 minutes), yielding about 50 ml of high quality concentrated adipose tissue. This was abruptly mixed with 50 ml collagenase digestion solution (Collagenase NB 6 GMP Grade 17458; Serva GmbH, Heidelberg, Germany), previously diluted with sterile phosphate-buffered saline (PBS) as follows: 1 g of collagenase was suspended in 10 ml PBS, and 1 ml of the obtained solution was further diluted with 49 ml of PBS. The solution obtained (lipoaspirate + collagenase digestion solution) was then incubated for 30 minutes at 37°C in a shaker-incubator (Celltibator; Medikhan) and centrifuged at 200 relative centrifuge force for 4 minutes. Subsequently, the 10 ml of SVF obtained was washed 2 times with 45 ml saline solution. After each wash, the syringes containing SVF were centrifuged at 200 relative centrifuge force for 4 minutes. The cellular pellet obtained at the bottom of the syringe was ready for use, vehiculated by 5 ml of saline solution. Several alternative isolation methods have been proposed, which avoid enzymatic digestion completely. Raposio et al (95, 96, 102) also described an effective alternative mechanical procedure in which the isolation process was performed using a vibrating shaker (Multi Reax; Heidolph, Schwabach, Germany) and a centrifuge (MPW 223; Johnson & Johnson Medical, New Brunswick, N.J.), both placed in a laminar air flow bench (1200 FLO; FIMS, Concorezzo, Italy;). After liposuction, the harvested fat tissue (80 ml) was collected in eight 10-ml plastic test tubes, positioned in the vibrating shaker at 6000 vibrations/minute for 6 minutes, and immediately centrifuged at 1600 rpm for 6 minutes. Subsequently, under the same laminar flow cabinet, the pellet at the bottom of each tube was collected by means of an automated pipetting system (Rota-Filler 5000; Heathrow Scientific, Nottingham, United Kingdom) and poured into a 10-ml Luer-Lock syringe. The entire isolation process lasted approximately 15 min (95,96,102). In 2006, Matsumoto et al. (103) provided evidence to support a novel method of autologous tissue transfer, which they named cell-assisted lipotransfer (CAL). CAL is the concurrent transplantation of aspirated fat and ASCs. In CAL, ASCs were supportively used to boost the efficacy of autologous lipoinjection (resulting in a higher survival rate and the persistence of transplanted fat) and to decrease the known adverse effects of lipoinjection, such as fibrosis, pseudocyst formation, and calcification (103). To date, approximately 130 active clinical trials investigating the potential of ADSCs are listed on the US National Institutes of Health Website (104). These clinical trials span a broad range of applications, such as soft tissue regeneration, skeletal tissue repair, ischemic injuries, myocardial infarction and immune disorders (including lupus, arthritis, Crohn's disease, multiple sclerosis, diabetes mellitus, and graft-versus-host disease). Other therapeutic targets that are being explored in clinical trials include intervertebral disc degeneration and pulmonary disease to name just a few (104).

Conclusion

Although adipose tissue is the main energy storage, when present in excess it is harmful to the body.

Liposuction remove excessive adipose tissue in pathological conditions such as obesity or refining the body contour in aesthetic surgery. In addition, liposuction makes it possible to get adipose tissue for use in aesthetic or reconstructive surgery and regenerative medicine. In fact, by exploiting the properties of adipose tissue, autologous fat transplantation allows to correct body contour, malformations and post-surgical outcomes. In addition, fat grafts can be used to treat damaged tissues such as burns, scars and radiodermatitis due to the regenerative properties of ASCs. By means the Isolation of ASCs from lipoaspirate it's possible use them in various fields of regenerative medicine and tissue engineering with encouraging results for the future.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Yoshimura K, Suga H, Eto H. Adipose-derived stem/progenitor cells: roles in adipose tissue remodeling and potential use for soft tissue augmentation. Regen Med 2009; 4: 265-273.
2. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002; 13: 4279-4295.
3. Coleman WP, Glogau RG, Klein JA, Moy RL, Narins RS, Chuang TY, Farmer ER, Lewis CW, Lowery BJ; American Acacemy of Dermatology Guidelines/Outcomes Committee. Guidelines of care for liposuction. J Am Acad Dermatol 2001; 45: 438-447.
4. Atiyeh B, Costagliola M, Illouz YG, Dibo S, Zgheib E, Rampillon F. Functional and Therapeutic Indications of Liposuction: Personal Experience and Review of the Literature. Ann Plast Surg 2015; 75: 231-245.
5. Berry MG, Davies D. Liposuction: a review of principles and techniques. J Plast Reconstr Aesthet Surg 2011; 64: 985-992.
6. Ma GE, Lei H, Chen J, Liu ZJ. Reconstruction of large hypertrophic scar on trunk and thigh by means of liposuction technique. Burns 2010; 36: 256-260.
7. Illouz YG. Body contouring by lipolysis: a 5-year experience with over 3000 cases. Plast Reconstr Surg 1983; 72: 591-597.
8. Grieco M, Grignaffini E, Simonacci F, Raposio E. Analysis of Complications in Postbariatric Abdominoplasty: Our Experience. Plast Surg Int 2015; 2015: 209173.
9. Grieco M, Grignaffini E, Simonacci F, Di Mascio D, Raposio E. Post-bariatric body contouring: our experience. Acta Biomed 2016; 87:70-75.
10. Grignaffini E, Grieco MP, Bertozzi N, Gandolfi M, Palli D, Cinieri FG, et al. Post-bariatric abdominoplasty: our experience. Acta Biomed 2015; 86: 278-282.
11. Grignaffini E, Grieco MP, Bertozzi N, Gandolfi M, Palli D, Cinieri FG, et al. Quality of life in post-bariatric surgery patients undergoing aesthetic abdominoplasty: Our experience. Surg Chrn 2016; 21:5-8.
12. Tabit CJ, Slack GC, Fan K, Wan DC, Bradley JP. Fat grafting versus adipose-derived stem cell therapy: distinguishing indications, techniques, and outcomes. Aesthetic Plast Surg 2012; 36: 704-713.
13. Coleman SR. Structural fat grafting: more than a permanent filler. Plast Reconstr Surg 2006; 118: 108S-120S.
14. Daher SR, Johnstone BH, Phinney DG, March KL. Adipose stromal/stem cells: basic and translational advances: the IFATS collection. Stem Cells 2008; 26: 2664-2665.
15. Bertozzi N, Simonacci F, Grieco MP, Grignaffini E, Raposio E. The biological and clinical basis for the use of adipose-derived stem cells in the field of wound healing. Ann Med Surg 2017; 20: 41-48.
16. Gimble J, Guilak F. Adipose-derived adult stem cells: isolation, characterization, and differentiation potential, Cytotherapy 2003; 5: 362-369.
17. Pelosi MA, Pelosi MA 2nd. Liposuction. Obstet Gynecol Clin North Am 2010; 37: 507-519.
18. Klein JA. The tumescent technique for liposuction surgery. AM J Cosmetic Surg 1987; 4: 1124-1132.
19. Lawrence N, Coleman WP. Liposuction. J Am Acad Dermatol 2002; 47: 105-108.
20. Venkataram J. Tumescent liposuction: a review. J Cutan Aesthet Surg 2008; 1: 49-57.
21. Mordon S, Plot E. Laser lipolysis versus traditional liposuction for fat removal. Expert Rev Med Devices 2009; 6: 677-88.
22. Chia CT, Neinstein RM, Theodorou SJ. Evidence-Based Medicine: Liposuction. Plast Reconstr Surg. 2017; 139: 267e-274e.
23. Hunstad JP. Tumescent and syringe liposculpture: A logical partnership. Aesthetic Plast Surg. 1995; 19: 321-333.
24. Fodor PB, Vogt PA. Power-assisted lipoplasty (PAL): A clinical pilot study comparing PAL to traditional lipoplasty (TL). Aesthetic Plast Surg. 1999; 23: 379-385.
25. Graf R, Auersvald A, Damasio RC, Rippel R, de Araújo LR, Bigarelli LH, Franck CL. Ultrasound-assisted liposuction: an analysis of 348 cases. Aesthetic Plast Surg 2003; 27: 146-153.
26. Ingra H, Satur N. Tumescent liposuction versus internal ultrasonic-assisted tumescent lipsuction. A side-toside comparison. Dermatol Surg 1997; 23: 1213-1218.
27. Lawrence N, Cox SE. The efficacy of external ultrasound-assisted liposuction: a randomized controlled trial. Dermatol Surg 2000; 26: 329-332.
28. Zocchi ML. Ultrasonic-assisted lipoplasty. Technical

refinements and clinical evaluations. Clin Plast Surg 1996; 23: 575-598.
29. McBean JC, Katz BE. Laser lipolysis: an update. J Clin Aesthet Dermatol 2011; 4: 25-34.
30. Heymans O, Castus P, Grandjean FX, Van Zele D. Liposuction: review of the techniques, innovations and applications. Acta Chir Belg 2006; 106: 647-653.
31. Apfelberg DB. Results of multicenter study of laser-assisted liposuction Clin Plast Surg 1996; 23: 713-719.
32. Di Bernardo BE, Reyes J. Evaluation of skin tightening after laser-assisted liposuction. Aesthet Surg J 2009; 29: 400-407.
33. Di Bernardo BE, Reyes J, Chen B. Evaluation of tissue thermal effects from 1064/1320nm laser-assisted lipolysis and its clinical implications. J Cosmet Laser Ther 2009; 11: 62-69.
34. Badin AZ, Moraes LM, Gondek L, Chiaratti MG, Canta L. Laser lipolysis: flaccidity under control. Aesthetic Plast Surg 2002; 26: 335-339.
35. Grazer FM, de Jong RH. Fatal outcomes from liposuction: census survey of cosmetic surgeons. Plast Reconstr Surg 2000; 105: 436-446.
36. Simonacci F, Bertozzi N, Grieco MP, Grignaffini E, Raposio E. Procedure, applications, and outcomes of autologous fat grafting. Ann Med Surg 2017; 20: 49-60.
37. Billings E Jr, May JW Jr. Historical review and present status of free fat graft auto transplantation in plastic and reconstructive surgery. Plast Reconstr Surg 1989; 83: 368-381.
38. Katz AJ, Llull R, Hedrick MH, Futrell JW. Emerging approaches to the tissue engineering of fat. Clin Plast Surg 1999; 26: 587-603.
39. Raposio E, Guida C, Baldelli I, Benvenuto F, Curto M, Paleari L, Filippi F, Fiocca R, Robello G, Santi PL. Characterization and induction of human pre-adipocytes. Toxicol In Vitro 2007; 21: 330-334.
40. Raposio E, Guida C, Coradeghini R, Scanarotti C, Parodi A, Baldelli I, Fiocca R, Santi PL. In vitro polydeoxyribonucleotide effects on human pre-adipocytes. Cell Prolif 2008; 41: 739-754.
41. Kakagia D, Pallua N. Autologous fat grafting: in search of the optimal technique. Surg Innov 2014; 21: 327-336.
42. Pu LL, Coleman SR, Cui X, Ferguson RE Jr, Vasconez HC. Autologous fat grafts harvested and refined by the Coleman technique: a comparative study. Plast Reconstr Surg 2008; 122: 932-937.
43. Simonacci F, Bertozzi N, Grieco MP, Grignaffini E, Raposio E. Autologous fat transplantation for breast reconstruction: A literature review. Ann Med Surg (Lond) 2016; 12: 94-100.
44. Leong DT, Hutmacher DW, Chew FT, Lim TC. Viability and adipogenic potential of human adipose tissue processed cell population obtained from pump-assisted and syringe-assisted liposuction. J Dermatol Sci 2005; 37: 169-176.
45. Klein JA. Tumescent technique for local anesthesia improves safety in large volume liposuction. Plast Reconstr Surg 1993; 92: 1085-1098.
46. Ozsoy Z, Kul Z, Bilir A. The role of cannula diameter in improved adipocyte viability: a quantitative analysis. Aesthet Surg J 2006; 26: 287-289.
47. Campbell GL, Laudenslager N, Newman J. The effect of mechanical stress on adipocyte morphology and metabolism. Am J Cosmet Surg 1987; 4: 89-94.
48. Erdim M, Tezel E, Numanoglu A, Sav A. The effects of the size of liposuction cannula on adipocyte survival and the optimum temperature for fat graft storage: an experimental study, J Plast Reconstr Aesthet Surg 2009; 62: 1210-1214.
49. Mojallal A, Foyatier JL. The effect of different factors on the survival of transplanted adipocytes. Ann Chir Plast Esthet 2004; 49: 426-436.
50. Sommer B, Sattler G. Current concepts of fat graft survival: histology of aspirated adipose tissue and review of the literature. Dermatol Surg 2000; 26: 1159-1566.
51. Condé-Green A, de Amorim NF, Pitanguy I. Influence of decantation, washing and centrifugation on adipocyte and mesenchymal stem cell content of aspirated adipose tissue: a comparative study. J Plast Reconstr Aesthet Surg 2010; 63: 1375-1381.
52. Gir P, Brown SA, Oni G, Kashefi N, Mojallal A, Rohrich RJ. Fat grafting: evidence-based review on autologous fat harvesting, processing, reinjection, and storage. Plast Reconstr Surg 2012; 130: 249-258.
53. Coleman SR. Facial augmentation with structural fat grafting. Clin Plast Surg 2006; 33: 567-77.
54. Wilson A, Butler PE, Seifalian AM. Adipose-derived stem cells for clinical applications: a review. Cell Prolif 2011; 44: 86-98.
55. Tuin AJ, Domerchie PN, Schepers RH, Willemsen JC, Dijkstra PU, Spijkervet FK, Vissink A, Jansma J. What is the current optimal fat grafting processing technique? A systematic review. J Craniomaxillofac Surg 2016; 44: 45-55.
56. Hamza A, Lohsiriwat V, Rietjens M. Lipofilling in breast cancer surgery. Gland Surg 2013; 2: 7-14.
57. Cigna E, Ribuffo D, Sorvillo V, Atzeni M, Piperno A, Calò PG, et al. Secondary lipofilling after breast reconstruction with implants. Eur Rev Med Pharmacol Sci 2012; 16: 1729-34.
58. Ribuffo D, Atzeni M, Serratore F, Guerra M, Bucher S. Cagliari University Hospital (CUH) protocol for immediate alloplastic breast reconstruction and unplanned radiotherapy. A preliminary report. Eur Rev Med Pharmacol Sci 2011; 15: 840-4.
59. Fan J, Raposio E, Wang J, Nordström RE. Development of the inframammary fold and ptosis in breast reconstruction with textured tissue expanders. Aesthetic Plast Surg 2002; 26: 219-22.
60. Raposio E, Belgrano V, Santi P, Chiorri C. Which is the Ideal Breast Size? Some Social Clues for Plastic Surgeons. Ann Plast Surg 2016; 76: 340-345.
61. Porro I, Schenone A, Fato M, Raposio E, Molinari E, Beltrame F. An integrated environment for plastic surgery support: building virtual patients, simulating interventions, and supporting intraoperative decisions. Comput Med Imaging Graph 2005; 29: 385-394.

62. Raposio E, Cicchetti S, Adami M, Ciliberti RG, Santi PL. Computer planning for breast reconstruction by tissue expansion: an update. Plast Reconstr Surg 2004; 113: 2095-2097.
63. Raposio E, Caregnato P, Barabino P, Gualdi A, Orefice A, Spagnolo A, Capello C, Santi PL. Computer-based preoperative planning for breast reconstruction in the woman with unilateral breast hypoplasia. Minerva Chir 2002; 57: 711-714.
64. Raposio E, Calderazzi F. Fat grafting for chronic heel pain following surgery for adult flat foot deformity: Pilot study. Foot (Edinb) 2017; 31: 56-60.
65. Khouri RK, Smit JM, Cardoso E, Pallua N, Lantieri L, Mathijssen IM, Khouri RK Jr, Rigotti G. Percutaneous aponeurotomy and lipofilling: a regenerative alternative to flap reconstruction? Plast Reconstr Surg 2013; 132: 1280-1290.
66. Arno A, Smith AH, Blit PH, Shehab MA, Gauglitz GG, Jeschke MG. Stem cell therapy: a new treatment for burns? Pharmaceuticals 2011; 4: 1355-1380.
67. Linares HA, Larson DL. Early differential diagnosis between hypertrophic and nonhypertrophic healing. J Invest Dermatol 1974; 62: 514-516.
68. Bruno A, Delli Santi G, Fasciani L, Cempanari M, Palombo M, Palombo P. Burn scar lipofilling: immunohistochemical and clinical outcomes. J Craniofac Surg 2013; 24: 1806-1814.
69. William J, Berger T, Elston D. Andrews' diseases of the skin. Clin Dermatol 2005; 10: 789-790.
70. Bernier J, Bonner J, Vermorken JB, Bensadoun RJ, Dummer R, Giralt J, Kornek G, Hartley A, Mesia R, Robert C, Segaert S, Ang KK. Consensus guidelines for the management of radiation dermatitis and coexisting acne like rash in patients receiving radiotherapy plus EGFR inhibitors for the treatment of squamous cell carcinoma of the head and neck. Ann Oncol 2008; 19: 142-149.
71. Rigotti G, Marchi A, Galiè M, Baroni G, Benati D, Krampera M, Pasini A, Sbarbati A. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells. Plast Reconstr Surg 2007; 119: 1409-1422.
72. Peltoniemi HH, Salmi A, Miettinen S, Mannerström B, Saariniemi K, Mikkonen R, et al. Stem cell enrichment does not warrant a higher graft survival in lipofilling of the breast: a prospective comparative study. J Plast Reconstr Aesthet Surg 2013; 66: 1494-1503.
73. Smith P, Adams WP Jr, Lipschitz AH, Chau B, Sorokin E, Rohrich RJ, Brown SA. Autologous human fat grafting: effect of harvesting and preparation techniques on adipocyte graft survival. Plast Reconstr Surg 2006; 117: 1836-1844.
74. Chan CW, McCulley SJ, Macmillan RD. Autologous fat transfer: a review of the literature with a focus on breast cancer surgery. J Plast Reconstr Aesthet Surg 2008; 61: 1438-48.
75. Peer LA. The neglected free fat graft, its behavior and clinical use. Am J Surg 1956; 92: 40-47.
76. Sattler G, Sommer B. Liporecycling: a technique for facial rejuvenation and body contouring. Dermatol Surg 2000; 26: 1140-1144.
77. Spear SL, Wilson HB, Lockwood MD. Fat injection to correct contour deformities in the reconstructed breast. Plast Reconstr Surg 2005; 116: 1300-1305.
78. Coleman SR, Saboeiro AP. Fat grafting to the breast revisited: safety and efficacy. Plast Reconstr Surg 2007; 119: 775-85.
79. Gutowski KA. ASPS Fat Graft Task Force. Current applications and safety of autologous fat grafts: a report of the ASPS fat graft task force Plast Reconstr Surg 2009; 124: 272-280.
80. Gould L, Abadir P, Brem H, Carter M, Conner-Kerr T, Davidson J, DiPietro L, et al. Chronic wound repair and healing in older adults: current status and future research. Wound Repair Regen 2015; 23:1-13.
81. Raposio E, Bonomini S, Calderazzi F. Isolation of autologous adipose tissue-derived mesenchymal stem cells for bone repair. Orthop Traumatol Surg Res 2016; 102: 909-912.
82. Raposio E, Bertozzi N, Bonomini S, Bernuzzi G, Formentini A, Grignaffini E, et al. Adipose-derived stem cells added to platelet-rich plasma for chronic skin ulcer therapy. Wounds 2016; 28: 126-131.
83. Raposio E, Libondi G, Bertozzi N, Grignaffini E, Grieco MP. Effects of topic simvastatin for the treatment of chronic vascular cutaneous ulcers: A pilot study. J Am Coll Clin Wound Spec 2016; 7: 13-18.
84. Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med. 1999 2; 341: 738-746.
85. Tang W, Zeve D, Suh JM, Bosnakovski D, Kyba M, Hammer RE, Tallquist MD, Graff JM. White fat progenitor cells reside in the adipose vasculature. Science 2008; 322: 583-586.
86. Caruana G, Bertozzi N, Boschi E, Grieco MP, Grignaffini E, Raposio E. Role of adipose-derived stem cells in chronic cutaneous wound healing. Ann Ital Chir 2015; 86: 1-4.
87. Kapur SK, Katz AJ. Review of the adipose derived stem cell secretome. Biochimie 2013; 95: 2222-2228.
88. Salgado AJ, Reis RL, Sousa NJ, Gimble JM. Adipose tissue derived stem cells secretome: soluble factors and their roles in regenerative medicine. Curr Stem Cell Res Ther 2010; 5: 103-110.
89. Altman AM, Abdul Khalek FJ, Alt EU, Butler CE. Adipose tissue-derived stem cells enhance bioprosthetic mesh repair of ventral hernias. Plast Reconstr Surg 2010; 126: 845-854.
90. Makarov AV, Arutyunyan IV, Bol'shakova GB, Volkov AV, Gol'dshtein DV. Morphological changes in paraurethral area after introduction of tissue engineering construct on the basis of adipose tissue stromal cells. Bull Exp Biol Med 2009; 148: 719-724.
91. Coradeghini R, Guida C, Scanarotti C, Sanguineti R, Bassi AM, Parodi A, et al. A comparative study of proliferation and hepatic differentiation of human adipose-derived stem cells. Cells Tissues Organs 2010; 191: 466-477.
92. Aluigi MG, Coradeghini R, Guida C, et al. Pre-adipocytes commitment to neurogenesis 1: preliminary localisation of cholinergic molecules. Cell Biol Int 2009; 33: 594-601.

93. Scanarotti C, Bassi AM, Catalano M, Guida C, Coradeghini R, Falugi C, et al. Neurogenic-committed human pre-adipocytes express CYP1A isoforms. Chem Biol Interact 2010; 184: 474-483.
94. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 2006; 24:1294-1301.
95. Raposio E, Caruana G, Petrella M, Bonomini S, Grieco MP. A standardized method of isolating adipose-derived stem cells for clinical applications. Ann Plast Surg 2016; 76: 124-126.
96. Raposio E, Caruana G, Bonomini S, Libondi G. A novel and effective strategy for the isolation of adipose-derived stem cells: minimally manipulated adipose-derived stem cells for more rapid and safe stem cell therapy. Plast Reconstr Surg 2014; 133: 1406-1409.
97. Higuci A, Chuang CW, Ling QD, et al. Differentiation ability of adiposederived stem cells separated from adipose tissue by a membrane filtration method. J Memb Sci 2011; 366: 286-294.
98. Salibian AA, Widgerow AD, Abrouk M, Evans GR. Stem cells in plastic surgery: a review of current clinical and translational applications. Arch Plast Surg 2013; 40: 666-675.
99. Rodbell M. Metabolism of isolated fat cells. I. effects of hormones on glucose metabolism and lipolysis. J Biol Chem 1964; 239: 375-380.
100. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001; 7: 211-228.
101. Raposio E, Bertozzi N. Isolation of ready-to-use Adipose-derived Stem Cell (ASC) pellet for clinical applications and a comparative overview of alternate methods for ASC isolation. Curr Protoc Stem Cell Biol 2017; 41: 1F.17.1-1F.17.
102. Raposio E, Simonacci F, Perrotta R. Adipose-derived stem cells: Comparison between two methods of isolation for clinical applications. Ann Med Surg 2017; 20: 87-91.
103. Matsumoto D, Sato K, Gonda K, Takaki Y, Shigeura T, Sato T, Aiba-Kojima E, Iizuka F, Inoue K, Suga H, Yoshimura K. Cell-assisted lipotransfer: supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. Tissue Eng 2006; 12: 3375-3382.
104. Frese L, Dijkman PE, Hoerstrup SP. Adipose Tissue-Derived Stem Cells in Regenerative Medicine. Transfus Med Hemother 2016; 43: 268-274.

Received: 18 July 2017
Accepted: 20 July 2018
Correspondence:
Francesco Simonacci, MD
Department of Medicine and Surgery,
Plastic Surgery Division, Cutaneous, Regenerative,
Mininvasive and Plastic Surgery Unit,
Parma University and Maggiore Hospital,
Via Gramsci 14, 43126 Parma, Italy.
E-mail: francescosimonacci@hotmail.it

Acta Biomed 2019; Vol. 90, N. 2: 209-214 DOI: 10.23750/abm.v90i2.8345

Original article

Urban diabetes: the case of the metropolitan area of Rome

Antonio Nicolucci[1], Maria Chiara Rossi[1], Ketty Vaccaro[2], Roberta Crialesi[3], Stefania Rossetti[3], Stefano da Empoli[4], Lucio Corsaro[5], Lelio Morviducci[6], Marco G. Baroni[7], Simona Frontoni[8], Francesco Dotta[9]

[1] Center for Outcomes Research and Clinical Epidemiology - CORESEARCH, Pescara, Italy; [2] Fondazione CENSIS, Rome, Italy; [3] Istituto Nazionale di Statistica – ISTAT, Rome, Italy; [4] Istituto per la Competitività - I-COM and Health City Institute, Rome, Italy; [5] MEDIPRAGMA, Rome, Italy; [6] Servizio di Diabetologia, Ospedale S. Camillo Forlanini, Rome, Italy; [7] Dipartimento di Medicina Sperimentale, Endocrinologia e Diabete, Università Sapienza di Roma, Italy; [8] Dipartimento di Medicina dei Sistemi Università degli Studi di Roma Tor Vergata; Endocrinologia, Diabetologia e Malattie Metaboliche Ospedale Fatebenefratelli Isola Tiberina, Rome, Italy; [9] Health City Institute, Rome and Università di Siena, Siena, Italy

Summary. *Background:* The world is rapidly urbanizing, causing alarming health problems to their citizens. The Cities Changing Diabetes program aims to address the social factors and cultural determinants that can increase type 2 diabetes (T2D) vulnerability among people living in cities. *Methods:* Public data of Italian Institute for Statistics (ISTAT) and available scientific reports were reviewed and findings integrated. The prevalence of T2D in the 8 health districts of Rome was mapped and the correlation between prevalence and social and cultural determinants was assessed. *Results:* The metropolitan area of Rome has 4.3 million inhabitants. People over 65 has increased by 136,000 units in the last decade, reaching 631,000 citizens in 2015. Elderly people living alone are 28.4%. The obesity prevalence is 9.3%, as compared to 8.2% in the year 2000. The prevalence of T2D is 6.6%, varying in the different 8 health districts between 5.9% and 7.3%. A linear correlation exists between the prevalence of diabetes in the districts, unemployment rate and use of private transportation rate (Pearson R 0.52 and 0.60, respectively), while an inverse correlation is present with aging index, school education level, and slow mobility rate (Person R -0.57, -0.52, and -0.52, respectively). *Conclusions:* Important socio-demographic changes have occurred in Rome during the last decades with a raise in the prevalence of obesity and diabetes. A wide variation exists in the prevalence of T2D among the districts of Rome, associated with social and cultural determinants. This study model can help rethinking diabetes in an urban setting. (www.actabiomedica.it)

Key words: urban diabetes, social determinants, obesity, lifestyle, vulnerability

Introduction

Diabetes is rising at an alarming rate all over the world. The global prevalence of diabetes has almost doubled in the past 16 years – from 4.6% in 2000 (1) to 9.1% in 2017 (2). In the absence of specific interventions, the prevalence of diabetes is projected to rise to 11.7% – 736 million people – by 2045 (2). Given the devastating human and economic cost of diabetes and its complications, this growth is simply unsustainable for all Countries.

Established drivers of the increasing prevalence of type 2 diabetes include a growing ageing population and global trends such as urbanization, unhealthy diet and reduced physical activity (2).

Today, more than half the world's population (3.9 billion people) live in cities, and it is estimated that almost 6.3 billion people will live in urban areas by 2050 (3). Urban environments are already home to two-thirds of people with diabetes (2). This makes cities the front line in the fight against type 2 diabetes – and where we must take action to hold back the

alarming rise of the condition. Starting from these premises, in 2014 three global partners, Steno Diabetes Center Copenhagen, University College London and Novo Nordisk, launched the Cities Changing Diabetes program to accelerate the global fight against urban diabetes (4). Today, the program has established local partnerships in 15 cities to address the social factors and cultural determinants that can increase type 2 diabetes vulnerability among certain people living in cities. Rome joined the program in 2017, and a series of initiatives was launched with the aim of mapping the problem, sharing the learnings, and designing interventions. The first phase consisted in the clinical-epidemiological assessment of diabetes and its related risk factors in the metropolitan area of Rome.

Aims

To assess, using existing data sources, the prevalence of diabetes in the metropolitan area of Rome and its health districts, and to address the social factors and cultural determinants that can increase type 2 diabetes vulnerability.

Methods

Socio-demographic data on the metropolitan area of Rome were provided by the National Institute of Statistics (ISTAT) (5). The prevalence of diabetes was estimated by the Department of Epidemiology of the Regional Healthcare System of Lazio through record linkage between drug prescriptions, hospital discharge registry, and exemption from co-payments due to a diagnosis of diabetes (6).

The prevalence of obesity and physical activity was obtained by the national health examination surveys conducted by ISTAT (7).

The association between the prevalence of diabetes in the different health districts and several socio-economic and lifestyle indicators was assessed through linear regression analysis and Spearman correlation coefficient. The following indicators were considered: aging index (expressed as the ratio between residents over 65 years of age and those aged 14 years or less); school education level (expressed as high school/degree to middle school education ratio); unemployment rate; use of private transportation (car, motorcycle); slow mobility (walk, bicycle).

Results

Rome is the largest city in Italy and it is the fourth most populated city in the EU after Paris, London and Berlin, with 4.3 million inhabitants in the metropolitan area. Since the Second World War, the population doubled in the metropolitan area, rising from 2.1 million in 1951 to 4.0 million in 2011. In the city of Rome, the population raised from 1.65 million to 2.62 million. In the city, the number of citizens over the age of 65 has grown by 136,000 over the last 13 years, reaching a total of 631,000 in 2015. Furthermore, in 1971 one out of 10 families in Rome had only one component; in 2011 the rate increased to one in three. Today, elderly people (≥65 years) living alone are 28.4%.

In addition to the increasing age of the population, one of the largest contributors to the rise of diabetes incidence is represented by obesity. The prevalence of obesity among adults in the Lazio region, where Rome is located, was 9.3% in 2013, as compared to 8.2% in the year 2000. Furthermore, 43.8% of the adult residents in the Lazio region are physically totally inactive, and only 24.0% exercise on a regular basis.

The prevalence of diabetes in the Lazio region (6.6%) is amongst the highest in Italy, preceded only by Campania (6.7%) and Calabria (8.2%). Compared to the year 2000, the prevalence of diabetes has increased from 5.0% to 6.5% among males and from 4.2% to 6.8% among females.

In absolute terms, it can be estimated that about 189.500 citizens with diabetes live in the city of Rome and 286.500 residents with diabetes live in the metropolitan area. In other words, of all residents with diabetes in the Lazio region 50% live in Rome and 75% live in the metropolitan area.

Within the metropolitan area of Rome, the prevalence of diabetes varies among the different health districts between 5.88% and 7.32% (table 1). The prevalence in the city of Rome (6.53%) is lower than in the suburban areas (6.93%).

We found a strong, inverse correlation between the prevalence of diabetes in different neighborhoods

Table 1. Prevalence of diabetes in the health districts of the metropolitan area of Rome.

Health District	Population	No. of people with diabetes	Prevalence (%)
RM A	507,203	29,824	5.88
RM B	735,230	47,983	6.52
RM C	559,628	38,101	6.80
RM D	523,627	38,358	7.32
RM E	550,446	33,616	6.11
Rome	**2,876,134**	**187,882**	**6.53**
RM F	311,235	20,852	6.70
RM G	481,788	34,196	7.10
RM H	538,256	37,197	6.91
Suburbs	**1,331,279**	**92,245**	**6.93**
Metropolitan area	**4,207,413**	**280,127**	**6.66**

and aging index (figure 1): those districts with the highest prevalence of diabetes were characterized by a lower aging index. A strong correlation was also documented between the prevalence of diabetes and the level of school education. Districts characterized by a lower prevalence of diabetes also showed a higher high school/degree to middle school education ratio (figure 2). Similarly, a linear association was found between diabetes prevalence and unemployment rate (figure 3). Finally, those districts with higher diabetes prevalence showed higher percentages of residents who use motor vehicles to get around (figure 4), and lower percentages of people who choose to walk or cycle to their destination (figure 5).

Discussion

Cities offer unique chances for citizens to increase their income and benefit from education as well as health and social services. Despite these opportunities and benefits, the urban environment can also negatively impact the health of the citizens by exacerbating the relevant factors that lead to chronic non-communicable diseases such as diabetes (8). Cities thus provide tremendous opportunity for studying and understanding the drivers behind type 2 diabetes.

The analysis of the data relative to the metropolitan area of Rome provides relevant information. Of note, major socio-demographic changes occurred in Rome during the last decades with a raise in the prevalence of obesity and diabetes. The prevalence of

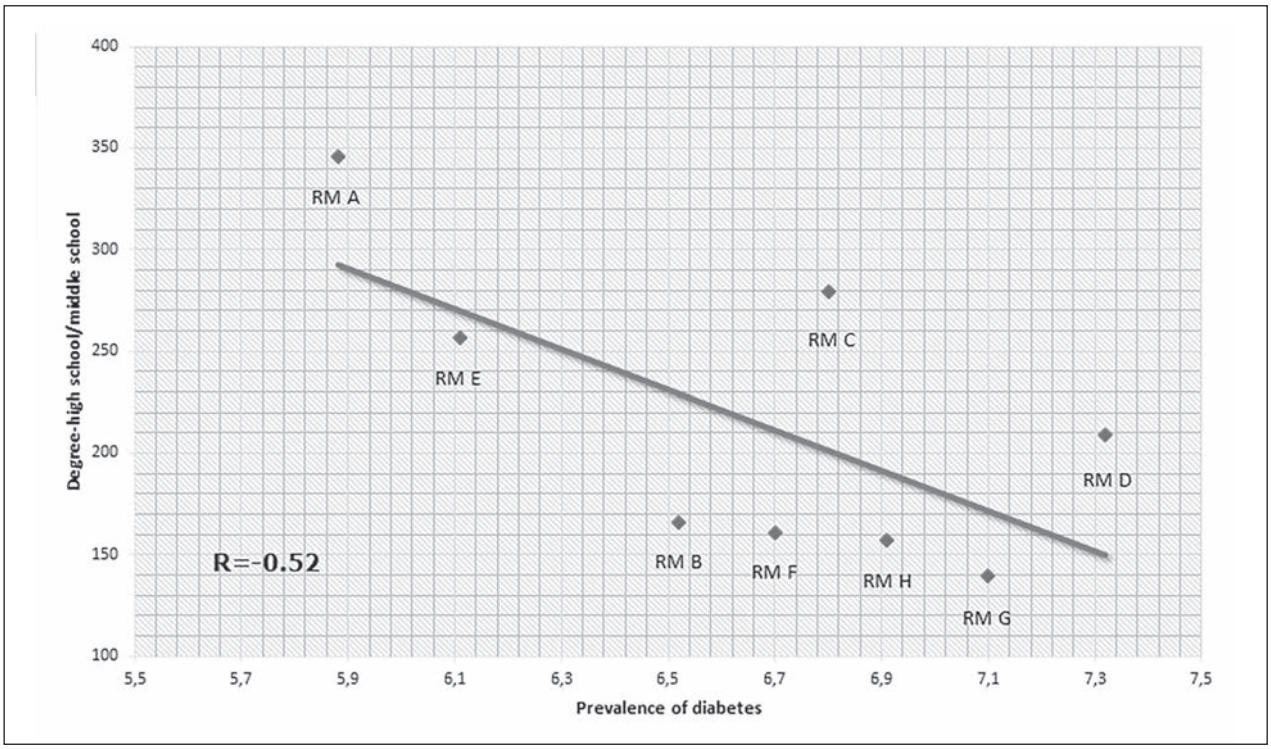


Figure 1. Correlation between prevalence of diabetes and ageing index in the health districts of the metropolitan area of Rome

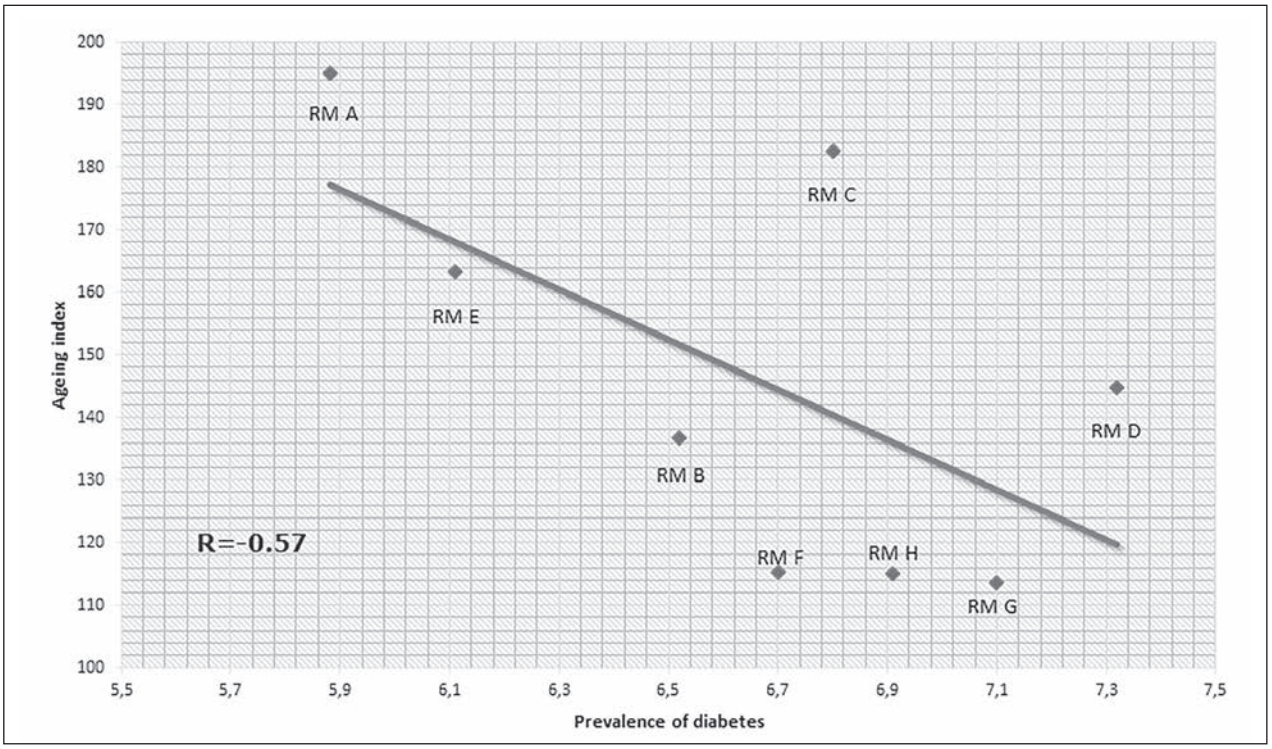


Figure 2. Correlation between prevalence of diabetes and level of school education in the health districts of the metropolitan area of Rome

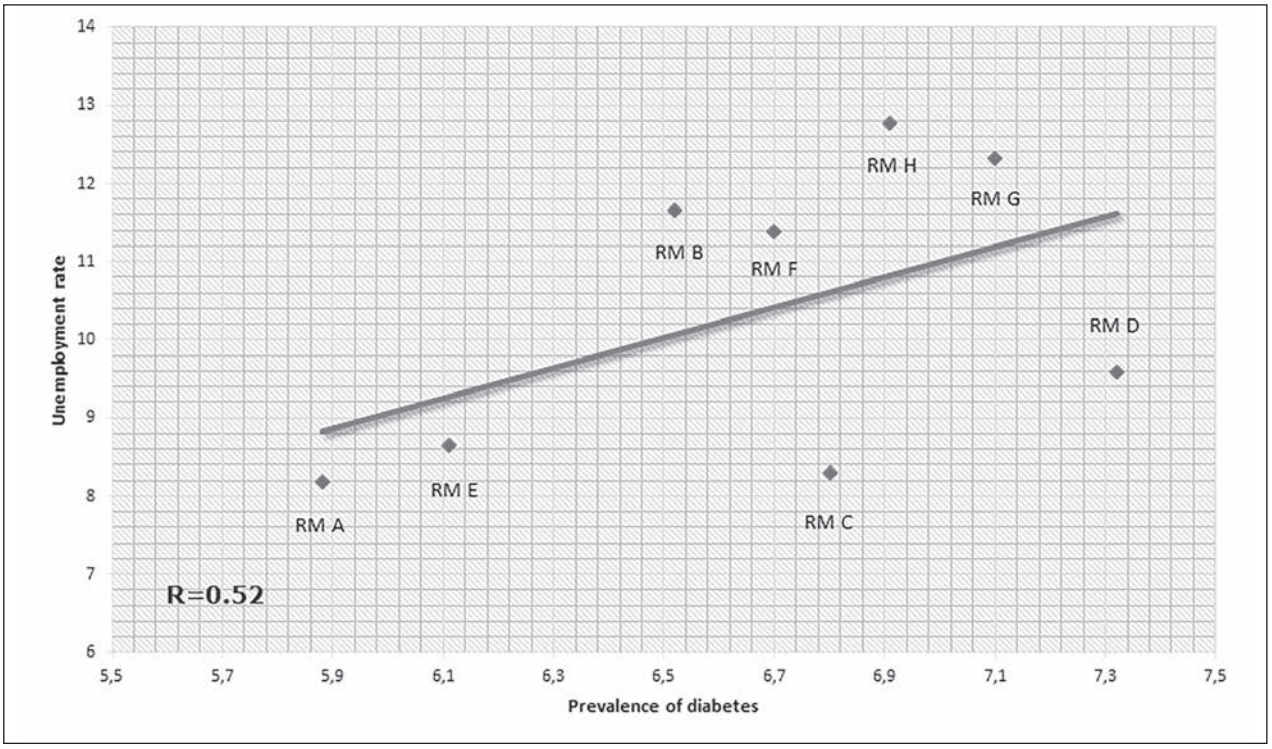


Figure 3. Correlation between prevalence of diabetes and unemployment rate in the health districts of the metropolitan area of Rome

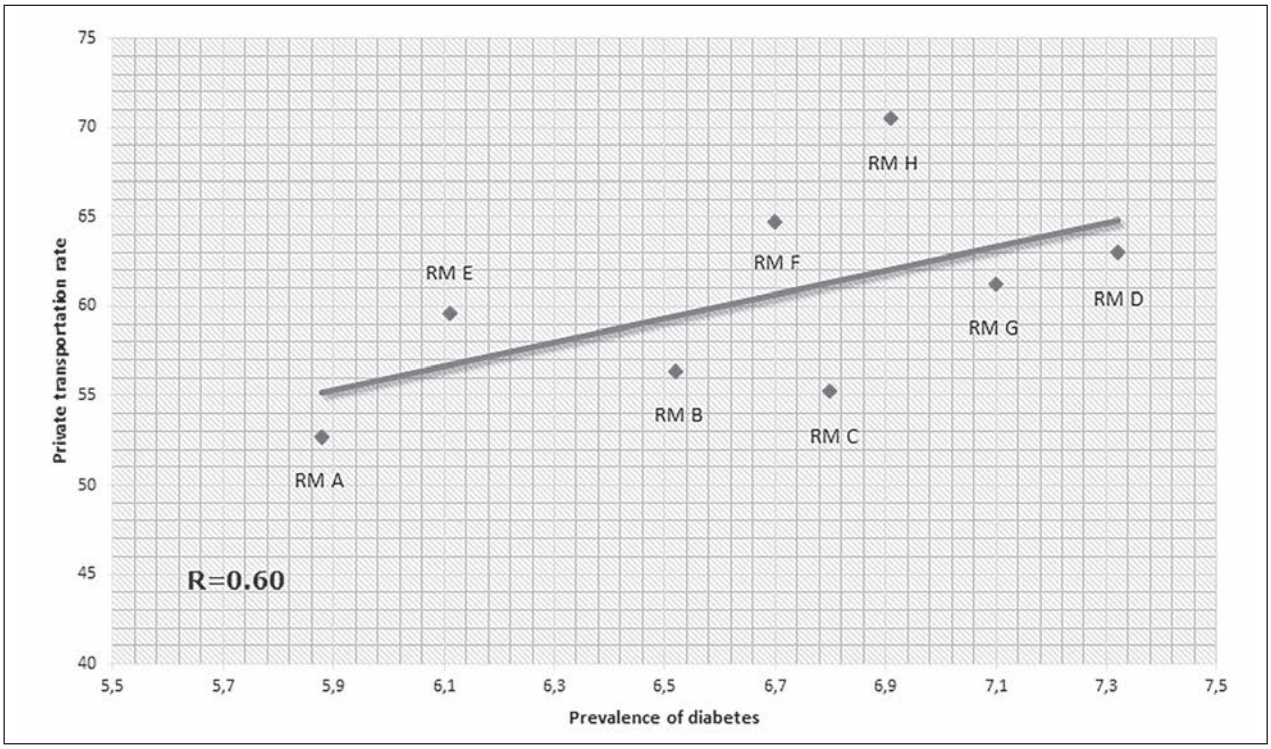


Figure 4. Correlation between prevalence of diabetes and use of private transportation in the health districts of the metropolitan area of Rome

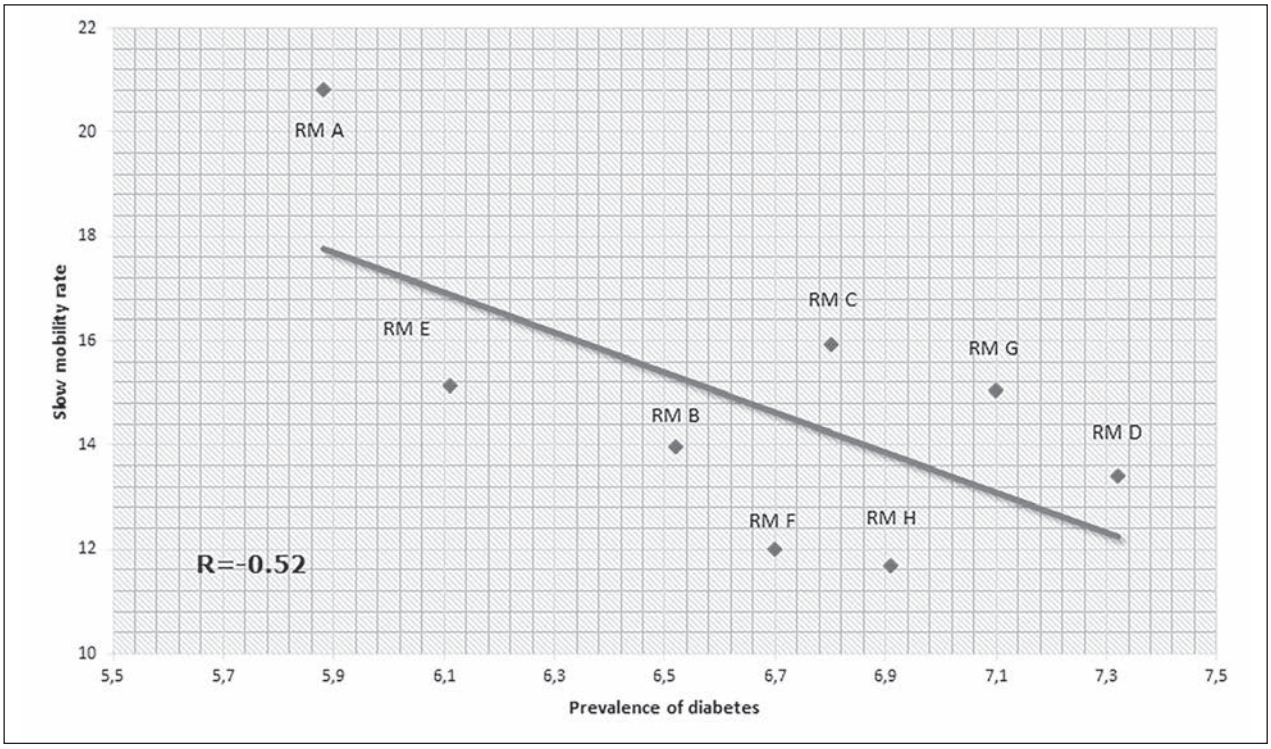


Figure 5. Correlation between prevalence of diabetes and use of slow mobility (walk, cycle) in the health districts of the metropolitan area of Rome

diabetes in the area is amongst the highest in Italy, and it markedly varies among the different health districts. We found that the areas with a higher prevalence of diabetes are those more disadvantaged from a socio-economical point of view. In fact, a strong association was evidenced between higher diabetes prevalence, higher rate of unemployment, and lower level of school education, the latter considered as a proxy for lower socio-economic status. Despite the growth of the aging population represents one of the major candidate drivers of diabetes, those neighborhoods characterized by a higher prevalence of diabetes also showed a lower ageing index. These findings suggest on one side that socio-economic factors are major determinants of the risk of diabetes; on the other they also indicate that in more disadvantaged areas diabetes develops at younger ages, thus increasing the number of life years lived with the disease. Use of inactive transport and lack of physical activity also play an important role, likely related to the increased risk of obesity. We documented that the use of motor vehicles was more frequent in those districts with a higher prevalence of diabetes, while the proportion of citizens walking or cycling was lower.

The analysis of current data sources allowed a first recognition of key factors playing a role in increasing the risk of diabetes in an urban environment. Our findings suggest the need to implement effective strategies to reach socially disadvantaged citizens by increasing their accessibility to preventive activities. Of particular importance will be to strengthen social networks and increase, in the more disadvantaged areas, low-cost, high impact structures allowing physical activity.

The information derived from this analysis will be further enriched with qualitative research, investigating local vulnerabilities associated with diabetes using the Urban Diabetes Risk Assessment. The findings will help give a more thorough understanding of how the sociocultural factors of diabetes come into play, and help to identify barriers and opportunities for successful diabetes prevention, care and management in Rome.

In conclusion, the world is rapidly urbanizing, changing not just where we live, but the way we live. Today, the way cities are designed, built and run poses important health challenges to their citizens. This implies new approaches to map the challenge, understand the areas of greatest risk and vulnerability and design interventions that can have a real impact. Through the Changing Diabetes Program, Rome and the other participating cities will help in developing new tools to rethink diabetes in an urban setting.

Acknowledgement

The authors wish to thank Novo Nordisk, Società Italiana di Diabetologia, Associazione Medici Diabetologi, Comune di Roma and Regione Lazio for the support to the initiative.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. International Diabetes Federation. IDF Diabetes Atlas, 1st edition (2000). https://www.idf.org/e-library/epidemiology-research/diabetes-atlas/24-atlas-1st-edition.html
2. International Diabetes Federation. IDF Diabetes Atlas, 8th edition (2017). https://diabetesatlas.org/resources/2017-atlas.html
3. UNDESA. World Urbanization Prospects: The 2014 Revision. New York: United Nations Department of Economics and Social Affairs, Population Division; 2014.
4. Cities Changing Diabetes. http://www.citieschangingdiabetes.com/home.html
5. Statistiche demografiche Roma 2016. http://demo.istat.it/pop2016/index1.html
6. Dipartimento di Epidemiologia del Servizio Sanitario Regionale Regione Lazio - Programma Regionale Valutazione degli Esiti degli Interventi Sanitari 2016 https://www.deplazio.net/attivita/94-programma-regionale-valutazione-esiti-prevale
7. Istat, Indagine Multiscopo, Aspetti della vita quotidiana 2015. https://www.istat.it/it/archivio/129956
8. WHO. Hidden Cities: Unmasking and overcoming health inequities in urban settings. Geneva, Switzerland: World Health Organization; 2010.

Received: 28 March 2019
Accepted: 29 April 2019
Correspondence:
Antonio Nicolucci, MD
Center for Outcomes Research and
Clinical Epidemiology - CORESEARCH
Via Tiziano Vecellio 2 - 65124 Pescara Italy
Tel. +39 085 9047114 - Fax +39 0875 9047113
E-mail nicolucci@coresearch.it

Acta Biomed 2019; Vol. 90, N. 2: 215-220 DOI: 10.23750/abm.v90i2.8260

Original article

Clinical utility of beta-hydroxybutyrate measurement in the management of physiological ketosis at home in children under 5

Maurizio Vanelli[1], Carla Mastrorilli[2], Valentina Fainardi[3], Brunella Iovane[4], Chiara Scarabello[4], Piero Veronese[4], Umberto Fanelli[4], Tiziana Incerti[4], Bertrand Tchana[4], Icilio Dodi[4], Dora Di Mauro[4]

[1] University Center for Health systems organization, quality and sustainability, University of Parma, Parma, Italy; [2] Pediatric and Neonatology Unit, Madonna delle Grazie Hospital - ASM Matera, Matera, Italy; [3] Research Doctorate in Medical Science, University of Parma, Parma, Italy; [4] Postgraduate school of Pediatrics, University of Parma, Parma, Italy

Summary. *Aim*: To verify the possible advantages of 3- β-hydroxybutyrate (3HB) measurement compared to urinary assay of ketones during an intercurrent disease managed at home. *Methods*: Twelve Pediatricians were asked to enroll at least 4 patients aged 3 to 5 years, affected by an intercurrent illness and showing at least one of symptoms reliable to ketosis. Recruited patients were submitted to the simultaneous assay of 3HB in capillary blood and ketones in urine at 3 (T3) and 6 hours (T6) from the first measurement (T0). For urinary and blood ketone detection commercial tests were used. *Results*: Thirty-eight children (4.36±2.60 years old; 25 boys) were enrolled into the study. At T0 all children showed 3HB levels (1.2-3.2 mmol/L), but only 10 of them (26.3%) associated also urinary ketone bodies (2 to 4+). In response to 3 hour treatment (T3) with a glucose solution, 3HB values decreased in 19 (0,8-1,8 mmol/L) and normalized in 13 children (<0.2 mmol/L); while ketonuria disappeared in only 2 patients, it was confirmed in 8 and appeared (4+) the first time in the remaining 28 children. At T6 3HB levels fell definitively within the normal range in all children, while ketonuria was still present (2+) in 9 patients (23%). The pediatricians reported two limitations about blood 3HB dosage compared to the urinary test: the invasiveness of capillary blood collection, and the cost of supplies for finger pricking, reagent strips and reflectance meter. *Conclusions*. 3HB monitor in capillary blood is more effective and clinically more useful in diagnosing and managing of an ongoing ketosis in children with a mild infective disease than ketones detection in the urine. These advantages are mitigated by the cost of 3HB measurement. (www.actabiomedica.it)

Key words: ketone bodies, physiological ketosis, 3-beta-hydroxybutyrate, ketonemia, ketonuria

Introduction

When the body does not have enough carbohydrates available to meet its demand for energy, this begins to utilize the stored fat for energy. These fatty acids are metabolized in the liver and this metabolism induces chemical products called ketones. The ketone bodies consist of acetone, acetoacetate (AcAc), and 3-β-hydroxybutyrate (3HB) (Figure 1).

Mild infections in children under 5, especially those complicated by vomiting and diarrhea, commonly cause elevated levels of ketone bodies. This kind of ketosis is known as "physiological ketosis" because it is a transient condition, reliable to the low availability of glycogen in children's liver, and because it responds promptly to glucose administration.

To diagnose ketosis Pediatricians can rely on clinical criteria (such as high fever, appetite loss, nausea,

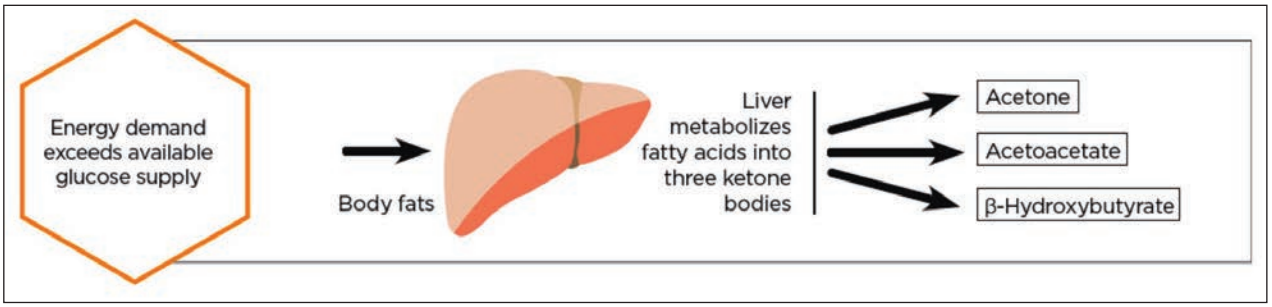


Figure 1. A schematic representation of ketogenesis process

vomiting, abdominal pain, dehydration signs, headache, somnolence), and on some tests to measure ketone bodies in either urine or blood. Urine test is the most used method in pediatric practice to investigate a state of ketosis even if this procedure does not detect the presence of all three ketone bodies. AcAc is the only measurable ketone since 3HB is not detectable by common urinary strips and acetone is eliminated through the breath.

New rapid enzymatic methods have been introduced since the nineties for the quantization of 3HB levels in small blood samples (1). These systems allow to measure within a few seconds on a fingerstick blood specimen 3HB which during the ketogenesis process accounts the majority of the total ketone body pool (78%) Figure 2) (2, 3).

Most information on advantages of 3HB dosage on ketosis management comes from the experience on ketoacidosis treatment in children with Type 1 diabetes (T1D). In response to insulin therapy, 3HB blood levels commonly decrease long before AcAc levels do in the urine. Our previous experience showed that in children with T1D at onset, monitored with 3HB measurement, it was possible to record ketoacidosis disappearance 4 to 9.5 hours earlier than in patients monitored with urine ketone bodies determination (4). Furthermore blood ketone tests help diabetic children to guide a sick day management more efficiently than urine test (5). Blood 3HB measurements may be especially valuable to prevent DKA in patients who use an insulin pump: elevations in blood 3HB levels may precede increases in urine ketones due to interrupted insulin delivery (6). 3HB measurement seems also to be able to improve clinical outcomes and enhance cost efficiency in ketoacidosis treatment (7).

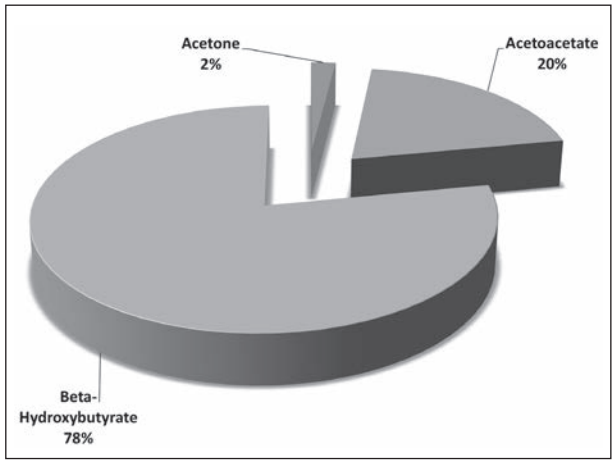


Figure 2. Distribution of the total ketone bodies pool

There is no evidence in literature on the use of blood 3HB test in early detection of "physiological ketosis" during a mild infection in healthy children. The lack of information about this topic led us to submit to some Pediatricians a protocol in order to verify the possible advantages of capillary blood test compared to urinary assay of ketones during an intercurrent disease managed at home. The results we have obtained are shown below.

Subjects and Methods

The study involved 12 Pediatricians working in the same area of Parma municipality, a district located in northern Italy. Pediatricians were asked to enroll each-one at least 4 patients 3 to 5 years old, affected by an intercurrent illness and showing at least one of the following symptoms reliable to ketosis, in addition to

fever (≥37.5°C): fruity-smelling breath, appetite loss, vomiting, abdominal pain, headache, alteration of consciousness.

All Pediatricians were equipped with devices for simultaneous measurement of ketonuria and capillary blood 3HB, supplies for reagent strips, finger pricking and a reflectance meter. One-hour meeting was organized to inform pediatricians on correct management of capillary blood 3HB and urine ketone tests, and to give them more information about ketogenesis and about the criteria for early diagnosis of ketosis. Pediatricians were invited to collect and storage clinical data of tested children in a database, and to obtain the written informed consent from the parents before patients enrollment.

Once a patient was selected, this was submitted to the simultaneous assay of 3HB in capillary blood and ketones in the urine (T0). Children who did not present ketone bodies either in the blood or in the urine were excluded from the study. T0 procedures were repeated at 3 (T3) and 6 hours (T6) from the first measurement. T3 and T6 tests were performed by an experienced nurse or by a resident of the Postgraduate School of Pediatrics of Parma who was performing the internship in an outpatient clinic involved in the study. Capillary blood assay was interrupted when 3HB values have reached an amount <0.2 mmol/L. Urine tests were repeated until the urine was not appeared cleaned from ketones. The decision to stop the tests in either urine or blood was agreed with the pediatrician. Children with ketones were orally treated by a spoon of 10% dextrose solution at 5 minutes of interval. As soon as 3HB levels were normalized or ketones were undetectable in urine, the children were fed normally. Body temperature was measured by an ear thermometer.

For urinary ketone detection the same commercial test was used. After urine collection, the strip was passed through the urine, and after 15 seconds AcAc in the of urine reacted with nitroprusside producing a purple-colored complex on the test strip. The color was compared to that displayed on the color scale of the strips bottle. Test results were read as negative or 1+ to 4+.

For the quantification of 3HB levels in the blood commercial systems available in Italy were unconditionally chosen. Adopted devices measured 3HB levels on a fingerstick blood specimen (0.8 µl) within 10 seconds, and were reported to be accurate for 3HB levels from 0.1 to 8.0 mmol/L. A value <0.2 mmol/L was considered as indicative of ketosis absence; and a value ranging from 0.1 to 2.5 mmol/L was assessed as expression of mild to moderate ketosis (8,9).

At the end of the study pediatricians were asked to report an opinion on clinical utility of the 3HB test and on any found limitations.

Analysis of variance and the χ^2 for the comparison of percentages were used. Linear regression analysis between fever degree and 3HB levels at T0 was calculated. Data were reported as mean±SD. P<0.05 was considered significant. No conflict of interest existed in relation to the subject matter of the present paper.

Results

Ten children dropped out of the study since they had no ketone bodies either in the blood or in the urine. Thirty-eight children (4.36±2.60 years old; n. 25 boys) were finally enrolled. All subjects showed body temperature ranging from 37.7 to 40.0°C. The symptoms reported by pediatricians to support the suspicion of a ketosis state are showed in the Figure 1. Otitis media (4/38), sinusitis (5/38), bronchitis (7/38), diarrhea (10/38), common cold (12/38) were the most frequent illnesses diagnosed by the Pediatricians at recruitment.

Measurement of ketone bodies in either urine or blood was performed in all selected children 24 hours after the first fever detection. At T0 all children showed detectable blood 3HB levels (1.2-3.2 mmol/L) meeting the criteria of a ketosis at onset, but only 10 of them (26.3%) showed also ketone bodies (2 to 4+) in the urine.

After 3 hour treatment (T3), 3HB values decreased in 19 (0,8-1,8 mmol/L) and normalized in 13 children (<0.2 mmol/L); in 6 children 3HB levels remained elevated (2.2-2.5 mmol/l). In contrast, ketonuria disappeared in only 2 patients, it was confirmed in 8 and it occurred for the first time (4+) in 28 children.

At the end of the study (T6), 3HB levels fell definitively within the normal range in all children, while ketonuria was still present (2+) in 9 (23%) patients An urine test arbitrarily performed by parents in the hours

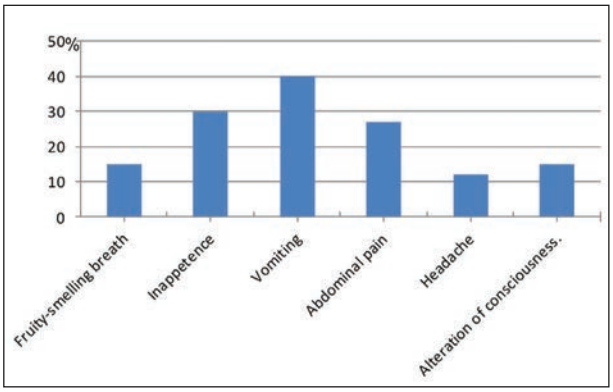


Figure 3. Prevalent symptoms reported by pediatricians to support the suspicion of a ketosis state

following T6 showed that ketonuria completely disappeared 2-6 hours after 3HB. All tests on capillary blood were performed at the scheduled times, while 36% of the urine tests underwent a delay ranging from 30 minutes to 2 hours since children did not urinate

Among symptoms reported by pediatricians to support the suspicion of a state of ketosis, vomiting (40.2%) and appetite loss (29.8%) recurred more frequently (χ^2=19.28 and 9.19; p<0.001) compared to the others symptoms (Figure 3). A strong correlation was found between body temperature and 3HB levels at diagnosis of ketosis (Figure 4).

Thirty-two children (84.2 %) responded promptly to glucose administration. The levels of 3 HB have begun to decrease as early as the third hour of treatment. Sixty-nine per cent of parents reported that the choice to give glucose with a spoon at short intervals proved to be a very effective tool to prevent vomiting and treatment rejection by patients. Thanks to this approach 25 children (65.8%) were able to restart normal feeding already at the end of T3.

No patient needed to be hospitalized. None of the pediatricians involved in the study had previously used 3HB measurement. All pediatricians recognized clinical utility of 3HB detection at home. However, two limitations have been reported compared to the urinary test: the invasiveness of capillary blood collection, and the cost of supplies for finger pricking, reagent strips and reflectance meter.

Discussion

We showed that 3HB levels in capillary blood is more effective and clinically more useful in diagnosing and managing an ongoing ketosis in children with a mild infective disease than ketones levels in urine. This is in agreement with previous reports on the use of 3HB both in treating ketoacidosis and in monitoring a sick day in children with T1D (3, 4, 5, 7).

The measurement of 3HB was considered as a reliable tool to recognize an incipient ketogenesis earlier than ketonuria detection (9). Ketonuria misses this

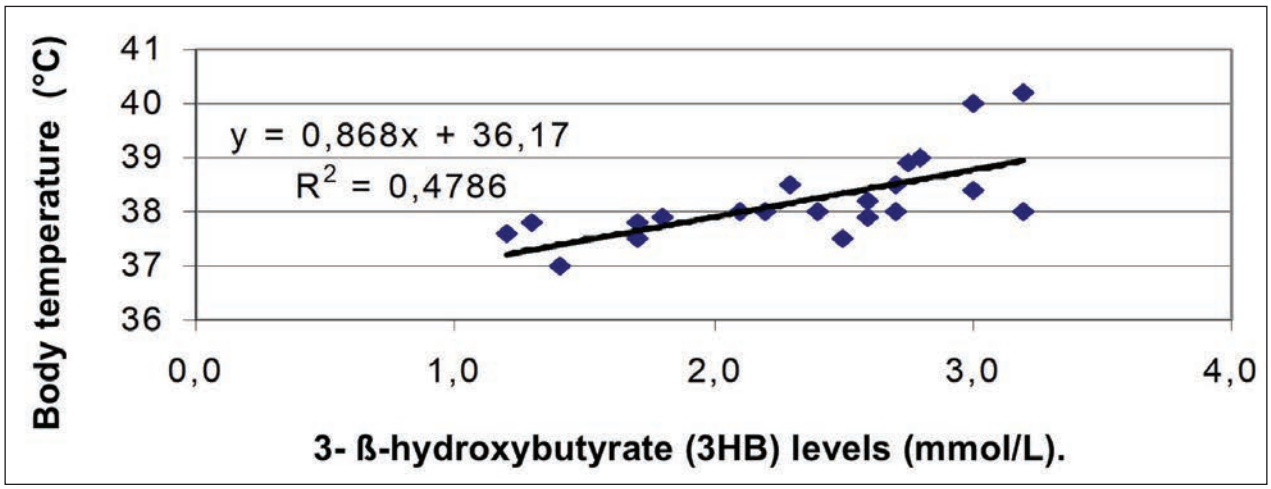


Figure 4. Fever is a factor increasing the levels of 3HB

goal because it provides a partial and delayed information about ketones during the ketogenesis process. Urine test assesses only AcAc and not 3HB levels, with a delay that is proportional to the interval between a bladder emptying and the next (4). Dehydration status that is frequently noticeable in a child with physiological ketosis affects the regular collection of urine specimens and may prolong the above delay. The gap in information between the two methods explains the high percentage of children we found who at T0 were already positive for 3HB but not yet for ketonuria, and of those children who at T6 continued to showing ketonuria despite 3HB levels had already came back to the normal range.

Thanks to 3HB detection, the pediatricians had the chance to start promptly the treatment established by the study protocol. Glucose solution administration stopped shortly the ongoing ketogenesis process, and children were enabled to resume within 3-6 hours of treatment the usual feeding. If the treatment had been modulated on the basis of ketonuria levels, in 23% of patients the treatment would have been prolonged beyond the planned conclusion of clinical observation, with discomfort for children and parents.

Pediatricians reported being encouraged in carrying out the 3HB test by three sentinel symptoms that are usually related to a physiological ketosis: vomiting, appetite loss and fever. Fever has proven to be a reliable indicator of ketosis at onset, and its reliability seems to grow with increasing the body temperature.

Vomiting, appetite loss and fever were reported by pediatricians more frequently than fruity-smelling breath due to acetone. Acetone is produced from AcAc and represents the least relevant ketone given its volatility, nevertheless it is often believed to be the first and the only sign of a ketotic state. The recognition of this ketone body through the breath depends on the investigator subjectivity, and not being an objective sign it could be liable to be both under- and overestimated. This remark could be retained one of the reasons why fruity-smelling breath was estimated by the pediatricians involved in the study as a unreliable sentinel symptom for ketosis.

At the end of the study, we found general satisfaction of pediatricians about blood ketone measurements compared to ketone determinations in urine. However, pediatricians reported that an important limitation on the use of blood ketone monitoring technology is the high costs compared to the determination in the urine. In fact, the cost of one test strip for ketonemia determination varies between 3 to 5 €, while the cost of one strip for ketonuria dosage ranges between 0.20 and 0.50 €, in other words ten times less. If the costs related to the puncture of the finger and to the meter of reflection are added, the gap becomes macroscopic.

Due to this difference we can speculate that it will take a lot time for blood 3HB monitoring to gain routine acceptance over urine testing in ketosis diagnosis and management. The delay could be shortened focusing on the benefits obtained with the measurement of 3HB in managing and monitoring diabetic ketoacidosis in terms of quality of care, and reduction in time and costs of treatment (4). A further impulse could result from promotional campaigns for pediatricians to update their outpatient clinics with new simplified and fast minimally invasive enzymatic methods for capillary blood measurement of some biological parameters such as glucose, cholesterol, glycated hemoglobin, and finally 3HB. We believe that these recently developed technological supports should enter without further delay in pediatric practice.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Koch DD, Feldbruegge DH. Optimized kinetic method for automated determination of beta- hydroxybutyrate. Clin Chem 1987; 33: 1761-1766.
2. Byrne HA, Tuesnon KL, Hollis S et al. Evaliation ofan Electromedical sensor for measuring blood ketone. Diabetes Care 2000; 23: 500-5003.
3. Sena SF. Beta-hydroxybutyrate: New test for ketoacidosis. Tehinically speaking, Danbury Hospital, A high level of care. July 2010, vol 4, n 8.
4. Vanelli M. Chiari G, Capuano C, et al. T. The direct measurement of 3-B-hydroxybutyrate enhances the management of diabetic ketoacidosis in children and reduces time and costs of treatment. Diab Nutr Metab 2003; I 6: 312-316.
5. Brink S, Joel D, Laffel L, et al. Sick day management in children and adolescents with diabetes. Pediatric Diabetes 2014: 15 (Suppl. 20): 193-202.

6. Umpierrez GE, Watts NB, Phillips LS. Clinical utility of beta-hydroxybutyrate determined by reflectance meter in the management of diabetic ketoacidosis. Diabetes Care 1995: 18: 137-138.
7. Vanelli M, Chiari G, Capuano C. Cost effectiveness of the direct measurement of 3-beta-hydroxybutyrate in the management of diabetic ketoacidosis in children. Diabetes Care 2003; 26: 959.
8. Mitchell GA, Kassovska-Bratinova S, Boukaftane Y, et al. Medical aspects of ketone body metabolism. Clin Invest Med 1995; 18: 193-216.
9. Laffel L. Ketone Bodies: a Review of Physiology, Pathophysiology and Application of Monitoring to Diabetes. Diabetes Metab Res Rev 1999; 15: 412-426.

Received: 1 March 2019
Accepted: 19 April 2019
Correspondence:
Maurizio Vanelli, MD
University Center on Health systems organization, quality and sustainability
University Hospital,
Viale A. Gramsci, 14 - 43122 Parma, Italy
E-mail: maurizio.vanelli@unipr.it

Acta Biomed 2019; Vol. 90, N. 2: 221-227 DOI: 10.23750/abm.v90i2.6681

Original article

Interleukin-10 and *Transforming Growth Factor Beta1* Gene Polymorphisms in Chronic Heart Failure

Mohammad Jafar Mahmoudi[1], *Mona Hedayat*[2], *Mohammad Taghvaei*[3], *Sara Harsini*[4, 10], *Ebrahim Nematipour*[5], *Elham Farhadi*[6], *Maryam Mahmoudi*[7], *Maryam Sadr*[3], *Nilufar Esfahanian*[3], *Keramat Nourijelyani*[8], *Ali Akbar Amirzargar*[3, 9], *Nima Rezaei*[3, 4, 9, 10]

[1] Division of Cardiology, Department of Internal Medicine, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; [2] Division of Immunology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA; [3] Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran; [4] Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran; [5] Tehran Heart Center, Tehran University of Medical Sciences, Tehran, Iran; [6] Hematology Department, School of Allied Medical Science, Tehran University of Medical Sciences, Tehran, Iran; [7] School of Nutrition and Dietetics, Tehran University of Medical Sciences, Tehran, Iran; [8] Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; [9] Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; [10] Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Tehran, Iran

Summary. *Background:* As cytokines, including interleukin-10 (IL-10) and transforming growth factor beta 1(TGF-β1) seem to contribute towards the pathogenesis of chronic heart failure (CHF), this study was performed to assess the associations of certain single nucleotide polymorphisms (SNPs) of these genes in a case control study. *Methods:* This investigation was carried out to determine the frequency of alleles, genotypes and haplotypes of *TGF-β1* and *IL-10* single-nucleotide polymorphisms (SNPs) in 57 Iranian patients with CHF compared with 140 healthy subjects using polymerase chain reaction with sequence-specific primers method. **Results:** Results of the analyzed data divulged a negative association for both *TGF-β1* GC genotype at codon 25 (*P*=0.047) and CT genotype at codon 10 (*P*=0.018) and CHF proneness. Although, *TGF-β1* CC genotype at codon 10 was found to be positively associated with CHF (*P*=0.011). Moreover, the frequency of *IL-10* (–1082, -819, -592) ATA haplotype and *TGF-β1* (codon 10, codon 25) TG haplotype were significantly lower in the patients group (*P*=0.004 and *P*=0.040, respectively), while *TGF-β1* (codon 10, codon 25) CG haplotype was overrepresented in patients with CHF (*P*=0.007). *Conclusions:* Cytokine gene polymorphisms might affect vulnerability to CHF. Particular genotypes and haplotypes in *IL-10* and *TGF-β1* genes could render individuals more susceptible to CHF. (www.actabiomedica.it)

Key words: heart failure; single nucleotide polymorphism; interleukin-10; transforming growth factor beta1

Introduction

Chronic heart failure (CHF) is an intricate public health problem, characterized by impaired contractile function and gradual ventricular dilation (1). It has been understood that several physiologic systems, including the immune system, engage in the pathogenesis of this complex multi-step disease (2). Considering high morbidity and mortality of CHF despite utilizing current treatment modalities, it stands to reason that identification of gene variations affecting underlying pathogenic mechanisms, seems necessary to improve the disease treatment strategies.

CHF is characterized by systemic inflammation, as evident by elevated circulating levels of multiple inflammatory cytokines with increasing levels in ac-

cordance with the extent of disease severity (3).Cytokines have been also implicated in the pathogenesis of underlying cardiovascular disorders such as atherosclerosis (4). Interleukin-10 (IL-10) is a significant immunoregulatory cytokine which exerts potent immunosuppressive functions by down-regulating the expression of co-stimulatory molecules and T helper 1 (TH1) cytokines (5). The other key immunoregulatory cytokine is transforming growth factor-beta1 (TGF-β1), to which certain vasculoprotective properties, comprising inhibition of the adhesion of neutrophils and T cells to the endothelium, transmigration of neutrophils through the endothelium, and production of pro-inflammatory adhesion molecules within endothelial cells, have been attributed (6-9).

It has been indicated that genetic polymorphisms within coding and promoter sequences of cytokine genes could modulate their production (10, 11). The association of certain cytokine gene polymorphisms and a number of diseases with possible underlying immune disturbances have already been studied (2, 12-21), whilst our understanding in CHF is restricted due to the scantiness of studies in this area. To the best of our knowledge, this is the first study exploring possible contributions of SNPs in *IL-10* and *TGF-β1* genes toward individual vulnerability to CHF in Iranian cases.

In order to evaluate the associations between the SNPs in *IL-10* gene at positions -1082, -819 and -592 and *TGF-β1* gene at codon 10 and codon 25 and CHF, this study was conducted in a group of Iranian patients and compared with healthy control subjects.

Patients and Methods

Subjects

In the current study, we investigated a total of 57 Iranian patients with chronic heart failure (43 male, 14 female) with the mean age 57.96±12.24. The control group is consisted of one hundred and forty unrelated individuals (mean age 45.63±10.84; 101 men, 39 women) who were randomly selected from healthy volunteers, as previously described (22). The diagnosis of chronic heart failure was based on thorough history taking, comprehensive physical examination, electrocardiography and impaired left ventricular (LV) systolic function (LV ejection fraction ≤40%) and LV dilation (LV end-diastolic diameter >5.5 cm) on echocardiography. We excluded all subjects with chronic lung disease, recent myocardial infarction, malignancies and acute decompensated HF within 3 months prior to enrollment. All the cases who fulfilled the inclusion criteria were in stable clinical condition and received conventional medical therapy for at least 3 months. Baseline clinical characteristics of patients with CHF are depicted in Table 1.

Written informed consents were taken from all participants before recruitment. This investigation was conducted according to the guidelines of the Ethics Committee of Tehran University of Medical Sciences.

Genotyping

For all of the entrants to the present study, amount of 5 milliliters (ml) of venous blood samples were obtained and kept with ethylenediaminetetraacetic acid

Table 1. Baseline clinical characteristics of patients with chronic heart failure

Characteristics	N (%)
Hypertension	21 (36.8%)
Diabetes	19 (36.8%)
Dyslipidemia	22 (38.6%)
Obesity	8 (14%)
History of smoking	
Current smoker	25 (43.9%)
Ex-smoker	4 (7%)
Non-smoker	28 (49.1%)
History of ACS	31 (54.4%)
Chronic kidney disease	5 (8.8%)
CVA	1 (1.8%)
History of CABG	5 (8.8%)
History of PCI	4 (7%)
NYHA classification	
I	15 (26.3%)
II	18 (31.6%)
III	15 (26.3%)
IV	9 (15.8%)

ACS acute coronary syndrome, CVA cerebrovascular accident, CABG coronary artery bypass grafting, PCI percutaneous coronary intervention, NYHA New York Heart Association

(EDTA) at -20°C until being investigated. Genomic DNA was extracted using the "salting out" technique (23). Cytokine typing was carried out on genomic DNA by polymerase chain reaction with sequence -specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany), as previously elucidated in detail (22). Briefly, amplification was performed using a thermal cycler Techne Flexigene apparatus (Rosche, Cambridge, UK). The availability of PCR products was visualized by 2% agarose gel electrophoresis.

We have determined the allele and genotype frequencies of *TGF-β1* (C/T at codon 10; rs1800470, and C/G at codon 25; rs1800471) and *IL-10* (A/G at -1082; rs1800896, C/T at -819; rs1800871, and A/C at -592; rs1800872) genes.

Statistical Analysis

Allele, genotype, and haplotype frequencies for all cytokine gene polymorphisms were calculated by direct counting and compared with the controls using both Fisher's exact test and *chi square* test. The frequencies of different genotypes were compared using the chi-square test so as to test the Hardy-Weinberg equilibrium. The odds ratio (OR) and 95% confidence intervals were estimated. The *P* value of less than 0.05 was considered to be statistically significant.

Results

Alleles and Genotype Frequencies

We observed a higher frequency of heterozygous GC in *TGF-β1* at codon 25 in controls compared to CHF cases (12.3% in controls *versus* 2.2% in patients, *P*=0·047). Moreover, heterozygous CT in *TGF-β1* at codon 10 was found to be more frequent in healthy controls compared to patients with CHF. The frequency of heterozygous CT at codon 10 reached 65.9 and 46% in these groups, respectively (*P*=0·018). However, the prevalence of homozygous CC in *TGF-β1* at codon 10 was lower in controls than in patients (14.5% in controls *versus* 32% in patients, *P*=0·011). Although the frequencies of *TGF-β1* TT genotype at codon 10 together with CG genotype at codon 25 were similar in patients and controls groups.

The allele and genotype frequencies of *IL-10* at positions -592, -819 and -1082 as well as the allelic frequency of *TGF-β1* at codon 10 and codon 25 were similar in two groups of patients and controls.

Allelic and genotype frequencies in patients with chronic heart failure and healthy subjects are shown in Table 2.

Haplotype Frequencies

IL-10 ATA haplotype at positions -1082, -819 and -592 was found to be more frequent in healthy controls in comparison with patients group (28.9% in controls *versus* 15.2% in patients, *P* = 0·004). Furthermore, a positive association was detected between *TGF-β1* CG haplotype at codon 10 and codon 25 and individual susceptibility to CHF (56.7% in patients *versus* 39.9% in controls, *P*=0·007), while *TGF-β1* TG haplotype at the same positions was significantly lower than controls (40% in patients *versus* 52.5% in controls, *P*=0·04).

We observed no significant differences between the two groups neither for ACC and GCC haplotypes at positions -1082, -819 and -592 of *IL-10* gene nor for CC and TC haplotypes at codon 10 and codon 25 of *TGF-β1* gene.

Haplotype frequencies in patients with chronic heart failure and healthy subjects are depicted in Table 3.

Discussion

Heart failure may results from a variety of underlying disorders, including ischemic heart disease, dilated cardiomyopathy and hypertension (24). Current thinking promotes the notion that multiple inflammatory elements intervene with hemostatic factors and endothelium, resulting in plaque formation, and in this way, these factors contribute towards the pathogenesis of heart failure. These inflammatory proteins, comprising IL-6 and C-reactive protein, take action through different mechanisms, one of which is down-regulation of atheroprotective cytokines, namely IL-10 and

Table 2. *IL-10* and *TGF-β1* allele and genotype polymorphisms in Iranian patients with CHF and healthy controls

Cytokine	Position	Alleles/Genotypes	Patients (N=57)	Controls (N=140) N (%)	Odds Ratio (95% CI) N (%)	p-value
			N=138		**N=50**	
TGF-β1	Codon 10	C	131 (47.5)	55 (55)	1.35 (0.85-2.14)	0.202
		T	145 (52.5)	45 (45)		
		CC	**20 (14.5)**	**16 (32)**	**2.78 (1.3-5.94)**	**0.011**
		CT	**91 (65.9)**	**23 (46)**	**0.44 (0.23-0.85)**	**0.018**
		TT	27 (19.6)	11 (22)	1.16 (0.53-2.56)	0.687
			N=138	**N=46**		
TGF-β1	Codon 25	C	21 (7.6)	3 (3.3)	0.41 (0.12-1.41)	0.221
		G	255 (92.4)	89 (96.7)		
		CC	2 (1.5)	1 (2.2)	1.51 (0.13-17.06)	1
		GC	**17 (12.3)**	**1 (2.2)**	**0.16 (0.02-1.22)**	**0.047**
		GG	119 (86.2)	44 (95.6)	3.51 (0.79-15.7)	0.108
			N=140	**N=57**		
		A	181 (64.6)	75 (65.8)	1.05 (0.66-1.66)	0.907
		G	99 (35.4)	39 (34.2)		
			N=110	**N=54**		
IL-10	-1082	AA	23 (40.3)	20 (33.8)	1.11 (0.59-2.08)	0.750
		GA	75 (53.6)	29 (50.9)	0.9 (0.48-1.66)	0.755
		GG	12 (8.6)	5 (8.8)	1.02 (0.34-3.05)	1
			N=140	**N=56**		
		C	199 (71.1)	74 (66.1)	0.79 (0.49-1.27)	0.333
		T	81 (28.9)	38 (33.9)		
IL-10	-819	CC	71 (50.7)	26 (46.4)	0.84 (0.45-1.57)	0.637
		CT	57 (40.7)	22 (39.3)	0.94 (0.5-1.77)	0.873
		TT	12 (8.6)	8 (14.3)	1.78 (0.68-4.62)	0.295
			N=140	**N=57**		
		A	81 (28.9)	26 (22.8)	0.72 (0.44-1.21)	0.261
		C	199 (71.1)	88 (77.2)		
IL-10	-592	AA	12 (8.6)	2 (3.5)	0.39 (0.08-1.79)	0.358
		CA	57 (40.7)	22 (38.6)	0.91 (0.49-1.72)	0.873
		CC	71 (50.7)	33 (57.9)	1.34 (0.72-2.49)	0.432

Table 3. IL-10 and TGF-β1 haplotype polymorphisms in Iranian patients with CHF and healthy controls

Cytokine	Position	Haplotype	Controls (n=140) N (%)	Patients (n=57) N (%)	Odds Ratio (95% CI)	p-value
TGF-β1	Codon10, Codon25	**CG**	**110 (39.9)**	**51 (56.7)**	**1.97 (1.22-3.19)**	**0.007**
		TG	**145 (52.5)**	**36 (40)**	**0.6 (0.37-0.98)**	**0.040**
		CC	21 (7.6)	2 (2.2)	0.28 (0.06-1.2)	0.08
		TC	0 (0)	1 (1.1)	-	-
IL-10	-1082, -819, -592	GCC	99 (35.4)	34 (30.3)	0.8 (0.5-1.28)	0.409
		ACC	100 (35.7)	33 (29.5)	0.75 (0.47-1.21)	0.288
		ATA	**81 (28.9)**	**17 (15.2)**	**0.44 (0.25-0.78)**	**0.004**

TGF-β1 (25). While cytokine production could be regulated by gene polymorphisms (26), we have evaluated the involvement of certain functional single nucleotide polymorphisms within *IL-10* and *TGF-β1* genes in CHF susceptibility.

TGF-β1 is a multifunctional cytokine participating in several physiological and pathological processes. Multiple mechanisms have been suggested through which TGF-β1 exerts its effects on cardiovascular pathophysiology. These mechanisms include interfering with the development of atherosclerosis, influencing endothelial function, along with affecting vascular and cardiac remodeling to name but a few (27). In particular, elevated levels of serum or plasma TGF-β1 have been reported in patients with dilated cardiomyopathy or hypertension (28). In the present study, we evaluated two cytokine single-nucleotide polymorphisms situated at codon 10 (T869C, rs1982073) and codon 25 (G915C, rs1800471) in the coding region of *TGF-β1* gene. These gene variants have been proven to be associated with the levels of cytokine production (29). It has been postulated that *TGF-β1* CC and CT genotypes at codon 10, as well as *TGF-β1* GG and GC genotypes at codon 25 would be associated with higher TGF-*β*1 production level (30). At the genotype level, we detected down-regulation of both *TGF-β1* CT genotype (codon 10) together with GC genotype (codon 25) in addition to notable overexpression of codon 25 for the CC genotype in our patients group. Therefore, TGF-β1 could act as a protective factor against CHF in Iranian population, as the low-producing *TGF-β1* genotypes have been associated with CHF in our study. The frequency of *TGF-β1* (codon 10, codon 25) TG haplotype was significantly decreased in our group of patients, whilst CG haplotype was overrepresented in patients with CHF. In a recent meta-analysis of the role of *TGF-β1* gene polymorphisms in relation to the CHD risk, it was suggested that minor allele carriers of rs1800469 and rs1982073 genetic variants in *TGF-β1* have a 15% increased risk of CHD, although no significant association was observed between rs1800471 variant and CHD susceptibility (31). The other meta-analysis of the possible contributions of *TGF-β1* gene variants towards the development of CHD complications, such as myocardial infarction, indicated the association of rs180047 C allele with CHD complications (32).

IL-10 is a potent anti-inflammatory cytokine with pleiotropic effects in inflammation and immunoregulation. It diminishes the expression of MHC class 2 antigens, TH1 cytokines as well as co-stimulatory molecules on macrophages. Additionally, it upregulates B cell survival, proliferation and antibody production (33). It has been speculated that IL-10 protects endothelial function following an inflammatory stimulus via restricting superoxide synthesis within the vascular wall (34). The production of IL-10 is modified through a promoter region containing three SNPs situated at positions –1082 (G/A), –819 (C/T) and –592 (C/A) upstream from the transcriptional start site (35). Presence of the A allele at –592 has been related to low IL-10 production. Moreover, presence of an A allele at position –1082 has been correlated with a low IL-10 production by T lymphocytes as compared to a G allele (35). It has been previously demonstrated by Edwards-Smith et al. that the *IL-10* promoter haplotypes (–1082, –819, and –592) ATA, ACC, and GCC were associated with low, intermediate, and high IL-10 production, respectively (36). In the current study, we investigated these three SNPs in both patients and controls groups. Statistical analysis of *IL-10* gene polymorphisms disclosed decreased frequency of *IL-10* (-1082, -819,-592) ATA haplotype in patient group in comparison with control category. The scarcity of the aforementioned low-producing haplotype in our patients group could suggest IL-10 as a susceptibility factor for CHF in Iranian population. Our results are in line with a previous study performed by Bijlsma et al. (35), which detected no correlation between the aforementioned genotypes and heart failure or heart transplant rejection in patients suffering from dilated cardiomyopathy or ischemic heart failure. Karaca et al. (37) also found no associations between *IL-10* -1082 G/A and -592 C/A polymorphisms and coronary heart disease in elder subjects, although they have suggested the probable role of *IL-10* -592 C/A polymorphism in CHD susceptibility in younger patients (37). Our findings are inconsistent with the results of a very recent meta-analysis study conducted by Chao et al., which revealed the association of *IL-10* -1082 AA genotype with increased atherosclerotic

risk (38). In addition, Wang et al. (39) suggested *IL-10* -1082G/A polymorphism genotypes (GA+AA) to be associated with an increased risk of coronary heart disease, especially in Caucasians, as a result of their meta-analysis study. In another recent study, Yu et al. (40) proposed C allele with SNPs at position –592C/A and –819C/T of *IL10* gene to be associated with ischemic heart disease (IHD) in the Korean population, but observed no correlation between -1082 G/A SNPs with IHD.

In closing, we believe this is the first study in which the assessment of the associations between certain SNPs in both *IL-10* and *TGF-β1* genes and individual vulnerability to CHF has been carried out in a group of Iranian patients. Our findings unveiled great contrasts in certain genotypic positions [TGF-β1 at codon 10 (CT and CC), TGF-β1 at codon 25 (GC)], and haplotypic positions [IL-10 (-1082, -819, -592) in ATA, TGF-β1 (codon 10, codon 25) in CG and TG], between case and control groups. This association study suggests the aforementioned gene variants as possible genetic risk factors for the initiation and progression of underlying cardiovascular disorders leading to CHF. However, considering the genetic heterogeneity in studies of HF susceptibility in different races, further investigations are advocated in divergent ethnic groups, using larger sample size, to authenticate such associations between *IL-10* and *TGF-β1* gene polymorphisms and CHF.

Acknowledgement

This study was supported by grant from Tehran University of Medical Sciences and Health Services (87-04-93-9584).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Lloyd-Jones DM, Larson MG, Leip EP, et al. Lifetime risk for developing congestive heart failure the Framingham Heart Study. Circulation 2002; 106(24): 3068-72.
2. Amirzargar AA, Bagheri M, Ghavamzadeh A, et al. Cytokine gene polymorphism in Iranian patients with chronic myelogenous leukaemia. Int J Immunogenet 2005; 32(3): 167-71.
3. Yndestad A, Damås JK, Øie E, Ueland T, Gullestad L, Aukrust P. Systemic inflammation in heart failure–the whys and wherefores. Heart failure reviews 2006; 11(1): 83-92.
4. Lesnik P, Haskell CA, Charo IF. Decreased atherosclerosis in CX3CR1–/–mice reveals a role for fractalkine in atherogenesis. Journal of Clinical Investigation 2003; 111(3): 333-40.
5. Spits H, de Waal Malefyt R. Functional characterization of human IL-10. International archives of allergy and immunology 1992; 99(1): 8-15.
6. Gamble JR, Vadas MA. Endothelial adhesiveness for blood neutrophils is inhibited by transforming growth factor-beta. Science 1988; 242(4875): 97-9.
7. Gamble JR, Vadas MA. Endothelial cell adhesiveness for human T lymphocytes is inhibited by transforming growth factor-beta 1. The Journal of immunology 1991; 146(4): 1149-54.
8. Smith WB, Noack L, Khew-Goodall Y, Isenmann S, Vadas MA, Gamble JR. Transforming growth factor-beta 1 inhibits the production of IL-8 and the transmigration of neutrophils through activated endothelium. The Journal of Immunology 1996; 157(1): 360-8.
9. DiChiara MR, Kiely JM, Gimbrone MA, Jr., Lee ME, Perrella MA, Topper JN. Inhibition of E-selectin gene expression by transforming growth factor beta in endothelial cells involves coactivator integration of Smad and nuclear factor kappaB-mediated signals. The Journal of experimental medicine 2000; 192(5): 695-704.
10. Miyake K, Nakashima H, Akahoshi M, et al. Genetically determined interferon-γ production influences the histological phenotype of lupus nephritis. Rheumatology 2002; 41(5): 518-24.
11. Ohtsuka K, Gray J, Stimmler M, Horwitz D. The relationship between defects in lymphocyte production of transforming growth factor-β1 in systemic lupus erythematosus and disease activity or severity. Lupus 1999; 8(2): 90-4.
12. Amirzargar A, Shahram F, Nikoopour E, et al. Proinflammatory cytokine gene polymorphisms in Behcet's disease. Eur Cytokine Netw 2010; 21(4): 292-6.
13. Mahdaviani SA, Rezaei N, Moradi B, Dorkhosh S, Amirzargar AA, Movahedi M. Proinflammatory cytokine gene polymorphisms among Iranian patients with asthma. J Clin Immunol 2009; 29(1): 57-62.
14. Rezaei N, Amirzargar AA, Shakiba Y, Mahmoudi M, Moradi B, Aghamohammadi A. Proinflammatory cytokine gene single nucleotide polymorphisms in common variable immunodeficiency. Clin Exp Immunol 2009; 155(1): 21-7.
15. Amirzargar AA, Rezaei N, Jabbari H, et al. Cytokine single nucleotide polymorphisms in Iranian patients with pulmonary tuberculosis. Eur Cytokine Netw 2006; 17(2): 84-9.
16. Tahghighi F, Ziaee V, Moradinejad MH, et al. Tumor necrosis factor-alpha single nucleotide polymorphisms in juvenile systemic lupus erythematosus. Human immunology 2015; 76(8): 533-6.
17. Mahmoudi M, Tahghighi F, Ziaee V, et al. Interleukin-4 single nucleotide polymorphisms in juvenile systemic lupus erythematosus. International journal of immunogenetics 2014; 41(6): 512-7.

18. Rezaei A, Ziaee V, Sharabian FT, et al. Lack of association between interleukin-10, transforming growth factor-beta gene polymorphisms and juvenile-onset systemic lupus erythematosus. Clinical rheumatology 2015; 34(6): 1059-64.
19. Ziaee V, Tahghighi F, Moradinejad MH, et al. Interleukin-6, interleukin-1 gene cluster and interleukin-1 receptor polymorphisms in Iranian patients with juvenile systemic lupus erythematosus. European cytokine network 2014; 25(2): 35-40.
20. Maddah M, Harsini S, Rezaei A, et al. Association of Interleukin-2, but not Interferon-Gamma, single nucleotide polymorphisms with juvenile idiopathic arthritis. Allergologia et immunopathologia 2016.
21. Ziaee V, Rezaei A, Harsini S, et al. Polymorphisms of genes encoding interleukin-4 and its receptor in Iranian patients with juvenile idiopathic arthritis. Clinical rheumatology 2016.
22. Amirzargar AA, Naroueynejad M, Khosravi F, et al. Cytokine single nucleotide polymorphisms in Iranian populations. Eur Cytokine Netw 2008; 19(2): 104-12.
23. Miller S, Dykes D, Polesky H. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic acids research 1988; 16(3): 1215.
24. Matsumori A, Sasayama S. The role of inflammatory mediators in the failing heart: immunomodulation of cytokines in experimental models of heart failure. Heart failure reviews 2001; 6(2): 129-36.
25. Ikonomidis I, Stamatelopoulos K, Lekakis J, Vamvakou GD, Kremastinos DT. Inflammatory and non-invasive vascular markers: the multimarker approach for risk stratification in coronary artery disease. Atherosclerosis 2008; 199(1): 3-11.
26. Hoffmann SC, Stanley EM, Cox ED, et al. Association of cytokine polymorphic inheritance and in vitro cytokine production in anti-cd3/cd28-stimulated peripheral blood lymphocytes1. Transplantation 2001; 72(8): 1444-50.
27. Cambien F, Ricard S, Troesch A, et al. Polymorphisms of the Transforming Growth Factor-β1 Gene in Relation to Myocardial Infarction and Blood Pressure The Etude Cas-Témoin de l'Infarctus du Myocarde (ECTIM) Study. Hypertension 1996; 28(5): 881-7.
28. Crobu F, Palumbo L, Franco E, et al. Role of TGF-β1 haplotypes in the occurrence of myocardial infarction in young Italian patients. BMC medical genetics 2008; 9(1): 13.
29. Grainger DJ, Heathcote K, Chiano M, et al. Genetic control of the circulating concentration of transforming growth factor type β1. Human molecular genetics 1999; 8(1): 93-7.
30. Crilly A, Hamilton J, Clark CJ, Jardine A, Madhok R. Analysis of transforming growth factor beta1 gene polymorphisms in patients with systemic sclerosis. Annals of the rheumatic diseases 2002; 61(8): 678-81.
31. Lu Y, Boer JM, Barsova RM, et al. TGFB1 genetic polymorphisms and coronary heart disease risk: a meta-analysis. BMC medical genetics 2012; 13(1): 39.
32. Morris DR, Moxon JV, Biros E, Krishna SM, Golledge J. Meta-analysis of the association between transforming growth factor-beta polymorphisms and complications of coronary heart disease. PloS one 2012; 7(5): e37878.
33. Prondzinsky R, Unverzagt S, Lemm H, et al. Interleukin-6, -7, -8 and -10 predict outcome in acute myocardial infarction complicated by cardiogenic shock. Clinical research in cardiology : official journal of the German Cardiac Society 2012; 101(5): 375-84.
34. Gunnett CA, Heistad DD, Berg DJ, Faraci FM. IL-10 deficiency increases superoxide and endothelial dysfunction during inflammation. American journal of physiology Heart and circulatory physiology 2000; 279(4): H1555-62.
35. Bijlsma F, Bruggink A, Hartman M, et al. No association between IL-10 promoter gene polymorphism and heart failure or rejection following cardiac transplantation. Tissue Antigens 2001; 57(2): 151-3.
36. Edwards-Smith CJ, Jonsson JR, Purdie DM, Bansal A, Shorthouse C, Powell EE. Interleukin-10 promoter polymorphism predicts initial response of chronic hepatitis C to interferon alfa. Hepatology 1999; 30(2): 526-30.
37. Karaca E, Kayikcioglu M, Onay H, Gunduz C, Ozkinay F. The effect of interleukin-10 gene promoter polymorphisms on early-onset coronary artery disease. Anadolu kardiyoloji dergisi : AKD = the Anatolian journal of cardiology 2011; 11(4): 285-9.
38. Chao L, Lei H, Fei J. A meta-analysis of interleukin-10-1082 promoter genetic polymorphism associated with atherosclerotic risk. Neurology India 2014; 62(2): 130.
39. Wang Y, Zheng J, Liu P, et al. Association between the Interleukin 10– 1082G> A polymorphism and coronary heart disease risk in a Caucasian population: a meta-analysis. International journal of immunogenetics 2012; 39(2): 144-50.
40. Yu G-I, Cho H-C, Cho Y-K, et al. Association of promoter region single nucleotide polymorphisms at positions– 819C/T and– 592C/A of interleukin 10 gene with ischemic heart disease. Inflammation Research 2012; 61(8): 899-905.

Received: 5 August 2017
Accepted: 20 July 2018
Correspondence:
Nima Rezaei, MD, PhD
Children's Medical Centre Hospital,
Dr Qarib St, Keshavarz Blvd, Tehran 14194, Iran
Tel. +9821-6692-9234
Fax +9821-6692-9235
E-mail: rezaei_nima@tums.ac.ir

Acta Biomed 2019; Vol. 90, N. 2: 228-232 DOI: 10.23750/abm.v90i2.8477

Original article

Adverse events during testosterone replacement therapy in 95 young hypogonadal thalassemic men

Vincenzo De Sanctis[1], Ashraf T Soliman[2], Shahina Daar[3], Salvatore Di Maio[4]

[1]Pediatric and Adolescent Outpatient Clinic, Quisisana Hospital, Ferrara, Italy; [2] Department of Pediatrics, Hamad General Hospital, Doha, Qatar; [3] Department of Hematology, College of Medicine and Health Sciences Sultan Qaboos University Muscat, Sultanate of Oman; [4] Emeritus Director in Pediatrics, Children's Hospital "Santobono-Pausilipon", Naples, Italy

Summary. *Background:* Hormonal treatment of hypogonadism in thalassaemia major (TM) is a complex issue due to the co-existence of other contributing factors such as severity of iron overload, associated chronic liver disease and other endocrine complications. *Objectives:* Data about adverse events (AEs) of testosterone replacement therapy (TRT) in hypogonadal males with TM is scarce.We report the adverse events registered during TRT in 95 young patients with TM. *Results:* These AEs included gynecomastia, documented in 41/95 (43.1%) TM patients of mild to moderate severity (90%). Persistent pain in the injection site and local reactions to testosterone (T) skin patch occurred in a third of patients. Priapism was reported in 2 patients on treatment with intramuscular T enanthate. In both patients, substitution with T gel was successful, and no recurrence during the following 24 months was observed. *Conclusions:* Clinicians should exercise caution when considering TRT for hypogonadal men with TM. (www.actabiomedica.it)

Key words: thalassemia major, testosterone therapy, adverse events, gynecomastia, priapism

Introduction

About 40% to 80% of male patients with thalassemia major (TM) experienced pubertal failure, sexual dysfunction and infertility due to hypogonadotropic hypogonadism (HH) (1, 2). Hormonal management of HH in thalassaemia is a complex issue due to the co-existence of other interfering factors such as severity of iron overload, associated chronic liver disease and other endocrine complications (3).

Over the past two decades, significant advances have been made for improving the understanding of the pathophysiology of endocrine complications in patients with TM. Cross-sectional and review papers reported that from 40% to 80% of male TM patients experienced pubertal failure, sexual dysfunction and infertility due to HH (1). However, testosterone replacement therapy (TRT) has numerous benefits that can greatly enhance a patient's quality of life.

Much of the controversy surrounding testosterone therapy stems from intense attention on recent reports suggesting increased risk of venous thromboembolism or cardiovascular events in young and aging men (4-6).

The aim of the present retrospective study was to analyze the adverse events registered during TRT in a group of hypogonadal TM patients, followed in the last 43 years by a single Centre.

An adverse event was defined as an unfavourable medical event that may present during treatment with a pharmaceutical product, which does not necessarily have a causal relationship with the product.

Patients and methods

This retrospective study analysed adverse events registered during TRT in 95 hypogonadal male TM patients with arrested puberty (AP:4.2%), HH

(84.3%) or acquired hypogonadotropic hypogonadism (AHH:11.5%), followed regularly or occasionally at the Pediatric and Adolescent Outpatients Clinic of Ferrara. All patients (age 17-42 years; mean 27.6) were of Italian ethnic origin.

An adverse event was defined as an unfavourable medical event that may present during treatment with a pharmaceutical product, which does not necessarily have a causal relationship with the product.

All patients were evaluated for pituitary-gonadal axis integrity. The diagnosis of HH was based on symptoms and signs of hypogonadism plus the presence of low serum testosterone level measured on at least two occasions, normal prolactin, and low basal pituitary gonadotropin levels (LH and FSH).

Virilization was the primary objective for these TM patients in order to ameliorate their psychological problems related to hypogonadism. Several testosterone (T) formulations were prescribed for their treatment: T intramuscular injections (51.7%), T gel preparations (25.2%), T transdermal patches (12.6%), and T undecanoate given orally (10.5%). The duration of TRT varied from 1-25 years (mean 8.5 years).

Results

Normalization of T levels improved most of the effects due to hypogonadism (sexual infantilism, decreased sense of well-being, loss of libido, erectile or ejaculatory dysfunctions.

The adverse events registered during long-term TRT in 95 patients with TM are reported in table 1. The commonest event was gynecomastia, documented in 41/95 TM patients (43.1%) of mild to moderate severity (90%). Forty six percent of them were HCV-RNA positive. No patient was treated with aromatase inhibitors.

Persistent pain in the injection site and local reactions to T skin patch occurred in some patients.

Priapism was reported in 2 patients on treatment with intramuscular T (100 mg testosterone enanthate, monthly and twice a month). Priapism was not due to supra-physiological levels of testosterone in the serum. One patient (32 years), responded to aspiration of blood from the corpora cavernosa and administration of a sympathomimetic. Acute lower-limb exercise (stair climbing) was effective in the second patient (26

Table 1. Adverse events registered during testosterone replacement therapy in 95 thalassemia major patients (TM) with hypogonadism

Adverse events	TM patients (Numbers and %)
Gynecomastia	41/95 (43.1%)
Persistent pain in the injection site	25/95 (26.3%)
Application site reactions where the skin patch was worn (redness, itching, burning, or hardened skin)	13/22 (13.6 %)
Acne and/or oily skin	8/95 (8.4%)
Mild elevation of liver enzymes	4/95 (4.2%)
Insomnia	3/95 (3.1%)
Frequent urination	3/95 (3.1%)
Excessive libido	2/95 (2.1%)
Priapism	2/95 (2.1%)
Mild elevation of lipids	2/95 (2.1%)
Associated recurrent mild fever	1/95 (1%)
Headache	1/95 (1%)
Deterioration of glucose tolerance (from normal to impaired glucose tolerance)	1/95 (1%)
Elevation of prostate-specific antigen (PSA)	none
Depression	none
Sleep apnea	none
Increased blood pressure	none
Increased appetite	none
Changes in taste or smell	none

years). In both, substitution with T gel was successful, and no recurrence during the following 24 months was observed.

None of the patients had symptoms or signs suggesting venous thromboembolism, associated with use of TRT.

Discussion

Despite recent advances in iron chelation therapy, excess iron deposition in pituitary gonadotropic cells remains one of the major problems in thalassemic patients (1, 7, 8).

Hypogonadism, mostly HH , is usually detected during puberty. In addition, the direct effect of iron, in particular that of Non-Transferrin-Bound Iron (NTBI), on the testes appears to be harmful (9).

Histological examination of testicular tissues from autopsies demonstrated testicular interstitial fibrosis with small, heavily pigmented, undifferentiated seminiferous tubules and an absence of Leydig cells (10).

Early diagnosis and treatment are crucial for normal pubertal development and to reduce the complications of hypogonadism (1, 2). The American Society of Andrology, concluded that men with serum testosterone concentrations below 264 ng/dL (9.2 nmol/L) will usually benefit from testosterone replacement therapy (TRT) (11).

The different goals of T therapy should be summarized by the following: 1. to induce sex-specific secondary sexual characteristics, and then maintain them in adulthood; 2. to optimize pubertal growth spurt and have physiologic body proportions (not to be eunuchoid); 3. to obtain adequate lean muscle mass, fat mass and optimal bone mineral mass accretion; 4. to develop adequate external genital appearance (penile size and scrotum) and internal genitalia growth; 5. to reduce cardio-vascular and metabolic syndrome risk by optimization of lipid profile, and 6. to induce sex-specific psychosocial and psychosexual maturation, and assure normal social/sexual life and well-being (12). However, the risks and benefits of TRT, remain a challenge for providers caring for thalassemic patients.

In this long-term retrospective study, the commonest side effect was gynecomastia, documented in 43.1% of patients. However, the degree of gynecomastia ranged between mild to moderate in severity (90%) and did not necessitate further management. None of the patients received aromatase inhibitor therapy. Aromatase is the enzyme responsible for converting androgens to estrogens and is widely distributed in several tissues such as brain, liver and reproductive tissue. In men, estrogen production occurs mainly by extra testicular aromatization of androstenedione to estrone and of testosterone to estradiol. Therefore, exogenous testosterone through aromatization may increase estradiol production leading to gynecomastia (13).

In addition, the associated presence of chronic liver disease, because of hepatic iron overload and previous viral liver infection (46.4% of TM patients were HCV-RNA positive), may exaggerate and contribute to the relatively high incidence of the gynecomastia in thalassemic patients on TRT. In chronic liver disease there is an increased production of androstenedione by the adrenal glands, increased aromatisation of androstenedione to estrogen, loss of clearance of adrenal androgens by the liver and a rise in SHBG, resulting in gynecomastia (14, 15) (figure 1).

Persistent pain at the injection site and local reactions to T skin patch occurred in about one third of patients. Successful management of cutaneous reactions has been shown to be successful through rotation of application sites, patch placement on the buttocks, trials of alternate brands, a shorter duration of occlusion, and pretreatment of the skin with topical corticosteroids (16).

Although priapism because of TRT is a rare complication, it was reported in 2 of our TM patients on treatment with intramuscular enanthate T. Intramuscular depot injection of testosterone is less expensive and can be administered every 2-4 weeks. A major disadvantage is the strongly fluctuating concentrations of plasma testosterone which are at least 50% of the time not in the physiological range. This also causes supraphysiologic serum testosterone concentrations within 2 to 3 days of injection and a slow decline to subnormal levels within 1 to 2 weeks (12, 17). However, in the two patients, priapism did not appear to be due to supra-physiological levels of testosterone in the serum because of the lower dose used. Treatment of priapism in one patient (32 years old) required aspiration

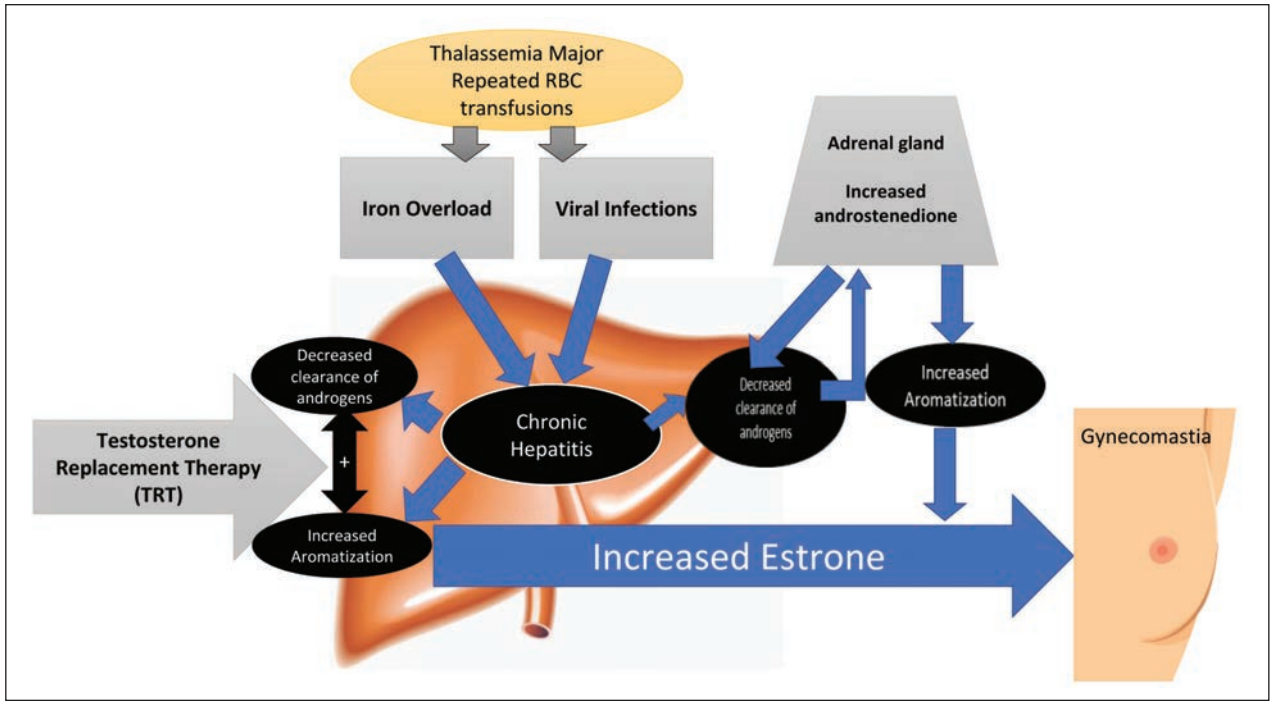


Figure 1. Pathophysiology of gynecomastia in thalassemic patients on testosterone replacement therapy (TRT)

of blood from the corpora cavernosa, to decompress the penis, and administration of a sympathomimetic. Conservative management using acute lower-limb exercise (stair climbing) was effective in the second patient (26 years old) on treatment with 100 mg testosterone enanthate, bimonthly. In both patients, replacement therapy with a different drug formulation (T gel) was successful, and no recurrence developed during the following 24 months.

None of our patients had symptoms or signs suggesting a hypercoagulable state or venous thrombosis. However, various studies reported incidence of thromboembolic event in thalassemia, ranging between 1–29% (18-20). Our group previously reported the incidence of left atrial thrombosis in a 19-year-old adolescent male with TM and diabetes mellitus (6).

In females, randomized controlled trials (RCT) demonstrated that oral estrogen increases venous thromboembolism (VTE) risk in women aged 50 to 59. However, observational studies and meta-analyses suggest that transdermal estrogen therapy does not increase VTE risk, even in women with thrombophilia (21). No RCTs were conducted in thalassemic patients on estrogen or testosterone transdermal therapy. Therefore, VTE is still potential serious side effect that should be kept in mind during TRT.

It appears that TRT improves many negative effects of hypogonadism in our thalassaemic hypogonadal males and in other studies but should be utilized with full awareness of its potential risks.

Measuring serum testosterone to avoid supraphysiological levels of T and monitoring for adverse effects from TRT should also be done. A prostatic specific antigen (PSA) concentration and digital rectal examination should be performed at baseline and at 3 months, 6 months, then yearly after TRT is initiated (11).

Proper and intensive iron chelation is important for decreasing the iron load in the liver and endocrine system. In 14 hypogonadal males on testosterone therapy, seven stopped treatment after intensive chelation of their body iron (22).

Conclusion

Clinicians should exercise caution when considering TRT for hypogonadal men with TM and discuss the benefits and potential risks prior to initiating treatment and monitor any side effects during their use.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. De Sanctis V, Soliman AT, Yassin MA, Di Maio S, Daar S, Elsedfy H, Soliman N, Kattamis C. Hypogonadism in male thalassemia major patients: pathophysiology, diagnosis and treatment. Acta Biomed 2018; 89: 6-15.
2. De Sanctis V, Soliman AT, Elsedfy H, Skordis N, Kattamis C, Angastiniotis M, Karimi M, Yassin MA, El Awwa A, Stoeva I, Raiola G, Galati MC, Bedair EM, Fiscina B, El Kholy M. Growth and endocrine disorders in thalassemia: The international network on endocrine complications in thalassemia (I-CET) position statement and guidelines. Indian J Endocrinol Metab 2013; 17: 8-18.
3. Soliman AT, elZalabany MM, Ragab M, Abdel Fattah M, Hassab H, Rogol AD, Ansari BM. Spontaneous and GnRH-provoked gonadotropin secretion and testosterone response to human chorionic gonadotropin in adolescent boys with thalassaemia major and delayed puberty. J Trop Pediatr 2000; 46: 79-85.
4. Martinez C, Suissa S, Rietbrock S, Katholing A, Freedman B, Cohen AT, Handelsman DJ.Testosterone treatment and risk of venous thromboembolism: Population based case-control study. BMJ 2016; 355: i5968. doi: 10.1136/bmj.i5968.
5. Glueck CJ, Prince M, Patel N, Patel J, Shah P, Mehta N, Wang P.Thrombophilia in 67 patients with thrombotic events after starting testosterone therapy. Clin Appl Thromb Hemost 2016; 22: 548-553.
6. De Sanctis V, Daar S, Soliman AT, Elsedfy H, Khater D, Di Maio S. Does testosterone replacement therapy promote an augmented risk of thrombotic events in thalassemia major male patients with hypogonadism? Indian J Endocr Metab 2017; 21: 636-537.
7. Christoforidis A, Haritandi A, Perifanis V, Tsatra I, Athanassiou-Metaxa M, Dimitriadis AS. MRI for the determination of pituitary iron overload in children and young adults with beta-thalassaemia major. Eur J Radiol 2007; 62: 138-142.
8. Lam WW, Au WY, Chu WC, Tam S, Ha SY, Pennell DJ. One-stop measurement of iron deposition in the anterior pituitary, liver, and heart in thalassemia patients. J Magn Reson Imaging 2008; 28: 29-33.
9. Siripunthana S, Sahakitrungruang T, Wacharasindhu S, Sosothikul D,Supornsilchai V. Testicular function in patients with regular blood transfusion for thalassemia major. Asian Biomed 2015; 9: 185-191.
10. Canale V, Steinherz P, New M, Erlandson M. Endocrine function in thalassemia major. Ann N Y Acad Sci 1974; 232: 333-345.
11. Bhasin S, Brito JP, Cunningham GR, Hayes FJ, Hodis HN, Matsumoto AM, Snyder PJ, Swerdloff RS, Wu FC, Yialamas MA. Testosterone Therapy in Men With Hypogonadism: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2018; 103: 1715-1744.
12. Soliman AT, De Sanctis V, Yassin M. Androgen Therapy in Adolescents: A Review. Andrology 2014, 3: 124. doi: 10.4172/2167-0250.1000124.
13. Johnson RE, Murad MH. Gynecomastia: pathophysiology, evaluation, and management. Mayo Clin Proc 2009; 4: 1010-1015.
14. Gordon GG, Olivo J, Rafil F, Southren AL. Conversion of androgens to estrogens in cirrhosis of the liver. J Clin Endocrinol Metab 1975; 40: 1018-1026.
15. Rahmani S, Turton P, Shaaban A, Dall B.Overview of gynecomastia in the modern era and the Leeds Gynaecomastia Investigation algorithm. Breast J 2011; 17: 246-255.
16. De Sanctis V, Vullo C, Urso L, Rigolin F, Cavallini A, Caramelli K, Daugherty C, Mazer N. Clinical experience using the Androderm testosterone transdermal system in hypogonadal adolescents and young men with beta-thalassemia major. J Pediatr Endocrinol Metab 1998; 11 (Suppl 3): 891-900.
17. Gooren LJ. Advances in testosterone replacement therapy. Front Horm Res 2009; 37: 32-51.
18. Taher AT, Otrock ZK, Uthman I, Cappellini MD. Thalassemia and hypercoagulability. Blood Rev. 2008; 22: 283-292.
19. Cappellini MD, Poggiali E, Taher AT, Musallam KM. Hypercoagulability in β-thalassemia: a status quo. Expert Rev Hematol 2012; 5: 505-511.
20. Elalfy MS, Andrawes NG, Sadek AM, Hussein O, Abdou A. D-dimer and portal vein status in splenectomized Egyptian β-thalassemia major patients: a prospective single-thalassemia center experience. Pediatr Hematol Oncol 2012; 29: 247-253.
21. Stuenkel CA, Davis SR, Gompel A, Lumsden MA, Murad MH, Pinkerton JV, Santen RJ. Treatment of symptoms of the menopause: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2015; 100: 3975-4011.
22. Farmaki K, Tzoumari I, Pappa C, Chouliaras G, Berdoukas V. Normalisation of total body iron load with very intensive combined chelation reverses cardiac and endocrine complications of thalassaemia major. Br J Haematol 2010; 148: 466-475.

Received: 14 April 2019
Accepted: 15 May 2019
Correspondence:
Vincenzo De Sanctis MD,
Pediatric and Adolescent Outpatient Clinic,
Quisisana Hospital - 44100 Ferrara, Italy
Tel. +39 0532 770243
E-mail: vdesanctis@libero.it

Acta Biomed 2019; Vol. 90, N. 2: 233-240 DOI: 10.23750/abm.v90i2.6831

Original article

The "Patient's Empowerment rights-duty Charter": new communication tools targeted at patient and professionals in a Hematology and Bone marrow transplant center

Cipriana Mengozzi[1], Giovanna Campaniello[1], Franco Aversa[2], Francesca Re[2], Barbara Calatafimi[2], Roberto Albertini[3], Francesca Montali[4]

[1] Clinical Government Unit of Parma University Hospital, Parma, Italy; [2] Hematology and Bone marrow transplant center Unit of Parma University Hospital, Parma, Italy; [3] Chiara Tassoni Onlus, Volunteer Association of Parma, for the research into leukemia and cancer, Parma, Italy; [4] Department of Primary Care of the Local Health Company of Reggio Emilia, Italy

Summary. *Background and aim of the work:* Empowerment is very important to keep high staff motivation and attention on patient safety. The aim of this study was to produce a "Charter of Rights-Duties for Patients' Empowerment", by developing empowerment both of patients and professionals of the Hematology and Bone marrow transplant center Unit of Parma University Hospital. *Methods:* The professionals were actively involved in meetings to complete the Italian version of the SESM Empowerment Questionnaire, draft the Charter and produce some communication tools to be implemented in the Unit. *Results:* All professionals had participated to the research. The level of empowerment in the unit, both for doctors and non-medical staff, is very high. This result, the Charter and the others communication tools are now known and shared by all. *Conclusions:* Stimulating empowerment seems to be a winning choice. It is important to involve professionals right away in the process because a high level of staff empowerment can generate a good field for high degree of patients' empowerment, that can increase patient safety and reduce the risk of dangerous health choices. This approach aims to increase patient safety through the collaboration of patients, volunteer associations and professionals. Communication programs must include the development of empowerment: it motivates citizens to engage and the literacy enables them to make informed and reasoned choices. General Management is now evaluating how to realize the program in each ward, discussing the importance of carefully conceptualizing this approach for the design of health interventions. (www.actabiomedica.it)

Key words: patient empowerment, professionals' empowerment, clinical risk management, patient safety and prevention, hematology, bone marrow transplant center

Introduction

As the WHO has repeatedly called, the citizen must be the first actor of the choices that affect his health and, because this is achieved, the active participation of citizens in the socio-sanitary processes involving them must be promoted, using a "shared" approach to the decision/management of pathways and health care: since 1994, WHO has defined the Principles of Patients' Rights in Europe (1) and in 2007 it underlined the primary importance of People at the Centre of Health Care (2). Many studies, scientific works and international reports, say that the National Health System need a more patient centered approach (3) and support that the way to drive change is working together for excellence, creating a continuously im-

proving consumer engagement framework for excellence in patient-centered care (4).

In Italy, the AgeNaS (the Italian national agency for regional health services) since 2007 has established an Interregional Empowerment Working Group, precisely to promote the development of individual, organizational and community empowerment processes. The importance of citizens' empowerment for the development of Health Care System is underline in many AgeNaS publications, which summarize the national experiences in this area (5) and underline the citizens' experience in improving regional and national health services (6). The Empowerment Working Group, which deals with empowerment of both patients and professionals, has adopted the following definition of empowerment: "(...) is a process of social action through which people, organizations and communities acquire competence over their own years, in order to change their social and political environment to improve equity and quality of life" (7).

The concept of empowerment, which has for some time now been part of the common language of those who care for health and care, translates, on the one hand, to the professional awareness, engagement and empowerment, and on the other side in the growing capacity and awareness of the citizen in taking care of himself. The citizen is empowered when acquiring knowledge, skills, attitudes and awareness to influence one's own and other behaviors, to improve the quality of their own life and their "well-being" even in critical conditions.

Schulz and Nakamoto, in a 2013 article on Patient Education and Counseling (8), insist on the importance of distinguishing mastery/mastery and health literacy/literacy: both are needed, and in cases where there is one without the other, problems arise. In summary, the single actor, whatever its role (citizen or professional), once empowered, becomes part of a system that involves and enhances that attitude, to be transferred to interpersonal relationships and care paths, and, more reason, to be the basis of the healthcare organization that delivers them.

When the healthcare provider first engages in patient and citizen empowerment programs, he needs to be supported in his professional empowerment path. First of all, it is to stimulate a cultural change, leaving behind the traditional way of understanding the profession to look at things with other eyes.

This research has stimulated professionals of the Hematology and Bone marrow transplant center Unit from University Hospital of Pama to actively engage the patient in the cure process by creating a new patient communication tool. The "Patient's Empowerment rights-duty Charter", which is the principal result of this work, has been written by professionals to make precise commitments to the patient and to feel part of the process of empowerment both of themselves and of patients, in a growth, personal and professional development, aimed at increasing the quality and safety of care.

Materials and methods

The project, supported by the "Chiara Tassoni Volunteer Association" of Parma (it is a volunteer association for the research into leukemia and cancer), started with a first phase of bibliographic research, which lasted from June 2016 to August 2016, with a view to compiling the studies already carried out on the empowerment of patients and professionals - and to find out which tools and methods - particularly in the field of oncology and hematology.

The second phase of sharing with Hematology and Bone marrow transplant center Unit from University Hospital of Parma, Italy, took place in September-November 2016, with the first planning and sharing meetings involving the Director of the Structure, the Nursing Officer and some professionals. The purpose of these preliminary meetings was to share design lines with the project headquarters structure, so as to lay the foundations for intervention based on involvement and active participation of staff.

The third stage of selection of survey tools was completed in November 2016.

The fourth phase of activities started and the implementation of the design phases began in December 2016 and lasted until March 2017.

The fifth and final phase of returning results and dissemination and implementation within the structure of communication tools implemented with the project was completed in May 2017.

With regard to future developments, the tools implemented with the project will be in use from June 2017 onwards in the Hematology and Bone marrow transplant center Unit and will evaluate the applicability and dissemination (with appropriate modifications) at the best practice level also in other departments and business structures, in order to optimize the resources used in the project and put the tools at the disposal of the largest number of professionals and patients.

The survey sample is made up of all the staff of the Hematology and Bone marrow transplant center Unit which respected the requirement of "work in the ward/outpatient clinic, mainly in contact with the patient". Samples were then excluded according to the following criteria: "poor interaction with the department", "membership of the stem laboratory", "transfer to another health company in progress", "secretarial duties only", "pregnancy".

For the data collection and the gathering of the opinions of the professionals, 6 meetings were held at the facility: making directly at the facility wanted to facilitate access to the initiative and give everyone the opportunity to participate, compatible with the shifts of the individuals and service needs.

Each meeting has got three distinct phases:

- Presentation of the goals of the project and tools that we're going to build;
- Discussion by Focus Group on the "Empowerment Citizens' Rights Charter";
- Compiling the "Empowerment Questionnaire".

Based on this kind of participative method, fundamental to the success of the project was the collaborative role played by the department's medical and nursing staff who promoted the initiative, actively supporting the Clinical Government Unit, for collect membership and equal distribution of operators inside the various editions of the meeting. During the meetings, the choice of the focus group methodology allowed us to look at the opinions and to gather concrete proposals from all the participants who participated (43 operators on the 6 editions), which they felt called contributing and expressing themselves first, as well as being directly involved and valued. We have made it possible to complete the empowerment questionnaire also to other 3 operators who could not participate in the meetings, so as to have everyone's answer.

The "Patient's Empowerment rights-duty Charter"

The focus group discussion forum was the "Patient Empowerment Patient Charter", prepared by the EPF (European Patients Forum) for their campaign to promote the empowerment of the patients in many European hospitals over the years (13). The Charter consists of 10 items, each briefly described and supported by the authors, which touch upon all the many aspects of the citizen's relationship with the public institution on health and safety of care in terms of the demands and rights that every citizen should/It would also like to have, at the level of law, the ability to play an active role in the decisions concerning its state of health and the process of care. Being born only from the bottom, the citizens and their representatives, the Charter in its original version presupposes a very high commitment from professionals to meet the demands and ensure the real application of the content and principles contained therein. The project did not want to assume that professionals felt able to take on such a commitment in all the points outlined in the Charter and wanted to explore the real possibilities of implementing the tool, giving the professionals the opportunity to Customize the Charter to their department and for Hematology and Bone marrow transplant center Unit. For this reason, the focus group aimed at examining in detail, together with the operators, every item of paper and evaluating the real possibilities of application within the daily activity with patients, both in terms of content expressed and, above all, based on the level of empowerment perceived in turn by the operators in relation to the structure and, more generally, of the national health system. During the meeting, after the introductory phase of project submission and data collection tools, it was to read, discuss and reformulate with each operator each item of the paper in its original version, to build a specific paper, totally Belonging to the department and filtered through daily experience.

The "Empowerment Questionnaire"

Another goal of the meetings was to strengthen the internal climate and the relationships among professionals, encouraging the exchange of views and directing the forces of everyone towards the creation of a

single instrument (the Charter), shared at the medical and nursing level, to be distributed to users, as a "basic document" for mutual engagement between the users and the multiprofessional team. In addition to arriving at the drafting of the Charter, it was in the intentions of the project to experiment with the use of a questionnaire that would reveal the level of empowerment first in the professionals, then (eventually) in the patients. In this logic, through the administration of the questionnaire to professionals, they wanted to give them a chance to reflect on the possible use of this tool with patients, starting with the emotions and reactions they themselves experienced in compiling. For this reason, the questionnaire chosen is the SESM - Italian version of the Scale of Users to Measure Empowerment in Mental Health Services (14), as it was considered that this instrument affected the main dimensions involved in demonstrating the difficulties of these types of patients and which would, however, allow to monitor the main variables involved in the process of empowerment at the individual level, regarding actually working conditions and ways of seeing life and making decisions. The questionnaire was anonymous, but at the time of compilation, operators were given the opportunity to enter their name and address if they were interested to receive privately their personal result.

Results

All the professionals have decided to take part in the study (N=46), so the results do not refer to a sample, but to the entire population of professionals operating in Hematology and Bone marrow transplant center Unit and the 67.39% of the operators signed the questionnaire also if anonymity was allowed. 41.3% were Doctors and 58.7% were non-medical personnel (nurses, assistant nurses and biotechnology laboratory technicians). About gender, 23.9% and 76.1% were male and female respectively. Regarding the age of the participants, the distribution was the following: 2.2% is <25 years; 47.8% is 26-35; 17.4% is 36-45, 28.3% is 46-55 and 4.3% is >56 years. Regarding the total working seniority in healthcare, the distribution is 4.3% was <1 year; 69,6% was 1-20; 17,4% was 21-30 and 8,7% >30 years. About the experience in an Hematology Unit, the result was as follows: 17,4% is <1 year,76,1% is 1-20, 2,2% is 21-30 and 4,3% is >30 years.

The survey results are reported in terms of:

a) Final version of the "Patient's Empowerment rights-duty Charter" in Hematology Unit;
b) Evaluation of the general level of professionals' empowerment and analysis of the individual aspects of the multi-professional team's work (working conditions and decision-making).

The "Patient's Empowerment rights-duty Charter" in Hematology Unit

The result of this part of the project is the editing and spreading of the Charter in Hematology and Bone marrow transplant center Unit, that is the outcome of the meetings with the operators and the subsequent revision and final approval carried out during the meeting with the Director of the Unit, the Nursing Officer, some professionals and the Volunteer Association promoting the project. The final version of the Charter (produced also as an information brochure and as posters to be posted on the walls in the department) is currently being tested at the Hematology and Bone marrow transplant center Unit and will be approved by the hospital's management at the end of the year. Next, the Charter will be published on the company's website for free use by those interested in using it in their Hospital Units.

The results of the empowerment questionnaire (SESM – Italian version)

Totally 46 questionnaires were collected, so all the operators of the Unit expressed their opinion. The information contained in the questionnaires was placed in a Microsoft Excel spreadsheet and subsequently treated statistically (Correlation analysis between groups; Scale analysis). The questionnaire contains 28 items, which provide the answer following the following Likert scale: 1=fully agree; 2=agree; 3=disagree; 4=absolutely disagree. The test author defines a low level of empowerment when you get a total score of 28 points and a high empowerment level when you get a final score of 112 points and therefore sets the cut-off (mean central empowerment value) on 70 points.

Only one value on the 46 questionnaire falls below 70 points. The overall result of the sample gives a middle-high/high empowerment level of all operators and, as can be seen from the diagram, the values are equally distributed between doctors and non-medical personnel (Empowerment Means Value: Doctors, 84.15; Non-Medical personnel, 82.15). The diagram below shows the empowerment values obtained by the operators (Figure 1).

About the Correlation analysis between groups, the gender variable did not appear to be significantly associated with empowerment scores (U of Mann-Whitney=171.5, p=.587) and the same thing was repeated with regard to professional qualification: in fact, the two subgroups of doctors/non-medical personnel do not show significant differences in their empowerment scores (U of Mann-Whitney=218, p=.389). Spearman's non-parametric correlation analysis showed that the participants' empowerment scores do not vary with age (Rho=-.217), professional seniority (Rho=-.183) and work at UO of the participants (Rho=-.046) significantly.

About the Scale analysis, in the validation of Rogers et al. were identified 5 underlying factors (self-esteem/self-efficacy, power/impotence, community activism and autonomy, optimism and control in the future, justified rage); in the present study the Direct Oblimin Rotation of Main Components (ACPs) revealed 9 components (with values above 1) that reached convergence with 14 iterations and account for 71.6% of total variance. The total empowerment scale is therefore not supported by the ACP, showing a low internal insufficiency score (α=.592). It would be interest in the future to set up a study with a larger number of participants, in order to have a large enough sample to be subjected to such statistical analysis and see if certain factors become significant.

Even if it is not provided by the original tool, a detailed analysis of the responses given to the individual items of the scale was also conducted. The mean values (Likert scale: 1=fully agree; 2=agree; 3=disagree; 4=absolutely disagree) are good for almost all aspects investigated, particularly for item 3 "people have more power if they join in groups" (1,5), item 11 "working together can benefit your community" (1,5), item 16 "usually I just feel alone" (3,0), item 28 "working with others in my community can help improve things" (1,4). There are, however, some possible improvements, in particular related to item 2 "I just keep doing things that I'm sure to do" (2,8), item 4 "getting angry at something needs nothing at all" (2,4), item 7 "I should be more capable of saying what I do not like about the behavior of others" (2,1), item 14 "when I make projects I'm almost certain to realize them" (2,3), item 17 "experts are in the best position to decide what people should do or learn" (2,5), item 18 "I am capable of doing things as well as most other people" (2,2) and item 21 "you cannot fight against the power of the institutions" (2,9). The graphic below shows the means obtained from each item (Figure 2).

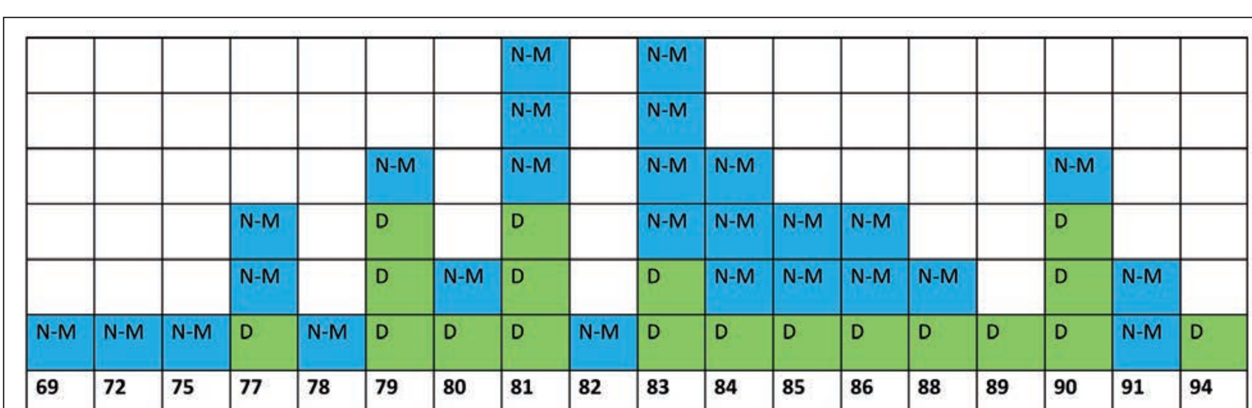


Figure 1. Empowerment values obtained by the operators of the Hematology Unit (N=46), pointing to the green color of Doctors (D) and in blue color the Non-medical personnel (N-M)

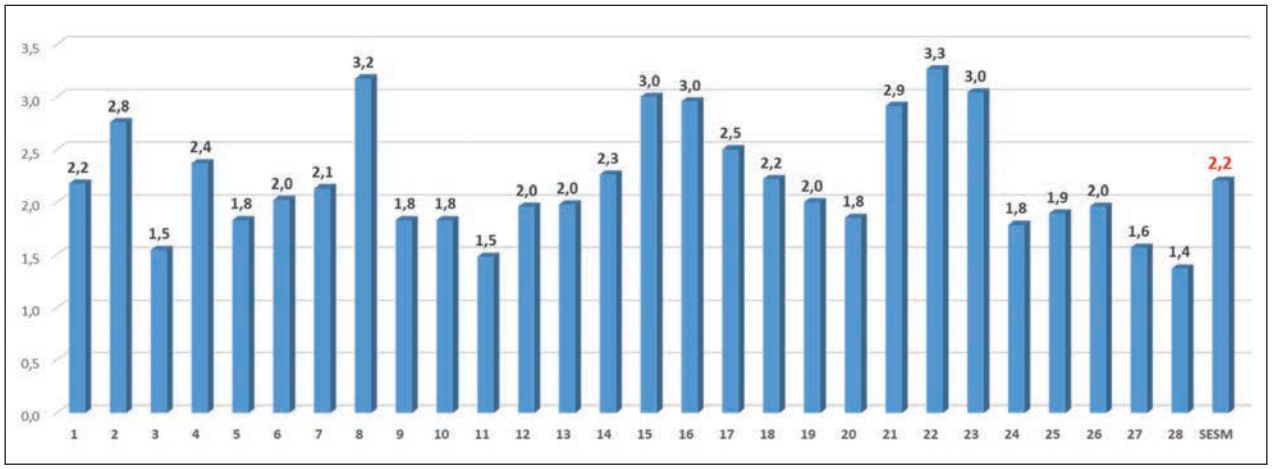


Figure 2. Mean values obtained by each item of the SESM empowerment questionnaire

Discussion

Data from the questionnaires are presented here in aggregate and anonymous form, but we have the 67.39% of the operators decided to sign the questionnaire: this figure, in addition to the maximum level of participation in the survey (which has reached 100%) is, in our view, a very encouraging sign for the continuation of the initiative, a participant of interest and adhesion convinced to the project by the professionals, who were obviously enthusiastic and felt involved in the topic being dealt with.

About the Scale analysis, we have not the same that there is in the validation of Rogers et al., but our research involved by virtue of things a few operators (all the staff present in the nematology unit), so it would be interest in the future to set up a study with a larger number of participants by a greater number of wards, in order to have a large enough sample to be subjected to such statistical analysis and see if certain factors become significant.

Even if it is not provided by the original tool, the detailed analysis of the responses given to the individual items of the scale and the means values obtained, appear as a resource that supports the staff and the individual during his professional activity: in that way professionals will be stimulated to improve these aspects to foster the development of life skills and continue in the process of empowerment undertaken with the project.

Conclusions

Many studies indicate that people with malignant hematomas (leukemia, lymphoma, and myeloma) tend to have less psychological control (9). Compared with what happens in solid tumors, there is a significantly lower percentage of patients who want to participate in clinical choices in hematologic cancer, and there are many more who want to have a passive role. Poor psychological mastery combines poor social mastery, and one favors the other. For a number of reasons, therefore, in the hematological field there is little propensity to empower the patient. For example, Ernst J. et al. in their studies seem to conclude that the desire to remain passive for hematologic patients must be respected without attempting anything to not force them (10). But to look at this, this way of thinking, at first very respectful of people, denies that a hematologic patient, made more capable of handling fear and approaching scientific knowledge and placed in a different clinical context, can modify your preferences and experience of the disease more prominently, working with the professional during the course of care. Sadak K.T. et al, presents innovative educational approaches to engage and empower the adolescent and young adult childhood cancer survivor, and they confirm that patients' empowerment is possible and recommended also with this kind of patients (11). Also Dubrovsky A.S. et al. underline that innovation in patient engagement and empowerment has been identified as a priority area,

and with the "We Should Talk" campaign at an academic pediatric hospital, by the use of a guiding theoretical framework and a multidisciplinary project team, they realize a multimedia campaign with a lot of new communication tools, specially designed to inspire at the same time staff, patients and families to effectively communicate to improve patient safety (12). The "We Should Talk" campaign provides a case study for how an organization can foster frontline improvement through the engagement of patient, families, staff and healthcare providers.

To achieve these goals is indispensable and fundamental that the patients and the citizens' commitment to patient empowerment be based on the abandonment of traditional paternalism that puts the patient into a state of passivity and relegated it to the role of a mere "recipient of benefits". Professionals need to develop the skills useful to create and maintain a dynamic and interpersonal relationship with the patient, providing valuable information and clinical indications about their health status, must continually access scientific literature and acquire knowledge based on the needs of the moment, and transfer knowledge to the practice. It is challenging to feel good operators, sure of themselves even in fluid situations, dynamic interaction, often dependent on others and the tools the world offers. In some cases, they are reconfiguring their professional identity, moving from the professional paradigm of "personal skills possession" (according to which "I am a good professional because I possess the necessary skills"), which impels to rely only on oneself in a state of isolation, to that of the "dynamic distribution of skills" ("I am a good professional because I have to do it continuously to have the skills they need when they serve, even by comparing and acquiring external aid").

The empowerment in literature is proposed under two different but increasingly integrated perspectives: as a tool and as a goal. In this study we used both approaches together: as an instrument, to obtain in the community the desired changes, and as a goal of intervention, to build and mobilize, through participated processes, the internal and external resources that are necessary to be able to put in place autonomous and responsible choices. The novelty of our approach is that there is no study in literature that had taken charge at the same time the empowerment of professionals and patients and it is also the first time that the Italian version of the SESM Empowerment Questionnaire (14) was used with professionals: with this survey we want to lay the foundations for a deepening in this regard.

The project, in addition to producing the Charter as a tool for communication to the users and a measurement of empowerment among the staff, allowed to discuss and reform together with the operators every aspect of their professional activity, stimulating their sense of belonging to the Hematology and Bone marrow transplant center Unit and increasing the level of participation and involvement in increasing the quality and safety of care.

The added value of this research lies also in improving indoor climate and close partnership between Clinical Governance Unit and wards, with a view to working towards excellence and improving service for the users. The great interest of the company's general management and the strong sponsorship of the project are other elements that will hope for a continuation of the initiative and for the widespread dissemination of this kind of work to all the other hospital Units. Investing in the process of empowerment of professionals and users, acting simultaneously on the two sides, has been a success factor in this work and has resolved and involved those who, when in contact with the users, send more to everyone's commitment to quality and safety of the hospital care. They now have an idea of the meaning and importance of empowerment, they have more consciousness and also an instrument to measure it with patients (that they are able to administer and process statistically by themselves).

Another goal of the project is certainly the consolidation of the great collaboration with volunteering associations in this area: the Charter obtained at the end of the meetings, which describes the citizen's rights and duties as well as the most closely related aspects of empowerment, is in fact the result of the commitment of all the operators of the ward, who are the authors and owners of the instrument, which we hope can become a useful and effective communication tool for the users, a support for daily work, shared and familiar to all the operators, precisely because they are born from them, with a view to preventing internal conflict (between operators) and external (with the users and their family/caregivers). The support of the

volunteer association, which has been involved since the design of the study, is a central point of contact between the professionals and the needs of the citizen. The empowerment of the citizen is possible if the professionals feel empowered and believe they can affect the relationship with the patients who so become allied to the care process in a total quality of care, characterized by equity, appropriateness and security of the received treatments.

Currently in the Unit, there are in use the communication tools of the project, the Chart, the empowerment questionnaire and another questionnaire to verify the principles of the Charter, that is administered to all patients at the end of the hospitalization/access to the surgery: we hope that this new survey and collecting data will give confirmation in the same direction and that soon, once assessed the outcomes on the nematology unit, we can extend the study to all hospital wards.

Acknowledgments

The authors would like to thank "Chiara Tassoni Onlus-Volunteer Association for the research into leukemia and cancer", Parma, Italy for its contributions.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. WHO - Regional Office for Europe (1994), Principles of Patients' Rights in Europe, a Common Framework (http://www.ctdmferrara.it/carta_dei_diritti_e_dei_doveri/dichiarazione_sulla_promozione_della_salute_umana_oms_europa.pdf), Dichiarazione sulla promozione dei diritti dei malati in Europa.
2. WHO - Western Pacific Region (2007), People at the Centre of Health Care (http://www.wpro.who.int/publications/PUB_139789290613169/en/index.html).
3. Richards N, Coulter A. Is the NHS becoming more patient centered? Trends from the national surveys of NHS patients in 2002-2007, Picker Institute, Oxford, 2007.
4. Ryan C. The Power to drive change: Working together for excellence. Creating a continuously improving consumer engagement framework for excellence in patient-centered care. World Hosp Health Serv 2016; 52(1): 37-41.
5. AA.VV. Il Sistema Sanitario e l'empowerment, Quaderno di Monitor n. 6, Anno IX, n. 25, 2010 - Trimestrale dell'Age.Na.S. (Agenzia Nazionale per i servizi sanitari regionali).
6. AA.VV. L'esperienza dei cittadini per il miglioramento dei servizi, Quaderno di Monitor n. 32, Anno XII, n. 32, 2013 - Trimestrale dell'Age.Na.S. (Agenzia Nazionale per i servizi sanitari regionali).
7. Wallerstein N. What is the evidence on effectiveness of empowerment to improve health? Copenhagen, WHO Regional Office for Europe (Health Evidence Network report; http://www.euro.who.int/Document/E88086.pdf, accessed 01 February 2006).
8. Schulz J, Nakamoto K. Health literacy and patient empowerment in health communication: The importance of separating conjoined twins. Patient Education and Counseling, January 2013; 90: 4-11.
9. Bulsara C, Ward A, Joske D. Hematological cancer patients: achieving a sense of empowerment by use of strategies to control illness. Journal of Clinical Nursing 2004; 13: 251-258.
10. Ernst J, Kuhnt S, Schwarzer A, et al. The desire for shared decision making among patients with solid and hematological cancer. Psych oncology 2011; 20 (2): 186-93.
11. Sadak KT, Connor C, DeLuca H. Innovative Educational Approaches to Engage and Empower the Adolescent and Young Adult Childhood Cancer Survivor. Pediatric Blood Cancer 2013; 60: 1919-21.
12. Dubrovsky AS, Bishop A, Biron A, et al. "We Should Talk"-Moving knowledge into action by learning to engage patients, families, and healthcare staff to communicate for patient safety, Health Manage Forum 2016; 29(4): 141-5.
13. EPF – European Patient Forum (2015). EPF Background Brief: Patient Empowerment, http://www.eu-patient.eu/globalassets/campaign-patient-empowerment/briefing_paperpatient-empowerment_final_external.pdf
14. Straticò E, Mirabella F, Degli Espositi M, Morosini P. Psychometric properties of the SESM, Italian version of the "Consumer constructed scale to measure empowerment among users of mental health services, Rogers et al (1997)". Epidemiology Psychiatry Soc 2007; 16(3): 256-64, Cambridge University Press 2007.

Received: 19 October 2017
Accepted: 20 July 2018
Correspondence:
Cipriana Mengozzi
Via Lorenzo il Magnifico, 11/11
50013 Campi Bisenzio (Florence) - Italy
E-mail: c.mengozzi@inwind.it

Acta Biomed 2019; Vol. 90, N. 2: 241-247 DOI: 10.23750/abm.v90i2.6939

Original article

Comparative study on anti-proliferative potentials of zinc oxide and aluminium oxide nanoparticles in colon cancer cells

Vimala Devi Subramaniam[1], Murugesan Ramachndran[1], Francesco Marotta[2], Antara Banerjee[1], Xiao Feng Sun[3], Surajit Pathak[1]

[1] Chettinad Hospital and Research Institute (CHRI), Chettinad Academy of Research and Education (CARE), Kelambakkam, Chennai-603103, India; [2] ReGenera R&D International for Aging Intervention, Milan and Beijing, China-Italy; [3] Department of Oncology and Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

Summary. *Background and aim of the study:* Use of commercial products containing nanoparticles formulated from zinc oxide (ZnO) and aluminium oxide (Al_2O_3) has increased significantly. These nanoparticles are widely used as ingredient in cosmetics, and also in food packaging industry although their toxicity status is yet to be studied. Here, we aimed to explore the effect of zinc oxide nanoparticles (ZnO-NPs) and aluminium oxide nanoparticles (ANPs) in human HT29 colon cancer cell line. *Methods:* In this study, ZnO-NPs were synthesized by chemical method and ANPs synthesized by sol-gel method and were characterized using UV-Vis spectroscopy, X ray diffraction and Transmittance electron microscopy. The effects of ZnO-NPs and ANPs was determined by cell viability, membrane integrity and colony formation potentials. *Results:* ZnO-NPs and ANPs inhibit HT29, colon cancer cell proliferation in a dose dependent manner, and affect the membrane potentials and also prevent the colony formation. *Conclusions:* The results suggest that ZnO NPs are found to be more effective than ANPs in reducing colon cancer cell proliferation. (www.actabiomedica.it)

Key words: cell proliferation, nanoparticles, anti proliferative, toxicity, colon cancer and cytotoxicity

Introduction

Nanoparticles have high volume to surface area and results in enhanced potentials. The use of ZnO and as well as other metal oxide nanoparticles in biomedical and cancer applications are acquiring importance due to the physical and chemical properties of these nanomaterials. Nanoparticles are similar in size as biological molecules so it can penetrate through the cells and interact with the biological system. Metal oxide nanoparticles, including ZnO-NPs and ANPs, has application in sunscreens, food packaging and as components of various cosmetics (1). Among five zinc compounds, ZnO is the one which is currently recognized as safe for the nutrients by the U.S. Food and Drug Administration (21CFR182.8991) (2). Nanoparticles endowed with targeting abilities offer a novel approach for site-specific delivery of chemotherapeutic agents (3). But, after treatment with nanoformulation, toxicity status are not yet studied.

Some studies have been conducted to investigate the cytotoxicity of ZnO-NPs in various cell lines, human hepatocytes and found that oxidative stress and lipid peroxidation play important role in cell disruption provoked by ZNO-NPs (4). Studies conducted in human lung epithelial cells (L-132) (5) and human alveolar adenocarcinoma cell line A549 (6), reveal that ZnO NPs can selectively penetrate the tumor cells and thereby interact with the cancerous cells and destroy them. In the case of ANPs, are found to inhibit the cell division in a dose dependent manner on CHO-K1 cells and UMR106 cells (7-8). The A549 cells after expo-

sure to various nanoparticles exhibited that ANPs induces minor cytotoxic effects, compared to nanometric titanium dioxide or carbon nanotubes (9). Despite the already existing studies on the toxicity of ANPs, the underlying mechanism of toxicity remains unclear. The genotoxicity effect of NPs is of specific concern because of the alteration in the genetic material that have potential for tissue malfunction, development of cancer and cell death (10). Recent *in-vitro* studies reveal metal oxide nanoparticles to selectively destruct cancer cells with relatively less toxicity against normal cells (11-12).

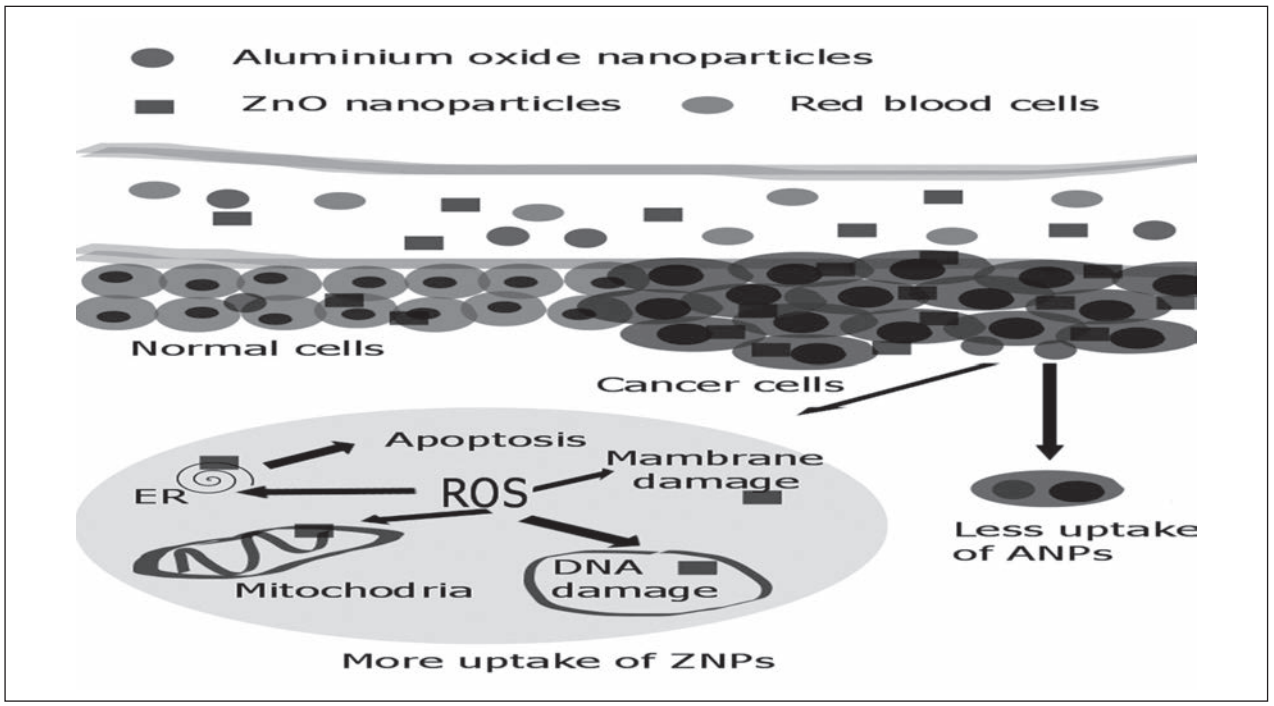
Some of the inorganic NPs, such as iron oxide NPs, titanium dioxide NPs, ZnO NPs, copper oxide NPs, silica NPs, show anti-cancer activity so that they can be used in anti-cancer therapy (8-9). Basically, nanoparticles have some unique features, which make them novel and efficient anticancer agent. Even these ZnO NPs based microfluidic immunosensor coupled with the laser instigated fluorescence is used for the detection of epithelial cell adhesion molecule (13). Studies have shown that ZnO NPs can very selectively instigate apoptosis in the cancer cells, which is mediated much likely by the reactive oxygen species through p53 pathway (14), although extensive studies were needed for assessment of anti-proliferative effect of ZnO NPs on cancer cells.

In this study, we have synthesized and characterized ZnO-NPs and ANPs, and investigated their anti-proliferative potentials in HT29 cells, a colon cancer cell line.

Methods

Synthesis of ZnO NP and ANPs

In this study, ZnO NPs were synthesized by the bottom-up approach. The atomic mixing of the constituents yields a final product of near stoichiometric perfection without high-temperature treatments. In the typical process, 1 g of $ZnCl_2 6H_2O$ was dissolved in 100 ml of double distilled water. It was neutralized with 20 ml of NaOH (1 M) solution under constant stirring at room temperature (pH.8). It led to the anionic and cationic interaction, followed by the nucleation and formation of crystallites together with the by-products



Graphical Abstract

of NH_4NO_3. In order to remove the by-product, the product was then washed with the distilled water for several times, and then air dried in room temperature for few days and grounded well to obtain an amorphous powder. To obtain different crystallite size, the prepared amorphous sample was calcined at 200±2°C for 30 mins.

Aluminum oxide nanoparticles were successfully synthesized by sol-gel method using aluminium chloride, ethanol, and Ammonia. 0.1M aluminium chloride solution was prepared in ethanol and under a stirring condition, 28% of ammonia was added dropwise to aluminium chloride solution. A white colored gel was formed and it was kept at room temperature for 30 h and then the nano gel was dried at 200°C for 10 min in the box furnace.

Characterization of the ZnO NPs and ANPs

The synthesized ZnO-NPs and ANPs were characterized using UV-visible spectroscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM), which are the widely being used techniques to examine the optical, structural and microstructural properties, of ANPs and ZnO NPs. The synthesized samples were characterized by XRD using focused monochromatized Cu $K\alpha^1$ source to determine the structure and average crystallite size of the calcined Al_2O_3 and ZnO. The detailed morphology studied under Transmission Electron Microscope (TEM) JEOL 3010). TEM images were taken on a JEOL 1200 EXII (IIT Madras) at 100 kV. Samples were prepared by evaporating single drop of ethanolic nanoparticle solution on a carbon-coated copper grid.

Cell lines and cell culture conditions

Colon cancer cell line (HT29) was obtained from National Centre for Cell Sciences, Pune, India. The cells were cultured in standard DMEM supplemented with 10% FBS and 1% antibiotic and incubated at 37°C with 5% CO_2. The cells were trypsinized using 0.025% trypsin and $1x10^5$ cells were re-plated on each well in a 96 well plate containing standard DMEM, incubated at 37°C with 5% CO_2 and allowed to adhere for 24 h before treatment.

In Vitro Cell viability assay

The anti-proliferative effect of ZnO NPs and ANPs in HT 29 was determined using MTT assay. MTT assay was performed by following the procedure of Mossman (1983) (15). Cells ($1x10^5$) were seeded in 96-well plates and were cultured in standard DMEM. The cells were allowed to grow until 80 % confluency was reached and cells were treated with different concentration of nanoparticles (0, 10, 25, 50, 100 and 250 µg/ml) in dose dependent manner, and incubated for 48 h. After incubation, the spent medium from each well was collected and were stored for further studies (LDH assay). The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was added to each of the well and incubated for around 4 h. After incubation with MTT, resulting formazan crystals were solubilized by the addition of dimethyl sulphoxide and were quantified by measuring absorbance at 570 nm in a multiwall plate reader (Biorad).

Assessment of cell morphology

Colon cancer cells (HT29) were plated in 6-well culture plates (5000 cells/well) and cultured in DMEM supplemented with 10% FBS for 48 h and treated with Zn NPs and ANPs at a 100 µg/ml. After incubation, nanoparticle induced morphological changes were assessed using inverted phase contrast microscope (Olympus CKX41) connected with Optika B5 digital camera.

Lactate dehydrogenase assay

Cells treated with ZnO NPs and ANPs were incubated for 48 h in the CO_2 incubator, and after the incubation medium was collected as lysis solution. In a 96 well plate, 10 µl of lysis solution was added to LDH control wells, 10 µl of test compounds to the experimental wells, 10 µl of PBS to untreated control wells and 10 µl of solvent was added to the vehicle wells respectively. The plate was incubated in a CO_2 incubator at room temperature for 40-45 min. 50 µl of supernatant from each well was taken in another 96 well plate and to each well, the LDH reagent was added, followed by 20-30 mins of incubation at room

temperature. Finally, absorbance was measured at 490 nm which was used as the main wavelength and 600 nm as a reference wavelength.

Lipid peroxidation

The colorimetric assay of thiobarbituric acid (TBA) was performed based on Buege and Aust (1978) method with minor modifications (16). 1 ml of TBA–TCA–HCl reagent was mixed with 1 ml of cell lysate and the mixture was heated in boiling water bath for 15 min. The reaction mixture was centrifuged at 4000 rpm for 10 mins and the absorbance of the supernatant was measured at about 535 nm against blank that contained all the reagents except cell lysate. The MDA concentration was calculated using molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and was reported in nmol/mg protein.

Colony formation assay

Clonogenic assays serve as a very useful tool to test whether the given cancer therapy can reduce the clonogenic survival of the tumor cells. This method is used to determine the cells reproductive health after treatment with nanoparticles. $5x10^3$ cells were seeded into 6-well plate and were cultured in DMEM with 10% FBS. After incubation, the cells were harvested and treated with ZnO NPs and ANPs (100 µg/ ml) followed by incubating for one week in the CO_2 incubator. The spent medium was removed and cells were rinsed with PBS. It was followed by the addition of 2-3 ml of fixation solution [Acetic acid/MeOH 1:7] and left at room temperature for 5 min. 0.5% Crystal violet solution was added after removing fixation solution and incubated at the room temperature for 30 min. Crystal violet was removed carefully by immersing the 6 well plates in tap water. The plates were allowed to air dry and the numbers of colonies were counted.

Results and discussion

The size of the nanoparticles plays a significant role in altering the properties of materials in entirety. UV-visible spectroscopy is widely utilized technique to examine the optical absorbance of NPs. The absorbance spectra of ZnO NPs and ANPs exhibit a strong absorption band at about 367 nm and 260 nm respectively. It is also evident that the significant sharp absorption of these NPs indicates the monodispersed nature of the NP distribution. The UV-visible spectra are shown in Figure 1.

Diffraction pattern of ANPs were shown in Figure 2a. The obtained pattern was compared with JCPDS standard files of (46-1212) aluminium oxide which indicates hexagonal structure of ANPs. The sharp peak pattern indicates the nanoscale size with high purity. The peaks were indexed as planes (012), (104), (113), (024), (116) and (300) of ANPS. The estimated average crystalline size is 50.89 nm. Diffraction pattern of ZnO NPs were shown in Figure 2b. The obtained pattern was compared with JCPDS standard files of

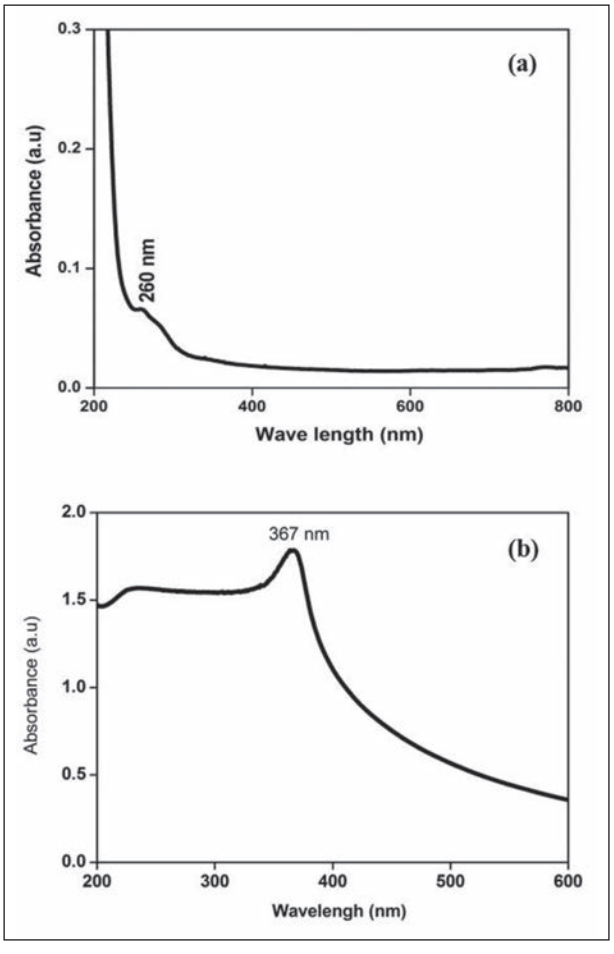


Figure 1. UV vis spectrum of nanoparticles (a) UV vis spectrum of ANPs (b) ZnO NPs

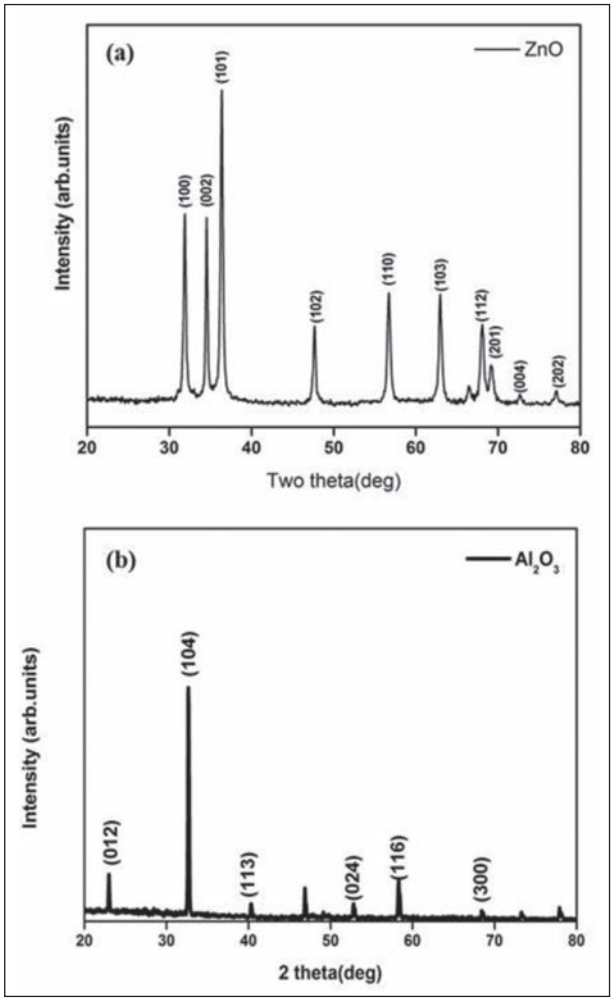


Figure 2. X- ray diffraction pattern of nanoparticles (a) ZnO NPs (b) ANPs

(36-1451) zinc oxide which indicates hexagonal structure. The sharp peak patterns indicate the nanoscale size with high purity. The peaks were indexed as planes (101), (002), (101), (102), (110), (103), (112), (201), (004) and (202) of ZnO. Diffraction pattern corresponding to impurities was found to be absent. The estimated average crystalline size is 32.11 nm.

The detailed morphology of ANPs and ZnO NPs were analyzed using HRTEM. The average crystal size of ANPs and ZnO NPs was matched to the microscopic images. TEM images of ANPs and ZnO NPs are given in Figure 3. These images showed high homogeneity on the surface of the samples. The morphology of the particles was viewed under Transmission Electron Microscope (TEM) and found to be spherical and less agglomerate. TEM analysis results were used to find the actual size of the particles and distribution of the crystallites.

The *in vitro* cytotoxic effect of ZnO NPs and ANPs on colon cancer cells was explored by treatment of the colon cancer cells with different concentrations of nanoparticles. The results are presented in Figure 4. The anti-proliferative effect was determined using MTT assay. The results showed that ZnO-NPs significantly inhibit cell proliferation in a dose dependent manner. ZnO NPs at concentration of 50, 100 µg/ml showed only 49% and 33% of cell viability respectively in colon cancer cells, The toxic effect to colon cancer cells by ANPs showed 65% and 41% of cell viability respectively at concentration of 50, 100 µg/ml .

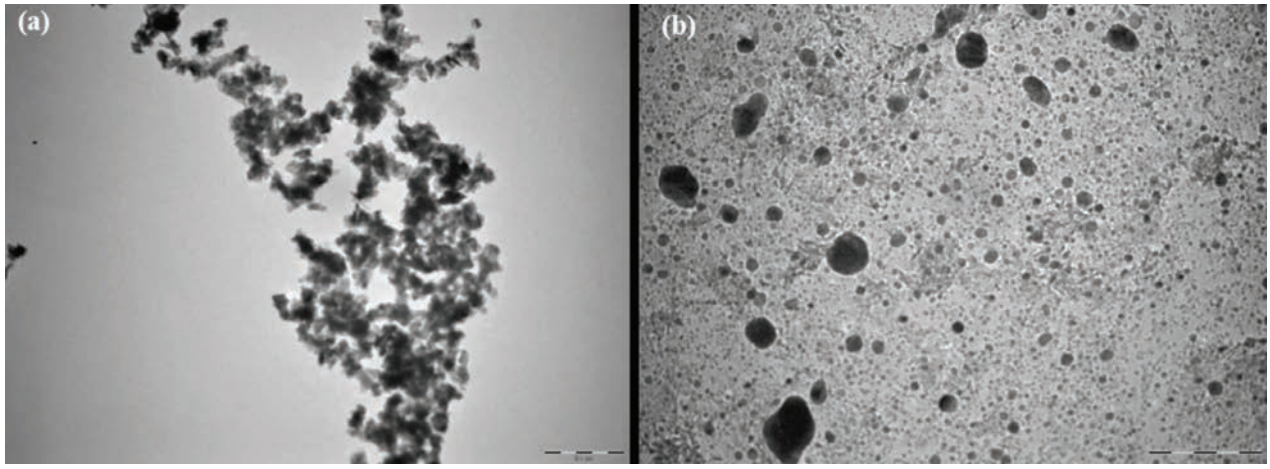


Figure 3. Transmission electron microscopic images (a) ZnO NPs (b) ANPs

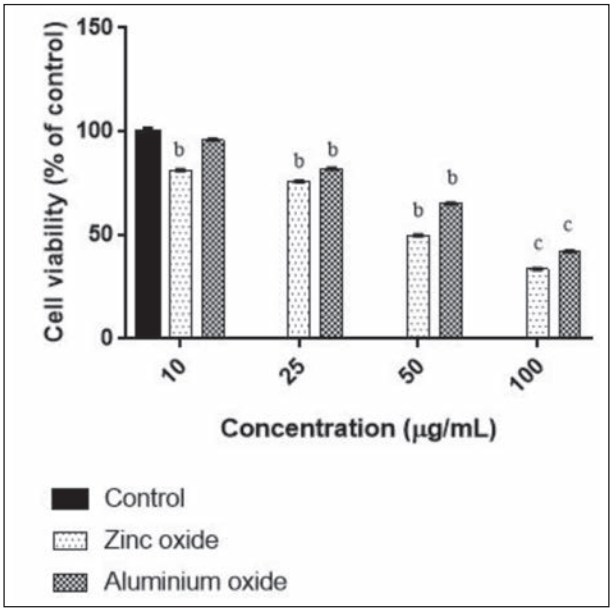


Figure 4. Concentration dependent Cytotoxicity of ZnO NPs and ANPs in HT 29 cells. Cells were exposed to different concentrations of ZnO NPs and ANPs for 48 h and the viability was determined by MTT assay. Unexposed cells as a control and run it in parallel to the exposed groups. Values were the mean±SD from three independent experiments. Significance was indicated by [b]p<0.001 and [c]p<0.01 verus control

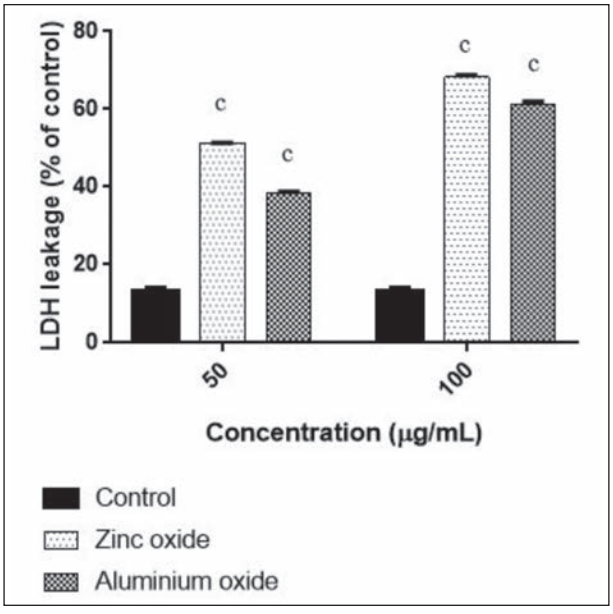


Figure 5. Effect of ZnO NPs and ANPs on LDH leakage in HT 29 cells. Cells were exposed to 50 and 100 µg/mL for 48 h. Control cells cultured in nanoparticles free medium run in parallel to the exposed groups. Values were the mean±SD from three independent experiments. Significance was indicated by [c]p<0.01 verus control

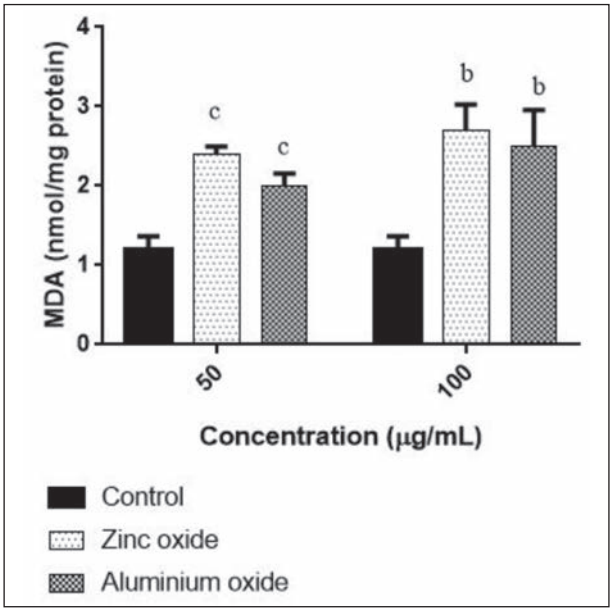


Figure 6. Effect of ZnO NPs and ANPs on MDA level in HT 29 cells. Cells were exposed to 50 and 100 µg/mL for 48 h. Control cells cultured in nanoparticles free medium run in parallel to the exposed groups. Values were the mean±SD from three independent experiments.Significance was indicated by [b]p<0.001 and [c]p<0.01 verus control

ROS generation contribute to cell killing mechanism of ZnO NPs and ANPs in cancer cells. However, the induced morphological changes by treatment with ANPs were not observed in the present study. To explore the effect of ZnO-NPs and ANPs on membrane integrity, LDH leakage assay was performed. Normally, even minor damage to the plasma membrane can lead to the release of LDH enzyme throughout the cytosol. For LDH leakage study, colon carcinoma cells incubated with ZnO-NPs and ANPs and the results are represented in Figure 5. The highest LDH release was detected from colon carcinoma cells treated with ZnO-NPs (50 and 100 µg/ml) for 48 h. The LDH release caused by ZnO-NPs was found to be in a dose dependent manner of nanoparticles treatment. These results reveal that ZnO-NP is highly effective to kill colon cancer cells when its compared to ANPs treatment.

ZnO-NPs and ANPs (50 and 100 µg/ml) induced oxidative stress, was assessed by determining the lipid peroxidation level. Figure 6 shows induced by increased level of lipid peroxidation in colon cancer cells. Alarifi et al also reported the similar result with

heptocarcinoma cells (14). The long-term cytotoxicity of ZnO-NPs and ANPs was studied by clonogenic assay, which was employed to determine the capability of a single cell to grow into a colony. ZnO-NPs treatment affected the cell survival of colon cancer cells. The colony formation was reduced about 60% in the concentration range of 100 μg/ml. The difference in ROS production was found to be statistically significant between the treatments which contribute the cell killing of ZnO NP in cancer cells.

Conclusions

Anti cancer effect of ZnO NPs and ANPs was evaluated in human colon carcinoma cells. Both ZnO NPs and ANPs showed reduced cell proliferation. LDH leakage and colony formation were assessed and the results also supports the anti-proliferative effects of ZnO NPs and ANPs. However, as compared to ANPs, ZnO NPs is a potential anti-proliferative agent in case of reducing cellular growth in colon carcinoma cells. Although, mechanistic studies of the action of ZnO NPs on more human colon carcinoma cells, may throw more light on its potential anti cancer activity.

Acknowledgements

The authors are thankful to Chettinad Academy of Research and Education (CARE), Chennai, India for the research facilities and support.

Funding: This study was partially financially supported by grants from Chettinad Hospital & Research Institute (CHRI), Chettinad Academy of Research and Education (CARE), Kelambakkam, Chennai-603103, India.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Serpone N, Dondi D, Albini A. Inorganic and organic UV filters: Their role and efficacy in sunscreens and suncare products. Inorg. Chim Acta 2007; 360: 794–802.
2. Palanikumar L, Ramasamy S, Hariharan G, Balachandran C. Influence of particle size of nano zinc oxide on the controlled delivery of Amoxicillin. Appl Nanosci 2013; 3(5): 441-451.
3. Banerjee A, Pathak S, Vimala DS, Vasan D, Murugesan R, Verma RS. Strategies for targeted drug delivery in treatment of colon cancer: current trends and future perspectives. Drug Discovery Today 2017; 22: 1224-1232.
4. Guan R, Kang T, Lu F, Zhang Z, Shen H, Liu M. Cytotoxicity, oxidative stress, and genotoxicity in human hepatocyte and embryonic kidney cells exposed to ZnO nanoparticles. Nanoscale res Let 2012; 7(1): 602-609.
5. Sahu D, Kannan GM, Tailang M, Vijayaraghavan R. In Vitro Cytotoxicity of Nanoparticles: A Comparison between Particle Size and Cell Type. J Nanosci 2016; 2016.
6. Ahamed M, Akhtar MJ, Raja M, et al. ZnO nanorod-induced apoptosis in human alveolar adenocarcinoma cells via p53, survivin and bax/bcl-2 pathways: role of oxidative stress. Nanomed Nanotechnol Biol Med 2011; 7(6): 904-913.
7. Wahab R, Dwivedi S, Umar A, et al. ZnO nanoparticles induce oxidative stress in Cloudman S91 melanoma cancer cells. J biomed Nanotech 2013; 9(3): 441-449.
8. Bhattacharyya S, Kudgus RA, Bhattacharya R, Mukherjee P. Inorganic nanoparticles in cancer therapy. Pharma Res 2011; 28(2): 237-259.
9. Vinardell MP, Mitjans M. Antitumor activities of metal oxide nanoparticles. Nanomat 2015; 5(2): 1004-1021.
10. Alarifi, Saud, Ali D, Alkahtani S. Nanoalumina induces apoptosis by impairing antioxidant enzyme systems in human hepatocarcinoma cells. Int J nanomed 2015; 10: 3751.
11. Hanley C, Layne J, Punnoose A, et al. Preferential killing of cancer cells and activated human T cells using ZnO nanoparticles. Nanotech 2008; 19 (29): 295103-295114.
12. Wang H, Wingett D, Engelhard MH, et al. Fluorescent dye encapsulated ZnO particles with cell-specific toxicity for potential use in biomedical applications. J material sci mater med 2009; 20(1): 1409-1420.
13. Baldo F, Martín A, Ortega FG, et al. Nanostructured platform integrated into a microfluidic immunosensor coupled to laser-induced fluorescence for the epithelial cancer biomarker determination. Microchem J 2016; 128: 18-25.
14. Akhtar MJ, Ahamed M, Kumar S, et al. Zinc oxide nanoparticles selectively induce apoptosis in human cancer cells through reactive oxygen species. Int J Nanomedicine 2012; 7: 845-857.
15. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods. J Immunol Methods 1983; 65: 55-63.
16. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods in enzymology 1978; 52: 302-310.

Received: 27 November 2017
Accepted: 20 July 2018
Correspondence:
Surajit Pathak
Chettinad Hospital and Research Institute (CHRI),
Chettinad Academy of Research and Education (CARE),
Kelambakkam, Chennai-603103, India
Tel. +91-44-47429050 - E-mail: surajit.pathak@gmail.com

Acta Biomed 2019; Vol. 90, N. 2: 248-250 DOI: 10.23750/abm.v90i2.6945

Original article

Safety and efficacy of Rotigotine in hospedalized patients with Vascular Parkinsonism aged 75 and older: effects on movement, praxis capacities, time-space orientation, quality of life and adherence to medical therapy

Nicola Marchitto[1], Francesco Sindona[2], Alberto Pannozzi[3], Serenella Gioia Dalmaso[1], Sabrina Anticoli[4], Gianfranco Raimondi[5]

[1] Department of Internal Medicine, Alfredo Fiorini Hospital, Terracina, Latina, Italy; [2] Department of Internal Medicine, Sapienza University of Rome, Italy; [3] Nursing student of "Sapienza" University of Rome, Italy; [4] Stroke Unit Department, Azienda Ospedaliera San Camillo/Forlanini, Roma, Italy; [5] Internal Medicine, Dept. of Medico-surgical Sciences and Biotechnologies, "Sapienza" University of Rome, Italy

Summary. In hospitals without stroke unit Department, the patients with acute ischemic stroke are stabilized in First Aid and sent to the Department of Internal Medicine. During the hospedalization period the patients undergo medical therapy for the stabilization of hemodynamic parameters and instrumental examinations for the determination of cardiovascular risk and thromboembolic evaluation. All patients are subjected to multidimensional evaluation of cognitive, praxis capacities, spatial-temporal orientation, quality of life and adherence to medical therapy. The aim of this study is evaluate the effect of Rotigotine patch on the impairment of neuro-cognitive capacity throught a continuous dopaminergic stimulation with transdermal administration. We have observed 19 patients (10 male and 9 female with range age 75-92 yrs) with Acute Ischemic Stroke stabilized in First Aid Depatment. The outcomes were the neurological changes from the baseline to 7 days in the clinical summury score on MMSE (on a scale from 0 to 30, with higher scores indicating fewer symtoms and lower physical limitations), Morinsky scale (on scale from 0 to 8, indicating adherence to therapy) and swallowing test (acts/minute). During the first week the patients were undergone to treatment with rotigotine 2 mg/24 hours. At the end of the treatment we obtained a statistically significant correlation about improvement of MMSE, Morinsky scale and swallowing test from a basal value. Rotigotine transdermal patches could be a new useful approach in the treatment of elderly hospetalized patients with acute ischaemic stroke correlated with cognitive impairment. Data shown that low dose of rotigotine patch could improves cognitive and praxis functions and therefore the quality of life of the hospitalized elderly patients. Rotigotine was effective and well-tolerated when used in routine clinical practice. Our data gave comfortable results but further evaluation are needed to have conclusive results. (www.actabiomedica.it)

Key words: Acute Ischaemic Stroke, cognitive evaluation, praxis capacities, adherence to therapy Rotigotine

Introduction

Acute Ischemic Stroke is the leading cause of neurologic disability in adults; 200,000 deaths annually in the United States. Much can be done to limit morbidity and mortality through prevention and acute intervention. Stroke patients have sudden neurologic deficit due to vascular mechanism that is ischemic in 85% or primary hemorrhages 15% (subarachnoid or intraparenchymal). An ischemic deficit that resolves

rapidly (24 h) is termed a *transient ischemic attack* (TIA). Stroke disease causes cognitive damage worsening the quality of life of the hospetalized patients: decline of praxis capacities, time-space orientation, swallowing (1, 2) and quality of life. Our observation shows the effect of low dose of Rotigotine patches (a complete dopamine agonist D3>D2>D1) on cognitive impairment (3) in 19 elderly patients with acute ischemic stroke. We used a trandermal dopaminergic therapy for the low compliance to oral therapy and the frequent presence of liver and kidney failure. Some recent data in literature underline that rotigotine could improve the cognitive impairment (4) and swallowing.

Materials and methods

A total of 19 patients are observed from july 1, 2016, through December 17, 2016. All patients fulfil the criteria for the study. No patients underwent randomization erroneously or were enrolled at sites that were closed owing to serious Good Clinical Practice violations. Most patients received recommended pharmacologic therapy for hypertension disease. Elegibility requirements at screening included age of at least 18 yrs. Our observation is caratherized by a run-in period (24 hours after the admission to the Internal Medicine Department) during wich all patients received rotigotine 2 mg/24h transdermal patch after a basal tests evaluation. After 24 hours no patient showed side effects due to dopaminergic therapy and therefore are underwent to follow up. We have treated 19 patients (10 male and 9 female with range age 70-92 yrs) with recent acute ischemic stroke wich determined hemiparesis or hemiplegia associated to a decline of praxis capacities, time-space orientation and swallowing (3), with high cardiovascular risk profile in 90% of cases. It was evaluated the adherence to therapy and the cognitive function using MMSE test, Morinsky Scale and swallowing test (acts/minute) before and post-administration of Rotigotine 2 mg/24h. Patients were subjected to multi-parametric evaluation. Data were collected, managed, and analyzed for statistical analysis using Sigmastat Analysis Program (version 3.5). Statistical analysis was performed with Wilcoxon singed rank test to compare data in small group. Sigmastat Analysis Program (version 3.5) is also used for graphic representation.

All the patients provided written informed consent.

Results

We obteined a statistical evaluation on all enrolled patients with cognitive impairment due to acute ischemic stroke after 7 days of treatment with Rotigo-

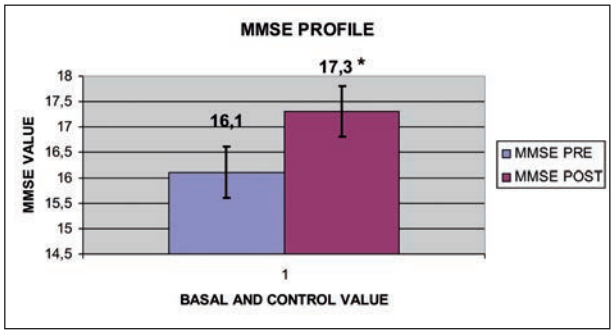


Figure 1. Mini Mental State Examination Profile (pre and post treatment)

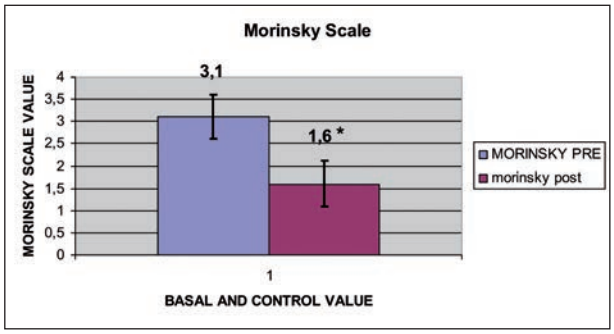


Figure 2. Morinsky Scale Profile (pre and post treatment)

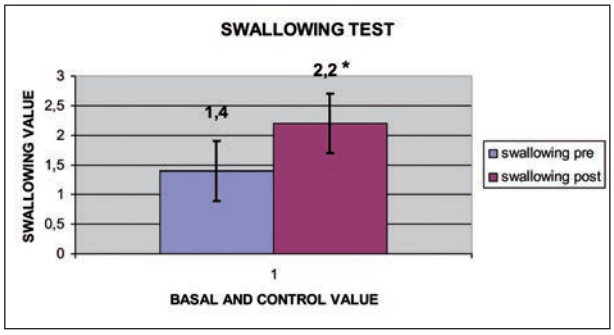


Figure 3. Swallowing Test Profile (pre and post treatment)

tine 2 mg/24h transdermal patch. At the end of the treatment, tha data have showed a statistically significant correlation about improvement of MMSE test (6-8) swallowing test and Morinsky scale from a basal value. Further evaluation are needed to have conclusive results.

Conclusion

Rotigotine could be a new useful approach in the treatment of elderly hospetalized patients with recent stroke correlated with cognitive impairment, expecially about praxis capacities, time-space orientation and swallowing. This observation led us to use rotigotine transdermal patches 2 mg/24 h in patients with cognitive degeneration related to acute ischemic stroke (5) treated in Internal Medicine Department. Rotigotine was effective and well-tolerated when used in routine clinical practice. Our data give comfortable results but further evaluation are needed to have conclusive results.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Hirano M, Isono C, Sakamoto H, Ueno S, Kusunoki S, Nakamura Y. Rotigotine Transdermal Patch Improves Swallowing in Dysphagic Patients with Parkinson's Disease. Dysphagia 2015 Aug; 30(4): 452-6.
2. Schirinzi T, Imbriani P, D'Elia A, Di Lazzaro G, Mercuri NB, Pisani A. Rotigotine may control drooling in patients with Parkinson's Disease: Preliminary findings. Clin Neurol Neurosurg 2017 May; 156: 63-65.
3. Gorgoraptis N, Mah YH, Machner B, Singh-Curry V, Malhotra P, Hadji-Michael M, Cohen D, Simister R, Nair A, Kulinskaya E, Ward N, Greenwood R, Husain M. The effects of the dopamine agonist rotigotine on hemispatial neglect following stroke. Brain 2012 Aug; 135 (Pt 8): 2478-91.
4. Martorana A, Di Lorenzo F, Esposito Z, Lo Giudice T, Bernardi G, Caltagirone C, Koch G. Dopamine D2-agonist rotigotine effects on cortical excitability and central cholinergic transmission in Alzheimer's disease patients. Neuropharmacology 2013 Jan; 64: 108-13.
5. Koch G, Di Lorenzo F, Bonnì S, Giacobbe V, Bozzali M, Caltagirone C, Martorana A Dopaminergic modulation of cortical plasticity in Alzheimer's disease patients. Neuropsychopharmacology. 2014 Oct; 39(11): 2654-61. doi: 10.1038/npp.2014.119. Epub 2014 May 26.
6. Semprini R, Lubrano A, Misaggi G, Martorana A. Apathy as marker of frail status. J Aging Res 2012; 2012: 436251.
7. Blundo C, Gerace C. Dopamine agonists can improve pure apathy associated with lesions of the prefrontal-basal ganglia functional system. Neurol Sci 2015 Jul; 36(7): 1197-201.
8. Brusa L, Pavino V, Massimetti MC, Bove R, Iani C, Stanzione P. The effect of dopamine agonists on cognitive functions in non-demented early-mild Parkinson's disease patients. Funct Neurol 2013 Jan-Mar; 28(1): 13-7.

Received: 27 November 2017
Accepted: 2 May 2018
Correspondence:
Dr Nicola Marchitto
Specialist in Geriatry and Gerontology, Medical Assistant,
Department of Internal Medicine, Alfredo Fiorini Hospital,
Terracina (Latina), Italy
Tel. +393277064979
Fax +390773708752
E-mail: n.marchitto@ausl.latina.it

Acta Biomed 2019; Vol. 90, N. 2: 251-258 DOI: 10.23750/abm.v90i2.7145

Original article

Abdominal pain and internal hernias after Roux-en-Y Gastric Bypass: are we dealing with the tip of an iceberg?

Francesco Tartamella[1], Antonio Ziccarelli[1], Stefano Cecchini[1], Michelina Ferro[2], Matteo Riccò[3], Edoardo Baldini[4], Rudj Mancini[5], Marco Anselmino[5], Federico Marchesi[1]

[1] Dipartimento di Medicina e Chirurgia, Sezione di Clinica Chirurgica Generale, Università degli Studi di Parma, Parma, Italia; [2] Unità Operativa di Anestesia e Rianimazione e Terapia del Dolore, Azienda ospedaliero Universitaria di Parma, Parma, Italia; [3] Azienda Provinciale per i Servizi Sanitari della p.a di Trento, UOPSAL, Trento, Italia; [4] Unità Operativa Chirurgia Generale, Ospedale "G. da Saliceto", Piacenza, Italia; [5] UOC Chirurgia Bariatrica e Metabolica, Azienda Ospedaliero Universitaria Pisana, Pisa, Italia

Summary. *Background:* Abdominal pain is the most frequent cause of hospital admission after Roux-en-y gastric bypass (RYGB). Among numerous possible underlying causes, internal hernias represent one of the most peculiar and insidious conditions, setting challenging diagnostic and therapeutic problems for the surgeon. The aim of this study is to analyze aspecific abdominal pain incidence and characteristics after RYGB, discriminating peculiar aspects suggestive of internal hernias. *Methods:* 13 patients submitted to internal hernia repair after laparoscopic antecolic RYGB and a group of 49 controls (non-complicated RYGB) have been assessed using a specific questionnaire. Overall aspecific abdominal pain incidence and characteristics have been analysed. Typical pain traits and predisposing conditions for internal hernias have been investigated. *Results:* 33% of controls reported aspecific abdominal pain after RYGB, mainly early postprandial, deep, remittent, colicky, located in the upper left abdomen. 77% of the case patients reported prodromal episodes of pain similar to the controls. The only significant differences between prodromal and acute episodes were pain intensity and quality (continuous). Excess weight lost at 3 months significantly correlated with internal hernia occurrence (p: 0.002). *Conclusions:* Based on abdominal pain characteristics, we can reasonably postulate the presence of remittent bowel torsions (remittent internal hernia) in many patients after antecolic RYGB, only occasionally complicating. Therapeutic management of these cases remains controversial, being laparoscopic exploration a reasonable option when symptomatology is suggestive. (www.actabiomedica.it)

Key words: abdominal pain, gastric bypass, obesity, Peteresen hernia, internal hernia

Introduction

Roux-en-Y gastric bypass (RYGB) has become the most used bariatric procedure worldwide (1), given its high effectiveness for weight loss and comorbidities resolution, with a well-demonstrated improvement in quality of life (2-6).

While standardization of the surgical techniques and amelioration of postoperative care have reduced postoperative complications, long-term morbidity still remains an important issue for bariatric surgery and, in particular, for RYGB (7-9).

Internal hernias represent the most peculiar late complication of RYGB, being related to altered anatomy which the different types of limb reconstruction entail.

The extremely variable clinical presentation and low reliability of diagnostic imaging (10) make the management of patients with suspected internal her-

nias extremely challenging, with high risk of unnecessary explorations or delayed treatments (11).

Abdominal pain, which is the key symptom of internal hernias, is reported quite frequently by patients after RYGB: its variable presentation and the wide range of possible causes make differential diagnosis extremely challenging (12).

This study aims at identifying possible specific features in the clinical presentation of patients with suspected internal hernias after RYGB, with specific focus on abdominal pain.

Materials and methods

A total of 13 cases of internal hernias after RYGB, 4 operated on at Parma University Hospital, 6 at Pisa University Hospital, 3 at Piacenza Hospital between 2007 and 2013, were retrospectively assessed. Indication for surgery was based on symptoms characteristics (severity and frequency) and diagnostic procedures findings. All the patients had previously undergone antecolic-antegastric laparoscopic RYGB, with posterior hand-sewn/mechanical gastrojejunal anastomosis (right oriented alimentary limb) and side-to-side jejuno-jeujonostomy; no mesenteric defect closure was performed.

Abdominal pain was investigated by having the patients answer a novel specific questionnaire (Bariatric Postoperative Pain Assessment - BAPPA), by phone or direct interview. The 15 items non validated test was designed based on the Rome III test (13) and changed upon advice of an expert panel including a neurologist, a psychiatrist, a gastroenterologist and an anaesthesiologist; it aims at identifying pain location and frequency, its correlation with meals and bowel movements, any presence of reflex events (nausea and vomiting) and use of analgesics. Pain intensity has been measured by the visual analogic scale (VAS). For patients reporting recurrent pain episodes before surgery, pain characteristics in prodromal and acute episodes were separately examined.

A group of 49 patients having undergone laparoscopic RYGB at Parma University Hospital answered the same questionnaire, in order to identify two subgroups: positive controls (patients reporting abdominal pain) and negative controls (patients not reporting abdominal pain). The set pain cut-off criterion was the presence of abdominal pain other than occasional and as already experienced before surgery. As potentially confounding factors, the following were considered as exclusion criteria:

- Less than 6 months from bariatric procedure
- Patients linguistic difficulties;
- Presence/persistence of psychopathologic traits and eating disorders
- Presence of specific causes of abdominal pain (gallstones, GERD, anastomotic ulcers, incisional hernias, …)

Biometric data were collected from the follow-up registries of the Centres.

Continuous variables were compared throught Student's t test or ANOVA, when appropriate. Univariate analysis of discrete variables was conducted using the chi-square with Yates' correction. All tests were two-tailed, and statistical significance was set at $p<0.05$. All statistical analyses were performed using IBM SPSS Statistics 22.0 for Macintosh (IBM Corp. Armonk, NY).

The study was approved by the Institutional Review Board, and informed consent was obtained from all participants.

Results

The overall incidence of Petersen hernias, estimated across the three centers was 3.3%.

Among the 60 controls recruited for the interview, 7 were excluded for the presence of typical pain (2 biliary colics, 2 renal colics, 2 dysmenorrhea, 1 diverticular disease) and 4 did not complete or refused the interview. Out of 49 enrolled patients, 16 (33%) reported abdominal pain and 34 no abdominal pain (69%). As reported in table 1, case patients and controls were similar as to demographics, biometric data and comorbidities.

Internal hernia clinical/pain characteristics

Mean time lapse from RYGB to reintervention was 27±17 months. Diagnostic imaging (Tc scan) re-

Table 1. Demographics and clinical data

		Cases	Controls	
n		13	49	p
Gender	M/F	3/10	5/44	0,347
	(%)	(30% / 70%)	(11% / 89%)	
Mean age at 1st operation		38,33 ± 3,8	38,59 ± 9,8	0,938
BMI - T_0		43,2 ± 6,7	46,2 ± 6,4	0,142
Comorbidities	Diabetes II	3	12	0,682
	Depression	1	5	1,000
	OSAS	1	6	1,000
	Arthrosis	1	8	1,000
	Hypertension	0	4	1,000
	Endometriosis	1	0	0,155
	Polycystic ovaries	1	2	0,403
	Hiatal hernia	1	3	0,501
	Asthma	1	0	0,155
	Thyroid disease	1	0	0,155
	Thrombocytosis	1	0	0,155
	Cauda equina syndrome	1	0	0,155
	Complications	0	0	1,000

vealed signs of internal hernias in 8/13 case patients (61.5%), being the swirl sign the most common finding. At surgical exploration, 11 Petersen hernias (85%) and 2 mesenteric hernias were found (15%). All interventions were carried out with a laparoscopic approach: the defect involved was repaired with direct suture; no bowel resection was necessary.

Only 1 out of 13 patients (7.7%) was in shock.

The majority of the patients (77%) reported prodromal episodes of abdominal pain, similar but less intense than the one leading to the operation.

During the acute episode, pain was defined as deep (100%), continuous rather than intermittent (colicky) (69%), with gradual onset (92%).

The left upper abdominal quadrant was the most common pain site (61.5%) (figure 1). Acute/prodromal episodes normally started during the first 6 months after RYGB (54%), occurred 2-3 times/month (54%) and usually lasted less than 24 hours (92%). In 9/13 patients (69%) pain appeared or worsened after a meal, most of the times within the first 20 minutes (78%). Vomiting was reported in 38% of the case patients

Table 2. Pain characteristics

Pain characteristics	Cases	C +	p
Onset (% within 6 month after RYGB)	7/13-53.8%	10/16-62.5%	0.927
< 1/week	11/13-85%	13/16-81%	
Frequency			
>1/week	2/13-15%	3/16-19%	0.798
Quality: Intermittent (colicky)	4/13-31%	14/16-87.5%	0.008
Continuous	9/13-69%	2/16-12.5%	0.024
Quality: deep (%)	13/13 -100%	13/16-81.2%	0.699
VAS (mean)	8.9	6.2	0.0049
Analgesic use	13/13-100%	3/13-18.7%	<0.0001
Correlation with meals	9/13-69%	8/16-50%	0.505
Changing in bowel movements	4/13-30.7%	6/16-37.5%	0.704
Alleviation by defecation	3/13-23%	2/16-12.5%	0.798
Vomiting	5/13-38%	3/16-18.7%	0.445

Table 3. Peculiar traits of internal hernias pain presentation

	Internal hernias	p
Continuous pain	9/13-69%	0.024
VAS (mean)	8.9	0.0049
Analgesic use	13/13-100%	<0.0001
>EWL% T3	49.3%	0.002

only and in most cases did not alleviate the symptoms. Bowel frequency did not usually change during the episodes (69%) and pain was not alleviated by defecation (77%). Pain intensity was reported with an average of 8.9/10 on VAS scale. No typical antalgic position was identified. NSAIDs were reported as the most effective analgesics. In addition to lower intensity, prodromal episodes, when present, were different from the acute ones as to location (epigastric 86%) and quality (intermittent 86%) only.

No patient reported abdominal pain after reintervention.

Differential diagnosis

As well as the case patients, positive controls mainly reported deep (81.2%) abdominal pain, with gradual onset (81.2%), starting in the first 6 months after surgery (62.4%). Episode frequency was not significantly different between the groups. Epigastrium was more frequently involved (37.5%, p: 0.185) (figure 1), and, differently from the case patients, the pain was defined mainly as intermittent (87.5%, p: 0.008). Pain intensity was reported significantly higher in case patients (8.9 vs 6.2, p: 0.0049), causing the patients to use analgesics more frequently (100% vs 18.6%, p: <0.0001). When was correlated with meal pain occurred more frequently in the first 20 minutes. In positive controls, as for the case patients, bowel movements were not changed by pain (62.5%) and pain was not alleviated by defecation (68.8%). Vomiting was more frequent in case patients (38% vs. 18.8%), even though the difference was not found to be statistically significant.

Predictive conditions

As shown in figure 2, EWL at 3 months significantly correlated with internal hernias occurrence (p: 0.002), while no correlation was found with comorbidities.

Discussion

The exponential increase in minimally-invasive bariatric procedures over the last decades contributed

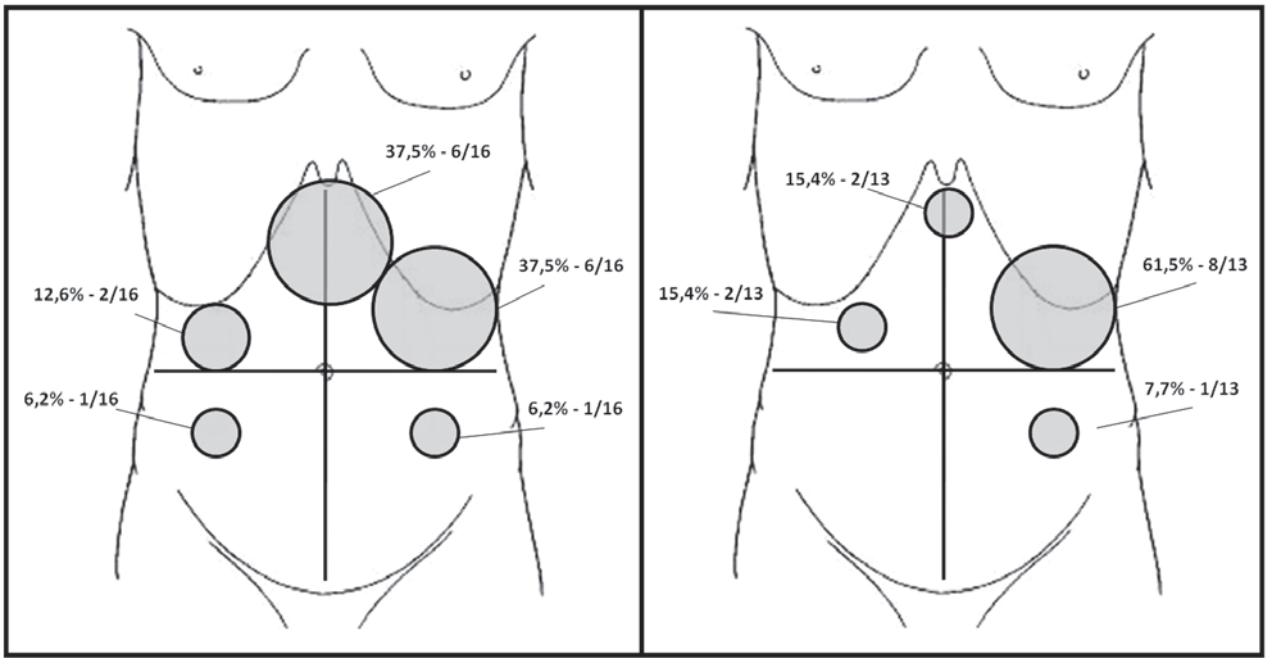


Figure 1. Pain site

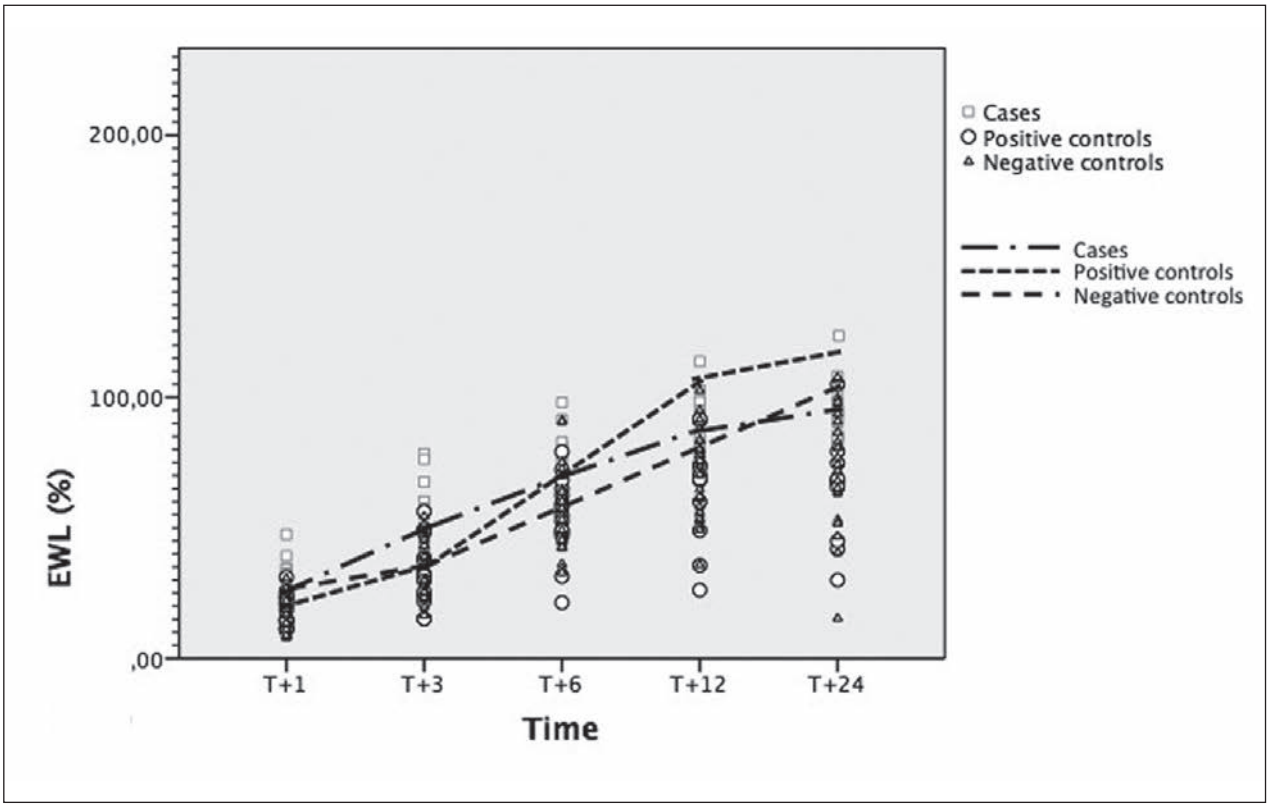


Figure 2. Weight loss

to make internal hernias a new and relevant pathology chapter in general surgery units.

Indeed, while alimentary tract reconstructive techniques and rapid weight loss after bariatric surgery create wide internal hernias defects, the reduction of bowel adhesions provided by the laparoscopic approach created the conditions (increased bowel mobility) for the significant increase in this threatening complication (14).

RYGB is the bariatric procedure more frequently complicated by internal hernias, although the real incidence is extremely variable among series, ranging from 0.2% to 9% (15). However, many are the reasons why the above data might be underestimated: rather than the patients lost at follow-up, quite common in bariatric surgery, it is the extremely variable clinical presentation that makes internal hernias incidence hard to quantify.

Beside a small number of life-threatening cases, with bowel occlusion and ischemia, it is plausible that the majority of internal hernias after RYGB is represented by paucisymptomatic spontaneously resolved cases, with abdominal pain as the only clinical symptom. This is typical of antecolic, reconstruction, where internal defects are wide, allowing spontaneous reduction of the herniated bowels.

Moreover, imaging low sensitivity (15-17), especially in absence of occlusive or ischemic scenarios, makes surgical exploration the most reliable diagnostic tool for internal hernias (11).

Differently from other series (18), none of the patients operated on in our series showed a clear occlusive syndrome and only 8 (61.5%) had specific CT signs. This is why we believe that a precise and scrupulous definition of abdominal pain after RYGB is mandatory, especially for patients for whom internal hernia suspicion is less clear.

Abdominal pain was quite frequent in our series: up to 43% of the patients, excluding the ones operated on for internal hernia. Our data are even higher than the ones reported by recent literature (12, 19), and most of the times (70%) the causes were aspecific. The lower pain threshold of obese patients (20) is an additional confounding factor.

Most of the patients operated on in our series (77%) reported a history of worsening abdominal pain. Pain characteristics were similar in case patients and positive controls: pain intensity and frequency, along with radiological findings, where present, were indications for surgical exploration.

For the above reasons, it is hard to determine whether prodromal pain episodes in patients and recurrent episodes in positive controls could amount to clinical expression of non-complicated spontaneously-resolved internal hernias, but it certainly is a plausible hypothesis. In this sense, pain characteristics reported by most patients (early postprandial, deep, epigastric/left hypochondrial) can suggest a bowel/vascular torsion mechanism, with some traits of angina abdominis (21). Moreover, when asked about prodromal episodes, the patients also reported a mainly intermittent (colicky) (86%), epigastric abdominal pain, which became continuous and located in the left hypochondrium during the acute episode leading to surgical exploration.

The entity and velocity of weight loss has been already reported as a predisposing condition for internal hernias after RYGB (22). Our data confirm this trend (figure 2), especially during the first months after surgery; the different performance at 1 year, with positive control showing higher weight loss, can be explained by impairment in eating caused by the persistent pain, which is expected to be solved in the cases that already had surgical repair.

Laparoscopic exploration has been advocated as a reasonable option in case of unexplained abdominal pain after RYGB (12, 17). In addition to its low morbidity and high diagnostic and therapeutic effectiveness, laparoscopic exploration proved very rarely an unnecessary measure (11, 23), as confirmed by our series (no false positives in our experience). Moreover, in absence of treatable causes, a prophylactic suture of internal hernia defects could be more safely and easily performed than during RYGB, owing to the lower amount of visceral adiposity.

Most of the series (15, 19, 24, 25) report higher incidence of internal hernias in patients undergoing retrocolic rather than antecolic RYGB. Because of the smaller defects of retrocolic reconstruction (mesocolic defect), a plausible cause is, rather than overall higher incidence of internal hernia, a higher number of severely symptomatic cases. As previously commented,

the operated patients could represent just the tip of the iceberg of internal hernias, especially for antecolic RYGB.

In this sense, the threshold for laparoscopic exploration should be lower for retrocolic reconstructions.

Conclusion

Diagnostic limitations make the real incidence of internal hernias after RYGB hard to determine. In presence of recurrent and worsening episodes of early postprandial, epigastric, colic, deep abdominal pain after antecolic RYGB, an explorative laparoscopy could be a correct option, even with no radiological signs and with no severely compromised clinical conditions.

Informed consent was obtained from all individual participants, for whom identifying information is included in this article.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Buchwald H, Oien D. Metabolic/bariatric surgery worldwide 2011. Obes Surg 2013; 23(4): 427-36.
2. Sanchez-Santos R, Del Barrio MJ, Gonzalez C, Madico C, Terrado I, Gordillo ML, et al. Long-term health-related quality of life following gastric bypass: influence of depression. Obes Surg 2006 May; 16(5): 580-5.
3. Torquati A, Lutfi RE, Richards WO. Predictors of early quality-of-life improvement after laparoscopic gastric bypass surgery. Am J Surg 2007; 193: 471-5
4. Marchesi F, De Sario G, Reggiani V, Tartamella F, Giammaresi A, Cecchini S, et al. Road running after gastric bypass for morbid obesity: rationale and results of a new protocol. Obes Surg 2015; 25(7): 1162-70.
5. Marchesi F, Giacosa R, Reggiani V, De Sario G, Tartamella F, Melani E, et al. Morphological Changes in the Carotid Artery Intima after Gastric Bypass for Morbid Obesity. Obes Surg 2017; 27(2): 357-63.
6. Riccò M, Marchesi F, Tartamella F, Rapacchi C, Pattonieri V, Odone A, et al. The impact of bariatric surgery on health outcomes, wellbeing and employment rates: analysis from a prospective cohort study. Ann Ig 2017; 29(5): 440-452.
7. Encinosa WE, Bernard DM, Du D, Steiner CA. Recent improvements in bariatric surgery outcomes. Med Care 2009; 47(5): 531-5
8. Marchesi F, Tartamella F, De Sario G, Forlini C, Caleffi A, Riccò M, Di Mario F. The Sleeping Remnant. Effect of Roux-En-Y Gastric Bypass on Plasma Levels of Gastric Biomarkers in Morbidly Obese Women: A Prospective Longitudinal Study. Obes Surg 2017; 27(7): 1901-5.
9. Tartamella F, Petracca G, Romboli A, Marchesi F. Laparoscopic gastric bypass with remnant gastrectomy in a super-super obese patient with gastric metaplasia: a surgical hazard? Acta Biomed 2018; 88(4): 491-5.
10. Lockhart ME, Tessler FN, Canon CL, Smith JK, Larrison MC, Fineberg NS, et al. Internal hernia after gastric bypass: sensitivity and specificity of seven CT signs with surgical correlation and controls. AJR Am J Roentgenol 2007; 188(3): 745-50.
11. Agaba EA, Gentles CV, Shamseddeen H, Sasthakonar V, Kandel A, Gadelata D, et al. Retrospective analysis of abdominal pain in postoperative laparoscopic Roux-en-Y gastric bypass patients: is a simple algorithm the answer? Surg Obes Relat Dis 2008; 4(5): 587-93.
12. Greenstein AJ, O'Rourke RW. Abdominal pain after gastric bypass: suspects and solutions. Am J Surg 2011; 201(6): 819-27. Review.
13. Drossman DA, Dumitrascu DL. Rome III: New standard for functional gastrointestinal disorders. J Gastrointestin Liver Dis 2006; 15(3): 237-41. Review.
14. Steele KE, Prokopowicz GP, Magnuson T, Lidor A, Schweitzer M. Laparoscopic antecolic Roux-en-Y gastric bypass with closure of internal defects leads to fewer internal hernias than the retrocolic approach. Surg Endosc 2008; 22(9): 2056-61.
15. Champion JK, Williams M. Small bowel obstruction and internal hernias after laparoscopic Roux-en-Y gastric bypass. Obes Surg 2003; 13: 596-600.
16. Iannuccilli JD, Grand D, Murphy BL, Evangelista P, Roye GD, Mayo-Smith W. Sensitivity and specificity of eight CT signs in the preoperative diagnosis of internal mesenteric hernia following Roux-en-Y gastric bypass surgery. Clin Radiol 2009; 64: 373-80.
17. Goudsmedt F, Deylgat B, Coenegrachts K, Van De Moortele K, Dillemans B. Internal hernia after laparoscopic Roux-en-Y gastric bypass: a correlation between radiological and operative findings. Obes Surg 2015; 25(4): 622-7.
18. Ximenes MA, Baroni RH, Trindade RM, Racy MC, Tachibana A, Moron RA, et al. Petersen's hernia as a complication of bariatric surgery: CT findings. Abdom Imaging 2011; 36(2): 126-9.
19. Higa KD, Ho T, Boone KB. Internal hernias after laparoscopic Roux-en-Y gastric bypass: incidence, treatment and prevention. Obes Surg 2003; 13(3): 350-4.
20. Foster A, Richards WO, McDowell J, Laws HL, Clements RH. Gastrointestinal symptoms are more intense in morbidly obese patients. Surg Endosc 2003; 17: 1766-8.

21. Brunton L, Williams WE. Angina Abdominis. Proc R Soc Med 1912; 5(Med Sect): 53-9.
22. Ahmed AR, Rickards G, Husain S, Johnson J, Boss T, O'Malley W. Trends in internal hernia incidence after laparoscopic Roux-en-Y gastric bypass. Obes Surg 2007; 17(12): 1563-6.
23. Pitt T, Brethauer S, Sherman V, Udomsawaengsup S, Metz M, Chikunguwo S, et al. Diagnostic laparoscopy for chronic abdominal pain after gastric bypass. Surg Obes Relat Dis 2008; 4: 394-8.
24. Comeau E, Gagner M, Inabnet WB, Herron DM, Quinn TM, Pomp A. Symptomatic internal hernias after laparoscopic bariatric surgery. Surg Endosc 2005; 19: 34-9.
25. Steele KE, Prokopowicz GP, Magnuson T, Lidor A, Schweitzer M. Laparoscopic antecolic Roux-en-Y gastric bypass with closure of internal defects leads to fewer internal hernias than the retrocolic approach. Surg Endosc 2008; 22: 2056-61.

Received: 28 February 2018
Accepted: 6 April 2018
Correspondence:
Federico Marchesi
Università degli Studi di Parma
Dipartimento di Medicina e Chirurgia
Sezione di Clinica Chirurgica Generale
Via Gramsci, 14 - 43100 Parma (Italy)
Tel. +39 0521 702156 - Fax +39 0521 940125
E-mail: fede53@lycos.com

Acta Biomed 2019; Vol. 90, N. 2: 259-264 DOI: 10.23750/abm.v90i2.7148

Original article

Urethrovaginal space during the third trimester of pregnancy is not related to vaginal orgasm

Giovanni Sisti[1], *Mauro Cozzolino*[2,7], *Flavia Sorbi*[2], *Carlo Maria Oranges*[3], *Maria Isabel Pachon Romero*[4], *Riccardo Landi*[5], *Andrea Sisti*[6], *Massimiliano Fambrini*[2]

[1] Department of Science for Woman and Child Health, University of Florence, Florence, Italy; [2] Department of Biomedical, Experimental and Clinical Sciences - Division of Obstetrics and Gynecology, University of Florence, Italy; [3] Department of Plastic, Reconstructive, Aesthetic, and Hand Surgery, Basel University Hospital, Basel, Switzerland; [4] Bronx Lebanon Hospital Center, New York, USA; [5] School of Medicine, Bologna, Italy; [6] Montecatini Terme, Pistoia, Italy;[7]Instituto Valenciano de Infertilidad, IVI-RMA Madrid, Spain.

Summary. *Introduction:* Sexologists have described the urethrovaginal space (UVS) as a region of the body involved in the female orgasm. Recently certain authors have described the UVS via ultrasound (US). Pregnancy is associated with a myriad of physiological, anatomical and biochemical changes. To measure the UVS thickness in the third trimester of pregnancy and to investigate the relationship between the UVS thickness and the presence of vaginal orgasm. *Material and Methods:* Sexually active pregnant patients in the third trimester were included. We measured the UVS via US. Each patient compiled a modified female sexual function index (FSFI) questionnaire and was categorized in group with or without vaginal orgasm. Association between vaginal orgasm and UVS thickness was evaluated via t-test and ROC curve analysis. *Results:* UVS thickness resulted greater than 15 mm (average) in the third trimester, and was not related to the presence of vaginal orgasm ($p>0.05$). *Conclusion:* UVS thickness is high in the third trimester of pregnancy but it is not related to the presence of vaginal orgasm. (www.actabiomedica.it)

Key words: G spot, pregnancy, orgasm, urethrovaginal space

Introduction

Since the 1950s (1) sexologists have described the urethrovaginal space (UVS) as a region of the body involved in the female orgasm. In non pregnant women UVS has been described via 2-D Ultrasound (US) and in greater detail via 3-D US. In both cases an association between UVS thickness and the presence of vaginal orgasm (2, 3) was found.

Controversy still exists about the independence of the UVS (therefore vaginal orgasm) from the more classical clitoral orgasm. Recently certain authors have inserted the UVS in a wider anatomophsyiological structure called the "clitoris-urethra-vagina (CUV) complex" (4-6). Within this complex vaginal orgasm is caused by contact of the internal clitoris and the anterior vaginal wall.

Unfortunately pregnancy has often been a period of the women's life neglected by medical literature in regard to sexual health (7). Given this paucity of information found in literature, it is not surprisingly that many physicians confess to lacking specific knowledge and communication skills to deal with their patients' sexual dysfunction (8). Therefore, there are many misconceptions among women regarding sexual activities and sexual satisfaction during pregnancy (9). Not long ago some physicians prohibit vaginal intercourse during pregnancy (10). Recent scientific evidence of the relative safe nature (11. 12) of sexual activity during all trimesters of pregnancy has lead to changes in

counseling. However, patient's fear of fetal harm remains in a considerable percentage of patients (13).

Pregnancy is associated with a myriad of physiological, anatomical and biochemical changes and though poorly understood the majority of them seem to be regulated by changes in hormone levels. Pregnancy is characterized by elevation levels of circulating estrogens, progesterones and androgens, all of which increase with advancing gestational age (14, 15). Intra and extracellular water retention, tissue hypertrophy and hyperplasia lead to several anatomical changes during normal pregnancy (1). Almost all maternal tissues and organs are invested by this "hormonal storm". These physiological hormonal changes during pregnancy also provide an opportunity to examine the UVS. Studies have demonstrated that a reduction in estrogen secretion, due to menopause, can lead to decreasing UVS thickness and suggest a hormonal influence of this structure (16).

In light of the current evidence in the literature supporting increased vaginal orgasm with increased UVS, we decided to examine this anatomical structure during the third trimester of pregnancy were available evidence is lacking. Given the demonstrated change of UVS thickness with hormonal changes we chose the third trimester of pregnancy because of the height of increased hormonal secretion. We hypothesize that in the third trimester of pregnancy, when the estrogen and androgen peak is highest, the UVS thickness will be greater. Furthermore, we investigated the relationship between the UVS thickness and the presence of vaginal orgasm.

Materials and methods

A cohort of volunteers was recruited from the population of women who underwent routine clinical examination, at Careggi University Hospital, during pregnancy. Approval for this study was obtained from the Internal Ethical Committee. All the patients provided informed consent.

Inclusion criteria were singleton pregnancy in the third trimester (from 24th to 40th weeks of gestation), absence of any illness arising before or during pregnancy, and sexual activity at least twice a week during the month before the interview. All women underwent a clinical interview at our pregnancy outpatient clinic. For this purpose, a modified female sexual function index (FSFI-6) was used, assessing sexual function (SF) in the last 4 weeks (17). A total score of less than 19 was considered suggestive of female sexual dysfunction (SD). All women with SD were excluded from the study.

Vaginal orgasm was ascertained by the following question: "Have you experienced a vaginal orgasm in the last month?". Responses were categorized as "yes" or "no".

US was performed with a Voluson model 730 Expert, 8 MHz (General Electric Medical Systems, Milwaukee, WI, USA) using a transvaginal probe. There are 2 different US methods to obtain UVS visualization: the introital and the translabial approach. We decided to use the former because the trans-vaginal probe is easily available in almost every ambulatory outpatient clinic. The superiority of the translabial approach over the introital has not been uniformly demonstrated. Transvaginal probe is a well validated instrument for urodynamic studies (18, 19). The procedure was performed following the indications appearing in the academic literature (3). Investigators were blinded to the patient FSFI-6 results. All procedures were performed with patients in a modified Trendelemburg position and with a full bladder filled to a volume of 300 mL.

The ultrasound evaluation was obtained by an introital approach with the transducer placed over the external urethral orifice and the transducer axis corresponding to the body axis.

Total urethral length and vaginal lumen were visualized in the midsagittal plane. One investigator measured the thickness of the urethrovaginal space obtained from the ultrasound images.

The anatomical border between the inner smooth muscle and mucosa submucosa layer of the urethral wall can be distinguished by US, as can the border between the vaginal wall and its lumen, seen as a strip of low echogenicity (Fig. 1). All the measurements were made in millimeters, using a line from the boundary of the smooth muscle and mucosa-submucosa layer of the urethral wall and the border of the vaginal wall and its lumen (Fig. 2). Measurements were taken at

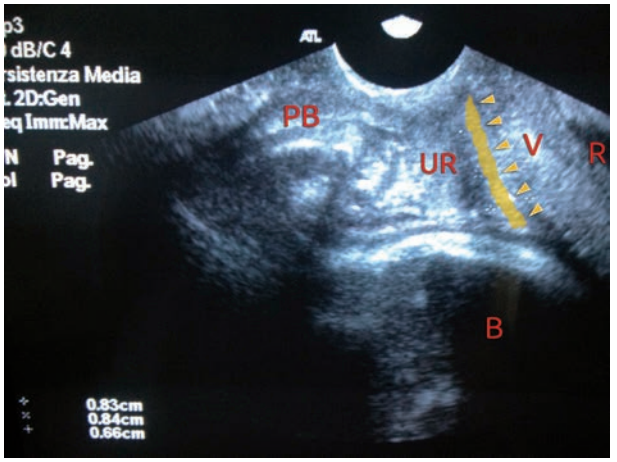


Figure 1.

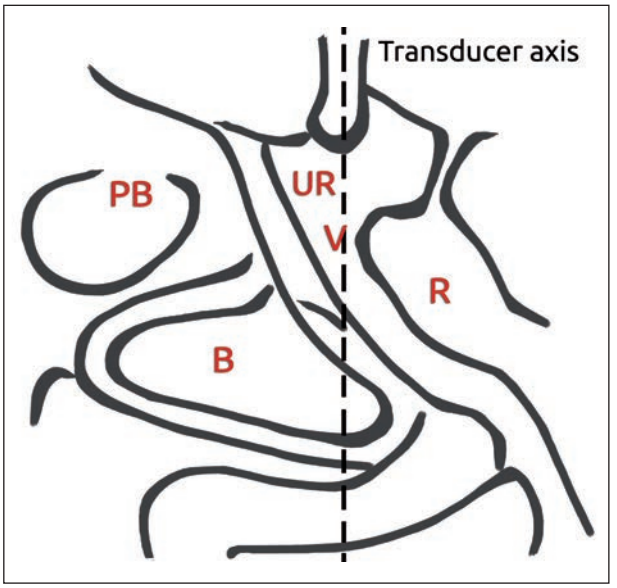


Figure 2.

various percentiles of the urethra length. The internal urethral meatus was considered as the zero point and the external meatus as the 100th percentile (Fig. 3). In the midsagittal plane, we measured the thickness of the UVS at the 10th (proximal segment), 50th (middle segment), and 90th percentile (distal segment) of the urethra (Fig. 3).The thickness was measured three times at each location and the median value was considered for statistical purposes.

We kept a distance of at least 5 cm between the internal uterine orifice (IUO) and the presenting part.

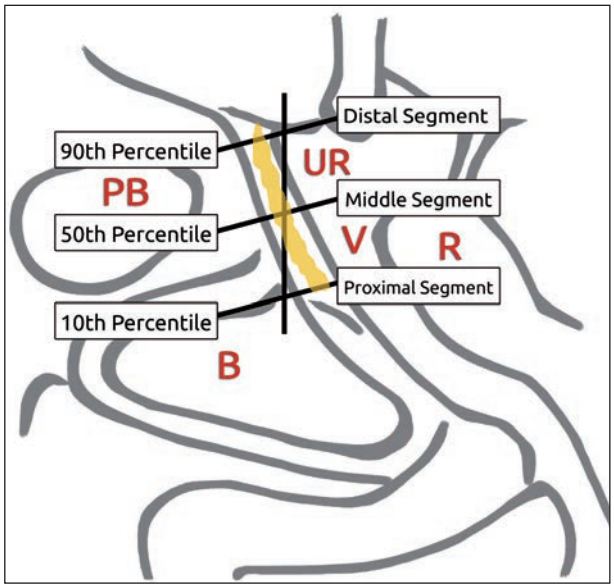


Figure 3.

The subjects were divided into 2 groups according to the presence of vaginal orgasm.

Statistical analysis

Statistical analysis was performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA) software. All *P* values less than 0.05 were considered to indicate significance. Normal distribution of the variables was checked with Shapiro-Wilk test. Normally distributed variables were presented as average ± SD and were analyzed with t test. The chi-square test was used to analyze categorical variables.

Based on available literature (2), a target sample size of 13 patients in each group was estimated to allow us to detect a minimum difference with a power of 80% using α=0.05.

Results

A cohort of 26 consecutive volunteers who met the inclusion criteria was recruited.

Demographic and obstetrical data are shown in table 1.

Table 1. Demographic and obstetrical data

	All the patients	Group 1 (with vaginal orgasm) n=12	Group 2 (without Vaginal 4 orgasm) n=1	p value
Age (years)	31.7±4.7	31.3±5.7	32±3.4	0.7375*
BMI (kg/m^2)	20.9±1.96	20.9±2.2	20.93±1.7	0.9964 *
Race	Caucasian (100%)	Caucasian (100%)	Caucasian (100%)	1
Smoking	0	0	0	1
Gestational Age (weeks±days)	36.56±0.36	36.53±0.24	36.59±0.44	0.7078*
Para ≥1	13 (50%)	7	6	0.6951 **
FSFI-6	21.8±2.1	21.3±2.0	22.2±2.2	0.3039*

*t test; **Fisher exact test

None of the patients smoked, all the patients were of Caucasian race (tab. 1). The mean age was 31.7±4.7 years. Twelve patients experienced at least one vaginal orgasm in last month and were considered group 1 and the other 14 patients group 2.

All the demographic and obstetrical data analyzed (tab. 1) were similar in the two groups (p>0.05).

FSFI did not differ between the two groups (tab. 1).

Mean UVS thickness was 18.4±6.7 at 10th centile, 17.7±6.6 at 50th centile, 18.8±7.8 at 90th centile.

We found no statistically significant regarding UVS thickness difference between the group 1 and 2. UVS thickness was similar in all the 3 segments. ROC curve analysis (Fig. 4) did not find a cut off value for each percentile (p>0.05), and AUC did not differ significantly between the three percentiles (AUC 0.485, 0.491, 0.497; p>0.05).

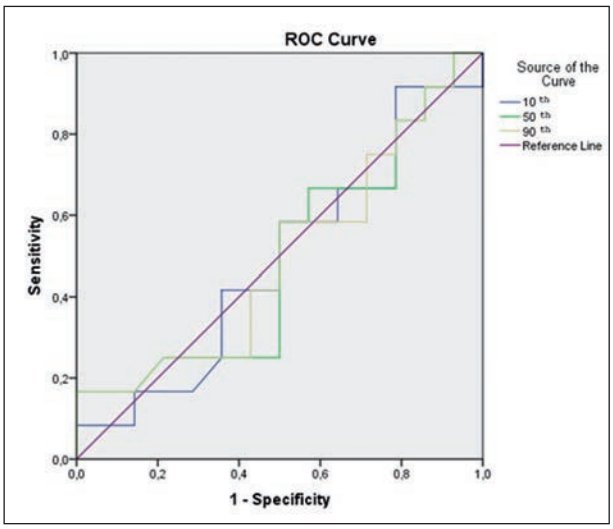


Figure 4.

Discussion

In our study we found a mean UVS thickness of more than 15 mm in all the segments considered along the anterior vaginal wall, indicating a large increase in UVS thickness as compared to other studies in fertile non-pregnant women (2, 3) and in menopausal women (20). Therefore this result confirmed our hypothesis of an increased UVS thickness during pregnancy. Other studies have demonstrated that a reduction in estrogen secretion, due to menopause, can lead to decreasing UVS thickness (16). Maternal estrogen levels rise during pregnancy up to more that 100-fold the value pre-pregnancy (1). Battaglia et al. (21) demonstrated an enlargement of UVS in iper-androgenic women with PCOS, especially in the middle part of the urethra. Maternal androgen levels increase as early as during the conception cycle and remain elevated throughout pregnancy (22, 23). There is an increment of androgen levels during the three trimesters (24, 25).

It is plausible that UVS enlargement is a consequence of the complex hormonal balance in women, dominated by estrogens and androgens in the third trimester (1, 26). As with other anatomical modification during pregnancy, the modification of the UVS is probably due to a mix of water retention, cellular hyperplasia and hypertrophy.

In our study, UVS was not significantly different between patients with or without vaginal orgasm during third trimester of pregnancy. This is not in accordance with the previous studies of the UVS in non-pregnant women (2, 3). This could be explained by rejecting the hypothesis of localizing the UVS in the anterior wall of the vagina previously reported by other studies outside pregnancy (2, 3). It is also possible that

the UVS does not function independently but instead as part of the CUV complex, as outlined by certain authors (4, 5). Even admitting the independence of the vaginal orgasm from the clitoral orgasm, some authors highlighted the importance of taking into account the response by other part of the vaginal cavity (27). In fact penile length has been independently associated with the achievement of vaginal orgasm (28), through the stimulation of deep vaginal region and cervical site. However these studies did not visualize via US the deeper vaginal or cervical zone, but relied upon a questionnaire (27, 28). We did not analyze the clitoral response and neither the incidence of the penile length to the achievement of vaginal orgasm, and these could be considered limitations of the study.

One may argue that the UVS is not properly functional during pregnancy, especially in the third trimester. Many studies have found a decline in SF, sexual arousal and frequency of orgasm in the third trimester (29). The reasons for this decline are not yet fully understood, though some authors (30) have suggested patient concerns such as exhaustion, fatigue, fear of harming the fetus, changes in body image (31) or sexual hormonal levels (26). While the majority of hormonal and physiological changes take place during the first trimester, the increase in body weight and body habitus is more prone to occur during the second and third trimesters.

Alternatively there could be a histological explanation. If the number of relevant nerve endings is proportional to the tissue mass, which could be the basis for the correlation in non-pregnant women, and if the tissue enlarges due to hormonal changes of pregnancy, this might not increase the numbers of relevant nerve endings and actually reduce the density of relevant nerve endings, and thereby reduce the correlation with vaginal orgasm. One may even speculate that the transformation of the UVS itself influences the decline of SF during the third trimester, but we consider this hypothesis less probable.

The limitation of our study was the absence of longitudinal multiple observations before the pregnancy and during all the three pregnancy trimesters and the inclusion of a control group of non-pregnant women.

Conclusions

This is the first study that analyses SF during pregnancy with the support of the ultrasound and not solely a questionnaire or blood hormonal levels. We failed to find an association between the UVS thickness and the achievement of vaginal orgasm or SF. Further studies are needed to assess whether this lack of correlation is merely casual or it is somewhat connected with the decline of SF in the third trimester with a cause-effect relationship.

Furthermore, we confirmed that in women this anatomical space is easily measurable with minimal discomfort to the patient. Whether the UVS thickness is related any adverse outcomes in pregnancy warrants further study. Further studies are needed to assess longitudinally the modification of UVS during each pregnancy trimester and post-partum, making a comparison with pre-pregnancy status in the same patients.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Grafenberg E. The role of the urethra in female orgasm. Int J Sexol 1950; 3: 145-8.
2. Battaglia C, Nappi RE, Mancini F, Alvisi S, Del Forno S, Battaglia B, Venturoli S. 3-D volumetric and vascular analysis of the urethrovaginal space in young women with or without vaginal orgasm. J Sex Med 2010a; 7(4 Pt 1): 1445-1453. doi: 10.1111/j.1743-6109.2009.01650.x
3. Gravina GL, Brandetti F, Martini P, Carosa E, Di Stasi SM, Morano S, Jannini EA. Measurement of the thickness of the urethrovaginal space in women with or without vaginal orgasm. J Sex Med 2008; 5(3): 610-618. doi: 10.1111/j.1743-6109.2007.00739.x
4. Buisson O, Jannini EA. Pilot Echographic Study of the Differences in Clitoral Involvement following Clitoral or Vaginal Sexual Stimulation. J Sex Med 2013. doi: 10.1111/jsm.12279
5. Foldes P, Buisson O. The clitoral complex: a dynamic sonographic study. J Sex Med 2009; 6(5): 1223-1231. doi: 10.1111/j.1743-6109.2009.01231.x
6. O'Connell HE, Eizenberg N, Rahman M, Cleeve J. The anatomy of the distal vagina: towards unity. J Sex Med 2008; 5(8): 1883-1891. doi: 10.1111/j.1743-6109.2008.00875.x
7. Lowenstein L, Mustafa S, Burke Y. Pregnancy and normal sexual function. Are they compatible? J Sex Med 2013; 10(3): 621-622. doi: 10.1111/jsm.12098
8. Vieira TC, de Souza E, Abdo CH, Torloni MR, Santana

TG, Leite AP, Nakamura MU. Brazilian residents' attitude and practice toward sexual health issues in pregnant patients. J Sex Med 2012; 9(10): 2516-2524. doi: 10.1111/j.1743-6109.2012.02809.x
9. Malarewicz A, Szymkiewicz J, Rogala J. Sexuality of pregnant women. Ginekol Pol 2006; 77(9): 733-739.
10. White SE, Reamy K. Sexuality and pregnancy: a review. Arch Sex Behav 1982; 11(5): 429-444.
11. Bartellas E, Crane JM, Daley M, Bennett KA, Hutchens D. Sexuality and sexual activity in pregnancy. BJOG 2000; 107(8): 964-968.
12. Fok WY, Chan LY, Yuen PM. Sexual behavior and activity in Chinese pregnant women. Acta Obstet Gynecol Scand 2005; 84(10): 934-938. doi: 10.1111/j.0001-6349.2005.00743.x
13. Tan PC, Andi A, Azmi N, Noraihan MN. Effect of coitus at term on length of gestation, induction of labor, and mode of delivery. Obstet Gynecol 2006; 108(1): 134-140. doi: 10.1097/01.AOG.0000223229.83920.af
14. Abduljalil K, Furness P, Johnson TN, Rostami-Hodjegan A, Soltani H. Anatomical, physiological and metabolic changes with gestational age during normal pregnancy: a database for parameters required in physiologically based pharmacokinetic modelling. Clin Pharmacokinet 2012; 51(6): 365-396. doi: 10.2165/11597440-000000000-00000
15. Kuijper EA, Ket JC, Caanen MR, Lambalk CB. Reproductive hormone concentrations in pregnancy and neonates: a systematic review. Reprod Biomed Online 2013; 27(1): 33-63. doi: 10.1016/j.rbmo.2013.03.009
16. Ibe C, Simon JA. Vulvovaginal atrophy: current and future therapies (CME). J Sex Med 2010; 7(3): 1042-1050; quiz 1051. doi: 10.1111/j.1743-6109.2009.01692.x
17. Isidori AM, Pozza C, Esposito K, Giugliano D, Morano S, Vignozzi L, Jannini EA. Development and validation of a 6-item version of the female sexual function index (FSFI) as a diagnostic tool for female sexual dysfunction. J Sex Med 2010; 7(3): 1139-1146. doi: 10.1111/j.1743-6109.2009.01635.x
18. Beco J, Leonard D, Lambotte R. Study of the artefacts induced by linear array transvaginal ultrasound scanning in urodynamics. World J Urol 1994; 12(6): 329-332.
19. Yang SH, Huang WC, Yang SY, Yang E, Yang JM. Validation of new ultrasound parameters for quantifying pelvic floor muscle contraction. Ultrasound Obstet Gynecol 2009; 33(4): 465-471. doi: 10.1002/uog.6338
20. Chae JY, Kim JH, Bae JH, Lee JG. (Relationship between Proximal Urethrovaginal Space Thickness and Detrusor Overactivity in Women with Stress Urinary Incontinence. Korean J Urol 2011; 52(10): 687-692. doi: 10.4111/kju.2011.52.10.687
21. Battaglia C, Nappi RE, Mancini F, Alvisi S, Del Forno S, Battaglia B, Venturoli S. PCOS and urethrovaginal space: 3-D volumetric and vascular analysis. J Sex Med 2010b; 7(8): 2755-2764. doi: 10.1111/j.1743-6109.2009.01651.x
22. Carlsen SM, Jacobsen G, Bjerve KS. Androgen levels in pregnant women decrease with increasing maternal age. Scand J Clin Lab Invest 2003; 63(1): 23-26.
23. Castracane VD, Stewart DR, Gimpel T, Overstreet JW, Lasley BL. Maternal serum androgens in human pregnancy: early increases within the cycle of conception. Hum Reprod 1998; 13(2): 460-464.
24. Kerlan V, Nahoul K, Le Martelot MT, Bercovici JP. Longitudinal study of maternal plasma bioavailable testosterone and androstanediol glucuronide levels during pregnancy. Clin Endocrinol (Oxf) 1994; 40(2): 263-267.
25. Schubring C, Englaro P, Siebler T, Blum WF, Demirakca T, Kratzsch J, Kiess W. Longitudinal analysis of maternal serum leptin levels during pregnancy, at birth and up to six weeks after birth: relation to body mass index, skinfolds, sex steroids and umbilical cord blood leptin levels. Horm Res 1998; 50(5): 276-283.
26. Erol B, Sanli O., Korkmaz D, Seyhan A, Akman T, Kadioglu A. A cross-sectional study of female sexual function and dysfunction during pregnancy. J Sex Med 2007; 4(5): 1381-1387. doi: 10.1111/j.1743-6109.2007.00559.x
27. Brody S, Klapilova K, Krejcova L. More frequent vaginal orgasm is associated with experiencing greater excitement from deep vaginal stimulation. J Sex Med 2013; 10(7): 1730-1736. doi: 10.1111/jsm.12153
28. Costa RM, Miller GF, Brody S. Women who prefer longer penises are more likely to have vaginal orgasms (but not clitoral orgasms): implications for an evolutionary theory of vaginal orgasm. J Sex Med 2012; 9(12): 3079-3088. doi: 10.1111/j.1743-6109.2012.02917.x
29. Pauleta JR, Pereira NM, Graca LM. Sexuality during pregnancy. J Sex Med 2010; 7(1 Pt 1): 136-142. doi: 10.1111/j.1743-6109.2009.01538.x
30. Eryilmaz G, Ege E, Zincir H. Factors affecting sexual life during pregnancy in eastern Turkey. Gynecol Obstet Invest 2004; 57(2): 103-108. doi: 10.1159/000075582
31. Pauls RN, Occhino JA, Dryfhout VL. Effects of pregnancy on female sexual function and body image: a prospective study. J Sex Med 2008; 5(8): 1915-1922. doi: 10.1111/j.1743-6109.2008.00884.x

Received: 19 March 2018
Accepted: 24 July 2018
Correspondence:
Giovanni Sisti
Department of Science for Woman and Child Health,
University of Florence, Italy
E-mail: gsisti83@gmail.com

Acta Biomed 2019; Vol. 90, N. 2: 265-268 DOI: 10.23750/abm.v90i2.7611

Original article

Pru p 3 sensitization in children with allergy to Parietaria pollens

Giorgio Ciprandi[1], Paolo Del Barba[2], Michela Silvestri[3], Salvatore Barberi[4], Maria Angela Tosca[3]

[1]Casa di Cura Villa Montallegro, Genoa, Italy; [2]Pediatric School, Università Vita Salute San Raffaele, Milan, Italy; [3]Pediatric Allergy, IRCCS Istituto Giannina Gaslini, Genoa, Italy; [4]Pediatric Department, ASST Fatebenefratelli-Sacco, Milan, Italy

Summary. *Background:* Pru p 3 is the major allergen of the peach and belongs to the LTP family. Pru p 3 sensitization has been associated with severe allergic symptoms after eating LTP-containing foods. However, a previous experience partially downsized the potential danger of Pru p 3 sensitization in a group of adult rhinitics. This study aimed to evaluate the real impact of Pru p 3 sensitization in children in a real-world setting. *Methods:* 82 consecutive paediatric patients (55 males and 27 females, mean age 8.19±4.23 years) with allergic rhinitis due to Parietaria pollen allergy and sensitization to Pru p 3, documented by ISAC test, were evaluated. Serum IgE was measured by ImmunoCap method. Allergic symptoms occurring after ingesting LTP-containing foods were considered and scored as oral allergy syndrome, food allergy, and anaphylaxis. *Results:* About one-quarter of Pru p 3-sensitized children reported anaphylaxis after ingesting LTP-containing foods, about half reported food allergy or oral allergy syndrome. Only ¼ was merely sensitized. *Conclusions:* Pru p 3 sensitization deserves careful attention in children contrary to what might occur in adult patients. It could depend on the age and the serum IgE level. Thus, Pru p 3 sensitization should be adequately interepreted and managed in clinical practice. (www.actabiomedica.it)

Key words: Pru p 3, sensitization, allergy, pollen food syndrome, pollen allergy, children

Introduction

Lipid transfer protein (LTP) belongs to the prolamins super-family and is implicated in cuticle formation and defence against pathogens (1). LTP is present in several plant food sources, such as fruits, vegetables, nuts, pollens, and in latex. LTP is a genuine food allergen as can sensitize throughout the digestive tract and may be a potent food allergen as resistant to thermal and acid processing (2, 3). LTP is the most frequent cause of both primary food allergy and food-dependent anaphylaxis in Italy (2, 4), although of course cow's milk and egg are the most frequent offending foods in Italian children (5). The LTP allergen Pru p 3 is the major peach allergen and is primarily present in the peel (1). Pru p 3 is commonly considered a pan-allergen as it is shared by several foods (4). It is well known that the peach may be considered the primary sensitizer to LTP in the Mediterranean area. It has been reported that high levels of serum IgE to Pru p 3 were associated with increased probability to have systemic allergy to peach (6). A further study partially confirmed these outcomes, as high levels could not be associated with true allergy (7). In addition, it has been reported that only 20% of children with LTP sensitization showed symptoms after LTP ingestion (8).

Pollen-food syndrome (PFS) is defined by the symptom occurrence after eating fruits or vegetables in patients with pollen allergy, because of a primary pollen sensitization due to cross reactivity between pollen and food allergen proteins. The severity of symptoms may vary from mild intensity, such as symptoms confined to

oral cavity (the so-called oral allergy syndrome, OAS) to life-threatening reactions. In this regard, LTP sensitization has been considered as a potential risk factor for severe allergic reaction after eating LTP-containing foods. Consequently, there are some allergists who prescribe restricted dietary regimen suggesting avoidance of many fruits and vegetables potentially cross-reacting with Pru p 3 and sometimes deliver auto-injectable epinephrine also in patients with Pru p 3 sensitization alone, fearing potential severe reaction. In this regard, a recent study, conducted in 3,937 Italian subjects, reported that the prevalence of Pru p 3 sensitization was 16.7% (6). More interestingly, Pru p 3 IgE production depended on age concerning both positive test and serum level: older age lower level (9, 10). As this matter is particular intriguing, we would like to test the hypothesis about the clinical and pragmatic relevance of Pru p 3 sensitization in clinical practice. So, a real-world study was conducted in a group of adult patients suffering from allergy to Parietaria pollen exploring this issue (11). In those clinical cases, no subject had anaphylactic reaction after eating LTP-food, even though those patients were a selected group, such as suffering from allergic rhinitis (11). Therefore, we retrospectively analysed a consecutive series of children with pollen allergy and Pru p 3 sensitization with the aim of defining the clinical relevance of IgE production toward LTP and comparing findings with those adult subjects.

Materials and Methods

A retrospective analysis of the medical records of 82 consecutive paediatric patients (55 males and 27 females, mean age 8.19±4.23 years) with allergic rhinitis due to Parietaria pollen allergy and sensitization to Pru p 3, documented by ISAC test, has been performed. All of them referred to the Allergy Center.

Inclusion criteria were the documented pollen allergy and Pru p 3 sensitization. In particular, all children had an IgE-mediated pollen allergy diagnosed on the consistency between positive skin prick test and symptoms occurrence after exposure to sensitizing allergen. Sensitization was defined as below described.

The study conformed to the ethic criteria concerning the management of personal data and was it was approved by the local Ethics Committee, consequently all the parents of the children gave a written informed consent to this pourpose.

Children were subdivided in 4 sub-groups: sensitized alone (such as without clinical reaction after eating LTP-containing foods), OAS (such as reporting oral symptoms alone after ingesting LTP-containing foods), food allergy (such as reporting systemic symptoms after eating LTP-containing foods, but without anaphylaxis), anaohylaxis (such as reporting anaphylactic reaction after eating LTP-containing foods).

Serum levels of specific IgE for Pru p 3 were detected by the IFMA (immunofluorimetric) procedure (ImmunoCAP Thermo Fisher Scientific, Uppsala, Sweden) in peripheral blood samples from patients. Serum was collected into gel-separator tubes, centrifuged and stored at -20°C until analysis. Measurement of circulating specific IgE antibodies was performed according to manufacturer's instructions (12). Specific Ig E levels were expressed in kUA/L (kilo Unit of Allergen) according to the traceable calibration to the 2nd IRP WHO (Implementation Research Platform of World Health Organization) for Human IgE and 0.35 kUA/L has been considered as a cut-off for defining sensitization (13).

In addition, a group of adult patients, with allegic rhinitis due to Parietaria allergy and sensitized to Pru p 3, and living in the same geographic area, were compared with the current pediatric cases. Details on these subjects were reported elsewhere (11).

Age was reported as mean with standartd deviation in parenthesis; IgE levels were non-normally distributed (as evaluated by the Shapiro-Wilk test), summarized as medians with lower and upper quartiles (LQ and UQ) and compared using the Mann U Whitney test (in case of comparison between two groups) or Kruskal Wallis test (in case of comparison among more groups). Categorical variables (i.e. groups of pediatric patients with clinical reaction after ingestion of LTP- containing foods) were reported as absolute frequency and percentage; comparison between or among absolute frequencies were made using chi-square test of Fisher's exact test in case of expected frequencies lower than 5. All the tests were two-sided and a p value <0.05 was considered as statistically significant. Statistica software 9.0 (StatSoft Corp., Tulsa, OK, USA) was used for all the analyses.

Results

Table 1 reports demographic and clinical characteristics of the paediatric and adult study populations. The frequency of reaction after ingestion of LTP-containing foods was almost double in children as compared to adults: 73.2% and 37.3% respectively (p=0.0007). Particularly, anaphylaxis to LTP- containing foods was only reported by children, whereas no adult referred this severe reaction; in contrast, about ¾ of adults reported oral allergic syndrome to LTP-containing foods, whereas only about ¼ of children referred this kind of reaction.

Notably, Pru p 3 levels were significantly higher in children as compared to adults being 4.91 (1.07-9.37) KuA/L and 1.62 (0.98-2.48) KuA/L, respectively (p=0.042). In children, serum IgE to Pru p 3 levels tended to become higher in relation with the severity of the reaction being lower in children with Oral Allergy Syndrome and higher in those with anaphylaxis (p=0.15), as reported in Table 2.

Analyzing the absolute frequency of anaphylaxis, food allergy and/or oral allergy syndrome in pediatric patients due to each specific LTP-containing food, we found that peach was the most common culprit food, followed by walnut, apple and nut. Peach was also the most frequent food able to induce anaphylaxis or oral allergy syndrome, whereas walnut was more frequently responsible for food allergy (Table 3).

Table 2. Serum IgE to Pru p 3 levels (KuA/L) in different groups of pediatric patients with clinical reaction after ingestion of LTP-foods

Anaphylaxis No. 20	Food Allergy No. 24	Oral Allergy Syndrome No. 16
6.05 (4.54-12.2)	4.06 (0.81-6.78)	2.84 (0.86-8.71)

Table 3. Absolute frequency of anaphylaxis, food allergy and/or oral allergy syndrome in pediatric patients due to each specific LTP-food

	Anaphylaxis	Food Allergy	Oral Allergy Syndrome	Whole
Peach	6	13	11	30
Walnut	4	15	5	21
Apple	0	4	9	13
Nut	4	4	4	11
Kiwi	1	1	8	10
Cherry	1	3	4	8
Peanut	2	3	1	6
Strawberry	0	3	3	6
Apricoat	1	1	3	5
Pear	0	3	1	4
Plum	1	1	0	2
Soy	1	1	0	2
Grapes	2	0	0	2
Orange	1	0	0	1
Chestnut	1	0	0	1
Wheat	1	0	0	1
Almond	0	1	0	1

Discussion

The current study demonstrates that Pru p 3 sensitization in children with allergic rhinitis due to Parietaria pollen allergy deserves adequate attention in this age group. Indeed, about one-quarter of children with Pru p 3 sensitization reported anaphylaxis ingesting LTP-containing foods. In addition, one half of these children had anyway clinical reaction after eating LTP-containing foods, including food allergy or OAS. Thus, about ¾ of all of these children had LTP allergy and only one-quarter were merely sensitized to Pru p 3. On the other hand, these outcomes are very differ-

Table 1. Demographic and clinical characteristics

	Children (No. 82)	Adults (No. 29)	P value
Age (yrs) [mean (standard deviation)]	8.19 (4.23)	39.54 (14.96)	-
Gender	55 m 27 f	15 m 14 f	0.14
Patients with reaction after ingestion of LTP-foods [No. (%)]	60 (73.2%)	11 (37.3%)	0.0007
Anaphylaxis to LTP-foods	20 (30.0%)	0	0.0071
Food Allergy to LTP-foods	24 (29.3%)	3 (27.2%)	
Oral allergic syndrome to LTP-foods	16 (26.7%)	8 (72.8%)	

ent in the adults with Pru p 3 sensitization, previously reported, as only about one third had clinical reaction after LTP-containing foods ingestion. Interestingly, peach and nuts were the most common LTP-containing foods accountable for anaphylactic reaction in our geographic area.

These conflicting findings could be dependent also on the level of serum IgE: in fact, IgE to Pru p 3 production significantly diminishes with age, as previously reported (9.10). Really, a noteworthy outcome is the relevance of serum IgE level: the higher is serum level the higher is the odds of true allergy, both in children and in adults. This outcome is consistent with Par j 2 IgE level assessment: in this regard, it was reported that also IgE to Parietaria significantly diminished over time (14). Interestingly, it has to be noted that Par j 2 belongs to LTP family, even though it is an allergen able to induce only respiratory allergy and not food allergy.

Of course, the current study has some limitations: the setting concerning respiratory allergy outpatients, the relatively restricted number of patients, the lack of a follow-up, the peculiarity of the considered geographic area. Anyway, a pragmatic message is that to find Pru p 3 sensitization does not automatically mean to diagnose true allergy to Pru p 3 food allergen. Only the demonstration of a close relationship between ingestion of a sensitizing Pru p 3 allergen and consistent symptom occurrence is mandatory for allergy diagnosis that should be confirmed by a double-blind food oral challenge. Thus, a thorough work-up is fundamental in Pru p 3 sensitized patients. Avoidance diet should be prescribed only to true food allergic patients and epinephrine should be deliver only when an anaphylactic reaction is undoubtedly documented.

On the other hand, the current study highlights the relevant difference between adult and paediatric subjects: in fact, severe reactions are more frequent in children.

In conclusion, Pru p 3 sensitization needs adequate interpretation and management in clinical practice. Indeed, children with pollen allergy to Parietaria and Pru p 3 sensitization deserve careful attention, as anaphylaxis may be not a rare occurrence at a young age.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Pastorello EA, Robino AM. Clinical role of lipid transfer protein in food allergy. MolNutr Food Res 2004; 48: 356-62.
2. Asero R, Piantanida M, Pinter E, Pravettoni V. The clinical relevance of lipid transfer protein. Clin Exp Allergy 2018; 48: 6-12.
3. Asero R, Antonicelli L, Arena A, et al. EpidemAAITO: features of food allergy in Italian adults attending allergy clinics: a multi-center study. ClinExp Allergy 2009; 39: 547-55.
4. Asero R, Mistrello G, Roncarolo D, et al. Immunological cross-reactivity between lipid transfer proteins from botanical unrelated plant-derived foods: a clinical study. Allergy 2002; 57: 900-6.
5. Caffarelli C, Coscia A, Ridolo E, et al. Parents' estimate of food allergy prevalence and management in Italian school-aged children. Pediatrics Internat 2011; 53: 505-10.
6. Rossi RE, Monasterolo G, Canonica WC, Passalacqua G. Systemic reactions to peach are associated with high levels of specific IgE to Pru p 3. Allergy 2009; 64: 1795-6.
7. Asero R, Arena A, Cecchi L, et al. Are IgE levels to foods other than Rosaceae predictive of allergy to lipid trabsfer protein-hypersensitivity patients? Int Arch Allergy Immunol 2011; 115: 149-54.
8. Mastrorilli C, Tripodi S, Caffarelli C, et al. Endotypes of pollen-food syndrome in children with seasonal allergic rhinoconjunctivitis: a molecular classification. Allergy 2016; 71: 1181: 91.
9. Ciprandi G, DeAmici M, DiMarino ML, Barocci F, Comite P. The impact of age on Pru p 3 IgE production in Italy. Asian Pacific Allergy 2017; 7: 42-7.
10. Tosca MA, Silvestri M, Olcese R, et al. Allergen-specific IgE to food molecular components and age: From early childhood to adulthood. Allergol Immunopathol 2017; 45: 87-92.
11. Ciprandi G, Ferrero P, Comite P. The pragmatic relevance of Pru p 3 sensitization in patients with pollen allergy. Rev Francaise Allergologie (in press)
12. Leimgruber A, Mosimann B, Claeys M, et al. Clinical evaluation of a new in-vitro assay for specific IgE, the immuno CAP system. Clin Exp Allergy 1991; 21: 127-31.
13. Seagroatt V, Anderson SG. The second international reference preparation for human serum immunoglobulin E and the first British standard for human serum immunoglobulin E. Journal of Biological Standardization 1981; 9: 431-7.
14. Tosca MA, Silvestri M, Olcese R, Pistorio A, Rossi GA, Ciprandi G. The impact of age on serum allergen-specific IgE to inhaled molecular components. Allergologia et Immunopathologia 2017; 45265-71.

Received: 8 August 2018
Accepted: 13 February 2019
Correspondence:
Giorgio Ciprandi, M.D.
Casa di Cura Villa Montallegro - Genoa, Italy
Tel. 00 39 10 35338120
E-mail: gio.cip@libero.it

Acta Biomed 2019; Vol. 90, N. 2: 269-278 DOI: 10.23750/abm.v90i2.6759

Original article

Tetanus vaccination status in construction workers: results from an institutional surveillance campaign

Matteo Riccò[1, 2], Luigi Vezzosi[3], Carlo Cella[1], Marco Pecoraro[1], Giacomo Novembre[1], Alessandro Moreo[1], Enrico Maria Ognibeni[1], Gert Schellenberg[1], Graziano Maranelli[1]

[1] Provincial Agency for Health Services (APSS) of the Autonomous Province of Trento, Department of Prevention, Operative Unit for Health and Safety in the Workplaces, Trento (TN); [2] IRCCS-AUSL of Reggio Emilia – Regional Health Service of Emilia Romagna, Department of Public Health, Service for Health and Safety in the Workplace, Reggio Emilia (RE); [3] University of Campania "Luigi Vanvitelli", Napoli (NA)

Summary. *Background:* Since 1963 Italian law (Law 292/1963, Legislative Decree n.81/2008), defines Tetanus Vaccination (TeV) as mandatory for defined occupational categories, including Construction Workers (CWs). *Materials and Methods:* An institutional survey on of CWs was performed in the Autonomous Province of Trento (Oct. 2016 - Apr. 2017). Vaccination booklets/certificate were retrieved recalling: TeV status (1), and TeV settings (2), i.e. basal schedule; year of last shot, healthcare providers who performed TeV, and TeV formulate(s). *Results:* Data about 205 CWs were collected (mean age 40.6±10.3 years; 78.0% <50 year-old, 71.7% born in Italy). Overall, 38.5% of CW had received last vaccination shot >10 years before the survey (mean: 8.8 ± 8.2 years). The majority of boosters had been administered by Vaccination Services of the Local Health Unit (47.3%), followed by Occupational Physicians (20.0%) and General Practitioners (11.2%). In 85.9% of CWs, a monovalent formulation was used. Combined TeV were mainly reported in CW who had received last vaccination shot in Vaccination Services (96.2%; p<0.001). *Conclusions:* TeV coverage rates in CWs are insufficient, and vaccination shots are frequently performed with inappropriate, monovalent formulates. As only professionals from Vaccination Services systematically employ combined vaccines and particularly Tdap, our results not only stress the opportunity for promoting TeV among CWs, but also the importance of improving reception of up to date official recommendations in Occupational Physicians, General Practitioner and professionals of Emergency Departments. (www.actabiomedica.it)

Key words: tetanus, combined vaccines, tetanus toxoid, occupational health, vaccination, vaccine hesitancy

Introduction

Tetanus (commonly known as "lockjaw") is a severe acute disease caused by toxinogenic strains of the bacterium *Clostridium tetani* and prevented by tetanus vaccine (TeV) and post-exposure prophylaxis (1-5). The spores of *C tetani* can enter the body through any injury contaminated with soils, street dust, human/animal faeces. Spores are nearly ubiquitous and, due to their continued presence in the environment, not only complete eradication is unlikely, but herd immunity plays no role in tetanus prevention (5, 6). In unvaccinated subjects, the case-fatality rate still remains significant, usually ranging from 10 to 80%, reaching 100% in absence of medical treatment (3, 5).

In the last decades, global incidence of Tetanus has decreased. In the majority of European Union (EU) countries, where most Member States have well-functioning immunization and surveillance systems, mortality of non-neonatal tetanus has declined by 85% between 1990 and 2015, recent estimated incidence being in 0.01 cases/100,000 inhabitants, with 65% of

cases aged ≥65 years (7-11). Italy is a well-known exception: since 2006 Italy reports the highest number of cases in Europe, with an annual notification rate that remains stable between 0.9-1.0/100,000. Case-fatality ratio, estimated to be 39% at the global level, has dropped less sharply in Italy as compared to other EU countries (i.e. -47% between 1990 and 2015) (2, 7, 8, 11, 12). Nearly 90% of reported cases occurred in unvaccinated or incompletely vaccinated subjects (2, 13), these figures stressing the inadequate protection rates of the Italian adult population; around 19% of Italian population is currently susceptible to tetanus (2, 13-15), and 10% of total population has only a basic, inadequate protection as a consequence of failing boost doses (2, 14).

Moreover, it is plausible that these figures may even deteriorate in the next decades. TeV was firstly introduced in 1938 for military personnel, becoming compulsory in 1963 for two-year-old children, and since 1968, for all newborns (L 292/1963). In the past, the rates of adequate protection in males were sustained by vaccination boosters received at conscription, but starting from 2003 compulsory military service has been discontinued for all subjects born after 1985 (2, 12, 13).

As a consequence, occupational TeV immunization has acquired an ever increasing relevance in order to sustain immunization rates (16, 17). In Italy, TeV is in fact the only vaccination whose status is legally defined as compulsory for workers engaged in activities considered to be at risk for interaction with tetanus toxin (e.g. construction, farming, waste collection and animal husbandry) (2, 12, 16, 18).

Starting with the National Immunization Prevention Plan (NIPP) in 1999, the Italian Ministry of Health has implemented reinforced vaccination policies in order to address falling vaccination rates, the increasing phenomenon of the vaccination hesitancy, and the re-emergence of anti-vaccination movements (19-23). Among the recommendations issued for TeV, NIPP strongly encourages the use of combined formulations for adult decennial boosters, initially (NIPP 2012-2014) with tetanus toxoid and reduced diphtheria toxoid (Td), whereas the more recent NIPP 2017-2019 officially recommends the active offer of trivalent formulations including tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) (4, 20, 24).

In the Autonomous Province of Trento (APT), the Operative Unit for Health and Safety in the Workplaces (UOPSAL, Italian acronym) represents the local governmental structure for the management and prevention of occupational injuries, occupational diseases, and work-related diseases in the workplaces. UOPSAL officers usually perform workplace interventions by visiting plants and/or construction sites without previous warning, evaluating whether parent companies comply with legislation concerning occupational health and safety conditions, and eventually establishing a deadline to solve any faults found (22, 25). Recently, National Plan for Health and Safety Prevention in Construction Settings 2014-2018 (in Italian, Piano Nazionale della Prevenzione in Edilizia, 2014-2018) has specifically included the assessment of health surveillance of CWs among the main objectives of UOPSAL's workplace interventions. Among groups considered at higher risk for tetanus, people working in construction industry are notable because of the frequency of injuries/wounds potentially contaminated with spores of *C tetani*, their large number (7, 26), and the high share of workers having inadequate protection against tetanus (7, 27, 28). Consequently, TeV coverage assessment among CWs was identified among the primary objectives of the aforementioned surveillance activity, whose results are here presented in details.

Materials and Methods

Settings

APT is located in the Italy's North East, covers a total area of 6,214 km^2 (2,399 sq. mi) and has a population of 537,416 habitants (2015 census). According to available labor force statistics, in the last decade construction industry employed around 9.2% of total, and 14.8% of male workforce (i.e. around 20,000 adult age subjects/year) (29,30).

Framework

Since October 2016 to April 2017, UOPSAL officers systematically inspected all construction sites notified to the Local Health Authority of the APT:

during the inspection, UOPSAL officers identified CWs who were working on the construction sites, and eventually acquired their institutional health and safety documentation, with specific focus on the TeV status of the workers. More in details, CWs were officially requested to provide a copy of the vaccine booklet, or a substitutive certificate.

Data analysis

Data about the type of vaccine received (T, Td, Tdap), the settings of the last vaccination shot (i.e. date; whether it was performed as a programmed/elective or an emergency shot after a penetrating injury), who actually performed it (i.e. General Practitioner, GP; Occupational Physician, OPh; or a healthcare professional from a Vaccination service of the Local Health Unit, Emergency Department, Military Service) were collected.

As recommended by Italian and the majority of international guidelines (3, 4, 15, 31-33), an "appropriate" TeV status was acknowledged for all patients who had completed the baseline schedule (i.e. for subjects vaccinated in infancy, a series of three tetanus-toxoid containing vaccine given in infancy, followed by two boosters at 6 and 11-15 years of age; for primary immunization in adults, 3 doses with a minimum of 1 month apart), plus a booster shot within the last 10 years.

A descriptive analysis was performed using means, standard deviation (SD and proportion as appropriate). Comparisons between CWs with an appropriate and not appropriate status, as well as between CWs whose last shot was performed with a monovalent formulation and those who had received a divalent/trivalent one, were performed through Student's t test for continuous variables and by means of chi-square test for discrete variables (i.e. ethnicity, occupational status, age categories, items derived from the vaccine booklet). All tests were two-tailed, and statistical significance was set at $p<0.05$. All statistical analyses were performed using IBM SPSS Statistics 22.0 for Macintosh (IBM Corp. Armonk, NY).

Ethics

This paper describes the results of a surveillance program put in place among institutional duties of the UOPSAL and was not primarily intended as a research project. The Italian legislation does not entail an ethical approval in this type of study and for this reason a formal ethical clearance was not required. Patient data are fully anonimized and no specific activity on human subjects was undertaken, other than that planned as regular surveillance activity.

Results

Characteristics of the sample (Table 1)

A total of 205 CWs were included in the study (1.1% of all employed in construction industry in the APT), all of male sex, with a mean age of 40.6±10.3 years. The majority of sampled CWs was of Italian origin (71.7%): of them, 27.2% were born before 1968 (when TeV was made compulsory for all newborns), and 21.9% after 1985, being exempted from military service and therefore did not receive vaccination booster at conscription.

Among Foreign-born people, the majority of them was of European origin (n=48, 82.8%), followed by people from India and South-West Asia (8.6%), South America (6.9%), North Africa and East Mediterranean (1.7%). Respectively to the occupational status, the majority of sampled CWs were salaried employees (86.8%), whereas 27 were either self-employed or employers (13.2%).

Vaccination status was available in 90.2% of assessed CWs and, at the time of the analysis, all had completed the basal schedule, with a mean time-lapse from the last vaccination shot of 8.8±8.2 years. More precisely, 106 of them (51.7% of the total sample) had received at least 1 shot in the previous 10 years and were consequently acknowledged as having an "appropriate" TeV status, whereas in 59 cases (38.5%) last vaccination shot was older than 10 years. However, all of them had spontaneously performed vaccination booster before delivering certification to UOPSAL officers.

Overall, 12.2% of sampled CWs had received a TeV that was performed during the validity of NIPP 2017-2019, 34.1% of NIPP 2012-2014, 32.2% of NIPP 2005, 11.7% of NIPP prior to 2005.

Table 1. Demographics of 205 Construction Workers (CWs) assessed during the survey, and characterization of their status regarding tetanus vaccine (TeV) and the recommendations issued by National Immunization Prevention Plans (NIPP)

Total number of assessed CWs (n)	205	
Age (years; mean±SD)	40.6±10.3	
Age group (years; n, %)		
<30	35, 17.1%	
30-39	64, 31.2%	
40-49	61, 29.8%	
≥50	45, 22.0%	
Occupational status (n, %)		
Employee	178, 86.8%	
Self-employed	27, 13.2%	
Migration Background (n, %)		
Italian-born people of them:	147, 71.7%	
Born before 1968 (compulsory tetanus vaccine for all newborns)		40, 27.2%
Born after 1985 (suspension of compulsory military service)		32, 21.9%
Foreign-born people of them:	58, 28.3%	
Europe		48, 82.8%
India and South-West Asia		5, 8.6%
South America		4, 6.9%
North Africa and East Mediterranean		1, 1.7%
Time since last TeV shot (years; mean ± SD)	8.8 ± 8.2	
TeV status (n, %)		
Basal schedule completed, at least 1 shot in the previous 10 years	106, 51.7%	
Basal schedule completed, last shot prior than 10 years	59, 38.5%	
Not available	20, 9.8%	
Last TeV shot, settings (n, %)		
Vaccination services of the Local Health Unit	97, 47.3%	
Occupational Physician	41, 20.0%	
General Practitioner	23, 11.2%	
Emergency Department	13, 6.3%	
Military service	11, 5.4%	
Unknown	20, 9.8%	
Last TeV shot was performed … (n, %)		
as a Monovalent formulation (T)	159, 77.6%	
as a Divalent formulation (Td)	9, 4.4%	
as a Trivalent formulation (TdaP)	17, 8.3%	
Unknown	20, 9.8%	
Official Recommendations for last vaccination shots (n, %)		
NIPP 2017-2019 (recommendation for Tdap)	25, 12,2%	
NIPP 2012-2014 (recommendation for Td)	70, 34.1%	
NIPP (Td/Tdap suggested, not recommended)	66, 32.2%	
NIPP prior to 2005 (neither suggestions or recommendations for Td/Tdap)	24, 11.7%	
Not Available	20, 9.8%	

Focusing on the vaccination settings, nearly half of the sample with a documented vaccination status (47.3%), had received last vaccination shot by healthcare providers from Vaccination Services of Local Health Units, followed by the OPhs (20.0%), GPs (11.2%), and professionals from Military Service at

conscription (5.4%). Eventually, in 13 CWs (5.9%) last vaccination shot was performed in the Emergency Department following a previous injury.

In the majority of workers, TeV was performed as a monovalent formulation (T, 77.6%), whereas 4.4% had received a divalent formulation (Td) and 8.3% a trivalent one (Tdap).

Factors associated with TeV status

As shown in Table 2, no significant differences in terms of age, age group, year of birth, ethnicity and occupational status was identified between CWs having an up-to-date TeV status and subjects lacking vaccination boosters.

Focusing on the formulates received by CWs (Table 3), no significant differences were identified in terms of demographics and occupational status for monovalent or combined vaccines. On the contrary, when focusing on the vaccination settings, the share of combined formulation was significantly higher in subjects who had received last vaccination shot in Vaccination Services (96.2% vs. 45.3%), whereas TeV by other professionals were more frequently performed as monovalent formulations. Again, a significant difference in the shares of formulation used for TeV was reported when focusing on the framework of reference recommendations: not only 42.3% of all combined formulations was reported after the enforcement of NIPP 2017-2019, but the ratio combined/monovalent vaccine increased from 0 in the years before NIPP 2005-2007, to 0.05 after its enforcement, to 0.21 after the enforcement of NIPP 2012-2014, and eventually to 0.79 after the enforcement of NIPP 2017-2019 (Figure 1).

Discussion

Tetanus immunization in Italy is a long-lasting problem (2, 7, 12-14): not only TeV rates appear to be largely unsatisfactory when compared to other European countries, but some reports suggests even worse estimates for certain occupational settings (34-36). More specifically, previous studies about Italian construction industry found that between 20% to 40%

Table 2. Comparisons of recalled demographic factors between CWs with an appropriate (i.e. a documented primary series of 3 doses with an interval of at least 4 weeks between the doses, plus a booster shot within the last 10 years) and a not appropriate TeV status

Variable	Vaccination status		Chi squared test p value
	Appropriate (n/106, %)	Not appropriate (n/99, %)	
Age	41.0±10.2	40.1±10.4	0.537
Age group			
<30	15, 13.9%	20, 20.6%	0.628
30–39	36, 33.3%	28, 28.9%	
40–49	33, 30.6%	28, 28.9%	
≥50	24, 22.2%	21, 21.6%	
Migration background			
Italian-born people	76, 70.4%	71, 73.2%	0.769
Foreign-born people	32, 29.6%	26, 26.8%	
Year of Birth (only Italian-born people)			
Born before 1968	22, 28.9%	18, 25.4%	0.761
Born in 1968 or thereafter	54, 71.1%	53, 74.6%	
Born before 1986	13, 17.3%	19, 26.8%	0.240
Born in 1986 or thereafter	62, 82.7%	52, 73.2%	
Occupational status			
Employee	14, 13.0%	13, 13.4%	1.000
Self-employed	94, 87.0%	84, 86.6%	

Table 3. Comparisons of recalled demographic factors and vaccination settings between CWs having received last shot of tetanus vaccine (TeV) as a monovalent formulation and as a combined tetanus-diphtheria (Td) or tetanus-diphtheria-acellular pertussis (Tdap) one

Variable	Formulation employed for last TeV shot		p value
	Monovalent (n/159, %)	Combined (Td / Tdap) (n/26, %)	
Age	40.6 ± 9.6	38.4 ± 9.6	0.418
Age group			
<30	29, 18.2%	4, 15.4%	0.126
30-39	58, 36.5%	4, 15.4%	
40-49	39, 24.5%	10, 38.5%	
≥50	33, 20,8%	8, 30.8%	
Migration background			
Italian-born people	108, 67.9%	21, 80.8%	0.275
Foreign-born people	51, 32.1%	5, 19.2%	
Year of Birth (only Italian-born people)			
Born before 1968	28, 25.9%	8, 38.1%	0.383
Born in 1968 or thereafter	80, 74.1%	13, 61.9%	
Born before 1986	25, 23.4%	4, 19.0%	0.883
Born in 1986 or thereafter	82, 76.6%	17, 81.0%	
Occupational status			
Employee	21, 13.2%	4, 15.4%	1.000
Self-employed	138, 86.8%	22, 84.6%	
Vaccination settings			
Vaccination Services	72, 45.3%	25, 96.2%	<0.001
Occupational Physicians	40, 25.2%	1, 3.8%	
General Practitioners	23, 14.5%	0, -	
Emergency Department	13, 8.2%	0, -	
Military service	11, 6.9%	0, -	
Vaccination references			
NIPP 2017-2019	14, 8.8%	11, 42.3%	<0.001
NIPP 2012-2014	57, 35.8%	12, 46.2%	
NIPP 2005-2007	60, 37.7%	3, 11.5%	
Prior NIPP 2005-2007	28, 17,6%	0, -	

Notes. NIPP: National Immunization Prevention Plan

of CWs may have an inadequate protection, either in terms of serology (7) or up-to-date vaccination status (16). Moreover, in a recent survey from the same geographical settings, i.e. APT, 41.6% of 707 agricultural workers (AWs) had either not completed basal schedule nor received a TeV booster in the last 10 years (18). This is particularly worrisome, as around 20% of tetanus cases usually follow an apparently minor and somehow unnoticed injury, and workers may therefore lack emergency catch-up vaccinations or even post-exposure prophylaxis with immune serum (4, 5, 33, 37). Even though TeV status is only a proxy of actual immunization status, and even intervals longer than 10 years may be eventually more cost-effective and represent a better estimate of physiological reduction of antibody levels (31, 36, 38-42), our results are consistent with available evidence, and collectively underscore the necessity for improving TeV rates and increase vaccine surveillance in adult population performing works at risk for burns and injuries potentially contaminated with soils, street dust, human/animal faeces (2, 16, 20, 24).

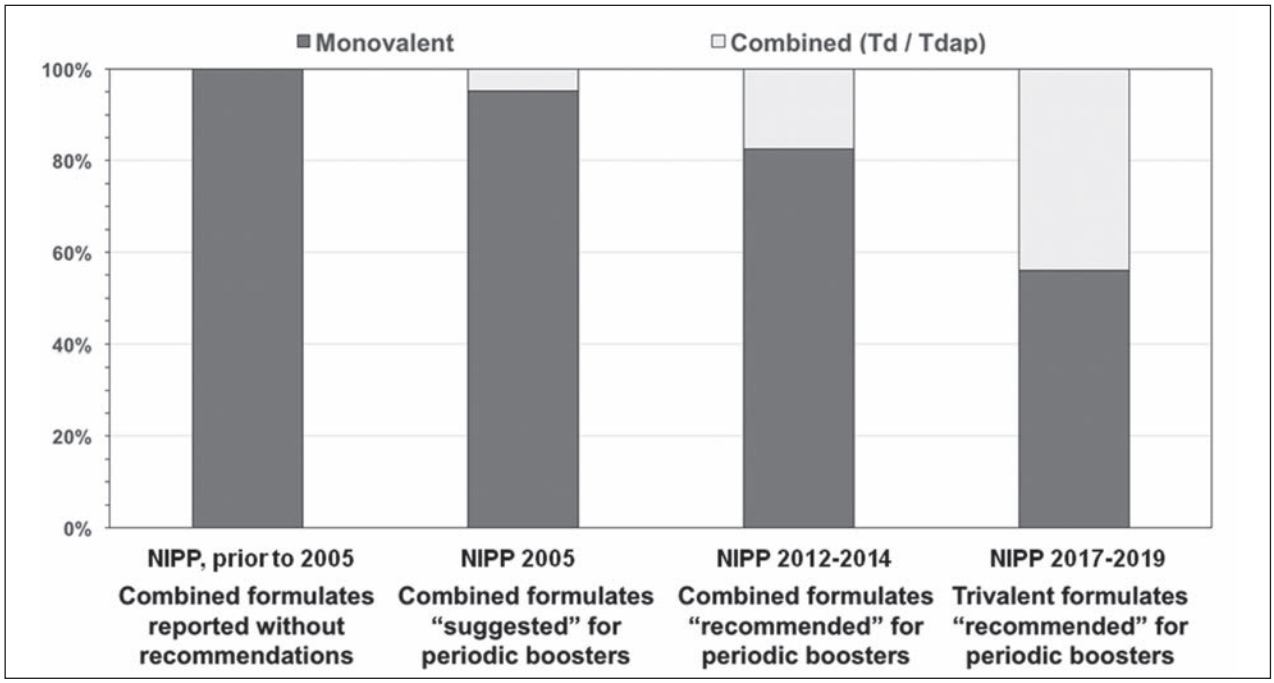


Figure 1.

Interestingly enough, the majority of CWs with inadequate TeV status spontaneously performed lacking vaccination shots, or even started the basal schedule when no documentation was available: these results are consistent with previous reports suggesting that workers from lower socio-economic status and education level (7, 43, 44), such as CWs and AWs, frequently assumed at higher risk for misconceptions and hesitancy (45, 46), actually may retain relatively low shares of vaccine hesitancy, and that the main reason for lacking a vaccination booster in these workers is usually the lack of time or even the simple forgetfulness (2, 10, 12).

Not coincidentally, NIPP 2017-2019 has stressed that every contact with a physician should be used to check vaccination status, and that decennial TeV boosters should be actively offered and performed by exploiting all the available opportunities (e.g. recertification for driving licence, periodic medical assessment etc.) (27). In other words, more recent guidelines design a proactive role for GPs and OPhs (47, 48), in particular for older age group. In this regard, even though some reports have suggested that TeV rates significantly decline with age, ratios of appropriate vs. not appropriate TeV status was similar across all age groups, and even cut-off potentially associated with differences in the immunization status, i.e. being born before the introduction of compulsory tetanus immunization for all newborns (i.e. 1968) or after suspension of conscription (i.e. 1985), did not affect vaccination rates. Actually, not only general attitude of CWs towards TeV might be diffusely better than previously supposed, but it should also be stressed that a significant share of sampled CWs had received last TeV shot in Emergency Departments, and such interventions have been described as significant opportunities to catch up with appropriate vaccination status in older workers, ultimately increasing general vaccination rates (2, 7, 16, 18, 49).

Aside assessing vaccination rates in CWs, this survey gave us the opportunity to evaluate the intervention of several healthcare providers in the vaccination practice, and our results are somehow disappointing. Although official recommendations have been issued in order to promote vaccination booster as combined formulates (since 2012 Td and, more recently Tdap), the large majority of CWs had received a monovalent formulate only including tetanus toxoid.

Moreover, we identified a significantly heterogeneous adaptation to the recommendations of NIPPs by different healthcare providers, as nearly all combined formulations were delivered in Vaccination Services of Local Health Units, and conversely GPs, OPhs as well as Emergency Departments still largely employ monovalent formulates. Such figures are therefore consistent with previous reports, and may found some presumptive explanations (16, 18). First and foremost, while workers considered at risk for tetanus may receive TeV without cost in Vaccination Services, when immunization is performed by other professionals such as GPs and OPhs, it is not compensated. Since the costs are on employer's charge, he may have some hesitancy towards the additional expenditures determined by the combined formulations (21-24).

Second, some previous reports have suggested that the knowledge of OPhs and GPs about vaccines and VPDs are neither regularly up-to-date or consistently based upon scientific evidence, rather frequently residing on personal beliefs and misconceptions (48, 50, 51). Consequently, our results may simply reflect the inappropriate reception of recently issued vaccine recommendations (21-24, 48-51).

Some limits of this survey should be considered. Firstly, the operative definition of inadequate vaccination status. As previously stated, a 10 years interval between the vaccine booster is a diffuse but arbitrary cut-off, as the excessive use of boosters in a restricted time frame could potentially result in anergy on the one hand, and in the severe side effects on the other hand, whereas a significant share of subjects lacking periodic booster may actually maintain an efficient protection (32). Moreover, the lack of vaccine booklet should not be automatically addressed as the lack of previous vaccinations or even as a lack of effective immunization (7, 12, 32, 49, 52).

Second, as a result of the primary aim of this survey, i.e. assess the vaccination rates among CWs in a restricted geographic area, we did not evaluate actual determinants of vaccination acceptance/hesitance/refusal among sampled workers. Even though our results are somehow consistent with previous reports that explicitly assessed a positive attitude of CWs towards TeV, we cannot rule out that the large share of workers spontaneously performing catch up vaccinations did it as they felt the request of documentation by UOPSAL's officers as a sort of informal warning, perceiving the possibility of significant fines whether the TeV status was ultimately ascertained as inappropriate, and ultimately representing a sort of "social desirability bias" (53, 54).

Third, it is important to underscore that the study population was not randomly selected. Moreover, although the sampling was somehow systematic, including all workers involved in active construction sites of the APT during the study period, it mainly included CWs from APT and nearby provinces from Northern Italy: as Italy is very heterogeneous in terms of tetanus vaccination rate, our results should be cautiously interpreted as representative of the National level (2, 7, 12-14, 44, 55).

Fourth, National setting of Italy on Occupational Health and Safety law is neither typical or representative of all developed countries. Actually, Italian law enforces occupational health surveillance, with occupational health services ultimately available to all workers, and defines specific occupational recommendations for TeV (16, 17, 22, 25). Consequently, our results cannot be easily generalized even at European level.

In conclusion, our study enlightens that TeV rates still remain unsatisfactory even among a high-risk occupational groups such as CWs, and that updated guidelines and recommendations encounter significant difficulties in their diffusion across all healthcare providers. Consequently, our results stress the opportunity for sustaining a more active role of GPs and OPhs in promoting and performing vaccinations, as well as in monitoring vaccine status of their patients, the latter being a critical aspect for a immunizations with very long recommended between-shots intervals. Therefore, it is also crucial to provide GPs and OPhs with up-to-date information about vaccines and their recommendations, assuring that they will be able to adequately advice and inform patients regarding vaccinations.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Luisto M, Seppalainen AM. Tetanus caused by occupational accidents. Scand J Work Environ Health 1992; 18: 323-326.
2. Filia A, Bella A, Hunolstein von C, Pinto A, Alfarone G, Declich S, et al. Tetanus in Italy 2001-2010: a continuing threat in older adults. Vaccine 2014; 32: 639-644.
3. World Health Oganization. Tetanus vaccines: WHO position paper: February 2017. Wkly Epidemiol Rec 2017; 92: 53-76.
4. World Health Organization. Tetanus vaccines: WHO position paper, February 2017 - Recommendations. Vaccine 2017: pii: S0664-410X(17)30228-1.
5. Thwaites CL, Loan HT. Eradication of tetanus. Br Med Bull 2015; 116: 68-77.
6. Thwaites CL, Beeching NJ, Newton CR. Maternal and neonatal tetanus. Lancet 2015; 385: 362-370.
7. Rapisarda V, Bracci M, Nunnari G, Ferrante M, Ledda C. Tetanus immunity in construction workers in Italy. Occup Med 2014; 64: 217-219.
8. European Center for Disease Prevention and Control (ECDC). Annual epidemiological report 2014. Stockholm: ECDC; 2015 pp. 1-45.
9. Tabibi R, Vaccalini R, Barassi A, Bonizzi L, Brambilla G, Consonni D, et al. Occupational exposure to zoonotic agents among agricultural workers in Lombardy Region, northern Italy. Ann Agric Environ Med 2013; 20: 676-681.
10. Öncü S, Önde M, Öncü S, Ergin F, Öztürk B. Tetanus seroepidemiology and factors influencing immunity status among farmers of advanced age. Health policy 2011; 100: 305-309.
11. Kyu HH, Mumford JE, Stanaway JD, Barber RM, Hancock JR, Vos T, et al. Mortality from tetanus between 1990 and 2015: findings from the global burden of disease study 2015. BMC Public Health; 2017; 17: 179.
12. Valentino M, Rapisarda V. Tetanus in a central Italian region: scope for more effective prevention among unvaccinated agricultural workers. Occup Environ Med 2001; 51: 114-117.
13. Pedalino B, Cotter B, Ciofi degli Atti ML, Mandolini D, Parroccini S, Salmaso S. Epidemiology of Tetanus in Italy in years 1971-2000. Euro Surveill 2002; 7: pii=357.
14. Stroffolini T, Giammanco A, Giammanco G, Maggio M, Genovese R, De Mattia D, et al. Immunity to tetanus in the 3-20 year age group in Italy. Public Health 1997; 111: 19-21.
15. Weinberger B. Adult vaccination against tetanus and diphtheria: the European perspective. Clin Exp Immunol 2017; 187: 93-99.
16. Riccò M, Cattani S, Veronesi L, Colucci ME. Knowledge, attitudes, beliefs and practices of construction workers towards tetanus vaccine in Northern Italy. Ind Health 2016; 54: 554-564.
17. Manzoli L, Sotgiu G, Magnavita N, Durando P; National Working Group on Occupational Hygiene of the Italian Society of Hygiene, Preventive Medicine and Public Health (SItI). Evidence-based approach for continuous improvement of occupational health. Epidemiol Prev 2015; 39: S81-S85.
18. Riccò M, Razio B, Panato C, Poletti L, Signorelli C. Knowledge, Attitudes and Practices of Agricultural Workers towards Tetanus Vaccine: a Field Report. Ann Ig 2017; 29: 239-255.
19. Tafuri S, Gallone MS, Cappelli MG, Martinelli D, Prato R, Germinario C. Addressing the anti-vaccination movement and the role of HCWs. Vaccine 2014; 32: 4860-4865.
20. Bonanni P, Ferro A, Guerra R, Iannazzo S, Odone A, Pompa MG, et al. Vaccine coverage in Italy and assessment of the 2012- 2014 National Immunization Prevention Plan Epidemiol Prev 2015; 39: 145-158.
21. Bonanni P, Bergamini M. Factors influencing vaccine uptake in Italy. Vaccine 2002; 20: S8-S12.
22. Signorelli C, Riccò M, Odone A. The Italian National Health Service expenditure on workplace prevention and safety (2006-2013): a national-level analysis. Ann Ig 2016; 28: 313-318.
23. Biasio LR, Corsello G, Costantino C, Fara GM, Giammanco G, Signorelli C, et al. Communication about vaccination: A shared responsibility. Hum Vaccin Immunother 2016; 12: 2984-2987.
24. Signorelli C, Odone A, Bonanni P, Russo F. New Italian immunisation plan is built on scientific evidence: Carlo Signorelli and colleagues reply to news article by Michael Day. BMJ 2015; 351: h6775.
25. Farina E, Bena A, Fedeli U, Mastrangelo G, Veronese M, Agnesi R. Public injury prevention system in the Italian manufacturing sector: What types of inspection are more effective? Am J Ind Med 2016; 59: 315-321.
26. Confindustria Servizi Innovativi e Tecnologici, Federcostruzioni. Il Sistema delle Costruzioni in Italia. Rome: Confindustria; 2013. [Italian] Available at: http://www.confindustriasi.it/files/FC%2020.11.13_Low%20Res%202_VERS_ONLINE.pdf Accessed on: September 15, 2017
27. Böhmer MM, Walter D, Krause G, Müters S, Gößwald A, Wichmann O. Determinants of tetanus and seasonal influenza vaccine uptake in adults living in Germany. Hum Vaccin 2014; 7: 1317-1325.
28. Lang P, Zimmermann H, Piller U, Steffen R, Hatz C. The Swiss National Vaccination Coverage Survey, 2005-2007. Public Health Rep 2011; 126: S96-S108.
29. The Labour Agency of the Autonomous Province of Trento. Local Job Creation: How Employment and Training Agencies can Help. Available at: https://www.oecd.org/cfe/leed/localjobcreationreporttrento.htm. Accessed on: September 15, 2017.
30. Agnolin C, Ioriatti C, Pontalti M, Venturelli MB. IFP Experiences in Trentino, Italy. Acta Hortic 2000; 525: 45-50.
31. Italian Ministry of Health. Tetanus prophylaxis measures. Nov 11, 1996. Available at: http://www.salute.gov.it/imgs/C_17_normativa_1529_allegato.pdf Accessed on: September 15, 2017.
32. Borella-Venturini M, Frasson C, Paluan F, De Nuzzo D,

Di Masi G, Giraldo M, et al. Tetanus vaccination, antibody persistence and decennial booster: a serosurvey of university students and at-risk workers. Epidemiol Infect 2017; 145: 1757-1762.
33. Dalal S, Samuelson J, Reed J, Yakubu A, Ncube B, Baggaley R. Tetanus disease and deaths in men reveal need for vaccination. Bull World Health Organ 2016; 94: 613-621.
34. Baratin D, Del Signore C, Thierry J, Caulin E, Vanhems P. Evaluation of adult dTPaP vaccination coverage in France: experience in Lyon city, 2010-2011. BMC Public Health 2012; 12: 940.
35. Poethko-Müller C, Schmitz R. [Vaccination coverage in German adults]. Bundesgesundheitsbl 2013; 56: 845-857. in German
36. Wagner KS, White JM, Andrews NJ, Borrow R, Stanford E, Newton E, et al. Immunity to tetanus and diphtheria in the UK in 2009. Vaccine 2012; 30: 7111-7117.
37. Alici DE, Sayiner A, Unal S. Barriers to adult immunization and solutions: Personalized approaches. Hum Vaccin Immunother. 2017; 13: 213-215.
38. Quinn HE, McIntyre P. Tetanus in the elderly - An important preventable disease in Australia. Vaccine 2007; 25: 1304-1309.
39. Ölander RM, Aureanen K, Härkänen T, Leino T. High tetanus and diphtheria antitoxin concentrations in Finnish adults - Time for new booster recommendations? Vaccine 2009; 27: 5295-5298.
40. Gardner P. Issues related to the decennial tetanus-diphtheria toxoid booster recommendations in adults. Infect Dis Clin North Am 2001; 15: 143-153.
41. Gardner P, LaForce MF. Protection against Tetanus. N Eng J Med 1995; 333: 599-600.
42. Centers for Disease Control and Prevention (CDC). Assessing Adult Vaccination Status at Age 50 Years MMWR Morb Mortal Wkly Rep 1995; 44: 561-563.
43. Hassan HA, Houdmont J. Health and safety implications of recruitment payments in migrant construction workers. Occup Med 2014; 64: 331-336.
44. Abbate R, Di Giuseppe G, Marinelli P, Angelillo IF, De Stefano A, Ferrara M, et al. Appropriate tetanus prophylaxis practices in patients attending Emergency Departments in Italy. Vaccine 2008; 26: 3634-3639.
45. Larson HJ, Jarrett C, Eckersberger E, Smith DMD, Paterson P. Understanding vaccine hesitancy around vaccines and vaccinations from a global perspectives: a systematic review of published literature, 2007-2012. Vaccine 2014; 32: 2150-2159.
46. Bloom BR, Marcuse E, Mnookin S. Addressing Vaccine Hesitancy. Science. 2014; 24; 344: 339.
47. Fortunato F, Tafuri S, Cozza V, Martinelli D, Prato R. Low vaccination coverage among italian healthcare workers in 2013. Hum Vaccin Immunother 2015; 11: 133-139.
48. Betsch C, Wicker S. Personal attitudes and misconceptions, not official recommendations guide occupational physicians' vaccination decisions. Vaccine 2014; 32: 4478-4484.
49. Orsi GB, Modini C, Principe MA, Di Muzio M, Moriconi A, Amato MG, et al. Assessment of tetanus immunity status by tetanus quick stick and anamnesis: a prospective double blind study. Ann Ig 2015; 27: 467-474.
50. Riccò M, Cattani S, Casagranda F, Gualerzi G, Signorelli C. Knowledge, attitudes, beliefs and practices of Occupational Physicians towards vaccinations of Health Care Workers: a cross sectional pilot study from North-Eastern Italy. Int J Occup Med Environ Health 2017; 30: 775-790.
51. Riccò M, Cattani S, Casagranda F, Gualerzi G, Signorelli C. Knowledge, attitudes, beliefs and practices of Occupational Physicians towards seasonal influenza vaccination: a cross-sectional study from North-Eastern Italy. J Prev Med Hyg 2017; 58: E141-54.
52. Veronesi L, R V, Bizzoco S, Colucci ME, Affanni P, Paganuzzi F, et al. Vaccination status and prealence of enteric viruses in Internationally adopted children. The case of Parma, Italy. Acta Biomed 2011; 82: 208-213.
53. Oh SS, Mayer JA, Lewis EC, Slymen DJ, Sallis JF, Elder JP, et al. Validating outdoor workers' self-report of sun protection. Prev Med 2004; 39: 798-803.
54. Riccò M, Razio B, Poletti L, Panato C. Knowledge, attitudes, and sun-safety practices among agricultural workers in the Autonomous Province of Trento, North-Eastern Italy (2016). G Ital Dermatol Venereol 2017; forthcoming.
55. Signorelli C, Riccò M. [The health-environment interaction in Italy]. Ig Sanita Pubbl 2012; 68: 374-380. In Italian.

Received: 21 September 2017
Accepted: 20 July 2018
Correspondence:
Dr. Matteo Riccò,
IRCCS-AUSL of Reggio Emilia
Via Amendola 2 - 42122 Reggio Emilia (RE)
Tel. 0039.3392994343 – 0039.522.837587;
E-mail: matteo.ricco@ausl.re.it / mricco2000@gmail.com

Acta Biomed 2019; Vol. 90, N. 2: 279-287 DOI: 10.23750/abm.v90i2.6818

Original article

Alexithymia in adults with brittle type 1 diabetes

Lorenzo Pelizza[1], Simona Pupo[2]

[1]Reggio Emilia Department of Mental Health and Pathological Addiction, Reggio Emilia Public Health Care Centre, Reggio Emilia, Italy; [2]Intensive Care Unit, Guastalla Civil Hospital, Reggio Emilia Public Health Care Centre, Guastalla, Reggio Emilia, Italy

Summary. *Background:* The term "brittle" is used to describe an uncommon subgroup of patients with type 1 diabetes whose lives are disrupted by severe glycaemic instability with repeated and prolonged hospitalization. Psychosocial problems and emotional disturbances are the major perceived underlying causes of brittle diabetes. Aim of this study is (a) to assess alexithymia in patients with brittle and non-brittle diabetes, and (2) to examine its relationship with specific parameters of general psychopathology. *Methods:* Participants comprised 44 patients with brittle diabetes and a case-control group of 88 individuals with stable (non-brittle) diabetes, matched for age, gender, years of education, and diabetes duration. Alexithymia and general psychopathology were assessed using the "20-item Toronto Alexithymia Scale" (TAS-20) and the "Symptom Checklist-90-Revised" (SCL-90-R). *Results:* Patients with brittle diabetes were more alexithymic than the control group. Alexithymia scores showed significant correlations with SCL-90-R anxiety and somatization subscales, but were relatively independent from gender, education, diabetes duration and complications, depression and glycaemic control. *Conclusions:* Given the impact of alexithymia on type 1 diabetes, the early detection and intervention of alexithymic subjects are very important for a better outcome of diabetes. (www.actabiomedica.it)

Key words: alexithymia, brittle diabetes, glycemic control, glycemic instability, psychopathology, type 1 diabetes

Background

Alexithymia is a multifaceted dimension encompassing a cluster of cognitive and affective characteristics relating to a difficultly in identifying and describing emotions in the self, an inability in differentiating them from bodily sensations, and an externally-oriented thinking style with poor fantasy and imagination (1, 2). A dichotomy was proposed to distinguish between primary and secondary alexithymia (3). Primary alexithymia is considered an "innate" and stable over time personality trait of neurobiological origin. It is though as a predisposing risk factor for the onset and/or maintenance of several psychiatric and medical disorders (such as anxiety, depression, somatoform disorders, hypertension, and functional gastrointestinal disorders) (4, 5). Secondary alexithymia is defined as a state reaction resulting from psychological stress, anxiety, depression, trauma, or even somatic illness during adolescence or adulthood (6).

Diabetes mellitus is also a stressful condition that generates negative emotional reactions, such as anxiety and depression (6). Indeed, in the face of a complex set of self-care directives (e.g. understanding the disease and its composite therapy, self-monitoring and self-adjustment of treatment), patients may become frustrated, angry, overwhelmed, and/or discouraged, while they have to cope with life-threatening risk of metabolic dyscontrol (7). Most studies investigating the relation between alexithymia and diabetes mellitus were conducted both in children/adolescents and in adult patients with type 1 diabetes and showed a high-

er prevalence of alexithymia in individuals with type 1 diabetes than in healthy controls, with values varying between 14.4% and 50% (6-12). Additional findings also showed an association between alexithymic features and worse glycaemic control (5, 8, 9, 12).

People affected by "brittle" type I diabetes are characterized by severe instability of glycaemic values with frequent and unpredictable fluctuations between hypoglycaemic and/or diabetic ketoacidosis episodes (13). Their quality of life is dramatically compromised because of very frequent acute complications (leading to hospital admissions) and premature chronic complications (14). Studies for hormonal and metabolic causes for brittle diabetes were not conclusive (15). More frequent "psychosocial" problems have been broadly demonstrated and are the major perceived underlying causes of brittle diabetes (16). Those have more often been described as non-specific anxious-depressive disorders, family dysfunction, marital disharmony, unsatisfactory relations with parents or spouse, bad-tempered separation or divorce, "life chaos", adolescent crises, unhappiness at school, and poor outside resources with no family support (17). Additional findings showed clinical features belonging to personality disorders, such as a history of manipulative behaviour, low frustration tolerance, difficulty in verbalizing emotions, obsessional glycaemic self-control, and poor impulse control (18). More recently, we observed that individuals with brittle diabetes showed no difference in terms of global severity of psychopathological distress and specific symptoms of axis I psychiatric diagnosis in comparison with subjects affected by "stable" (non-brittle) type 1 diabetes. Differently, they were more frequent affected by specific (cluster B) personality disorders (i.e. borderline, histrionic, and narcissistic type) (19, 20).

To the best of our knowledge, no study on alexithymia in brittle diabetes has been published in the literature to date. Aim of the current study was (1) to compare the presence and the levels of alexithymia in patients with brittle and non-brittle type 1 diabetes, and (2) to examine its relationships with glycaemic control and specific parameters of general psychopathology.

Methods

Subjects

All the participants (n=44) were patients affected by brittle type 1 diabetes recruited at the Diabetes Clinic of the Guastalla Civil Hospital (Reggio Emilia Public Health-Care Centre) between April 2009 to July 2010. They were all outpatients of at least 5-year duration of diabetes. Their age was comprised between 18 and 40 years. They were all native Italian-speakers. In order to avoid any attempt to selection, individuals with brittle diabetes had to fulfil the Tattersall's diagnostic criterion of "severe life-disrupting glycaemic instability of any kind" (21), as well as later accepted characteristics including "recurrent and/or prolonged hospitalization" (interfering with work and leisure) (22) and "glycaemic instability despite intensive subcutaneous insulin therapy (including subcutaneous pump treatment)" (23). To date, this is the most universally accepted working definition of "brittleness" (24). However, in all cases, infective, endocrine and therapeutic causes of glycaemic instability had been carefully excluded. Finally, according to the "Diagnostic and Statistical Manual of Mental Disorders, IV Edition, text revised" (DSM-IV-TR) criteria (25), all the participants had no previous history of major depression or any other axis I psychiatric disorder.

In order to create a case-control group, 88 individuals with "stable" (non-brittle) type 1 diabetes (i.e. patients who did not meet the universally accepted definition of brittle diabetes) were recruited at the same diabetic clinic. They were matched for age, gender, ethnic group, years of education, and diabetes duration. They were also all native Italian-speakers and had no previous history of any DSM-IV-TR axis I psychiatric disorder. Finally, both in groups with and without brittle diabetes, illiterate or markedly cognitively deteriorated patients and subjects suffering from mental retardation or organic mental disorders were excluded.

Full permission for the study was obtained from all participants, who specifically gave their written informed consent to the psychopathological assessment. Relevant ethical and local NHS research and development approvals were sought for the study. Finally, the

current study has been carried out in accordance with the Code of Ethics of the World Medical Assembly (Declaration of Helsinki) for experiments involving humans (Helsinki, 1964 and successive amendments).

Instruments and measures

A structured questionnaire was used to collect socio-demographic (i.e. age, gender, years of education, ethnic group, mother tongue, marital and employment status) and clinical data (i.e. diabetes duration, diabetes complications [retinopathy, nephropathy, macroangiopathy, microangiopathy, peripheral neuropathy, heart disease, and sexual dysfunction], personal psychiatric history, current use of psychotropic medications, and familiarity for diabetes and psychiatric disorders). To obtain a thorough evaluation, data were collected on the same day for each patient.

The "20-item Toronto-Alexithymia Scale" (*TAS-20*) (26) is the most widely used measure for the alexithymia construct. It is a self-report instrument that showed good psychometric properties (27) and consists of 20 items rating on a 5-point Likert scale (from1 to 5) along a "strongly disagree" to "strongly agree" continuum, with higher scores indicating more alexithymia. The total alexithymia score (ranging from 20 to 100) is the sum of all 20 item scores. The TAS-20 has conventionally a three-factor structure that assesses three different dimensions: (a) "Difficulty in Identifying Feelings" (DIF), (b) "Difficulty in Describing Feelings" to others (DDF), and (c) "Externally-Oriented Thinking" style (EOT). The first two factors match the emotional component of alexithymia, whereas the third one is linked to the cognitive component (26). Subjects with a TAS-20 total score of ≥ 61 were considered as "alexithymic" (26). In the present study, we used the Italian version of the TAS-20 (28).

The "Symptom Checklist-90-Revised" (*SCL-90-R*) (29) is a self-report questionnaire specifically designed to evaluate a broad range of psychopathological features. It is useful in a cross-sectional evaluation as a method for an overview of symptoms (and their intensity) at a specific point in time (i.e. in the last week) (30). It consists of 90 items (each one evaluated on a 5-point rating scale [from "0 = not at all" to "4 = extremely"]) and yields nine scores along primary symptom dimensions: "Somatization", "Obsessive-Compulsive" features, "Interpersonal Sensitivity" (corresponding to feelings of personal inadequateness and inferiority in comparison with others), "Depression", "Anxiety", "Hostility", "Phobic Anxiety" and agoraphobia, "Paranoid Ideation", and "Psychoticism" (corresponding to a continuous dimension of psychotic behavioural aspects from mild interpersonal alienation [such as withdrawal, isolation, and schizoid lifestyle] to dramatic evidence of first-rank schizophrenia symptoms [such as hallucinations or thought-broadcasting]). The SCL-90-R also generates three global indices of distress: "Global Severity Index" (GSI) (i.e. the average score of the 90 items of the questionnaire, designed to measure overall psychological distress); "Positive Symptom Distress Index" (PSDI) (i.e. the average score of the items scored above zero, designed to measure the intensity of symptoms); and "Positive Symptom Total" (PST) index (which corresponds to the number of items scored above zero). The GSI is suggested to be the best indicator of the current level of psychopathology (29). Good psychometric properties of the SCL-90-R have been widely demonstrated (30). In the current study, we used the Italian version of the SCL-90-R (31).

Glycaemic control was assessed according to HbA_{1c} levels. *HbA_{1c}* was measured by ion-exchange high performance liquid chromatography. The American Diabetes Association recommends an HbA_{1c} of ≤7% and careful reevaluation of treatment regiments for HbA_{1c} values consistently >8% (32). In the current study, we considered last HbA_{1c} measurement and the mean of the last three HbA_{1c} measurements.

Statistical analysis

Statistical analyses were performed using the "Statistical Package for Social Science" (SPSS), version 15.0. Descriptive data included mean value and standard deviation (SD) for quantitative variables, and absolute frequencies for categorical variables. Between-group comparisons on socio-demographic, clinical, and psychopathological parameters were performed using the Student's unpaired t-test for normally distributed quantitative variables. The Mann-Whitney's U test was used for quantitative variables

that were not normally distributed. Chi-squared (χ^2) test with Yates' correction or Fisher's exact test were employed for categorical variables. Fisher's exact test was used when any expected frequency was less than 1 or 20% of expected frequencies were less than or equal to 5. Spearman's rho (ρ) coefficients were performed to evaluate correlations of alexithymia with glycaemic control and psychopathological parameters.

Finally, hierarchical multivariable linear regression models were carried out to identify predictive socio-demographic, clinical, and psychopathological factors associated with TAS-20 scores. In details, socio-demographic variables (i.e. gender, age, years of education), clinical data (i.e. diabetes duration, glycaemic control [HbA_{1c} measurements], presence of diabetes complications, current use of psychotropic medications), and psychopathological parameters (i.e. SCL-90-R subscale scores) were entered as first, second and third block respectively. At each step, the increase of explained variance (ΔR^2) was computed. The rationale for the order of entry of the above-mentioned variables was that if some psychopathological factors still predicted alexithymia after the variance taken by more general socio-demographic and clinical variables, this will mean that these psychopathological features have made a unique contribution to the prediction of alexithymia. Statistical significance was set at a p-value ≤ 0.05.

Results

The *socio-demographic* and *clinical data* are shown in table 1. In comparison with patients with non-brittle diabetes, individuals with brittle diabetes showed significantly higher HbA1c levels and higher percentages of unemployed subjects and diabetes complications (in particular, retinopathy, nephropathy, and peripheral neuropathy). No differences were detected between the two groups in terms of gender, age, years of education, ethnic group, marital status, diabetes duration, current use of psychotropic medications, and familiarity for diabetes and/or psychiatric disorders.

Patients with brittle diabetes showed significantly higher *TAS-20* total score than individuals with non-brittle diabetes, as well as significantly higher scores in the DDF factor (table 1). In comparison with subjects affected by non-brittle diabetes, individuals with brittle diabetes were also more likely to be alexithymic (18.2% vs 2.3%) (table 1).

The comparison for *SCL-90-R* psychopathological parameters between individuals with brittle and non-brittle diabetes revealed no differences both in all primary symptom dimensions and in the three global distress indices (table 1).

With regard to clinical and psychopathological parameters, patients with brittle diabetes had significant positive correlations between TAS-20 and SCL-90 subscales scores (table 2). In detail, TAS-20 total score showed positive correlations with SCL-90-R "Somatization", "Anxiety", and "Phobic anxiety" subscale scores. Moreover, TAS-20 DIF factor score had significant positive correlations with all SCL-90 subscale scores. No significant correlations between SCL-90 subscale scores and TAS-20 DDF and EOT factor scores were detected. No significant correlation was also found between alexithymia and glycaemic control (table 2).

Hierarchical regression analysis results in brittle diabetes group are shown in table 3. No association between alexithymia scores and socio-demographic or clinical variables was found. The block of psychopathological parameters was the only one which made a significant contribution to the prediction of alexithymia. In particular, this effect was explained by SCL-90-R "Obsessive-Compulsive" subscale score (which had a significant positive association with TAS-20 total score [$F = 10.19$; adjusted $R^2 = 0.176$; $p = 0.003$] and EOT factor score [$F = 6.51$; adjusted $R^2 = 0.114$; $p = 0.014$]), and SCL-90-R "Somatization" subscale score (which had a significant positive association with DIF factor score [$F = 22.68$; adjusted $R^2 = 0.335$; $p = 0.001$).

Discussion

Socio-demographic and clinical data

In the present study, higher percentages of unemployed subjects were found in brittle diabetes group. This finding may involve both biological and psycho-

Table 1. Comparison of socio-demographic, clinical and psychopathological parameters, and alexithymia between patients with brittle and non-brittle diabetes.

Socio-demographic, clinical and psychopathological variables	Non-Brittle diabetes (n=88)	Brittle diabetes (n=44)	χ^2/t/z
Gender (♀)	56 (66.6%)	28 (66.6%)	.000
Age	32.91±4.80	32.07±3.21	.966
Ethnic group (Caucasian)	88 (100%)	44 (100%)	.000
Education (years)	12.59±2.62	12.82±3.38	-.353
Marital status (married)	52 (59.1%)	20 (45.5%)	1.139
Employment status (unemployed)	8 (9.1%)	14 (31.8%)	5.657[a]
Diabetes duration (years)	10.55±6.74	12.59±5.48	-1.562
HbA_{1c} (last measurement)	7.29±0.99	8.60±1.29	-5.387[c]
Mean HbA_{1c} (last three measurements)	7.15±1.28	8.35±1.20	-5.005[c]
Presence of diabetes complications	12 (13.6%)	26 (59.1%)	17.728[c]
Retinopathy	8 (9.1%)	16 (36.4%)	7.829[b]
Nephropathy	4 (4.5%)	12 (27.3%)	6.880[b]
Neuropathy	8 (9.1%)	14 (31.8%)	5.667[a]
Familiarity for diabetes	8 (9.1%)	8 (18.2%)	2.32
Familiarity for mental disorder	16 (18.2%)	14 (31.8%)	1.515
Current use of psychotropic medications	12 (13.6%)	8 (18.2%)	.085
Presence of *alexithymia*	2 (2.3%)	8 (18.2%)	5.993[a]
TAS-20 total score	39.00±13.89	55.05±12.83	-4.746[c]
TAS-20 "Difficulty identifying feelings" (DIF)	12.86±4.75	14.05±6.80	-.452
TAS-20 "Difficulty describing feelings" (DDF)	11.36±4.32	22.50±3.98	-7.766[c]
TAS-20 "Externally-oriented thinking" (EOT)	17.14±4.97	18.59±6.07	-1.110
SCL-90-R subscales			
Somatization	0.54±0.36	0.71±0.73	-.40
Obsessive-compulsive	0.47±0.26	0.56±0.39	-.29
Interpersonal sensitivity	0.39±0.33	0.42±0.35	-.25
Depression	0.48±0.44	0.56±0.28	-.24
Anxiety	0.46±0.42	0.47±0.37	-.17
Hostility	0.29±0.28	0.37±0.31	-.48
Phobic anxiety	0.65±0.38	0.89±0.73	-,43
Paranoid ideation	0.42±0.41	0.45±0.42	-.30
Psychoticism	0.15±0.11	0.18±0.12	-.20
Global severity index (GSI)	0.33±0.23	0.41±0.32	-.35
Positive symptom distress index (PSDI)	1.30±0.22	1.37±0.28	-1.45
Positive symptom total (PST) index	28.30±15.43	30.50±14.46	-1.60

Legend: [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$. Frequencies (percentages), mean ± standard deviation, chi-squared (χ^2) test, Student's t test, Fisher's exact test, and Mann-Whitney's U test (Z) values are reported

logical factors: i.e. (a) a biological factor associated with primary severe glycaemic instability, which could lead to repeated and prolonged hospitalization (because of more frequent episodes of unpredictable hypoglycaemia, diabetic ketoacidosis, and/or premature chronic complications [such as nephropathy, retinopathy, and neuropathy]), and (b) a psychological factor related to emotional disturbances observed in patients with brittle diabetes. Indeed, subjects affected by brittle diabetes with a history of low frustration tolerance, difficulty in verbalizing emotions (that is an alexithymic feature), and poor impulse control have been previously

Table 2. Spearman's correlations between alexithymia, glycaemic control and psychopathological parameters in patients with brittle diabetes (n=44)

Clinical and psychopathological variables	TAS-20 total score	TAS-20 DIF	TAS-20 DDF	TAS-20 EOT
HbA_{1c} (last measurement)	.041	.150	.034	-.020
Mean HbA_{1c} (last three measurements)	.097	.209	.013	.011
SCL-90-R subscales				
Somatization	.341[a]	.574[c]	.137	-.001
Obsessive-compulsive	.263	.371[a]	-.026	.231
Interpersonal sensitivity	.269	.515[c]	.176	-.037
Depression	.221	.403[b]	.221	-.090
Anxiety	.301[a]	.482[c]	.255	-.050
Hostility	.285	.488[c]	.191	-.025
Phobic anxiety	.302[a]	.419[b]	.161	.082
Paranoid ideation	.086	.415[b]	-.050	-.090
Psychoticism	.026	.346[a]	.005	-.181

Legend: [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$. Spearman's correlation ρ coefficient values are reported

Table 3. Hierarchical regression analysis predicting alexithymia by socio-demographic, clinical, and psychopathological variables in patients with brittle diabetes (n=44)

Model predictors	TAS-20 total score		TAS-20 DIF		TAS-20 DDF		TAS-20 EOT	
	β	t	β	t	β	t	β	t
Step 1								
Age	.08	.57	-.58	-.38	.09	.60	-.31	-2.20
Gender (male)	.27	1.92	.07	.47	.35	1.99	.35	2.22
Education (in years)	-.02	-.17	.09	.60	.13	.55	-.19	-.88
Step2								
Diabetes duration (in years)	.30	1.97	.33	1.99	.34	1.98	.04	.24
HbA_{1c} (last measurement)	.07	.48	.04	.25	.08	.61	.11	.73
HbA_{1c} (last three measurement)	.12	.86	.10	.69	.09	.63	.17	.82
Diabetes complications	-.31	-1.99	-.32	-1.98	-.01	-.15	-.14	-.78
Psychotropic medications	.06	.40	.10	.74	.12	.76	.14	.97
Step3								
Somatization	.23	.93	.59	4.76[a]	.19	.88	-.28	-1.10
Obsessive-compulsive	.44	3.19[b]	.02	.10	.03	.11	.46	3.20[a]
Interpersonal sensitivity	.11	.62	.22	1.21	.24	1.25	-.34	-1.87
Depression	.14	.77	.05	.21	.04	.20	-.29	-1.35
Anxiety	.10	.52	.04	.15	.03	.13	-.25	-1.23
Hostility	.09	.45	.03	.11	.04	.14	-.26	-1.22
Phobic anxiety	.09	.45	-.12	-.52	-.11	-.50	.-.15	-.67
Paranoid ideation	.05	.25	.02	.11	.06	.17	-.21	-1.13
Psychoticism	-.01	-.05	.01	.04	.02	.06	-.35	-1.84

Legend: [a]$p < 0.05$; [b]$p < 0.01$. β standardized coefficients and t values are reported

described (14, 16, 33, 34). Emotional disturbance of brittleness could interfere with work, inducing a bad self-management of the stress.

Consistently with other studies, our findings also showed significantly higher HbA_{1c} levels and percentages of diabetes complications (i.e. retinopathy,

nephropathy, and peripheral neuropathy) in patients with brittle diabetes. These results confirm that people affected by brittle type 1 diabetes suffer from a poor metabolic control characterized by severe instability of glycaemic values (13). Moreover, their quality of life is also more often compromised because of very frequent and premature chronic complications (14).

In the current study, no difference across the two groups in terms of current use of psychotropic medications, presence of past and/or current mental disorder, and familiarity for psychiatric disorder was detected. These findings confirm that only few patients with brittle diabetes result to have been seen by psychiatrists and/or psychologists (34, 35).

Psychopathological data

In the present study, between-group comparisons for SCL-90-R psychopathological parameters showed no significant difference in terms of intensity of all the primary specific symptom dimensions of psychopathology and global severity of psychopathological distress. This means that patients affected by brittle diabetes did not suffer more frequently or intensively from symptoms of major (axis I) psychiatric disorder compared to subjects with stable (non-brittle) diabetes. Our findings are not in line with those of previous studies, in which subjects with brittle diabetes showed to be more frequently affected by anorexia nervosa and unspecified anxious-depressive disorders (18, 34, 36).

Alexithymia

This is the first study exploring alexithymia in brittle type 1 diabetes. In our brittle subsample, alexithymic construct had a prevalence of 18.2%, a value significantly higher than in subjects with non-brittle diabetes. The association between alexithymia and brittle diabetes could be explained by (a) an overrepresentation of primary alexithymia (source of greater vulnerability to emotional stress that interferes in both the onset and the course of diabetes) (7) and/or (b) a higher level of secondary alexithymia. Indeed, the onset of brittle type 1 diabetes is often acute and symptomatic, requiring an immediate and mandatory treatment and change in life-style (34). Other factor that could be a source of secondary alexithymia include the impact of the onset of diabetes at a younger age (6), as well as the exposure to stressors such as hospitalization and serious complications leading to chronic stress, emerging psychiatric symptoms (e.g. anxiety, depression, somatoform features), and psychopathological distress (10).

Our findings also showed that patients with brittle diabetes had significantly higher TAS-20 total scores than individuals with non-brittle diabetes, as well as significantly higher scores in DDF factor. These findings seem to confirm that emotional disturbance of brittleness may include alexithymic features, especially a difficulty in verbalizing and describing feelings to others. Recently, we found that individuals with brittle diabetes were more frequently affected by DSM-IV-TR cluster B personality disorders (i.e. borderline, narcissistic, and histrionic type), which are specifically characterized by a deficit in emotional regulation and stress management (19, 20). Our findings make psychological assessment extremely necessary in brittle diabetes, especially in terms of personality traits. Our results also suggest that psychological intervention (i.e. psychotherapy) could be very useful to obtain a better glycaemic control. Therefore, everyone must be made aware that the treatment of brittle diabetes is likely to be prolonged and that the responsibility for successful outcomes does not lie with the diabetologist alone. Indeed, the patient, psychotherapist, and the family members must be prepared to cooperate (19). This alliance is a key-determinant for treatment adherence and appropriate metabolic control (7). Given the importance of therapeutic compliance in the course and the prognosis of brittle diabetes, an optimal psychological support becomes an absolutely necessary part of a multidisciplinary approach (37).

In the present study, we found no association between alexithymia and worse glycaemic control in brittle diabetes group. In the literature, results are not univocal. Most studies showed a positive correlation between alexithymia and the quality of glycaemic control. In this sense, Topsever et al. (12) observed that alexithymic patients with type 1 diabetes had a less balanced diabetes than their non-alexithymic peers. On the contrary, but consistent with our finding, Friedman et al. (10), Chatzi et al. (6), and Mnif et al.

(7) showed no relationhip between glycaemic control and alexithymia in subjects affected by type 1 diabetes.

In our research, individuals with brittle diabetes showed significant positive correlation between alexithymia (taken as a whole) and SCL-90-R subscales related to anxiety and somatization. This finding is in line with that reported in a sample of 50 patients with type 1 diabetes by Minf et al. (7), who observed a strong association between alexithymia and anxiety. Links between anxiety (and/or somatization) and alexithymia raise the question of the similarity of symptoms. Indeed, the inability to distinguish between feelings and bodily sensation in anxiety is also referred to as emotional component of the TAS-20. The existence of a conceptual overlap between alexithymia and anxiety (and/or somatization) does not help to explain whether alexithymia is a cause or a consequence of this symptomatology. Through hierarchical multivariable regression analysis, we found that SCL-90-R "Obsessive-Compulsive" and "Somatization" subscale scores were associated with alexithymia scores (particularly DIF and EOT factors) in patients with brittle diabetes. Therefore, the alexithymia observed in brittle diabetes could be in part considered as a secondary consequence of the distress associated with anxiety, reflecting a coping strategy to face a stressful situation. This type of alexithymia could decline over time once the triggering factors disappear (7). However, in subjects with chronic debilitating disease (such as brittle type 1 diabetes), secondary alexithymia may become permanent and cannot be distinguished from primary alexithymia (38).

In our study, the alexithymic difficulty in describing feelings (DDF), which specifically distinguished patients with brittle and non-brittle diabetes, showed no association with SCL-90-R psychopathological subscales. Therefore, it appears to be a primary and clinically independent component of alexithymia. On the contrary, the alexithymic difficulty in identifying feelings (DIF), which correlated with all SCL-90-R subscales (including depression), seems to be more dependent on severity of the psychopathology experienced by individuals with brittle diabetes. Links between some aspects of alexithymia and depression raise the same question of the similarity of symptoms. Indeed, affective flattening was found both in alexithymia and depression, making one a reflection of the other (7). However, perspective studies in patients with psychiatric or chronic medical illness showed that alexithymia observed during depression remained stable even following remission of depressive symptoms (2).

Conclusions

Our findings suggest that alexithymia in brittle diabetes may be a multicomponent dimension including both specific traits and state factors. Future studies should attempt to differentiate these components by assessing premorbid and developmental functioning.

We finally should mention some *limitations* of the current study. Firstly, the diagnostic criteria of brittleness are mainly based on a clinical observation of the diabetes course and thus could suffer from the arbitrary subjectivity of the clinician. According to us, it could be useful to draw up more objective criteria based on blood glucose parameters and their course over time (19, 20). Secondly, our sample of patients with brittle diabetes was numerically small (n=44). Therefore, further studies in a larger population are needed. Furthermore, in the present study, we only used self-report instruments to assess psychopathology (i.e. TAS-20 and SCL-90-R). These scales could suffer from an excessively subjective point of view. Therefore, further studies without self-report assessment tools are needed. Finally, the cross-sectional nature of our research hinders the real distinction between primary and secondary alexithymia, as well as the direction of the association between alexithymia and psychopathological parameters. Thus, further perspective studies are needed. Moreover, if causality is searched, some other mediator variables (such as distress or coping strategies) are necessary.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Sifneos PE. The prevalence of "alexithymic" characteristics in psychosomatic patients. Psychother Psychosom 1973; 22: 255-62.
2. Taylor GJ, Bagby RM, Parker JD. Disorders of affect regu-

lation: alexithymia in medical and psychiatric illness. Cambridge University Press, Cambridge, 1997.
3. Mikolajczek M, Luminet O. Is alexithymia affected by situational stress or is it a stable trait related to emotion regulation? Pers Individ Dif 2006; 40: 1399-408.
4. McGillivray L, Becerra R, Harms C. Prevalence and demographic correlates of alexithymia: a comparison between Australian psychiatric and community samples. J Clin Psychol 2016; 1: 1-12.
5. Luminet O, De Timary PH, Buysschaert M, Luts A. The role of alexithymia factors in glucose control of persons with type 1 diabetes: a pilot study. Diabetes Metab 2006; 32: 417-24.
6. Chatzi L, Bitsios P, Solidaki E. Type 1 diabetes is associated with alexithymia in non-depressed, non-mentally ill diabetic patients: a case-control study. J Psychosom Res 2009; 67: 307-11.
7. Mnif L, Damak R, Mnif F. Alexithymia impact on type 1 and type 2 diabetes: a case-control study. Ann Endocrinol (Paris) 2014; 75: 231-19.
8. Abramson L, McClelland DC, Brown D, Kelner Jr S. Alexithymic characteristics and metabolic control in diabetic and healthy adults. J Nerv Ment Dis 1991; 179: 490-4.
9. Fukunishi I. Alexithymic characteristics of bulimia nervosa in diabetes mellitus with end-stage renal disease. Psychol Rep 1997; 8: 627-33.
10. Friedman S, Vila G, Even C. Alexithymia in insulin-dependent diabetes mellitus is related to depression and not to somatic variables or compliance. J Psychosom Res 2003; 55: 285-7.
11. Manfrini S, Bruni R, Terminio N. Alexithymia in subjects with type 1 diabetes. Diabetologia 2005; 48 (suppl. 1): s.323-4.
12. Topsever P, Filiz TM, Salman S. Alexithymia in diabetes mellitus. Scott Med J 2006; 51: 15-20.
13. Voulgari C, Pagoni S, Paximadas S, Vinik A. Brittleness in diabetes: easier spoken than broken. Diabetes Technol Ther 2012; 14: 834-48.
14. Tattersall R, Gregory R, Selby C, Kerr D, Heller S. Course of brittle diabetes: 12-year follow-up. Br Med J 1991; 302: 1240.
15. Bertuzzi F. Brittle type 1 diabetes mellitus. Curr Med Chem. 2007; 14: 1739-44.
16. Gill G, Lucas S, Kent L. Prevalence and characteristics of brittle diabetes in Britain. Quart J Med 1996; 89: 839-43.
17. Gill G, Lucas S. Brittle diabetes characterised by recurrent hypoglycaemia. Diabetes Met 1999; 25: 308-11.
18. Brosig B, Leweke F, Milch W, Eckhard M, Reimer C. Psychosocial predictors of metabolic instability in brittle diabetes: a multivariate time series analysis. Psychother Psychosom 2001; 51: 232-8.
19. Pelizza L, Bonazzi F, Scatriti S, Milli B, Chierici G. Brittle diabetes: psychopathological aspects. Acta Biomed 2014; 85: 18-29.
20. Pelizza L, Pupo S. Brittle diabetes, psychopathology and personality. J Diabetes Complication 2016; 30: 1544-7.
21. Tattersall R. Brittle diabetes. Clin Endocr Metab 1977; 6: 403-19.
22. Kent L, Gill G, Williams G. Mortality and outcome of patients with brittle diabetes and recurrent ketoacidosis. Lancet 1994; 344: 778-81.
23. Pickup J, Williams G, Johns P, Keen H. Clinical features of brittle diabetic patients unresponsive to optimised subcutaneous insulin therapy (continuous subcutaneous insulin infusion). Diabetes Care 1983; 6: 279-84.
24. Cartwright A, Wallymahmed M, MacFarlane I, Wallymahmed A, Williams G, Gill G. The outcome of brittle type 1 diabetes: a 20-year study. Quart J Med 2011; 104: 575-9.
25. American Psychiatric Association. Diagnostic and statistical manual for mental disorders. IV edition, text revised. APA Press, Washington D.C., 2001.
26. Bagby RM, Parker JDA, Taylor GJ. The twenty-item Toronto Alexithymia Scale-I: item selection and cross validation of the factor structure. J Psychosom Res. 1994; 38: 23-32.
27. Bagby RM, Parker JDA, Taylor GJ. The twenty-item Toronto Alexithymia Scale-II: convergent, discriminant, and concurrent validity. J Psychosom Res 1994; 38: 33-40.
28. Bressi C, Taylor GJ, Parker JDA. Cross validation of the factor structure of the 20-item Toronto Alexithymia Scale: an Italian multicenter study. J Psychosom Res 1996; 41: 551-9.
29. Derogatis LR. Symptom Checklist-90-R: administration, scoring, and procedures manual. 3rd edition. National Computer Systems, Minneapolis, 1994.
30. Derogatis LR. Symptom Checklist-90-Revised. APA Press, Washington D.C., 2000.
31. Conti L. Repertorio delle scale di valutazione in psichiatria. SEE, Firenze, 1999.
32. American Diabetes Association. Tests of glycaemia in diabetes. Diabetes Care, 1999; 22 (suppl. 1): s.77-9.
33. Tattersall R. Brittle diabetes. Br Med J 1985; 291: 555-6.
34. Gill G. The spectrum of brittle diabetes. J Roy Soc Med 1992; 85: 259-61.
35. Tattersall R. Psychiatric aspects of diabetes: a physician's view. Br J Psychiatry 1981; 139: 485-93.
36. Steel J, Young R, Lloyd G, Clark B. Clinically apparent eating disorders in young diabetic women, associations with painful neuropathy and other complications. Br Med J 1987; 294: 859-62.
37. Sauvanet JP. Observance ou alliance therapeutique? Diabetes Met 2005; 31: 214-6.
38. Lumley MA, Stetner I, Whemer F. How are alexithymia and physical illness linked? A review and critique of pathways. J Psychosom Res 1996: 41: 505-18.

Received: 12 October 2017
Accepted: 1 August 2018
Correspondence:
Dr. Lorenzo Pelizza c/o CSM Petrella
Via Petrella n.1/A - 42100 Reggio Emilia (RE), Italy
Tel. +39 – 0522/339501 - Fax +39 – 0522/339523
E-mail: lorenzo.pelizza@ausl.re.it

Acta Biomed 2019; Vol. 90, N. 2: 288-299 DOI: 10.23750/abm.v90i2.8340

Original article

The requirements for manufacturing highly active or sensitising drugs comparing Good Manufacturing Practices

Fabio Petrelli[1], Alessandro Caraffa[1], Stefania Scuri[1], Iolanda Grappasonni[1], Elena Magrini[1], Aldo Cocchini[2]

[1] School of Medicinal and Health Products Sciences, University of Camerino, Camerino, Marche, Italy; [2] Site Compliance Manager Pfizer

Summary. *Background:* To date there exist no internationally recognised Good Manufacturing Practices (GMP) that clearly outline universally accepted standards for manufacturing highly active or sensitising ingredients. The pharmaceutical industry is faced with a twofold problem: determining which drugs need dedicated production areas and identifying the different regulations required in different countries. The aim of this paper is to find, by comparing the current regulations of the various Regulatory Agencies, the differences between containment requirements for the production of highly active or sensitising ingredients. *Methods:* An analysis of the following Regulatory Agencies' GMPs was performed: Europe (EMA), China (CFDA), Mexico (COFEPRIS), United States (FDA), Canada (Health Canada) Brazil (ANVISA), India (CDSCO), PIC/S and WHO in order to examine the differences in terms of containment requirements set by the different Regulatory Authorities for the manufacture of highly active or sensitising ingredients. *Results:* Our analysis found that the majority of Regulatory Agencies require that beta-lactams (sensitising materials) be produced in dedicated and segregated facilities. For "certain" highly active pharmaceutical ingredients (APIs), COFEPRIS, FDA, HC, EMA, PIC/S and WHO require that they be produced in facilities similar to those required for beta-lactams, while CDSCO, CFDA and ANVISA require that production takes place in segregated areas. Further differences between the Agencies have emerged regarding classes of highly APIs that require dedicated production. *Conclusion:* A study of GMP adopted by Regulatory Agencies has uncovered significant differences, in particular concerning containment requirements for the production of APIs. For this reason, the harmonisation of GMP following up-to-date quality standards based on cutting-edge science which are globally applicable is fundamental and will benefit companies and patients alike. Pharmaceutical companies would not be obliged to follow requirements enforced by the State in which they intend to manufacture a product, and patients would benefit from high-quality drugs regardless of their place of production. (www.actabiomedica.it)

Key words: Good Manufacturing Practices, highly active or sensitising drugs, cross-contamination, mix-ups, Regulatory agencies, Quality Risk Management

1. Introduction

The overmedication is often associated with an increase in the likelihood of making mistakes, abuse or non-compliance (1). Some people take medication without a prescription (2) or simply after consulting social media, and consequently risk being exposed to misleading and even dangerous information (3-6).

Highly active or sensitising ingredients require special rules for their production as accidental contamination with other materials could have serious consequences for the health of patients and because

they could also represent an occupational hazard to personnel who come into direct contact with these substances during all phases of production (7-9). Just as for regulations that concern the production and handling of some foodstuffs, even more care must be taken for medicines. Among the greatest dangers related to the manufacturing of any drug, and in particular during the production of those classed as highly active or sensitising, we find cross-contamination and mix-ups. Above all, GMPs stress the issue of contamination by substances which could be damaging to health, therefore the production of some products (in particular, sensitising and/or highly active ones) is required to take place in dedicated areas in order to ensure a clear-cut separation of these substances from other materials (10).

However, as there are no global GMP harmonising measures, some states require specific operating conditions for the manufacture of "certain" substances, while other states have different requirements, so that the only way to understand exactly what is meant by "certain" is by relying on an efficient Pharmaceutical Quality System within the organisation. Even in pharmaceutical companies, personnel can exhibit superficial behaviour caused by improper conservation practices or the inappropriate handling of substances during the production of medicines or supplements (11-13). At times this can be attributed to work-related stress, high levels of which have often been observed in these professions or linked to bad lifestyle choices that can increase improper behaviour (14, 15).

To assess the danger of cross-contamination and mix-ups, especially during the production of highly active or sensitising ingredients, pharmaceutical companies should resort to ICH Q9 Quality Risk Management guidelines. Quality Risk Management (QRM) is an essential instrument that all companies should use to perform a broad spectrum study aimed at determining any possible risks related to the production cycle of each drug. In the case of highly active or sensitising ingredients, if acceptable risk levels are not achieved for their production in multi-product plants, it is the duty of the quality assurance system to adopt precautionary measures during the production process (i.e. dedicated or partially isolated areas), especially when there is a high risk for cross-contamination and mix-ups which could be dangerous for the patient's health and/or if the Acceptable Daily Exposure (ADE) of a drug has values which could be hazardous to personnel.

The ADE shows the highest dose for each substance that is unlikely to cause an adverse health event or undesirable physiological effects if an individual is exposed to this dose or a lower dose every day for a lifetime (16, 17).

GMP in various countries do not list all the drugs that require dedicated production areas, nor the different levels of isolation required for processing certain drugs. In Europe, the European Medicines Agency (EMA) harmonises GMP belonging to the twenty-eight members of the European Union, though internationally there are still no universally harmonized guidelines; in fact, the *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use* (ICH), the *Pharmaceutical Inspection Cooperation/Scheme* (PIC/S) and the *World Health Organization* (WHO), were created precisely with the intent to harmonise different existing guidelines. Hence the pharmaceutical industry is faced with a twofold problem: it must determine which drugs need dedicated production and identify for each one regulatory differences required by different countries (10).

This study will examine issues concerning the manufacture of highly active and sensitising drugs in various Regulatory Authorities and will also analyse and compare GMP in order to highlight similarities and differences with the aim of identifying the most recent practices. Data may be useful to create a universally accepted standard for the production of these types of drug.

2. Materials and methods

GMPs issued by the following regulatory agencies were studied: Europe (EMA), China (CFDA), Mexico (COFEPRIS), United States (FDA), Canada (Health Canada), Brazil (ANVISA), India (CDSCO), PIC/S and WHO. The study was carried out in order to assess differences regarding containment requirements by different regulatory authorities for the production of the following categories of drugs: hormones, immu-

nosuppressants, cytotoxic agents, highly active pharmaceutical ingredients (APIs), biological preparations, steroids, sensitising pharmaceutical materials, antibiotics, cephalosporins, penicillin, carbapenems, beta-lactam derivatives. On the basis of this, we compared the different classes of drugs and the type of containment required by each regulatory agency.

3. Results

3.1 Segregation Requisites for producing highly active or sensitising ingredients according to the different regulatory authorities

Table 1 shows the results of the study with the categories of drugs for which different regulatory agencies require production in dedicated and/or segregated facilities.

The regulatory requirements adopted by various countries worldwide only partially define the different segregation levels essential for the production of certain classes of drugs and, as can be seen in Table 1, in classifying them adjectives such as: "*Certain*", "*Some*", "*Others*" are often used, the meaning of which is not always clearly given.

3.2 Requisites for the production of highly active or sensitising ingredients imposed by various regulatory authorities.

Brazil. The *Agência Nacional de Vigilância Sanitária* (ANVISA), provides the following guidelines, "*Technical Regulation of Good Manufacturing Practices of Drugs (2010), Resolution - RDC n. 17*", requires that production takes place in dedicated facilities for: sensitising pharmaceutical materials, biological preparations (eg: live microorganisms), cephalosporins, penicillin and carbapenems and "*other*" beta-lactam derivatives; therefore, monobactams and carbapenems could be included in this category. But it is specified that: "*In some cases, such as highly sensitising materials, segregation should also occur between them*". The production of certain highly APIs, such as some antibiotics,

Table 1. Regulatory Authorities and classes of drugs requiring segregation for production

Pharmaceutical substances	Brazil ANVISA	China CFDA	Mexico COFEPRIS	USA FDA	Europe EMA	Canada HC	India CDSCO	WHO	PIC/S
Hormones	X certain	X certain (contraceptives)	X (of biological origin)	X certain	X certain	X certain	X (sexual)	X certain	X certain
Immunosuppressants			X		X certain	X certain			X certain
Cytotoxic agents	X	X certain	X	X certain	X certain	X certain	X	X certain	X certain
Highly API	X certain	X certain	X others	X certain	X certain	X certain	X	X certain	X certain
Biological preparations	X	X	X	X	X certain	X certain	X	X	X certain
Steroids				X certain	X certain	X certain		X certain	X certain
Sensitising pharmaceutical materials	X	X	X	X certain	X certain	X certain	X	X	X certain
Antibiotics	X some				X certain	X certain	X certain	X some	X certain
Cephalosporins	X	X	X	X	X	X	X	X certain	X
Penicillin	X	X	X	X	X	X	X	X	X
Carbapenems	X	X		X	X	X certain	X		X
Beta-lactam Derivatives	X others	X		X	X	X certain	X		X

certain hormones and cytotoxic substances, should be carried out in segregated areas (18). For some classes of drugs (hormones and highly APIs), the terms "*some*" and "*certain*" are used but the meaning of these words has not been clarified by ANVISA. Therefore to determine which compounds should be included among the "some" and "*certain*" QRM must be consulted, which means resorting to case-by-case risk evaluation.

China. The *State Food and Drug Administration* (CFDA) is the Chinese regulatory authority that oversees safety in the management of food, cosmetics and pharmaceutical products. The GMPs are provided by the CFDA in the "*Good Manufacturing Practice 2010*" (19). This revised version of the Chinese GMPs, published in 2010, has been updated and is more comprehensive compared to the previous edition. For example, it has introduced the principles of QRM based on ICH Q9 standards. Despite the latest revision, Chinese GMPs remain mainly focused on the phases of production of the finished product. According to Chinese GMPs, there must be dedicated and self-contained premises available for the production of medicines like: sensitising pharmaceutical materials, biological preparations, beta-lactam antibiotics, contraceptive hormones (Art 46.3), certain cytotoxic agents and certain highly APIs.

Mexico. The regulatory authority that publishes Mexican GMPs is the *Comisión Federal Para la Protección contra Riesgos Sanitarios* (COFEPRIS) in the NOM-059-SSAI-2006 "*Buenas prácticas de fabricación para establecimientos de la industria químico farmacéutica dedicados a la fabricación de medicamentos*" (20). The rules set out in the guidelines are based on both European and FDA standards (both are quoted in the list of references). In 2014, COFEPRIS added Annex 20 to the GMPs, which is essentially the same as the ICH-Q9 in QRM; this attachment provides instructions for a systematic approach to QRM that facilitates compliance with GMPs and other quality requirements (21).

According to these GMP, the production of penicillin, cephalosporins, cytotoxic agents, immunosuppressants and hormones of biological origin must be completely independent and self-contained; furthermore, biological and microbiological processing must be physically separate. Regarding hormones, only those of biological origin must be produced in self-contained areas and there is no mention of those of synthetic origin. Cephalosporins and penicillin must be produced in areas that are completely independent and self-contained but there is no reference to the type of production necessary for beta-lactam derivatives. Highly active or sensitising ingredients, although not explicitly mentioned, are definitely among those at high risk mentioned in Art 8.2.16 (20).

USA. GMP regulations in the United States are enforced by the FDA through the Federal Register (mainly CFR Title 21, parts 210 and 211) and numerous industry guidelines. Areas of application of the American GMP is defined in the FDA regulations 21 CFR 210.1 where it is established that the rules cited must be considered the "*Minimum current GMP*" (22, 23). According to the FDA's GMP, the industrial production of penicillin must take place in totally dedicated areas of several buildings or even in the same building (CFR 21 Part 211.42-d). The "*Guidance for Industry Non-Penicillin Beta-Lactam Risk Assessment: a CGMP Framework for Preventing Cross Contamination*" states that the production of sensitising non-penicillin beta-lactams must be treated in the same way (24). The FDA has published internationally harmonized guidelines for highly potent APIs (*FDA, 2016, Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients Guidance for Industry*). In the FDA Q7 Guidelines published in 2016 it is specified that dedicated production areas should be used for manufacturing sensitising materials such as penicillin or cephalosporins, and that this should also be considered when infectious, highly active or toxic materials are involved (for example certain steroids or certain cytotoxic anti-cancer drugs) (25). In 2018, the FDA published an additional handbook: "*Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients: Questions and Answers Guidance for Industry*"(26); this document was written in collaboration with PIC/S and its aim was to clarify doubts concerning the interpretation of several sections of the FDA Q7 guidelines published in 2016. Regarding the highly active or sensitising ingredients, the ideas that can be found in the FDA Q7 Guidelines from 2016 are repeated. Further information can be obtained

from the "*Food and Drug administration compliance program guidance manual program*" (Ch.56) (27). Although GMP specify in numerous sections which rules must be followed for the production of penicillin and biological drugs, there are no specific manufacturing rules for other classes of drugs (as, for example, in the case of highly potent APIs). In the FDA's GMP it has not been clearly stated that the production of "*certain*" highly potent APIs must be carried out in dedicated areas, but this can be deduced by numerous references made in other documents, such as, for example, in the FDA's answer P-0069 to *Foley & Lardner's petition* (28).

Europe. In Europe, the EMA harmonises GMPs from the twenty eight members of the European Union in *Eudralex Volume* 4, which due to the number and complexity of its clauses should be considered a combination of the best and most current manufacturing procedures. The latest version, published in 2015, leaves the manufacturer a wider margin with which to assess risks (29).

The decision whether or not to use dedicated facilities covers all the categories that pose a risk and is still required in the following cases:

"a. The risk cannot be adequately controlled by operational and/ or technical measures,

b. Scientific data from the toxicological evaluation does not support a controllable risk (e.g. allergenic potential from highly sensitising materials such as beta lactams) or

c. relevant residue limits, derived from the toxicological evaluation, cannot be satisfactorily determined by a validated analytical method" (Chap. 3.6).

Therefore, beta-lactam antibiotics remain the only type of drugs for which the EMA requires *a priori* production in dedicated areas. Regarding all other drugs, the manufacturers must carry out a careful analysis of the risks involved in their production in order to assess whether they can be produced in multi-product plants or whether dedicated facilities must be used for their manufacture. This assessment must take into account the toxicological as well as the pharmacological parameters of the product (30).

Canada. The regulatory agency that promotes GMP in Canada is Health Canada (HC) through the "*Good manufacturing practices guide for drug products - GUI-0001, 2018*" (31). The introduction specifies that the handbook was written with the intent of harmonising GMP regulations with those of other countries as well as the WHO, PIC/S and ICH; indeed, some of the definitions in the glossary have been taken from the regulations of these Agencies. The Guidelines for active pharmaceutical substances are dealt with in a separate section:"*Health Products and Food Branch Inspectorate; Good Manufacturing Practices (GMP) Guidelines for Active Pharmaceutical Ingredients (API) - GUI-0104*" (32). In the Canadian GMP, which are similar to *Eudralex* guideline, the fact that any sort of cross-contamination poses a certain risk for patients is considered noteworthy and consequently the need to pay particular attention to any substance that is produced is emphasised. Moreover, it is clearly stated that the production of certain classes of sensitising materials, including penicillin and cephalosporins, must occur in dedicated areas (31). Hence other classes of beta-lactam antibiotics including carbapenems and "*other beta-lactam deivatives*" need to be further assessed by the manufacturer to establish the most suitable type of containment. In the "*Good Manufacturing Practices (GMP) Guidelines for Active Pharmaceutical Ingredients (API) - GUI-0104*" it is stated that for the production of certain classes of drugs, such as steroids and cytotoxic anti-cancer agents, dedicated areas should be considered, unless validated inactivation and/or cleaning procedures are established and maintained, but there is no obligation to carry out production in segregated areas when certified procedures that guarantee the quality of the products and prevent the risk of cross-contamination are in place (32).

In short, the latest Canadian Guidelines repeat the topics already addressed in the *Eudralex* Guidelines regarding the need to perform a recorded risk assessment (*QRM*) concerning both the pharmacological and the toxicological aspect for any substance that is produced in order to control the risk of cross-contamination. But the Canadian Guidelines mention only two categories of materials for which production in dedicated areas is compulsory.

India. The *Central Drugs Standard Control Organization* (CDSCO) is the Indian Regulatory Agency which, with the promulgation of SCHEDULE

M "*Drug and Cosmetics Act*", contains GMP for both drugs and cosmetics. Furthermore, the guidelines combine requirements for highly APIs with those of other medicines (33). The rules are also divided according to the different ways in which products are administered. It is obvious that the complexity and number of sections included in SCHEDULE M are fewer compared to other GMPs studied. The fact that the one volume contains all the regulations regarding the production of such different substances (medicines and cosmetics) underlines how Indian GMPs are much less specific than the EMA GMP. The way in which production plants are designed is fundamental in order to avoid any chance of contamination between the different products. Indian GMPs call attention to the location of the pharmaceutical plant in order to avoid contamination from outside agents. Regarding the use dedicated and self-contained areas, Indian practices state the need to design separate areas for: beta-lactams (with no exceptions), highly active materials, sex hormones, some antibiotics, cytotoxic and oncology products.

World Health Organisation (WHO). The WHO is a specialized UN Agency that deals with international public health. The requirements for the production of drugs containing highly active or sensitising substances are listed in the "*WHO Technical Report Series, No. 957, Annex 2 (2010)*", in the "*WHO Technical Report Series, No. 957, 2010 Annex 3 (2010)*" and in the "WHO *Technical Report Series, No. 986, Annex 2 (2014)*" (34-36). The version of GMP developed by the WHO is used by the pharmaceutical industries and by the sector's supervising authorities in over 100 countries worldwide. These GMP are to be considered general rules, as it is clear that they are not exhaustive in the treatment of topics such as safety precautions for the health of personnel and the environment, aspects that are normally covered by national laws. In its "*Technical Report Series No. 986 (2014)*", the WHO specifies which elements are essential in an effective quality management system, stressing aspects like self-inspection and quality audits similar to those required by the EMA. Regarding ingredients that require production in dedicated areas, neither the "*WHO's Technical Report Series, No. 986, Annex 2 (2014)*" nor the "*No. 957 Annex 3*" mention Beta-lactam derivatives, only penicillin and cephalosporins. According to "*WHO Technical Report Series, No. 986, Annex 2 (2014)*" and "*WHO Technical Report Series, No. 957, Annex 2 (2010)*", the production of sensitising materials and biological preparations must be carried out in dedicated areas; furthermore, it is stated that certain highly active materials, such as some antibiotics, hormones, some steroids, cytotoxic substances and other non-pharmaceutical products should not be manufactured in the same building. Further instructions can be found in the "*WHO Technical Report Series, No. 957, 2010 Annex 3*": these are Guidelines that regulate the good practices that should be applied to facilities that deal with pharmaceutical products (including active pharmaceutical ingredients that contain dangerous substances such as some hormones, steroids or cytotoxic substances). The handbook underlines the fact that the production of certain products containing dangerous substances should generally occur in separate, dedicated and independent buildings. The "*WHO Technical Report Series, No. 986, Annex 2 (2014)*" states that dedicated facilities are required for the production of "certain" hormones, whereas in the "*Guideline to the inspection of hormone product manufacturing facilities*" (2008) the word "certain" in paragraph 3.4 is not used. Indeed it says that: *"Hormone facilities should be separate, dedicated facilities and should not form part of any other non-hormone facility. They may be in the same building as another facility but should be separated by a physical barrier and have separate entrances, staff facilities, air-handling systems, etc"* (37). On the other hand, in terms of GMP requisites, paragraph 4.1 of the same handbook reads that not all hormone products are equally potent and that a risk assessment should be carried out to determine the potential hazards to operators and to the environment.

Pharmaceutical Inspection Convention (PIC) and Pharmaceutical Inspection Co-operation Scheme (PIC/S). PIC and PIC/S are two international tools created to improve the cooperation between the regulatory authorities and the pharmaceutical industry in the field of GMP. The aim of PIC/S is, fundamentally, to achieve the following goals: mutual recognition of the inspections, harmonisation of the GMP requisites, uniform control systems, training

inspectors, facilitating networking and mutual confidence. EMA and PIC/S cooperate to better harmonise the GMP at an international level, sharing resources and avoiding the duplication of efforts. To date 54 members, including Europe, the United States, Canada and both WHO and EMA adhere to the PIC/S and the number continues to increase. PIC/S develops and maintains a GMP guide that must be used by its members and is the PIC/S' main tool for harmonisation. In terms of GMP requisites, the PIC/S GMP handbook is identical to the EU GMP guidelines. The "*Guide to Good Manufacturing Practice for Medicinal Products PART I (PIC/S; 2018)*" contains the PIC/S' requirements for the production of highly active or sensitising ingredients (38). In PIC/S Guidelines, just as in the *Eudralex* Guidelines, the question of whether or not to use dedicated facilities is extended to all those categories which might present a risk and this remains a requirement in the cases listed in paragraphs a, b and c in Chapter 3.6, which cite exactly what is written in the *Eudralex volume 4* (2015) (29). In short, the PIC/S GMP Guidelines reiterate the topics already presented both in the *Eudralex* Guidelines and the Canadian Guidelines regarding the need for a recorded risk assessment (*QRM*) for both pharmacological and toxicological aspects in order to control the risk of cross-contamination. Unlike the *Eudralex* Guidelines, though, in the "*Guide to Good Manufacturing Practice for medicinal products PART II (PIC / S; 2018)*" the PIC/S GMPs list other categories of drugs which should be produced in dedicated areas, in particular: some steroids and cytotoxic anti-cancer agents (39).

3. Discussion

In order to clarify the different types of containment required by Regulatory Agencies to avoid the possible risks associated with the production of the aforementioned classes of drugs, it is important to understand the meanings of the terms used in the regulations adopted by different States. The *International Society for Pharmaceutical Engineering* (ISPE), an international society of engineers that supports pharmaceutical companies in designing and manufacturing pharmaceutical products, provides specific definitions concerning the different types of facilities and the types of containment required in the "*Risk-Based Manufacture of Pharmaceutical Products*" Handbook. In particular, this publication gives precise definitions for: dedicated area, dedicated building/facility, self-contained area and segregation (40).

All the GMP studied clearly forbid the production of penicillin in multipurpose areas, that is to say where other classes of drugs are manufactured. GMPs clearly state that this class of drugs must be manufactured in physically separate and dedicated facilities, although COFEPRIS, HC, FDA and WHO specify that it is not necessary to use separate buildings (20, 23, 31, 35). Hence, production can take place according to the option shown in Fig. 1. The GMP adopted by EMA, PIC/S, China, Mexico, USA, Canada and India regulate the production of cephalosporins in dedicated and separate facilities, designed in the same way as illustrated in Fig. 1 (19, 20, 25, 29, 31, 33, 38). In its Technical Report Series the WHO, No. 957

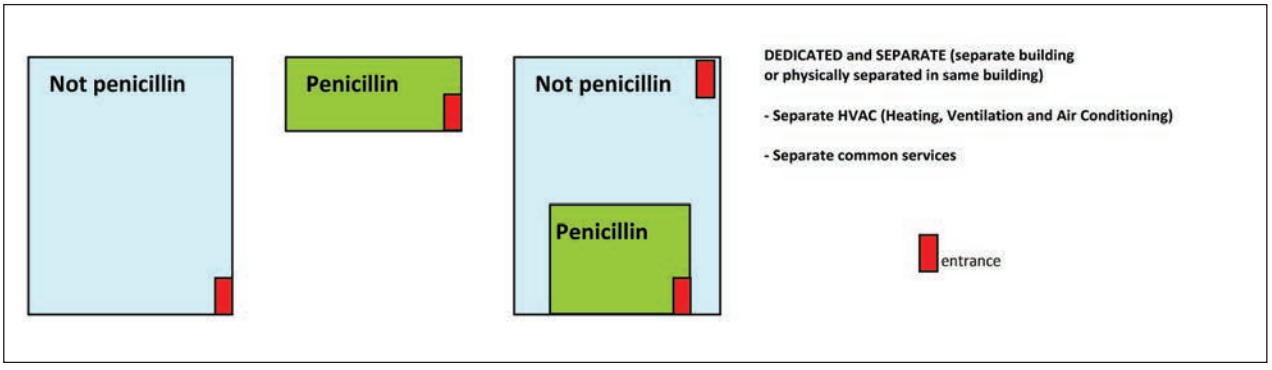


Figure 1. Facilities used for the production of sensitising materials

(2010, Annex 2), states that dedicated areas should be used for the production of cephalosporins; therefore, all compounds that belong to the latter class of drugs do not require segregation during the manufacturing process (34). Mexican and WHO regulations do not specify what sort of containment is necessary for the production of all beta-lactam derivatives, even though these classes of medicines are included among ones classed as sensitizing, consequently requiring production in facilities similar to those described for penicillin (20, 34-36).

3.3.2 *Production for highly potent APIs*

The GMP we studied provide specific indications for the production of "certain" highly potent APIs, except in the case of the Indian Regulatory agency which does not use the adjective "certain" (33). The type of containment required during the entire productive cycle of some classes of drugs belonging to this category varies depending on the Regulatory agency studied. The Mexican, USA, Canadian, European PIC/S and WHO Regulatory Organisations for example, require that the production of "certain" highly potent APIs take place in facilities designed in the same way as those meant for sensitising materials, that is to say totally dedicated and separate facilities (20, 25, 28, 29, 32, 34-39). In particular, EMA, PIC/S and WHO require this type of containment if QRM procedures prove that it is the only sort of containment capable of preventing events such as mix-ups and cross-contamination; otherwise it could be sufficient to adopt another form of control like, for example, dedicating areas and equipment to particular production phases and/or packaging. On the contrary, the Indian, Chinese and Brazilian Regulatory agencies regulate the production of "certain" highly potent APIs in dedicated manufacturing areas meant for the production of a given class of drugs (within the same building/facility where other medicines are produced), but designed in such a way as to prevent cross-contamination and guarantee that the product is not exposed to the adjoining areas, without having to be completely separate from other buildings (18, 19, 33).

Differences found between the GMP do not only involve the type of containment required but, as can be seen in Table 1, also refer to which class of highly potent API needs appropriate containment.

The Chinese, Brazilian, Mexican, Canadian, European, USA, PIC/S and WHO GMP regulate the production of "certain" hormones; among those classed as "certain", the CFDA (Chinese Regulatory Authority) mentions that contraceptives must be produced in dedicated and self-contained facilities; this is a significant distinction required by the CFDA and is not expected for the manufacturing of "other" hormones, which, on the contrary, can be produced in segregated areas within the same facility (18-20, 29,

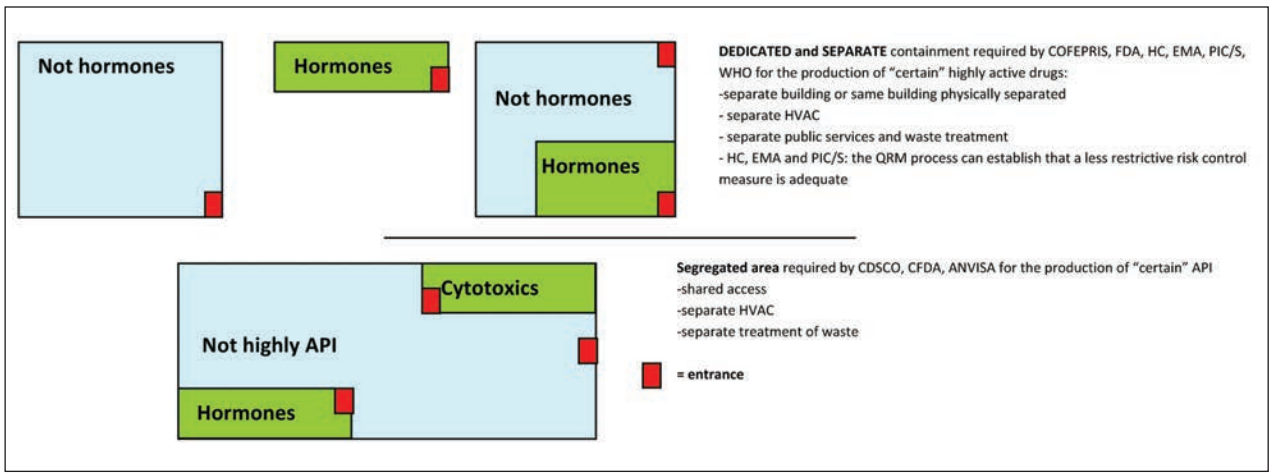


Figure 2. Facilities used for the production of highly APIs

31, 36-38). COFEPRIS is the only agency specifying that hormones of biological origin must be produced in separate and dedicated facilities, whereas the CDSCO only requires production in segregated areas for sex hormones (20, 33).

All GMP studied have specific containment rules for the production of cytotoxic medicines, although differences can be observed between them. CFDA, EMA, FDA, HC, PIC/S and the WHO only regulate the type of production required for "certain" cytotoxic agents (19, 25, 29, 32, 34, 36, 39). On the contrary, China, Mexico and India require that this type of drug be produced in a totally dedicated and separate facility (19, 20, 33). The USA, Brazil, Canada, PIC/S and WHO clarify that dedicated areas are sufficient, this does not imply a complete separation from other adjacent facilities (18, 25, 32, 34-36, 39). According to the EMA the choice of containment must be established by the manufacturer through adequate risk assessment, thus not necessarily requiring that these drugs be manufactured in contained facilities/areas (29).

Only the Mexican Regulatory Agency (COFEPRIS) requires that all immunosuppressants be manufactured in independent and self-contained facilities (20). ANVISA requires special containment rules for immunosuppressants exclusively when the active ingredient (raw material) is being produced (18). The European, Canadian and PIC/S GMP, which extend the risk assessment to any type of substance produced, refer the type of containment required for this class of drug to the manufacturer (29, 31, 38).

4. Conclusions

In various parts of the world the regulatory framework regarding GMP has evolved, but there are still significant differences between the various regulatory agencies concerning segregation requirements for the production of highly active or sensitising ingredients. Few scientific papers on this topic have been published and no critical analysis of the various regulations has yet been performed. A comparative study of GMP of some of the most important Regulatory agencies has shown that there are points of agreement and others of discord regarding the segregation required for the production of highly active or sensitising ingredients. In particular, regarding sensitising ingredients and especially penicillin, all GMP studied clearly forbid their production in areas where other classes of drugs are produced, necessitating their manufacture in dedicated and segregated facilities. According to most of the aforementioned regulatory agencies, cephalosporins, carbapenems and "*Other beta-lactam derivatives*" represent categories of sensitising ingredients which must be produced in facilities designed in a similar manner to those for the production of penicillin.

Regarding highly APIs, the type of containment required by each product varies in the Regulatory Authorities we studied. As can be observed in Figure 2, COFEPRIS, FDA, HC, EMA, PIC/S and WHO require that "certain" highly potent APIs be manufactured in facilities which have similar characteristics to those required for the production of sensitising ingredients, whereas CDSCO, CFDA and ANVISA require that "certain" highly potent APIs be manufactured in segregated areas, without requiring total separation from other facilities. EMA, PIC/S and HC in particular require that the above-mentioned type of containment be adopted only if the QRM procedures guarantee that it is the only type of containment capable of preventing mix-ups and cross-contamination; otherwise it could be sufficient to use another form of control such as dedicated areas and equipment for particular stages of the production and/or packaging. The Agencies also show some differences regarding the classes of API that require dedicated production areas.

In defining the levels of segregation for the different categories of drugs, and in particular for many of the classes of highly APIs, the Regulatory Agencies we studied used terms like "certain", "some", "others", often without any reference to their specific meaning. In these cases, it is up to the pharmaceutical industry to choose the most suitable containment measure by means of risk analysis. The use of these adjectives without providing a specific definition of their meaning obviously leaves too much room for individual interpretation which could potentially have a negative impact on both the safety and efficiency of the production processes. The problem should be overcome using unambiguous terminology with precise refer-

ences. In 2015, the EMA and later on PIC/S and HC updated their GMP by adding the need to use QRM procedures to their regulations in order to choose the most suitable facility for the production of all classes of drugs that could represent a risk. Moreover, they have introduced new criteria for the risk analysis of products based on toxicological and pharmaceutical parameters.

It became obvious that up to this moment, unlike other Regulatory Authorities only EMA, PIC/S and HC have regulations that define a specific operative method in order to assess the type of facility which should be used in the production of the highly active or sensitising ingredients mentioned in this paper.

The other regulations, as can be seen in Table 1, do not provide specific indications concerning the type of segregation required for these cases. As previously mentioned, in the case of sensitising ingredients such as beta-lactams, the majority of Regulatory Authorities require dedicated production, though in truth, even if a beta-lactam production plant were to be abandoned, its re-use would be nearly impossible. Nevertheless, advanced cleaning techniques could solve this problem. Takahashi et al., in their article "*Case Study: Beta-Lactam Decontamination and Cleaning Validation of a Pharmaceutical Manufacturing Facility*", describe the successful transformation of a facility previously used for manufacturing cephalosporins (41).

Various Regulatory Agencies require that some categories of highly potent APIs be manufactured in a dedicated or segregated facility from the very start, creating further costs for the manufacturing company, which would be forced to build this type of facility in order to comply with the regulations required by the Authority. This would encourage delocalization of productive activities, as a company might choose to manufacture in States where GMP are less restrictive, substantially cutting production costs. Unfortunately, this might result in a loss of quality for the finished product and an increase in risks for personnel who could be exposed to the effects of reduced quality standards and last but not least the end users.

The constant evolution of pharmaceutical legislation is aimed at finding innovative solutions for the production of highly active and sensitising materials, an approach that is not limited to the application of strict rules but that includes a risk analysis able to identify critical guidelines to focus on adopting appropriate safety measures for individual drugs and not just their class.

Consequently there is a need to bring legislation that does not support this approach up to date. Harmonising different regulations would be desirable, since by following a single QRM procedure a company could produce and market its products in every nation in the world in accordance with the best quality standards. A further problem is represented by the lack of official translations of the regulations, which facilitates mistakes due to an incorrect interpretation of regulations (7). Besides the language barrier, there is an obvious need to achieve a "Common scientific language", with which to standardise requisites enforced by the various Authorities according to available scientific evidence.

It is clear from the above that it is possible to improve the current status of drug production for the benefit of companies and users, reducing costs and increasing benefits and safety for all those involved.

The present study focuses on significant differences between some of the major GMP Regulation Agencies concerning the production of highly active and sensitizing pharmaceutical products, placing a special emphasis on the consequences of these differences and the urgent need to harmonise GMP to guarantee that the standards they provide are clear, up-to-date and globally applicable. A first step towards this goal might be provided if pharmaceutical companies collaborate to create a network of companies who conform to a set of standards.

Harmonisation will not happen without complication but needs to be achieved as soon as possible to ensure quality standards are met for medicinal products and patient safety is guaranteed.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Spacilova L, Klusonova H, Petrelli F, Signorelli C, Visnovsky P, Grappasonni I. Substance use and knowledge among Italian high school students. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2009 Jun; 153(2): 163-8.
2. Grappasonni I, Scuri S, Tanzi E, Kracmarova L, Petrelli F. The economic crisis and lifestyle changes: a survey on frequency of use of medications and of preventive and specialistic medical care, in the Marche Region (Italy). Acta Biomed 2018 Mar 27; 89(1): 87-92. doi: 10.23750/abm.v89i1.7068.
3. Siracusa M, Grappasonni I, Petrelli F. The pharmaceutical care and the rejected constitutional reform: what might have been and what is. Acta Biomed 2017 Oct 23; 88(3): 352-359. doi: 10.23750/abm.v88i3.6376.
4. Signorelli C, Odone A, Gozzini A, Petrelli F, Tirani M, Zangrandi A, Zoni R, Florindo N. The missed Constitutional Reform and its possible impact on the sustainability of the Italian National Health Service. Acta Biomed 2017 Apr 28; 88(1): 91-94. doi: 10.23750/abm.v88i1.6408.
5. Petrelli F, Contratti CM, Tanzi E, Grappasonni I. Vaccine hesitancy, a public health problem. Ann Ig 2018 Mar-Apr; 30(2): 86-103. doi: 10.7416/ai.2018.2200.
6. Grappasonni I, Petrelli F, Klusoňová H, Kračmarová L. Level of understanding of medical terms among italian students. Ceska Slov Farm. Winter 2016; 65(6): 216-220.
7. Cioffi P, Marotta V, Fanizza C, Giglioni A, Natoli C, Petrelli F, Grappasonni I. Effectiveness and response predictive factors of erlotinib in a non-small cell lung cancer unselected European population previously treated: a retrospective, observational, multicentric study. J Oncol Pharm Pract 2013 Sep; 19(3): 246-53. doi: 10.1177/1078155212465994. Epub 2012 Dec 7. PubMed PMID: 23223401.
8. Nguyen CTT, Petrelli F, Scuri S, Nguyen BT, Grappasonni I, A systematic review of pharmacoeconomic evaluations of erlotinib in the first-line treatment of advanced non-small cell lung cancer. Eur J Health Econ 2019 Mar 6. doi: 10.1007/s10198-019-01040-7.
9. Cioffi P, Laudadio L, Nuzzo A, Belfiglio M, Petrelli F, Grappasonni I. Gemcitabine-induced posterior reversible encephalopathy syndrome: a case report. J Oncol Pharm Pract 2012 Jun; 18(2): 299-302. doi: 10.1177/1078155211424628.
10. Sargent EV, Flueckiger A, Barle EL, Luo W, Molnar LR, Sandhu R, Weideman PA. The regulatory framework for preventing cross-contamination of pharmaceutical products: History and considerations for the future. Regul Toxicol Pharmacol 2016 Aug; 79 Suppl 1: S3-S10. doi: 10.1016/j.yrtph.2016.05.029.
11. Siracusa M, Petrelli F. Trade of food supplement: food or drug supplement? Recenti Prog Med 2016 Sep; 107(9): 465-471. doi: 10.1701/2354.25224.
12. Grappasonni I, Marconi D, Mazzucchi F, Petrelli F, Scuri S, Amenta F. Survey on food hygiene knowledge on board ships. Int Marit Health 2013; 64(3): 160-7.
13. Grappasonni I, Petrelli F, Scuri S, Mahdi SS, Sibilio F, Amenta F. Knowledge and Attitudes on Food Hygiene among Food Services Staff on Board Ships. Ann Ig 2018 Mar-Apr; 30(2): 162-172. doi: 10.7416/ai.2018.2207.
14. Petrelli F, Grappasonni I, Peroni A, Kracmarova L, Scuri S. Survey about the potential effects of economic downturn on alcohol consumption, smoking and quality of life in a sample of Central Italy population. Acta Biomed 2018 Mar 27; 89(1): 93-98. doi: 10.23750/abm.v89i1.7059.
15. Grappasonni I, Paci P, Mazzucchi F, De Longis S, Amenta F. Awareness of health risks at the workplace and of risks of contracting communicable diseases including those related to food hygiene, among seafarers. Int Marit Health 2012; 63(1): 24-31.
16. ICH Expert Working Group, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. 2005. ICH Harmonised Tripartite Guideline: Quality Risk Management Q9.
17. Sussman, Robert G, et al. "Identifying and assessing highly hazardous drugs within quality risk management programs." Regulatory Toxicology and Pharmacology 2016; 79): S11-S18.
18. ANVISA, "Technical Regulation of Good Manufacturing Practices of Drugs", Resolution – RDC n. 17, 2010.
19. CFDA. "People's Republic of China: Good Manufacturing Practice for Drugs" (2010 Revision) (MOH Decree No. 79).
20. COFEPRIS, Norma Oficial Mexicana NOM-059-SSA1-2006, "Buenas Practicas de fabricacion para establecimientos de la industria quimico farmaceutica dedicados a la fabricacion de medicamentos", 2006.
21. COFEPRIS, "Anexos a la Norma Oficial Mexicana NOM-SSA1-059-2015; Buenas prácticas de fabricación de medicamentos", 2014.
22. US Food and Drug Administration. "CFR-code of federal regulations title 21." Current good manufacturing practice for finished pharmaceuticals Part 210 (e-CFR data is current as of December 21, 2018).
23. US Food and Drug Administration. "CFR-code of federal regulations title 21." Current good manufacturing practice for finished pharmaceuticals Part 211 (e-CFR data is current as of December 21, 2018).
24. "Guidance for Industry - Non-Penicillin Beta-Lactam Drugs: A CGMP Framework for Preventing CrossContamination" April 2013.
25. FDA, "Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients Guidance for Industry", 2016.
26. FDA, "Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients _ Questions and Answers Guidance for Industry", 2018.
27. FDA, "Compliance Program Guidance Manual", PROGRAM 7356.002F, Ch.56, 2015.
28. FDA/CDER to Foley & Lardner LLP - Petition Denial. Other by FDA on 07/21/2010. ID: FDA-2010-P-0069-0007.
29. EMA, "Eudralex - Volume 4. Good manufacturing practice (GMP) Guidelines", 2015.

30. EMA, "Guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities". EMA/CHMP/CVMP/SWP/169430/2012. 2014.
31. Health Canada, "Good manufacturing practices guide for drug products - GUI-0001" , 2018.
32. Health Canada, "Health Products and Food Branch Inspectorate; Good Manufacturing Practices (GMP) Guidelines for Active Pharmaceutical Ingredients (API) - (GUI-0104)", 2013.
33. CDSCO, Schedule M, "Good Manufacturing Practices and Requirements of Premises, Plant and Equipment for Pharmaceutical Products", The Drugs And Cosmetics Act And Rules, India, 2005.
34. WHO, "WHO Good Manufacturing Practices for Active Pharmaceutical Ingredients". Technical Report Series, No.957, Annex 2, 2010.
35. WHO, "Good Manufacturing Practices for Pharmaceutical Products containing hazardous substances", Technical Report Series, No. 957 Annex 3, 2010.
36. WHO, "WHO Good Manufacturing Practices for Pharmaceutical Products: main principles", Technical Report Series, No. 986, Annex 2, 2014.
37. WHO, "Guideline to the inspection of hormone product manufacturing facilities", QAS/08.256, 2008.
38. PIC/S, "Guide to good manufacturing practice for medicinal products", PE 009-14 (Part I), 2018.
39. PIC/S, "Guide to good manufacturing practice for medicinal products", PE 009-14 (Part II), 2018.
40. ISPE Baseline® Pharmaceutical Engineering Guide, "Risk-Based Manufacture of Pharmaceutical Products", International Society for Pharmaceutical Engineering (ISPE), First Edition, Vol. 7, 2010.
41. Takahashi H., Sakai H., Gold D. Case study: Beta-lactam decontamination and cleaning validation of a pharmaceutical manufacturing facility. Pharmaceutical Engineering. 2008 Nov-Dec; 28(6): 1-5.

Received: 27 March 2019
Accepted: 13 May 2019
Correspondence:
Stefania Scuri
School of Medicinal and Health Products Sciences,
University of Camerino,
Via Madonna delle Carceri, 9 - 62032 Camerino (MC), Italy
Tel. +39 0737402420
Fax +39 0737402416
E-mail: stefania.scuri@unicam.it

Acta Biomed 2019; Vol. 90, N. 2: 300-307 DOI: 10.23750/abm.v90i2.8284

ORIGINAL ARTICLE

Atypical use of pediatric flexible nails in the treatment of diaphyseal fractures in adults

Alessio Pedrazzini[1], Paolo Bastia[2], Nicola Bertoni[1], Bianca Pedrabissi[1], Henry Claudel Yewo Simo[1], Vanni Medina[1], Francesco Ceccarelli[2], Francesco Pogliacomi[2]

[1] Orthopaedic Unit, Oglio Po Hospital, Vicomoscano di Casalmaggiore (CR), ASST Cremona, Italy; [2] Orthopaedics and Traumatology Clinic, Department of Medicine and Surgery, University of Parma, Parma, Italy

Summary. *Background and aim of the work:* Elastic intramedullary nails are commonly used for the treatment of diaphyseal fractures in adolescents and children. The major advantages are the minimally invasive nature of the technique, the short operation time, and the preservation of the growth plate and periosteum thus allowing bone healing within a closed and intact biological environment. Elastic nails are rarely applied to the adult fractures. *Methods:* Five selected adult patients affected by diaphyseal fractures were treated using paediatric flexible nails T2 Kids (Stryker®, Mahwah, NJ, USA) as consequence of their poor clinical conditions, high risk of neurovascular injuries and skin/soft tissues problems. All patients were monthly clinically and radiographically evaluated after surgery until fracture healing. *Results:* Radiological and clinical outcomes were satisfying. All fractures healed after a mean period of 3 months. No losses of reduction as well as mobilization/breakage of implant were observed. *Conclusions:* Use of pediatric elastic nails is a valid surgical option in treatment of diaphyseal fractures in selected adult patients who request fast and minimally invasive surgery as consequence of precarious clinical or soft tissues conditions. (www.actabiomedica.it)

Key words: elastic nailing, diaphyseal fractures, adults

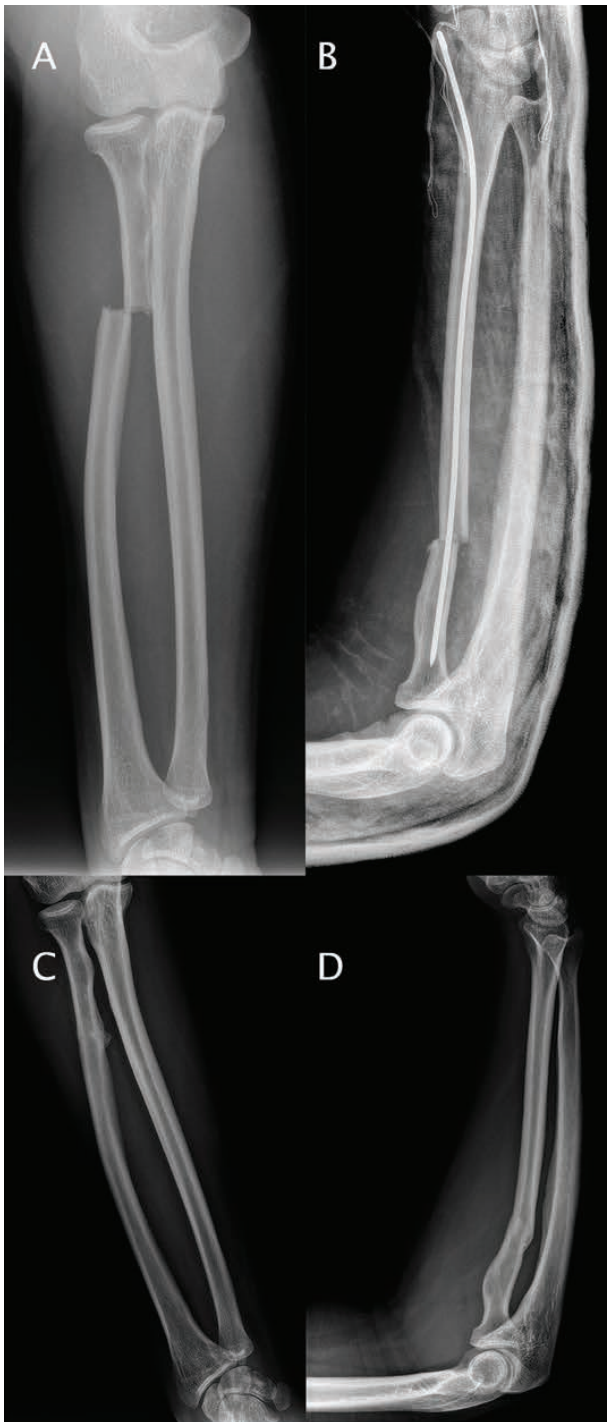
Introduction

Elastic intramedullary nailing is widely used to treat long bones fractures in children and adolescents (1-3). This technique preserves the integrity of the growth plates and the periosteum thus allowing bone healing within a closed and intact biological environment (4, 5). The use of elastic stable intramedullary nails is intended for fixation of diaphyseal fractures of long bones where the medullary canal is narrow, and flexibility of the implant is of paramount importance. The biomechanical principle of this technique is based on the symmetrical bracing action of two elastic nails inserted into the metaphysis, which bears against the inner bone in three points (6, 7). This method has the benefits of early immediate stability to the involved bone segment, which permits early mobilization and return to the normal activities of the patients, with very low complication rate (8). By contrast, adult bone healing properties are diminished compared with that of children. Osteoblasts in the inner cellular layer of the child's thick periosteum become thinner with age, and the bone healing process is also prolonged with aging.

For these reasons elastic nails are usually applied to pediatric population due to the thick periosteum and the increased potential for bone remodelling in children, but they are not routinely used in adults because of lack of resistance to rotational force and axial loading (4, 5).

Nevertheless, intramedullary elastic nailing offers a safe, rapid and minimally invasive surgical option for

the fixation of fractures of long bones in selected adult patients characterized by precarious general clinical or local soft tissues conditions. The aim of this study is to analyze the outcomes of 5 selected adult patients affected by long bones fractures and operated on with flexible nail T2 Kids (Stryker®, Mahwah, NJ, USA) in which open reduction and internal fixation (ORIF) or static intramedullary nailing (IM) was contraindicated.



Materials and methods

All subjects were treated during 2018; this surgical option was chosen for their low functional needs, high risk of neurovascular injuries and skin/soft tissues bad conditions in order to facilitate post-operative care. All patients were monthly clinically and radiographically assessed (mean follow-up of patients was 8.5 months) until fracture healing.

Case 1 (figure 1)

A 28 years-old male reported an isolated fourth proximal radial shaft fracture after a fall during a football match. The surgical approach had a high risk of posterior interosseous nerve injury. Therefore, the fracture was reduced using one elastic nail introduced

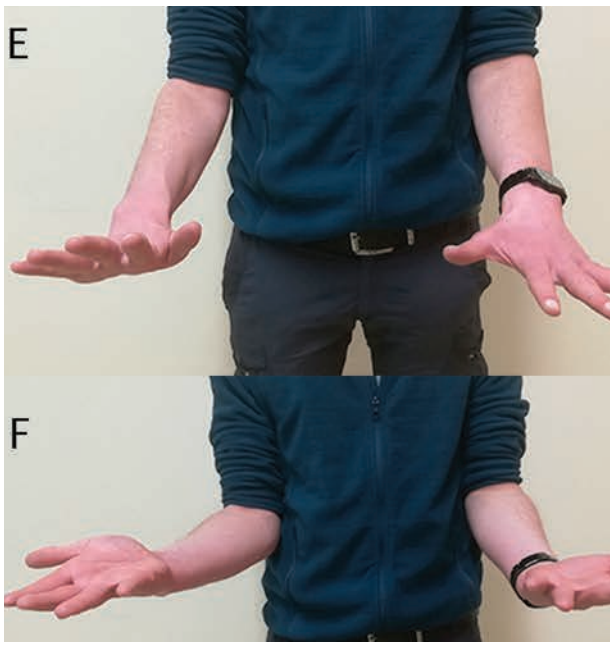
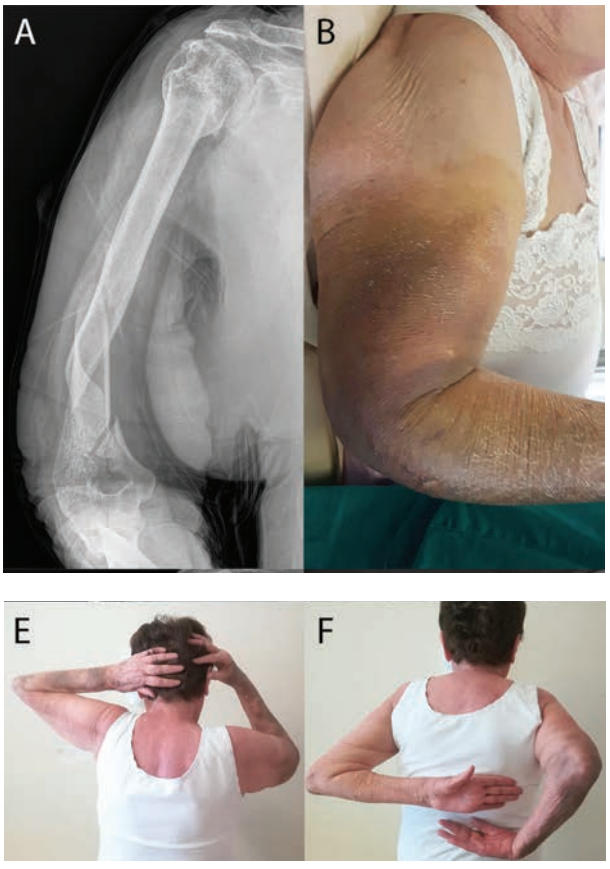


Figure 1. Pre-operative (A) and post-operative X-rays (B). Fracture's healing 7 months after surgery (C and D). No limitation of strength and of ROM at clinical evaluation (E and F)

through distal epiphyseal radius. Surgery lasted 45 minutes. The patient was discharged the following day with no evidence of peripheral vascular-nervous impairment. The elbow joint was initially immobilized with plaster cast for 1 month and after its removal the patient started rehabilitation program. Radiographic control performed 1.5 months after surgery showed initial bone consolidation and healing; T2 Kids nail was removed 4 weeks later. The patient was satisfied; nowadays, there are no limitations of strength and of range of motion (ROM) and no pain or vascular-nervous impairment has been reported. Last X-ray was performed 7 months after surgery and complete fracture's healing was observed.

Case 2 (figure 2)

A 70 years-old female came to our attention with a third distal humeral multifragmentary fracture. The patient had suffered a previous humeral head fracture 20 years before treated conservatively and for this reason shoulder mobility was already reduced. Management of the injury was very difficult because of severe concomitant pathologies [clinical history of reumathoid arthritis (AR) in treatment with corticosteroid and cardiac disease in treatment with Clopidogrel] and skin conditions that contraindicated open reduction and internal fixation (ORIF) even if a radial nerve impairment was present. The fracture was synthesized with a K-wire introduced through a percutaneous transolecranic access in order to stabilize distal fragments and a flexible intramedullary nail T2 Kids introduced proximally. Time of surgery was 1 hour . The post-operative course was uneventfully and the patient was discharged 1 week later. A gradual recovery of motility and sensitivity in the radial nerve area in the following weeks was reported. Cast was removed 30 days after surgery and nail and K-wire 1 month later. Despite the poor quality of bone tissue, following



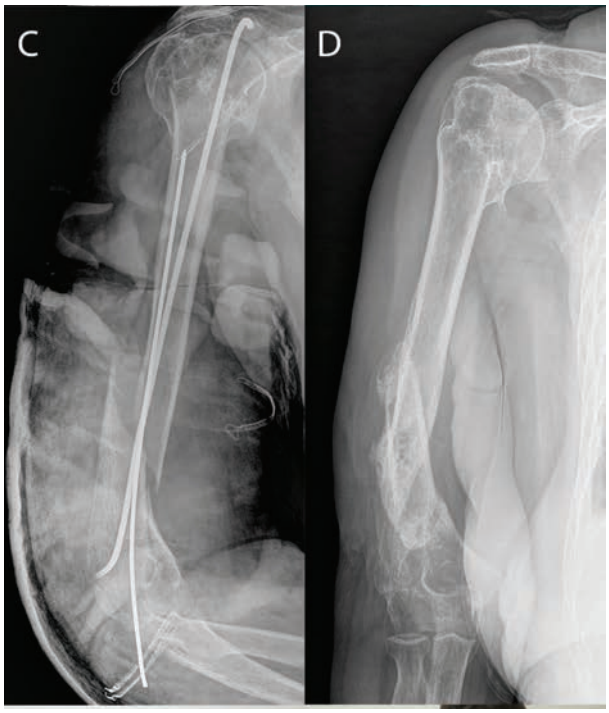


Figure 2. Pre-operative X-rays (A) and skin condition after the trauma (B). Stabilization of the fracture with flexible nail and K-wire (C) and radiographs 11 months after surgery (D). Acceptable clinical and functional outcomes (E and F)

controls showed acceptable clinical outcomes and 11 months after surgery the patient fully recovered. ROM of shoulder wasn't further reduced and she didn't refer pain or peripheric neurovascular or strength's deficits.

Case 3 (figure 3)

A 68 years-old female reported a complex fracture of proximal humerus. After admission blood exams revealed a persistent leukocytosis with no apparent causes. Due to the increased infectious risk a minimally invasive surgical option of treatment was performed using 2 paediatric flexible nails T2 Kids. Surgery lasted 50 minutes. Post-operative course was regular and the patient had no fever at discharge. The arm was immobilized with a Desault brace for 40 days and external rotation movements were initially forbidden. X-ray controls were performed monthly and showed bone consolidation and healing of the injury 2 months after treatment followed by nails removal and rehabilitation. Following controls confirmed a progressive good clinical and radiographic evolution as well as the last one, performed 11 months after surgery, in which the patient has minimal limitation of ROM in extreme external rotation and no pain or vascular-nervous deficit were reported.

Case 4 (figure 4)

A 76 years-old female with a history of AR and osteoporosis came to our attention with a diaphyseal humeral fracture with involvement of the surgical neck

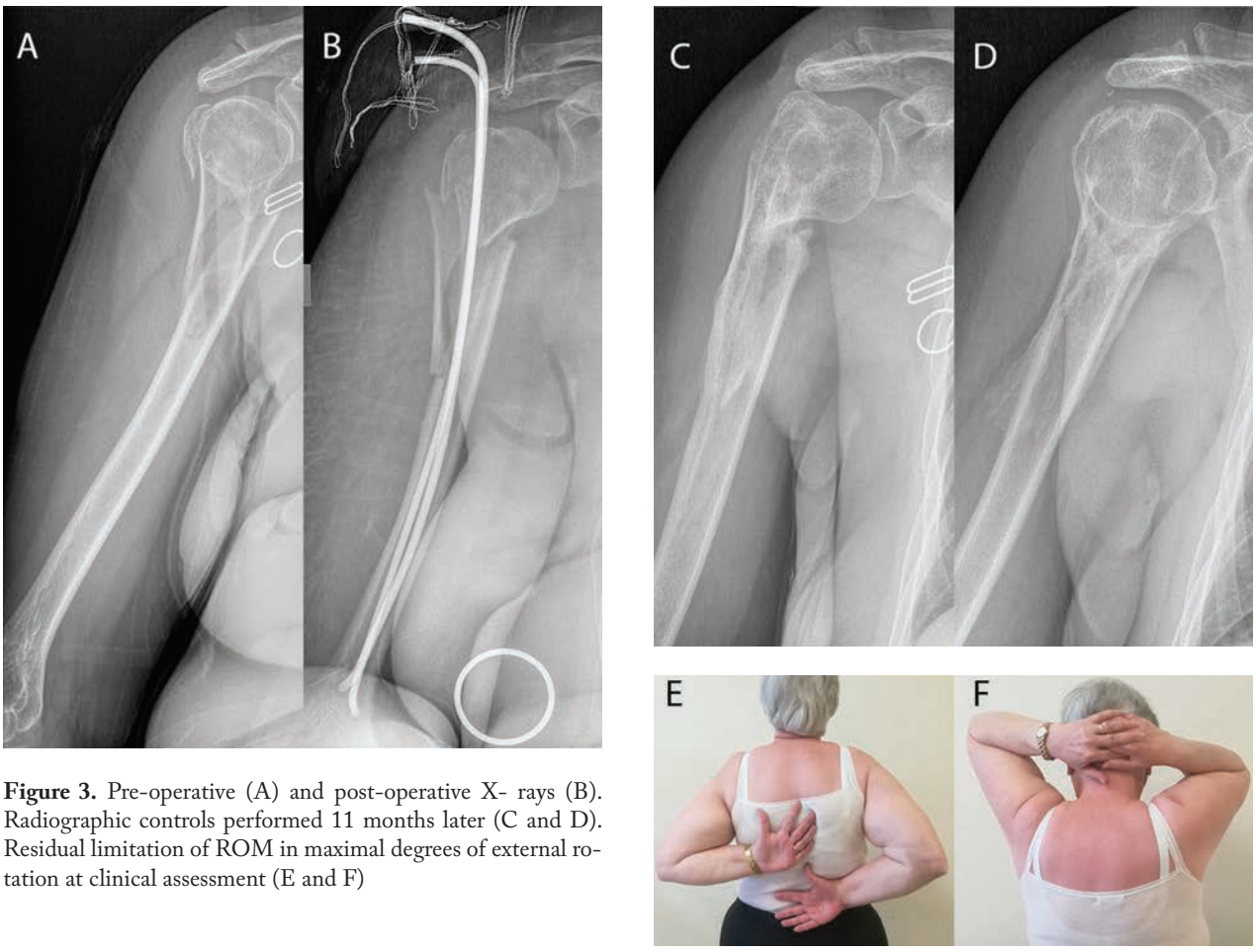


Figure 3. Pre-operative (A) and post-operative X- rays (B). Radiographic controls performed 11 months later (C and D). Residual limitation of ROM in maximal degrees of external rotation at clinical assessment (E and F)

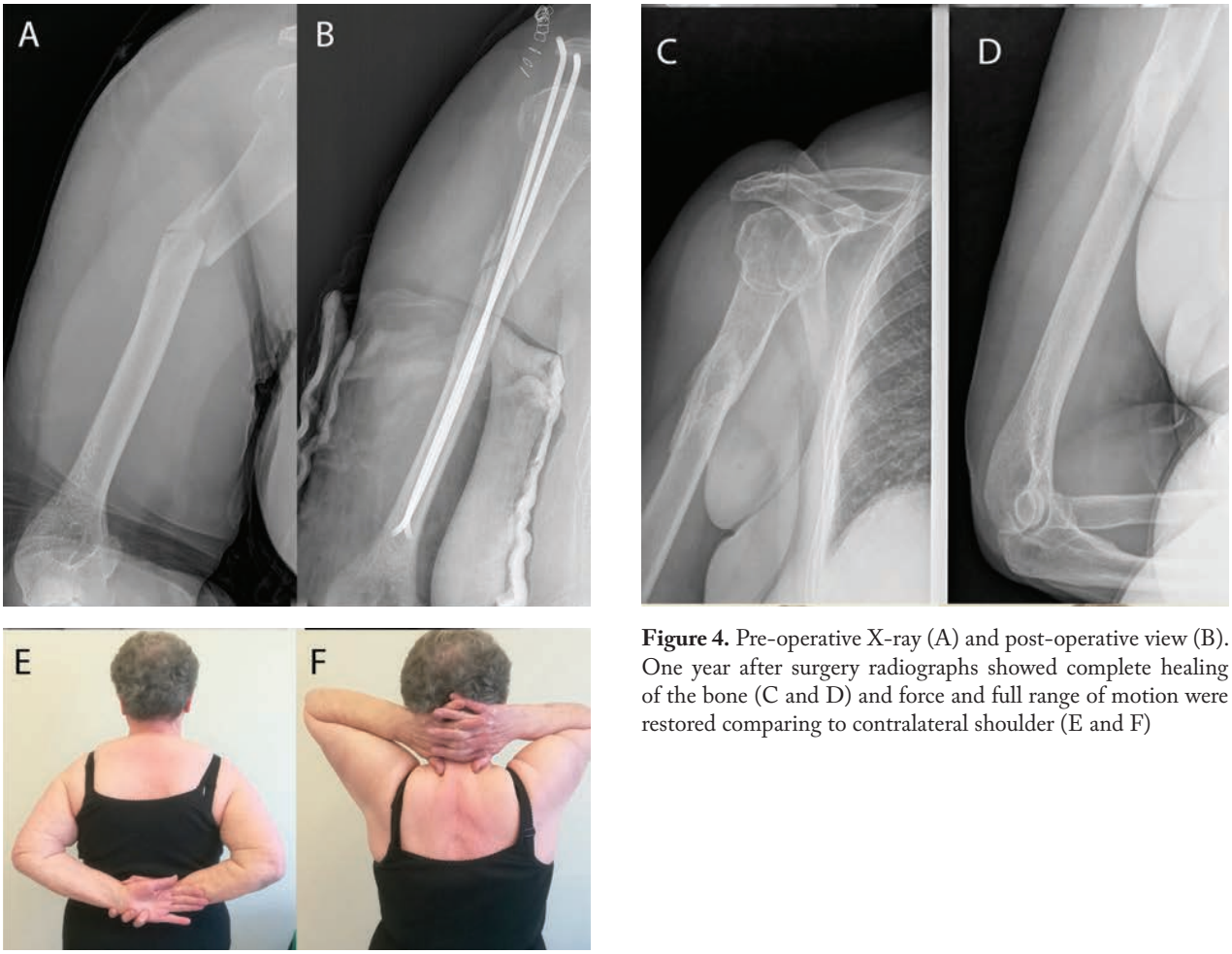


Figure 4. Pre-operative X-ray (A) and post-operative view (B). One year after surgery radiographs showed complete healing of the bone (C and D) and force and full range of motion were restored comparing to contralateral shoulder (E and F)

following a fall at home. The fracture was treated using 2 elastic nails T2 Kids introduced through proximal mini-access. In this case a minimally invasive surgical option was used because of higher low functional needs and in order to facilitate post-operative recovery. Time of surgery was 1 hour. The elbow joint was immobilized at 90° of flexion with plaster cast. After clinical stabilization the patient was discharged. Plaster was removed 2 months later and radiograph and clinical controls were performed monthly. Bone healing was observed 3 months after surgery followed by removal of the nails. The patient started active and passive rehabilitation 2 weeks after nails removal. X-ray and clinical follow-up continued with no evidence of complications and 12 months after surgical treatment the patient regained complete ROM without pain or any strength's deficit.

Case 5 (figure 5)

A 14 years-old male reported a third proximal humeral fracture after a car accident. Due to the type of fracture and the young age elastic nailing was performed. However, conventional distal entry point of nail (2-3 cm proximally to lateral humeral epicondyle) is very close to the radial nerve and caution is required in order to avoid it. Therefore, a single posterior access through triceps muscle was utilized to introduce T2 kids nails and to reduce the risk of nervous injury. The surgery lasted 70 minutes. The patient was discharged the following day and no post-operative complications were observed. Desault brace was applied to immobilize the operated arm for 40 days and pulsed electromagnetic fields was performed for 1 month in order to accelerate bone healing. After brace's removal the pa-

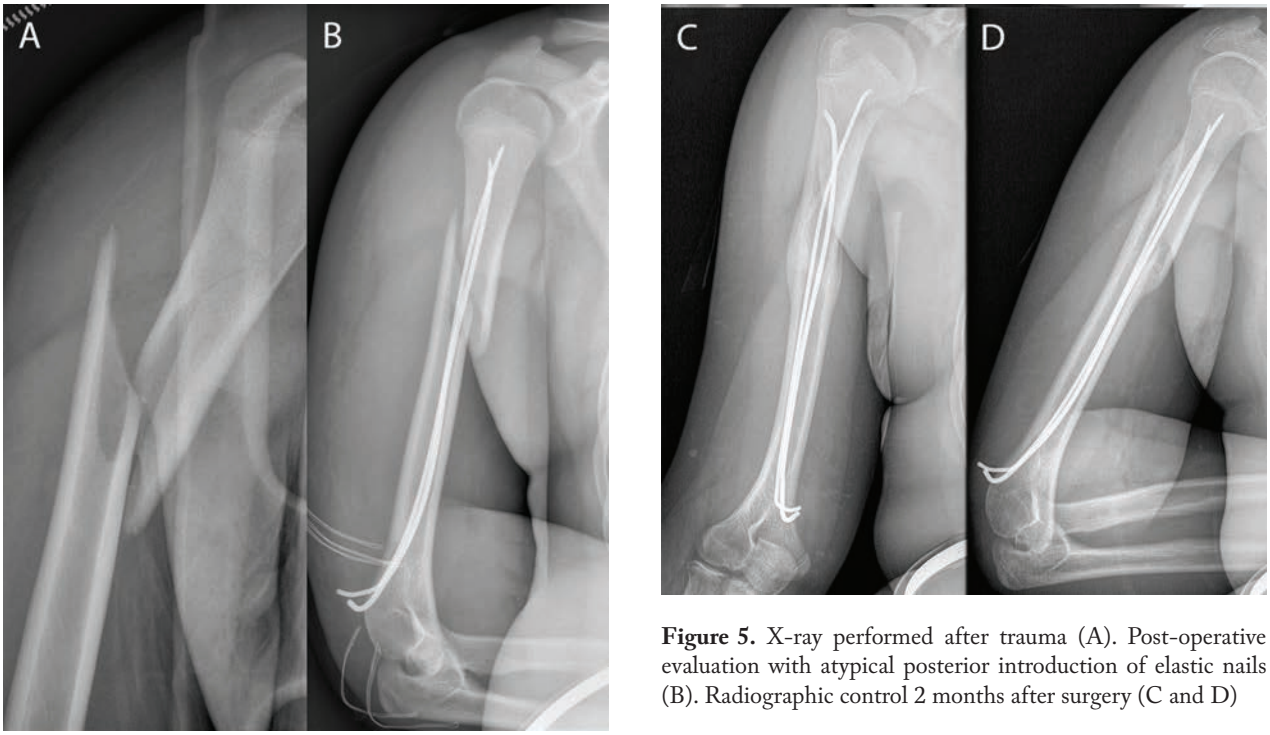


Figure 5. X-ray performed after trauma (A). Post-operative evaluation with atypical posterior introduction of elastic nails (B). Radiographic control 2 months after surgery (C and D)

tient started rehabilitation program. Last X-ray control performed 50 days after surgery from treatment shows complete bone consolidation. Nowadays, there are no limitations of strength and of ROM and no vascular-nervous deficits were reported. The patient does not report any pain and he is waiting for nails removal.

Discussion

The elastic nailing method was first described by Rush; it is based on the "3-point fixation principle" when introduced in the medullary canal of long bones. It was first used for the treatment of long bones fractures of lower extremities and soon became a very popular method for all long bone synthesis.

Elastic nails are rarely applied to the adult fractures because of lack of resistance to rotational force and axial loading (4, 5).

Elastic stable intramedullary nails (ESIN) are commonly used for the treatment of long bone fractures in adolescents and children (1-3). The major advantages are the minimally invasive nature of the technique, the short operation time, and the preservation of the growth plate and the periosteum thus favouring bone healing within a closed and intact biological environment. The aim of the treatment is to achieve a level of reduction and stabilization that is appropriate to the age of the child. The biomechanical principle of the technique is based on the symmetrical bracing action of two elastic nails inserted into the metaphysis, each of which bears against the inner bone at three points (6, 7). This produces immediate flexural, axial, translational and rotational stability thus permitting early mobilization and return to the normal activities of the patients, with low complication rate (6,9-11).

Nevertheless, in selected adult patients in which clinical and cutaneous conditions contraindicate ORIF or rigid IM nailing, this type of fixation may be still used as well demonstrated in the literature.

The effectiveness of flexible nailing as a treatment modality for humeral shaft fractures has been assessed by many studies (12-15). Most of these studies have used Ender's nail as a method of nailing (16, 17).

In a study to evaluate clinical outcome, Verma et al. have seen that titanium elastic nail system is a good alternative for treatment of diaphyseal fractures of humerus in adult age group as it requires minimum invasive approach, can achieve union without disturbing the biology of fracture site (18).

In 2014 Modi et al. compared the outcome of titanium elastic nail and plate in the adult shaft humerus fractures and concluded that results of titanium elastic nails in union rates and union time were good and comparable to plates with lesser rate of complications, but their usefulness is limited to middle third fractures of humerus. The results of titanium elastic nail depend greatly on jamming of canal, control of rotation, and proximity of fracture to entry point (19).

A preliminary study of 2011 by Kim et al. suggested that percutaneous flexible nailing and intramedullary cementing could be an optional treatment modality for surgical palliation of selected high-risk terminal cancer patients with metastases to the humerus (20).

Various nails such as elastic stable intramedullary nails (ESIN) and locking flexible clavicular nails were widely used in treatment of midclavicular fractures (21). In 2002, titanium elastic nail was firstly applied in the treatment of displaced midclavicular fractures and showed good clinical therapeutic outcomes, but commonly it might induce various complications, including hardware irritations, medial perforations, lateral penetrations, TEN breakage and dislocation (22, 23).

In the past, elastic Ender nails were used in the treatment of osteoporotic pertrochanteric fractures in aged people and of tibial shaft fractures with good results (24, 25).

In 2009 Khurana et al. treated a series of adult patients with humeral shaft fractures with retrograde Ender nails with satisfactory results and low rate of complications (26).

In a case report of 2015 Quiang et al. reported that application of the elastic nail in the fixation of the fibula can simplify the surgical procedure, maintain the stability, and lower-extremity alignment in the treatment of complicated tibia and fibula fractures. (27).

The use of elastic nails provides an alternative means for internal fixation of adult proximal radial fractures and there are few reports about this (28, 29). The proximal radius is surrounded by abundant forearm muscle and the posterior interosseous nerve (PIN) crosses this area. The intramedullary nail method has advantages such as closed application, less soft tissue injury, avoidance of nerve injury, and cosmetic benefits compared with the standard procedure of open reduction with plate and screw fixation. A recent study of Huang et al. (2018) evaluated the functional outcomes and efficiency of elastic nailing in the surgical treatment of adult proximal radial shaft fractures and most patients achieved desirable functional outcomes (30).

Results of this paper confirm these assumptions and demonstrate that intramedullary elastic nailing is a safe and minimally invasive surgical option in order to stabilize diaphyseal long bones fractures also in selected adult patients and in particular clinical conditions with satisfactory radiological and functional outcomes.

Conclusions

According to this study's assessment, the use of paediatric flexible nails T2 Kids (Stryker®, Mahwah, NJ, USA) is a good surgical option in treatment of selected adult patients who request fast and minimally invasive surgery due to precarious clinical or soft tissues conditions, not high functional requests, old age or when ORIF or static IM nailing is contraindicated.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted articlee

References

1. Nielsen E, Bonsu N, Andras LM, Goldstein RY. The effect of canal fill on paediatric femur fractures treated with titanium elastic nails. J Child Orthop 2018; 12(1): 15-19.
2. Popkov A, Foster P, Gubin A, Borzunov D, Popkov D. The use of flexible intramedullary nails in limb lengthening. Expert Rev Med Devices 2017; 14(9): 741-753.
3. Heare A, Goral D, Belton M, Beebe C, Trizno A, Stoneback J. Intramedullary implant choice and cost in the treatment of pediatric diaphyseal forearm fractures. J Orthop Trauma 2017; 31(10): e334-e338.

4. Sinikumpu JJ, Serlo W. The shaft fractures of the radius and ulna in children: current concepts. J Pediatr Orthop B 2015; 24(3): 200-206.
5. Antabak A, Luetic T, Ivo S, et al. Treatment outcomes of both-bone diaphyseal paediatric forearm fractures. Injury 2013; 44 (Suppl 3): S11-S15.
6. Li Y, Stabile KJ, Shilt JS. Biomechanical analysis of titanium elastic nail fixation in a pediatric femur fracture model. J Pediatr Orthop 2008; 28(8): 874-878.
7. Mahar A, Sink E, Faro F, Oka R, Newton PO. Differences in biomechanical stability of femur fracture fixation when using titanium nails of increasing diameter. J Child Orthop 2007; 1(3): 211-215.
8. Pogorelić Z, Kadić S, Milunović KP, Pintarić I, Jukić M, Furlan D. Flexible intramedullary nailing for treatment of proximal humeral and humeral shaft fractures in children: a retrospective series of 118 cases. Orthop Traumatol Surg Res 2017; 103(5): 765-770.
9. Pogorelić Z, Furlan D, Biocić M, Mestrović J, Jurić I, Todorić D. Titanium intramedullary nailing for treatment of simple bone cysts of the long bones in children. Scott Med J 2010; 55(3): 35-38.
10. Wall EJ, Jain V, Vora V, Mehlman CT, Crawford AH. Complications of titanium and stainless steel elastic nail fixation of pediatric femoral fractures. J Bone Jt Surg Am 2008; 90(6): 1305-1313.
11. Furlan D, Pogorelić Z, Biočić M, et al. Elastic stable intramedullary nailing for pediatric long bone fractures: experience with 175 fractures. Scand J Surg 2011; 100(3): 208-215.
12. Bong MR, Koval KJ, Egol KA. The history of intramedullary nailing. Bull NYU Hosp Jt Dis 2006; 64(3-4): 94-97.
13. Matityahu A, Eglseder WA Jr. Locking flexible nails for diaphyseal humeral fractures in the multiply injured patient: a preliminary study. Tech Hand Up Extrem Surg 2011; 15(3): 172-176.
14. Stannard JP, Harris HW, McGwin G Jr, Volgas DA, Alonso JE. Intramedullary nailing of humeral shaft fractures with a locking flexible nail. J Bone Joint Surg Am 2003; 85(11): 2103-2110.
15. Zatti G, Teli M, Ferrario A, Cherubino P. Treatment of closed humeral shaft fractures with intramedullary elastic nails. J Trauma1998; 45(6): 1046-1050.
16. Hall RF, Pankovich AM. Ender nailing of acute fractures of the humerus. A study of closed fixation by intramedullary nails without reaming. J Bone Joint Surg Am 1987; 69(4): 558-567.
17. Shazar N, Brumback RJ, Vanco B. Treatment of humeral fractures by closed reduction and retrograde intramedullary ender nails. Orthopedics 1998; 21(6): 641-646.
18. Verma A, Kushwaha SS, Khan YA, Mohammed F, Shekhar S, Goyal A. Clinical outcome of treatment of diaphyseal fractures of humerus treated by titanium elastic nails in adult age group. J Clin Diagn Res 2017; 11(5): RC01-RC04.
19. Modi D, Patel RR, Solanki RR, Mandaliya M, Chaudhary A. Comparison of results of intramedullary nailing with plate osteosynthesis in the treatment of fracture shaft of humerus. IJSR 2014; 3(8): 277-279.
20. Kim JH, Kang HG, Kim JR, Lin P, Kim HS. Minimally invasive surgery of humeral metastasis using flexible nails and cement in high-risk patients with advanced cancer. Surg Oncol 2011; 20(1): e32-e37.
21. Kaiser MM, Stratmann C, Zachert G, et al. Modification of elastic stable intramedullary nailing with a 3rd nail in a femoral spiral fracture model: results of biomechanical testing and a prospective clinical study. BMC Musculoskelet Disord 2014; 15: 3.
22. Frigg A, Rillmann P, Perren T, Gerber M, Ryf C. Intramedullary nailing of clavicular midshaft fractures with the titanium elastic nail problems and complications. Am J Sports Med 2009; 37(2): 352-359.
23. Fu B. Minimally invasive intramedullary nailing of clavicular fractures by a new titanium elastic nail. AOTT 2016; 50(5): 494-500.
24. Kedzierski M., Król R. Treatment of osteoporotic pertrochanteric fractures with use of elastic Ender nails in aged people. Ortop Traumatol Rehabil 2002; 4(2): 144-150.
25. Ando K, Yamaji T. Ender nailing for tibial shaft fractures. J Orthop Sci 2000; 5(3): 217-222.
26. Khurana A, Pendse A, Modi H, Diwanji S, Mathur H, Daveshwar R. Retrograde Ender nailing for humerus shaft fractures. Acta Orthop Belg 2009; 75(5): 599-605.
27. Wang Q, Xu HG, Zhang YC, Dong LJ. Elastic nails for fibular fracture in adult tibiofibular fractures. Int J Clin Exp Med 2015; 8(6): 10086-10090.
28. Sandmann GH, Crönlein M, Neumaier M, et al. Reduction and stabilization of radial neck fractures by intramedullary pinning: a technique not only for children. Eur J Med Res 2016; 21: 15.
29. Gadegone W, Lokhande V, Salphale Y. Screw elastic intramedullary nail for the management of adult forearm fractures. Indian J Orthop 2012; 46(1): 65-70.
30. Huang YC, Renn JH, Tarng YW. The titanium elastic nail serves as an alternative treatment for adult proximal radial shaft fractures: a cohort study. J Orthop Surg Res 2018; 13(1): 10.

Received: 13 March 2019
Accepted: 15 April 2019
Correspondence:
Alessio Pedrazzini
Orthopaedic Unit, Oglio Po Hospital
Via Staffolo 51 - 26041 Vicomoscano (CR), Italy
E-mail: alessio.pedrazzini@asst-cremona.it

Acta Biomed 2019; Vol. 90, N. 2: 308-315 DOI: 10.23750/abm.v90i2.6988

Pre-operative assessment of internal mucosal rectal prolapse in internal hemorrhoids: technical details and results from a single institution

Luigi Percalli[1], *Luigi Passalia*[2], *Renato Pricolo*[2], *Matteo Riccò*[3]

[1] UO Chirurgia Valtidone, Dipartimento Chirurgico AUSL Piacenza, Castel San Giovanni (PC), Italy; [2] UO Chirurgia del Presidio Ospedaliero di Codogno – ASST di Lodi, Codogno (LO), Italy; [3] IRCCS - AUSL di Reggio Emilia; Department of Public Health, Service for Health and Safety in the Workplace, Reggio Emilia (RE), Italy

Summary. *Background:* The aim of the study was to assess safety and efficacy of pre-operative assessment for internal mucosal rectal prolapse (IMRP) in internal hemorrhoids, in order to achieve a tailored transanal stapled surgery. *Methods:* All consecutive patients (January 2011 to December 2014; age 18-80 years), affected by prolapses with II-IV degrees hemorrhoids that underwent Longo procedure with EEA® Auto Suture stapler (Covidien) were included in the present study. *Results:* A total of 100 consecutive patients (38 females) were enrolled in the study. Preoperative Visual Analogue Scale pain assessment was 7.33±2.68. The mean duration of the procedure was 34.1±17.8 min, and the median hospital stay was 2 days (range 2-6). No major complication occurred, including relapses of mucosal prolapse. Preoperative prolapse measurement with EEA® EEA® Auto Suture stapler (2.3±0.5 cm) was well correlated direct assessment (2.4±0.6, p<0.001), but a proportional bias was identified, with significant preoperative underestimation of IMRP, particularly for lesions larger than 3 cm (around 10% of actual extent). *Conclusions:* EEA® Auto Suture stapler seems to be safe and effective for a tailored approach to anorectal prolapse due to hemorrhoids. However, it reasonable that its actual impact may have been overestimated, beneficing of the repetitive, direct assessment of the operatory field guaranteed by preoperative IMRP measurement. (www.actabiomedica.it)

Key words: hemorrhoids, hemorrhoidal prolapse, longo procedure, short-term follow-up

Introduction

Hemorrhoids are a common and underreported condition defined by the symptomatic enlargement and the distal displacement of the normal anal cushion (1): exact data on the hemorrhoid epidemiology are scant, but studies from high income countries suggest a prevalence ranging between 4.4% (United States) and 13-36% (United Kingdom), usually peaking between age 45 to 65 years (1, 2).

The traditional surgical approach is represented by the conventional excisional hemorrhoidectomy (CEH), performed either as an open procedure, as described by Milligan and Morgan in 1937 (M&M), or as a closed one following Ferguson and Parks (3). Both procedures are associated with similar complications: tissue trauma of the perianal skin and anoderm may elicit severe pain, bleeding and post-operative mucosal discharge, collectively requiring prolonged local care (2-4).

In order to avoid such complications, Antonio Longo in 1998 proposed stapled hemorrhoidopexy (SH) as a more efficient alternative to CEH (5, 6). In SH, hemorrhoidal tissue is not removed. Prolapsed internal hemorrhoids and anoderm are actually relocated and anchored by stapling and excising excess distal rectal mucosa. SH has therefore the potential to generate less post-operative pain than CEH. On the

one hand, the excised wound doesn't involve somatic innervation area. On the other hand, surgical procedure usually impairs the blood flow through superior hemorrhoidal vessels, ultimately enhancing symptom resolution (6, 7). As a consequence, SH would be associated with significant short- and long-term benefits, in particular a faster return to normal activities, which may offset its higher equipment costs (8-10).

Available evidence suggests that SH may be a safe, quick and less painful treatment also for symptomatic second and third degree hemorrhoids (2, 6, 8-12), whereas some uncertainties still remain for large external or thrombosed internal haemorrhoids (IHs) (8, 9, 11, 13, 14). Therefore, the preventive assessment of the patient, including an accurate measurement of the mucosa to be excised is critical for an appropriate selection of surgical procedure, as big full-thickness internal or external prolapses or even rectoceles should receive abdominal or perineal procedures, whereas for IHs without internal mucosal rectal prolapse (IMRP), a transanal hemorrhoidal artery ligation under Doppler control with mucopexy has been acknowledged as a more appropriate approach (4, 11, 15).

In such a setting, preliminary IMRP measurement has become the cornerstone for a more appropriate surgical treatment, and here we present the experience of our institution with a specifically designed instrumentation (EEA® Auto Suture stapler; Covidien) aimed to improve the reliability of the preliminary assessment.

Method

Aims

Primary endpoint of our study was comparing preliminary and intra-operative prolapse assessment. Secondary endpoints included post-operative pain, and incontinence symptoms.

Patients

All consecutive patients with age between 18 and 80 years, male or female, having a rectal prolapse from II to IV grade, that presented to the Surgical Department of Codogno Hospital (ASST of Lodi- Italy) between January 2011 to December 2014 were considered eligible for inclusion in the study. Written informed consent was obtained from all patients. Data including the patients' demographic characteristics, pre- and postoperative pain, and complications were recorded in a specifically designed database, whose content was ultimately retrieved and analyzed. Hospital discharge was approved when the patient was fully ambulant and analgesics were no longer required.

Exclusion criteria

Patients with significant comorbidities and patients having a poor understanding of the Italian language.

Surgical techniques (Figure 1)

We performed rectoscopy on each patient before surgery to establish stage of the disease and to eliminate any other pathology responsible for hemorrhoid-like syndrome. The IMRP was measured in order to tailor the exact quantity of mucosa to be excised. All measurement were performed by using the device EEA® Auto Suture (Covidien) that has an anvil detachable from the stapler with three holes on the rod. As indicated below, the surgical technique is simple, the only critical point is to fix the purse string at the corresponding hole on the rod of the anvil in relation to the length of the prolapse. i.e. for prolapses 1 to 2 cm long, at the first hole; for prolapses 2 to 3 cm long at the second hole; for prolapses longer than 3 cm to the third hole.

All patients received the same regimen of analgesia; analgesics were not routinely given unless the patient reported moderate or severe pain. An oral paracetamol table (1 g per dose) was initially given. The frequency of analgesic use during the first 24 h and days during which the patient required analgesics were recorded.

Pain

Before surgical procedures, the patients were instructed to record pain using a visual analogue scale (VAS), and such data were recorded at hospital admission (T0), at 24th hour post-surgery and 6 months after

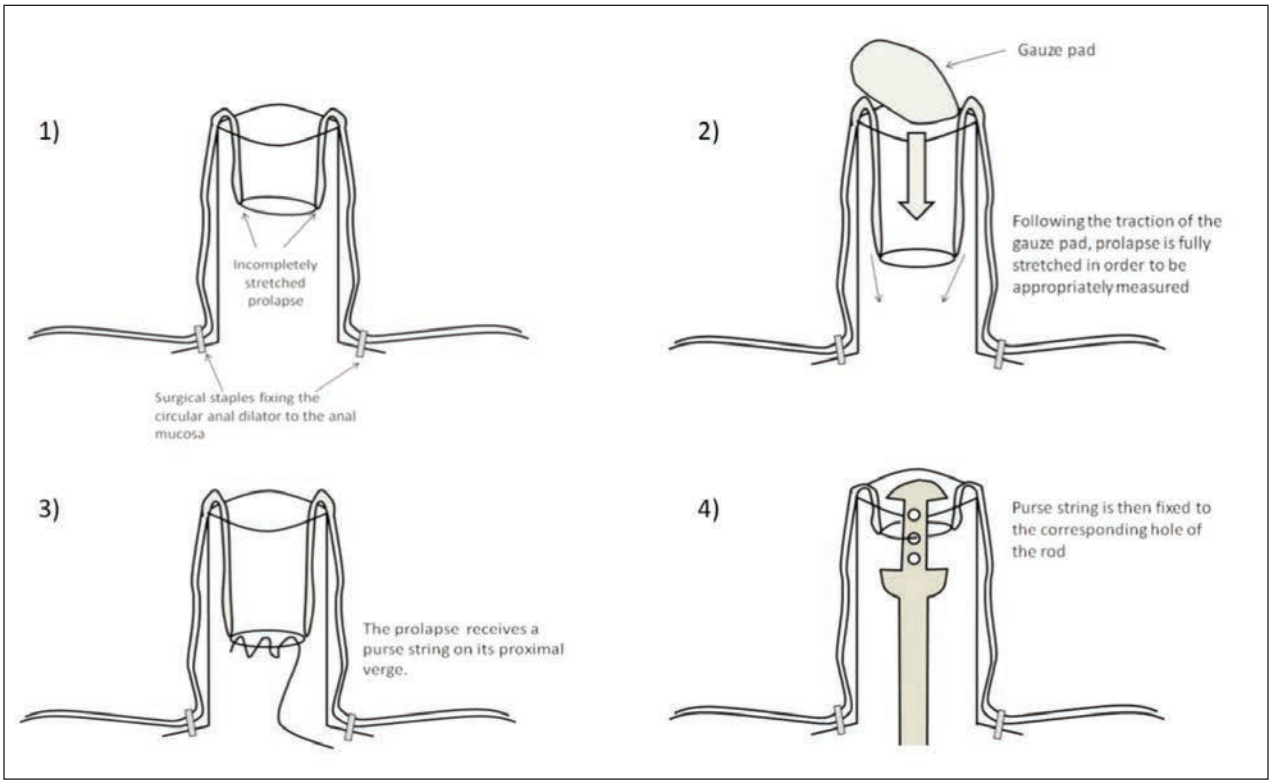


Figure 1. Surgical technique. Following the fixation of the circular anal dilator to the anal mucosa (1), a gauze pad is inserted into the rectum and then dragged downwards in order to stretch the prolapsed mucosa (2), allowing its appropriated measurement. The prolapse (3) then receives a purse string on its proximal verge, that is eventually fixed to the corresponding hole of the rod (4)

surgery (= end of follow up). The scale ranged from 0 to 10, with 0 corresponding to "no pain" and 10 to "the worst pain conceivable". Similarly, patients were requested to report the presence of itching, bleeding on defecation, soiling, and the need to reduce a mucosal prolapse manually after defecation. The aforementioned questions were graded according to the frequency of the symptoms similarly to pain data, and recorded at admission and at the end of the follow up.

Statistical analyses

Student's t test for two paired data was used for the comparison of continuous variables. Statistical relationship between preliminary and surgical prolapse measurements was assessed through correlation analysis and calculation of the Spearman's rho coefficient.

As correlation describes linear relationship between two sets of data but not the differences (i.e. their agreement) (16-18), the Bland-Altman method (BAM) was then applied in order to assess the comparability between methods. As Bland-Altman plot may produce a proportional bias (i.e. the methods do not agree equally through the range of measurement) a linear regression line was fitted (19-20): as stated by Ludbrook, the proportional bias was retained as absent whether the regression line fitted to the Bland-Altman plot was not significantly different from zero.

Sensitivity, Specificity and correspondent Positive and Negative Predictive Values (PNV and NPV, respectively) were calculated through a 2x2 table by assuming a cut-off value of 3 cm.

Ethics

Informed consent was obtained from all individual participants included in the study after detailed explanation of possible complications. The use of the device EEA® Auto Suture (Covidien) in common and well known.

Results

Patients characteristics

Eventually, 100 consecutive patients were included in the study, and their characteristics are summarized in Table 1. Mean age of the sample was 54.5±16.0 years, and 62% of the patients were males. Regarding the grade of prolapses, 73% had a grade 2 and 27% a grade 3. No one among participants had a previous anal surgery. Mean operation time was 34.1±17.8 min, with a median hospital stay of 2 days (range: 2 to 4).

Symptoms

Focusing on pre-operative symptoms, the most frequently reported was dyschezia (17%), followed by constipation (16%), and diarrhea (5%), for a preoperative mean symptom score of 7.33±2.68. All complaints significantly decreased at the end of the follow up, with no reported prolapse recurrence and a cumulative score assessment of 4.48±1.94 ($p<0.001$).

Staple Line

Preoperative height assessment of the staple line was 2.3±0.5 cm, compared to a intra-operative assessment of 2.4±0.5 cm, with a mean difference of 0.27±0.6 cm, and the difference was statistically significant (Student's t test p value <0.001) (Figure 2).

Focusing on the correlation of pre-operative and intra-operative values, they were significantly correlated (Pearson's $r=0.458$; $p<0.001$; Figure 3).

Agreement of the measurements is eventually represented in Figure 4: a bias of 0.27±0.6 cm was calculated, that is pre-operative assessment systematically underestimated height of the staple line of 9.9% ±22.5. As regression analysis identified a slope with a significant p value (<0.001), proportional bias was not ruled out.

Table 1. Clinical details of 100 consecutive patients included in the study sample

	Characteristics
Age (years; mean±S.D.)	54.4±16.0
Male/Female (No.)	62/38
Grade 2, 3 (No.)	73, 27
Operation time (min; mean±S.D.)	34.1±17.8
Hospital stay (days; median, range)	2, 2 to 4
Height of staple line (cm; mean±S.D.)	
Preoperative assessment	2.3±0.5
Direct assessment	2.4±0.6
Visual Analogue Scale pain assessment (0-10; mean±S.D.)	
Pre-operative	7.33±2.68
Post-operative	4.48±1.94
Complained pre-operative symptoms (No.)	
Constipation	16
Dyschezia	17
Diarrhea	5
Previous anal surgery	0
Postoperative complications (No.)	
Bleeding	2
Relapses	0
Surgical site hematoma	1
Thrombosis	0
Urinary retention	12

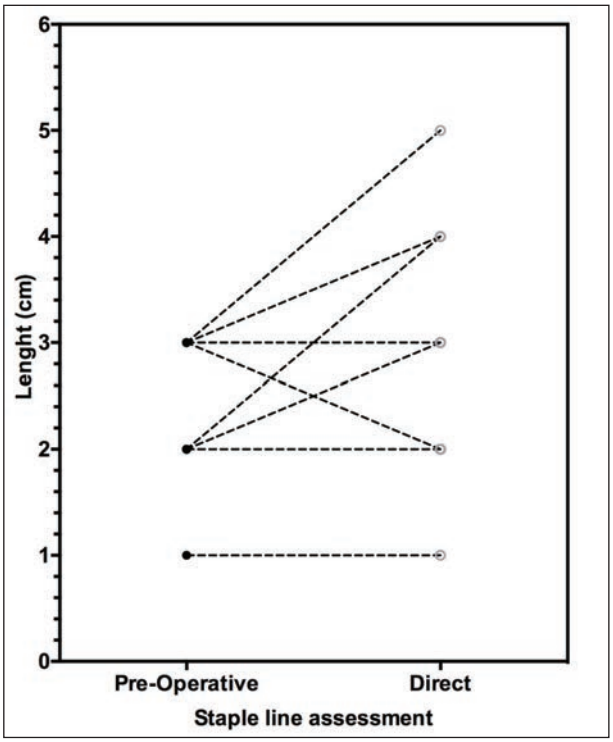


Figure 2. Comparison of pre-operative and operative assessment of staple line (cm). Overall, preoperative assessment significantly underestimate direct, intra-operative assessment of the height of the staple line (Student's t test p value<0.001)

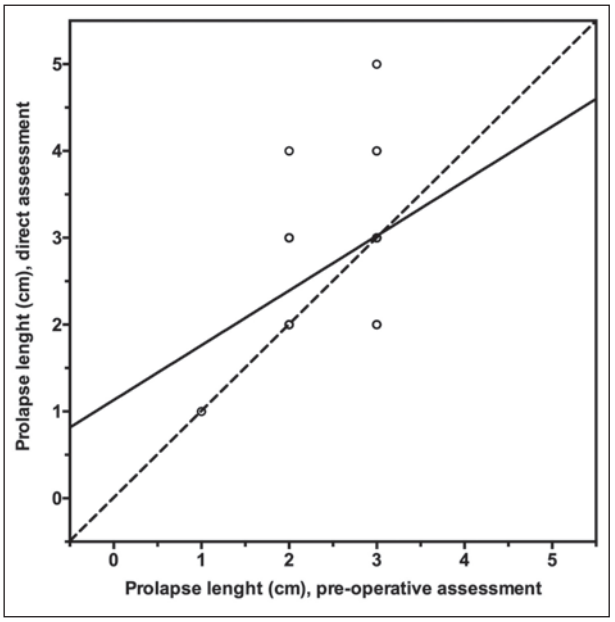


Figure 3. Correlation between pre-operative and direct assessment of the staple line (cm). Measurements well positively correlated (Pearson's r=0.458; p<0.001)

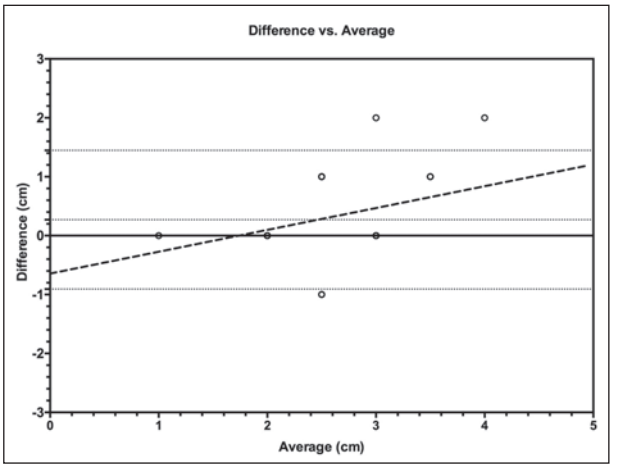


Figure 4. Bland-Altman plot of Difference vs. Average. Dotted line represent linear regression analysis of plotted data. As regression was not significantly different from zero, proportional bias was not ruled out, i.e. differences of measurements significantly increased across the measured heights. More specifically, we observed increased differences for values of 3 cm or more, as suggested by the low specificity observed

Eventually, sensitivity and specificity were calculated assuming the 3 cm cut-off value, and preoperative assessment through EEA® Auto Suture had a sensitivity of 0.870 (95%CI 0.737-0.951), whereas specificity was 0.519 (95%CI 0.378-0.657), with correspondent positive and negative predictive value of 0.824 (95%CI 0.655-0.932) and 0.606 (95%CI 0.478-0.724), respectively (Table 2).

Conclusions

In 1998, Longo proposed SH for treating hemorrhoidal prolapse in order to minimize postoperative discomfort, ultimately improving both short-term prognosis (5). Nowadays, SH is acknowledged as a safe and effective treatment for symptomatic second and third degree hemorrhoids (2, 6, 8-12), while its effectiveness for larger and complicated IHs, as well as on the long-term follow-up still remains largely disputed (8, 9, 11, 13, 14). More specifically, in their metanalysis Yang et al (21) reported a significantly lower incidence of residual skin tags and prolapse in hemorrhoidectomy than in SH patients (OR 0.17, 95%CI 0.06-0.45), with a significantly lower incidence of post-operative recurrence (OR 0.21, 95%CI 0.07-0.59). As recur-

Table 2. Sensitivity, specificity, positive and negative predictive values (PNV and NPV, respectively) for preoperative measurement of stapled line as <3 cm

	Preoperative assessment		Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
	≥ 3 cm (N = 54)	<3 cm (N = 46)				
Direct assessment						
≥ 3 cm	6 (13.0%)	28 (51.9%)	0.519 (0.378-0.657)	0.870 (0.737-0.951)	0.824 (0.655-0.932)	0.606 (0.478-0.724)
< 3 cm	40 (87.0%)	26 (48.1%)				

rence of mucosal prolapse is likely to be due to failures in removing an adequate volume of prolapsing tissue (21-22), preliminary IMRP measurement through specifically designed instrumentation has the potential to significantly improve surgical outcomes, particularly post-operative pain and recurrence rates (22).

In effect, we identified a mean VAS value of 4.84±1.84, that is in line with available reports, usually ranging from 3 to 5.3 (21), but the large majority of complaints was associated with symptoms such as urinary retention (12%), that is only partially related with the surgical procedure *per se*. Moreover, no cases of prolapse recurrence were reported at the end of follow up, that is significantly lower than the recurrence rate reported in literature about stapled surgery (i.e. 7.5%) (21-22), and even lower that that reported by Naldini et al in their study on the treatment of hemorrhoids with a similarly designed device (i.e. 1.9%) (22).

In times characterized by increasing attention to the costs-effectiveness of surgical procedures, the opportunity to complicate surgical procedure with a preventive assessment may be questioned. In fact, in our sample we identified a cumulative operating time of 34.1±17.8 minutes, that is significantly longer than that reported by available reports for conventional SH (i.e. ranging from 15 to 27 minutes) (1, 2, 7, 9, 10, 21), but somehow similar to the time range reported by Naldini et al (i.e. 15 to 60 minutes) with a modified stapler device (22).

However, the potential impact of preliminary IMRP measurement on clinical practice should cautiously assessed. First and foremost, it should be stressed that hemorrhoidectomy is steadily among the most frequently performed surgical procedures, and because of its limited sample size our study simply lacked the statistical power to provide reliable answers to the question whether this procedure may actually reduce complication and relapse rates (1-10).

Second, we identified a significant proportional bias, that specifically affected larger prolapses: even though preoperative assessment of prolapse was well correlated with intra-operative measurements, its sensitivity (i.e. 51.9%) was unsatisfying, and particularly for lesions larger than 3 cm. In fact, preliminary IMRP measurement of larger prolapses underestimated their extent in nearly half of cases. Therefore, it is reasonable to question whether preliminary measurement had a positive impact on post-operative issues, or other factors were actually involved. As previously stressed by previous reports (14, 23-25), accurate, direct vision of operatory field reduces the risk for severe complications. In other words, it is reasonable to assume that the outcomes recorded in our sample, and particularly the very low recurrence rate, have been extensively influenced by the repetitive assessment of the operatory field.

Third, despite we performed a direct clinical examination of patients, including VAS collection, symptoms were neither investigated through a validated questionnaire on the quality of life, nor appropriately blinded towards investigators (25). Therefore, we cannot rule out that our results have been affected by a significant social desirability bias, i.e. participants reporting the "socially appropriated" rather than their authentic complaints in order to satisfy the interviewing clinician (26-28).

In conclusion, the use of EEA® Auto Suture in mucoemorrohidal prolapse up to 3 cm allows an appropriate measurement of the prolapsed mucosa to be excised. Patient satisfaction both in short- and long-term was good. However, we have observed that the measurement of the excided tissue may be significantly underestimated for lesions larger than 3 cm, but this inaccuracies may have been compensated by the repetitive, direct assessment of the operatory fields. In summary, our results suggest that the use preliminary IMRP measurement through EEA® Auto Suture may be considered as a possible technical option for a tailored surgery. On the contrary, larger studies are required to accurately assess the actual impact of IMRP on surgical prognosis.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Lohsiriwat V. Hemorrhoids: From basic pathophysiology to clinical management. World J Gastroenterol 2012; 18(17): 2009.
2. Kaidar-Person O, Person B, Wexner SD. Hemorrhoidal Disease: A Comprehensive Review. J Am Coll Surg. 2007; 204(1): 102-117.
3. Milito G, Cadeddu F, Muzi MG, Nigro C, Farinon AM. Haemorrhoidectomy with Ligasure™ vsconventional excisional techniques: meta-analysis of randomized controlled trials. Colorectal Dis 2010; 12(2): 85-93.
4. Panarese A, Pironi D, Vendettuoli M, Pontone S, Arcieri S, Conversi A, et al. Stapled and conventional Milligan-Morgan haemorrhoidectomy: different solutions for different targets. Int J Colorectal Dis 2011; 27(4): 483-487.
5. Longo A. Treatment of hemorrhoids disease by reduction of mucosa and hemorrhoidal prolapse with a circular suturing device: a new procedure, in Proceedings of 6th World Congress of Endoscopic Surgery: Rome, Italy, June 3-6, 1998. In: Montori A, Lirici MM, Montori J, editors. Bologna; 1998. pp. 777-784.
6. Corman ML, Gravié JF, Hager T, Loudon MA, Mascagni D, Nyström PO, et al. Stapled haemorrhoidopexy: a consensus position paper by an international working party - indications, contra-indications and technique. Colorectal Dis 2003; 5: 304-310.
7. Huang W-S, Chin C-C, Yeh C-H, Lin P-Y, Wang J-Y. Randomized comparison between stapled hemorrhoidopexy and Ferguson hemorrhoidectomy for grade III hemorrhoids in Taiwan: a prospective study. Int J Colorectal Dis 2007; 22: 955-961.
8. Tjandra JJ, Chan MKY. Systematic Review on the Procedure for Prolapse and Hemorrhoids (Stapled Hemorrhoidopexy). Minerva Chir 2007; 50(6): 878-892.
9. Shao WJ, Li GCH, Zhang ZHK, Yang BL, Sun GD, Chen YQ. Systematic review and meta-analysis of randomized controlled trials comparing stapled haemorrhoidopexy with conventional haemorrhoidectomy. Br J Surg 2008; 95(2): 147-160.
10. Burch J, Epstein D, Baba-Akbari Sari A, Weatherly H, Jayne D, Fox D, et al. Stapled haemorrhoidopexy for the treatment of haemorrhoids: a systematic review. Colorectal Disease 2009; 11(3): 233-243.
11. Béliard A, Labbé F, de Faucal D, Fabreguette JM, Pouderoux P, Borie F. A prospective and comparative study between stapled hemorrhoidopexy and hemorrhoidal artery ligation with mucopexy. J Visc Surg 2014; 151(4): 257-262.
12. Lumb KJ, Colquhoun PHD, Malthaner R, Jayaraman S. Stapled versus conventional surgery for hemorrhoids. Jayaraman S, editor. Cochrane Database Syst Rev 2006; 4: CD005393.
13. Pescatori M. PPH stapled hemorrhoidectomy--a cautionary note. Dis Colon Rectum 2003; 46(1): 131.
14. Wong JC, Chung CC, Yau KK, HY C, Wong DC, C CO, et al. Stapled technique for acute thrombosed hemorrhoids: a randomized, controlled trial with long-term results. Dis Colon Rectum 2007; 51(4): 397-403.
15. Morinaga K, Hasuda K, Ikeda T. A novel therapy for internal hemorrhoids: ligation of the hemorrhoidal artery with a newly devised instrument (Moricorn) in conjunction with a doppler flowmeter. Am J Gastroenterol 1995; 90: 610-613.
16. Davide Giavarina. Understanding Blant Altman analysis. Biochem Men 2015; 25(2): 141-151.
17. Costi R, Riccò M, Negrini G, Wind P, Violi V, Le Bian AZ. Is CT Scan more Accurate than Endoscopy in Identifying Distance from the Anal Verge for Left Sided Colon Cancer? A Comparative Cohort Analysis. J Invest Surg 2018: Epub ahead of print.
18. Costi R, Le Bian AZ, Negrini G, et al. Preoperative localization of left-sided colon adenocarcinomas: comparing staging CT-scan and colonoscopy. Int J Colorectal Dis 2016; 31(8): 1533-1535.
19. Bland JM, Altman DG. Statistical method for assessing agreement between two methods of clinical measurement. Lancet 1986; 327: 307-310.
20. Ludbrook J. Confidence in Bland Altman plots: a critical review of the method of differences. Clin Exp Pharmacol Physiol 2010; 37(2): 143-149.
21. Yang J, Cui P, Han H, et al. Meta-analysis of stapled hemorrhoidopexy vs Ligasure hemorrhoidectomy. World J Gastroenterol 2013; 19(29): 4799-4807.
22. Naldini G, Fabiani B, Menconi C, et al. Tailored prolapse surgery for the treatment of hemorrhoids with a new dedicated device: TST Starr plus. Int J Colorectal Dis 2015; 30(12): 1723-1728.
23. Naldini G. Serious unconventional complications ofsurgery with stapler for haemorrhoidal prolapse and obstructed de-

faecation because of rectocele and rectal intussusception. Colorectal Dis 2011; 13(3): 323-327.
24. De Santis G, Gola P, Lancione L, et al. Sigmoid intramural hematoma and hemoperitoneum: an ear- ly severe complication after stapled hemorrhoidopexy. Tech Coloproctol 2012; 16(4): 315-317.
25. Sturiale A, Fabiani B, Menconi C, et al. Long-term results after stapled hemorrhoidopexy: a survey study with mean follow-up of 12 years. Tech Coloproctol 2018; 22(9): 689-696
26. Riccò M, Vezzosi L, Gualerzi G. Health and safety of pesticide applicators in a high income agricultural setting: a knowledge, attitude, practice, and toxicity study from North-Eastern Italy. J Prev Med Hyg 2018; 59(3): E200-E211.
27. Riccò M, Cattani S, Casagranda F, et al. Knowledge, attitudes, beliefs and practices of occupational physicians towards vaccinations of health care workers: A cross sectional pilot study in North-Eastern Italy. Int J Occup Med Environ Health 2017; 30(5): 775-790.
28. Riccò M, Cattani S, Casagranda F, et al. Knowledge, attitudes, beliefs and practices of Occupational Physicians towards seasonal influenza vaccination: a cross-sectional study from North-Eastern Italy. J Prev Med Hyg 2017; 58(2): E141-E154.

Received: 26 December 2017
Accepted: 11 February 2019
Correspondence:
Luigi Percalli MD, PhD
Dr. UO Chirurgia Valtidone,
Dipartimento Chirurgico AUSL Piacenza
Viale II Giugno, 92 - 29015 Castel San Giovanni (PC), Italy
Tel. 0039.346.852.8667;
E-mail: lpercalli@gmail.com

Acta Biomed 2019; Vol. 90, N. 2: 316-320 DOI: 10.23750/abm.v90i2.8485

Newborn screening in Nigeria: will incorporating congenital hypothyroidism with sickle cell disease improve neonatal screening programme?

Iroro Enameguolo Yarhere[1], *Tamunopriye Jaja*[1], *Datonye Briggs*[1], *Lorenzo Iughetti*[2]
[1]Department of Paediatrics, University of Port Harcourt, Nigeria; [2] Unit of Paediatrics, University of Modena, Italy

Summary. *Introduction:* Nigeria like many African countries has tried to start the newborn screening for congenital hypothyroidism and many failed. Since sickle cell disease is more common in Nigeria, the hypothesis is that incorporating it into a screening programme for congenital hypothyroidism will improve the uptake of the programme by parents and government. *Methods:* Different aspects of newborn screening with difficulties and challenges in running newborn screening were identified and discussed. *Result:* Identifying that for newborn screening to be successful, several key factors have to be put in place including but not limited to organizational structure, system thinking, finance, legislative and political will. A proper recall system for test positives and diagnostic/confirmatory test must be put in place before the programme starts. Since several other screening programmes like sickle cell disease, cervical and breast cancer have run successfully in Nigeria, incorporating one of them into the newborn screening for CH can make the programme succeed as there will be better uptake by the population and the policy makers. *Conclusion:* The difficulty in establishing a newborn screening programme in Nigeria stem from health care financing, organizing the programme from screening through to recall and treatment, and ultimately, prevention of diseases. (www.actabiomedica.it)

Key words: newborn screening, thyroid, sickle cell disease

Introduction

Many African countries, including Nigeria, have tried to do neonatal thyroid screening for congenital hypothyroidism and most have failed (1-3). Only some regions in South Africa have succeeded sparingly to do this continuously, however, South East Asia have succeeded over the last few decades to have newborn screening incorporated into the delivery services offered to all mothers (4). This success is largely due to individual and institutional commitments, advocacy and collaborations (5). For screening to occur, some basic principles and tenets must be met, including but not limited; test must be sensitive and specific, the condition must be prevalent and also treatable after diagnosis has been made.

In the black America population, while the prevalence of sickle cell trait and disease remains high at 1 in every 400 babies, the CH prevalence is actually less so, at 1 in 10,000 (6, 7). In Nigeria, many parents know more about sickle cell disease than they do about CH, and if they are given the option of having these two conditions diagnosed at birth, they are likely to agree for the process and even pay for them. It is the believe of the author that if Nigeria with a high sickle cell disease burden incorporates the screening into on-going efforts to have congenital hypothyroidism screening, there will be a better yield and uptake of both conditions.

State of Newborn screening in Africa

Newborn screening for any disease in Nigeria is non-existent and the need cannot be overestimated, as many such diseases can lead to long term devastating consequences and loss of economic power and lifestyle (2). Many developed countries have succeeded in screening their newborns for many metabolic and genetic diseases. In the Middle East and North Africa where there is high rate of consanguineous marriage, it stands to reason that many autosomal recessive disorders will be prevalent (3). This high rate of metabolic diseases was seen in Qatar (8), with 1 in 1327 children born with metabolic disorders. The diseases of interest in most of Sub Saharan Africa are mainly infectious and naturally, most of the resources for health are geared towards eradicating these. The HIV/AIDS scourge ravaged most of Sub Sahara Africa and the most effective means of reducing the incidence and prevalence was the introduction of Maternal to Child transmission of the disease and voluntary testing and counseling. The development of rapid diagnostic testing for HIV and malaria also improved the disease burdens of these conditions, reducing their prevalence and allowing for early treatment to prevent complications. Nutritional screening using weight, and height and other derivatives of these measurements as tools has also helped in reducing the burden and complications of under nutrition and over nutrition. These are done in schools, and well-baby clinics or hospitals just as the child makes contact with the health personnel. The most common noninfectious disease in Nigeria is sickle cell disease with up to 25% of the Nigeria population carrying the sickle cell gene (9). Many churches actually require that the intended couples have a Haemoglobin electrophoresis done and to present these results before they are wedded. Many debate the constitutional rights of such actions but it is done nonetheless. The purpose of this act is to reduce the prevalence of sickle cell disease and anaemia in the country because the resources to manage the conditions are thinning out not to mention the burden of having a child go through acute painful or anaemic crises. All these screening methods evolved over time when many ideas were brought forward and implemented with some of them failing and others succeeding.

Strategies for improving newborn screening uptake

For new programmes to be established and have public acceptability there has to be education as to the need for the programme from the health care institutions through to the general public that will benefit and the politicians that will make policies to see that there is universal coverage (1, 3-5). The insurance companies, media, government and legislatures have to understand why this kind of programme will benefit the whole country. Understanding that as little as $5 per infant screening can prevent a loss of intellectual ability in a child who will be dependent on the system could make the legislature enact laws that will make screening mandatory for all children (10). Community leaders who have better understanding of their people they lead should be incorporated ab initio to help mobilise all those to benefit from the programme. Production of flyers, handbills, posters and billboard advertisements should be used to disseminate information about the programme, and collaboration with bodies who have already done relatively similar programmes should be partnered with to help with the planning phase of the programme.

When establishing a newborn screening programme, consideration should be made primarily on the basis of improving the health outcomes of affected individuals, and the making sure the diagnosis is established in the shortest possible time. In this case, the screening should be specific and sensitive so the unaffected newborns are not erroneously labeled as being affected and having to go through a barrage of tests without end (1, 2). A newborn successfully screened and diagnosed will be treated immediately if the symptoms present early, or preventive strategies will be put in place at all levels of prevention to reduce the disease burden.

The model of community health insurance scheme being practiced in some parts of Rivers State, Nigeria under the supervision of Shell Petroleum Development Company should be approached for inclusion of newborn screening into their coverage (11). The success of such inclusion and establishment is likely to spur the nationwide National Health Insurance Scheme to include newborn screening in their health package. This not only reduces the cost of the screening programme, but it also increases uptake of the programme and also

uptake of insurance coverage by the general public. If the public knows that specific diseases can be detected long before symptoms start showing, and they do not have to pay extra from their pocket as the process is already covered in their insurance, there will be dissemination of information and more people will key into the scheme (10, 12).

Developing strict criteria for diseases to be screened is essential in the initial developing and planning stage (13). It makes no sense to include extremely rare and untreatable condition into a screening programme where resources will be expended and outcome will be dismal. The 2 conditions already mentioned in this article (sickle cell anaemia and congenital hypothyroidism) are quite common in Nigeria and should be included in any programme that seeks to screen newborns (1, 2, 6). Other conditions like HIV/AIDS and hearing loss can be included as the programme gets acceptance and structures are well developed as the system progresses. In Lagos state, advocacy for inclusion of hearing assessment for the newborn into the birthing package is ongoing and those with hearing losses are immediately referred to specialist for proper management (14).

The establishment of the structures that will manage the programme from sample collection to recall, diagnosis and treatment will take a long process that must be proper in the first instance (3). Identification of samples and filter papers should be done electronically and a system for archiving put in place. It thus stands to reason that the names on the filter papers with their parents' names and mobile numbers should be legible as was done by Yarhere et al (2). Before samples are sent for laboratory analyses, all details should be entered into a spreadsheet and any sample that is flagged after analysis should have the parents contacted immediately. The laboratory to be used for the screening should be domiciled in Nigeria, with the logistics of retrieving samples from every nook and cranny of the country. The possibility of getting samples to the center must be fully explored making sure samples arrive within 3-4 days of collection. Now, there are many problems and challenges in this aspect and having a system that overcomes these will make the programme succeed.

The cost of screening one newborn is not only in the machine and reagents to be used, but transportation, logistics of collecting samples during or soon after delivery should be factored in (12, 15). Electrical power should be constant, training, and retraining of support staff must meet contemporary requirements, legal and ethical team must sit regularly to improve the outcomes of the programme, and the recall process will be factored into the cost also (6, 13). Who bears the cost of all of these should be debated before the programme starts. The chances of success of the programme are slim if it is for free to the end users, and the charge should not be prohibitive to prevent continued payment by either the insurance or the end user (15). State governments that are interested in the programme should sign laws into place to make sure the programme is continuous even after their tenures expire. Understanding that the diseases to be screened for are of public health importance, the Ministry of health at Federal, and State levels should take up the responsibility of ensuring the success of the programme.

Partnering with organisations that have done screening before, like family Health International 360, World Health Organisation, United Nation International Children's Emergency Funds etc will improve the outcome of the programme as these bodies will give technical support and advice on how to proceed and where not to thread or to cautiously thread (7, 13). These partners that are well informed and experienced in managing these programmes can also serve as part of the external quality assurance team to ensure system continuity and audit. They will establish guiding principles and criteria for subsequent inclusion of other conditions into the screening panel as needs arise. It must be noted that the usual screening for diseases are voluntary, like the VCT and cervical cancer screening, which individuals usually pay for knowing the implications of not getting tested on time. The case is different in this instance because the end users, newborns, cannot give consent or assent so they do not have a say in the decision on whether or not to be screened.

Methods used for screening congenital hypothyroidism and sickle cell disease

For Congenital hypothyroidism screening, the samples are usually analysed for TSH, with levels greater than 50uIU/ml indicative of hypothyroidism, as

done in Europe and other Asian countries that screen (12, 13). In the USA however, samples are analysed for TSH and T4 immediately to ascertain whether the CH is primary or secondary. In the African and Nigeria model, authors advocate a primary TSH analysis in the first instance since a high level tells that the pituitary gland is functional and the thyroid is likely unresponsive (1, 2). A lower than normal TSH reveals a dysfunctional pituitary gland but does not tell if the thyroid gland is anatomically intact and functional. Now both models have their advantages but cost in the African and Nigerian model should make a primary TSH screening more feasible. The machines used are usually sensitive for TSH up to 0.005 μIU/ml.

For Sickle cell disease, High performance liquid chromatography is the most feasible method in the newborn period (6, 15). Specimens with HPLC profiles consistent with SCD, sickle cell trait, ß-thalassemia, or variant haemoglobins other than HbS can subsequently be analyzed by Capillary Electrophoresis as a confirmatory method. Other screening methods for HB genotype include Isoelectric focusing, cellulose acetate electrophoresis, Citrate ager electrophoresis, capillary zone electrophoresis and molecular methods.

The challenge of funding and overcoming this special barrier

For any programme to succeed, there has to be funding, and for a programme that the short and long term benefits are not monetary, getting government or organisations to fund them will be difficult (10, 15, 16). Now Nigeria and Africa face more peculiar problems as they are more into curative than preventive medicine and fighting infectious diseases with little devotion to metabolic or other systemic conditions. In countries where preventive medicine takes centre stage, the citizens rarely fall ill and many of their health care professionals devote their time to more innovative technologies and science. Asking the WHO or UNICEF to fund screening programmes will be over bearing because they are more concerned with infectious disease prevention and thus concentrate on vaccination as the main stay of prevention in these countries. Individual governments, at state and federal levels need to take up this challenge and increase budgetary allocations to primary health care and preventive medicine to allow for this type of screening. If health insurance were patronized and not shrouded in cesspool of corruption, many individuals will buy these insurance schemes and the insurance will cover for the screening of their newborns. Already, there is a sickle cell foundation in Nigeria that drives many charitable programmes geared at improving the care of these children and advocating for continued support. This organisation may be willing to get more financial aids to start screening all newborns for sickle cell disease and getting a system of recall for those who are positive. Foundational grants are a veritable source of funding for such programmes and this option should be explored.

Conclusion

The difficulty in establishing a newborn screening programme in Nigeria stem from lack of financing, organizing the programme from screening through to recall and treatment, and ultimately, prevention of diseases. Political will to improve the primary health care system and facilities where many of these children will be delivered and eventually cared for should be made priority so that actions can be backed by laws that govern the whole process. The cost effectiveness of this programme eventually translates to cost saving knowing the interventions that are available once there is early diagnosis and limitation of disability. The children diagnosed and treated early become less of a burden to the healthcare system and the society and ultimately economically productive and self-sufficient.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Adeniran K, Limbe M. Review Article on Congenital Hypothyroidism and Newborn Screening Program in Africa; the Present Situation and the Way Forward. J of Thyroid Disorders & Therapy 2012; 1.
2. Yarhere IE, Jaja T, Oduwole A, Ibekwe MU, Suwaid S Yahaya

A, Adeniran k, Fetuga b, Jarrett OO, Elusiyan JBE, Adesiyun O, Idris HW, Blankenstein O, Akani NA. Normative Thyroid-Stimulating Hormone values for Healthy Nigerian Newborns. Horm Res Paediatr 2016; 85: 22-28.
3. Therrell BL Jr, Padilla CD. Barriers to implementing sustainable national newborn screening in developing health systems. Interna J of Pediatr and Adolescent medicine 2014; 1: 49-60.
4. Therrell BL, Padilla CD, Loeber JG, Kneisser I, Saadallah A, Borrajo GJC, Adams JBA, Current status of newborn screening worldwide: 2015. Seminars in Perinatology 2015; 39: 171-1787.
5. Padilla CD, Therrell BL, Jr. Consolidating newborn screening efforts in the Asia Pacific region Networking and shared education. J Community Genet 2012; 3: 35-345.
6. Frömmel C, Brose A, Klein J, Blankenstein O. Newborn Screening for Sickle Cell Disease: Technical and Legal Aspects of a German Pilot Study with 38,220 Participants. BioMed Research International 20141-11.
7. Watson MS, Mann MY, Lloyd-Puryear MA, Rinaldo P, Howell RR. Newborn Screening: Toward a Uniform Screening Panel and System - Executive Summary. Pediatrics 2006; 117: S296-307.
8. Zadhoush MPAF. Newborn screening for inherited metabolic disorders;new and views. J Res Med Sci 2013; 18: 801-8.
9. Akodu SO Diaku-Akinwunmi IN, Njokanma OF. Age at diagnosis of sickle cell anaemia in Lagos, Nigeria. Meditterr J Hematol Infect Dis 2013; 5.
10. Stuart G Nicholls BJW, Holly Etchegary, Jamie C Brehaut, Beth K Potter, Robin Hayeems, Pranesh Chakraborty, Jennifer Milburn, Daryl Pullman, Lesley Turner, June C Carroll. Benefits and burdens of newborn screening: public understanding and decision-making. Per Med 2014; 11: 593-607.
11. Kelsey Vaughan NA, Babatunde Fakunle, Liezel Wolmarans. Who benefits from the Obio Community Health Insurance Scheme in Rivers State, Nigeria? A benefit incidence analysis. International Health 2016; 8: 405-12.
12. Aaron E. Carroll, MD, MSa,b, Stephen M. Downs, MD, MSa. Comprehensive Cost-Utility Analysis of Newborn Screening Strategies. Pediatrics 2005; 17: S287-95.
13. Léger J, Olivieri A, Donaldson M, Torresani T, Krude H, van Vliet G, Polak M, Butler, G. European Society for Paediatric Endocrinology Consensus Guidelines on Screening, Diagnosis, and Management of Congenital Hypothyroidism. Hormone Research in Paediatrics 2014; 81: 80-103.
14. BO Olusanya ASLW, b and LM Luxonb. Community-based infant hearing screening for early detection of permanent hearing loss in Lagos, Nigeria: a cross-sectional study. Bull World Health Organ 2008; 86: 956-963.
15. Andreas Kuznik, Abdulrazaq G. Habib, Deogratias Munube, Mohammed Lamorde. Newborn screening and prophylactic interventions for sickle cell disease in 47 countries in sub-Saharan Africa: a cost- effectiveness analysis. BMC Health Services Research 2016; 16: 304.
16. Uzochukwu B UMD, Etiaba E, Okwuosa C, Envuladu E, Onwujekwe OE. Health care financing in Nigeria: implications for achieving universal health coverage. Niger J Clin Pract 2015; 18: 437-4344.

Received: 15 May 2019
Accepted: 20 May 2019
Correspondence:
Lorenzo Iughetti
Unit of Paediatrics, University of Modena, Ital
lorenzo.iughetti@unimore.it

Acta Biomed 2019; Vol. 90, N. 2: 321-326 DOI: 10.23750/abm.v90i2.6944

The beginning of smallpox vaccination in the Duchy of Parma*

Raffaele Virdis
Former associate Professor of Pediatrics, Auxology and History of Medicine, University of Parma, Parma, Italy

Summary. Maria Luigia (Marie Louise) of Habsburg, daughter of the Austrian Emperor and, as Napoleon Bonaparte's second wife, Empress of the French, after the defeat of the husband in 1814 was relegated to role of Duchesse of Parma, Piacenza and Guastalla. She arrived in Parma in 1816 accompanied by several Austrian army and administrative officials, which were instructing and controlling her, and, willingly, she left to them most of the political and administrative decisions. On the contrary, since the first years she was interested and wanted to take decisions in the field of public health and charity. She opened new specialized hospitals and hospices for poor people, orphans and abandoned children, and, in February of 1820, promulgated the new «Regulations of the vaccinations», an exhaustive and specific code, that was taking into consideration the times, the places, and the people who had to vaccinate or to be vaccinated. Moreover, she fixed the modalities, the incentives, the sanctions, and she also nominated a series of people who had to publicize vaccinations and to help the general population inovercoming fears, prejudices and other causes of distrust. The new dispositions increased the number of vaccinated people in the Duchy, saving it from several epidemics that appeared in the following decades in the neighboring regions (Tuscany, Lombardy). In 1831 and 1832 she issued other two ordinances in which she urged the populations and the doctors to increase the vaccinations, probably after a decrease in interest of both, and introduced new practical arrangements to simplify and to facilitate the practice, ensuring and verifying the outcome. The effectiveness of the provisions of Maria Luigia has been shown by the marked decrease in smallpox epidemics throughout her whole reign, until 1847. Meanwhile after the end of the reign, in the second part of the nineteenth century, there was an increase of epidemics, because the following governments of the Bourbons Duchy (1847-1860) and of the united Italy after 1860 were not as diligent and active on spreading vaccinations. (www.actabiomedica.it)

Key words: immunizations, vaccination, smallpox, cowpox, measles, no-vax movements

When the smallpox vaccination started in the Duchy of Parma, on the first years of the nineteenth century, very short time after Jenner's successful inoculation of James Phipps (1796) and the appearance of his first publication, *An Inquiry into the Causes and Effects of the Variolæ Vaccinæ* (1798) (1), the local population was already aware of the preventive practice of inoculation. Indeed, the previous Duke, Ferdinand of Bourbon, dead in 1802, had undergone an inoculation when he was a young child (1764), saving his life, because the mother, a sister and the father, all died of this disease just before or after the practice on him (2). The ducal inoculation had a worldwide resonance because he was probably the first heir to an European throne doing it, and because sometime the result could be fatal. It is interesting to know that while the Bourbon

* In part presented to the 68th Scientific Session of "Archivio Storico per le province Parmensi", December 4th, 2016

courts of Spain and Naples were contrary to this medical maneuver (Ferdinand was also an Infant of Spain, that is a heir to Madrid throne), the Austrian Empress Maria Theresa send to the father, Duke Filippo, her congratulations, adding "that she wanted to do the same with her children, but the emperor consort did not want" (3).

The new Jenner's inoculation was much safer, because done with pus scraped from cowpox blisters and not from the more dangerous and often fatal smallpox. The practice of the new vaccination spread in all the European countries and rapidly also in the Americas and in all the British colonies in every continent. While in the Napoleonic Italian Republic, conterminous to the Parma Duchy, at the beginning of the nineteenth century under French influence, the new preventive maneuver was very popular (4), on the contrary in Parma only few people accepted to do it.

In 1805 a virulent smallpox outbreak in the Country and in all North-Italy pushed the French general administrator of Parmesan States; count Démérique Moreau de Saint Méry, to invite the population to vaccinate themselves and their children, overcoming prejudices and fears. The governmental warning was supported by explanatory invitations to vaccinate by the famous clinician Giacomo Tommasini and by the bishops of Parma and Placentia. The two clerical authorities invited all the priests of the dioceses to convince their parishioners, especially the "idiots":

thoughtful persuasion to overcome the prejudices, which could easily arise in the idiots, also to prevent stronger and disgusting measures that the Government would believe in having to take for the public health, (*premurosa persuasione di vincere i pregiudizi, che potrebbero facilmente insorgere negl'**idioti**, anche per prevenire misure più forti e disgustose che il Governo si crederebbe in dovere di prendere per il pubblico bene)* (5).

The word "idiot" in Italian means both "ignorant" and "feebleminded person", and today it is used predominantly with the second meaning, but the Parmesan bishop meant ignorant, even if with a disdainful meaning of presumptuous ignorant as the actual people contrary to vaccinations are (6). Criminals as well as idiots, because in this way not only endanger the health and sometimes the lives of their children but, lowering the global coverage of the immunes to less than 95%, let the diseases circulate, reaping, here and elsewhere, new victims, even hundreds of thousands of lives per year as it happens for measles (7).

In the following years the vaccinations augmented and when in 1809 the Duchy of Parma became Department of Taro, a province and prefecture of the French State, the situation was good and so it remained for a few years thanks to the recruitment of the clergy in advising and convincing the population, and to the awards given to the most intense vaccinators. In 1812, an epidemic interesting Tuscany, Cremona and Padua also touched slightly Parma but was stopped intensifying vaccinations and dividing the city into neighborhoods, each with his vaccine commissioner (5).

Probably in the following period of transition from French to Austrian influence, after the fall of Napoleon, and with the assignment of the Duchy to Maria Luigia of Austria, there was a decline in vaccinations, also favored by an outbreak of epidemic (petechial) typhus that struck the State in 1817, moving attention from smallpox to the other pathology.

Maria Luigia was born in 1791 as Maria Ludovica Leopoldina Franziska Therese Josepha Lucia von Habsburg-Lothringen, first daughter of the future Austrian Emperor Francis II and of Maria Teresa Bourbon of Naples-Sicily. For political reasons, she became the second wife of Napoleone Bonaparte when she was only nineteen, and as such Empress of the French from 1810 to 1814 with the name of Marie Louise. When she arrived in Parma she assumed officially the Italian name of Maria Luigia and so was always cited in the governmental affairs. The new Duchess was young and inexperienced, therefore, the Emperor father, had put good advisers to her side in order to support and guide, but also control her. She willingly leaved to them the state affairs, especially to the future morganatic husband Count Adam Albert von Neiperg, but she wanted to personally follow the charitable and health issues.

On February 25, 1820, she promulgated the *"Regulations on Vaccinations",* ordinance No. 25 in the *Collection of Laws for the States of Parma, Placentia and Guastalla* of that year, a comprehensive and specific rules that took into account the times, the places and the people who had to vaccinate and to be vaccinated (8). The regulations also provided modalities,

awards and penalties, and all the corollary of people who, while not being active vaccinators (because they were not doctors, surgeons, or the like), had to act to let the population inoculate herself, overcoming fears, preconceptions or other reasons of distrust. The new Government, thanks to the precise Austrian experience in the bureaucratic field, also envisaged all the administrative aspects so that the situation, the statistics, and the progress of the health campaign were always monitored (8).

What were the reasons for this new regulation, what had brought the new Reign to drafting a new legislation? Certainly the fall in the number of immunizations observed in the passage period from French to Austrian influence, moreover the risk, always present, of new outbreaks. Alfredo Frassi, chief health officer of the city at the beginning of the twentieth century, in an interesting "*History of vaccination in Parma*" published in 1913, stated that the Duchess had complained of the falling of the vaccinations, calculating also that in the Country there were at least forty thousand subjects to be inoculated and she hoped that at least half of them should be immunized. Spotting the State and City archives, the health officer noted an immediate increase in the number of vaccinations after the decree that continued throughout the next decade and

perhaps contributed to avoiding the epidemics appeared in many parts of Italy: Bologna (1822), Turin and Chioggia (1823), Urbino and Vicenza (1824), Upper Italy (1826) and Genoa (1829) (5).

He also calculated that at that time the performed vaccinations interested 50% of the newborns, without taking into account the infants dead in the first year of life, and thus a great result for those times.

The ducal decree was divided in 8 parts and started with the heading (8):

We Maria Luigia, Imperial Princess, Archiduchess of Austria, for the grace of God Duchess of Parma, Piacenza and Guastalla, etc, etc, etc, (Noi Maria Luigia, Principessa Imperiale ed Arciduchessa d'Austria, per la grazia di Dio Duchessa di Parma, Piacenza e Guastalla, ecc. ecc. ecc.).

The eight parts concerned: 1. Places and times of vaccinations; 2. Vaccinators; 3. Inoculation fluid; 4. Promoters; 5. Organization; 6. Awards and incentives; 7. Fines and punishments; 8. General provisions. In particular:

1. Each city and village had to find a vaccination room, for Parma the hall was located in the maternity hospice, recently established by the Duchess. The vaccine sessions were two per year, one in the spring and the other in the autumn. The "*Protomedicato*" (the public health management) could promote other sessions in case of danger of new outbreaks.

2. Physicians and surgeons were preferred for the role of vaccinator but, in case of need, also medical students of the last years and midwifes with their students.

3. The inoculation fluid had to be preserved all the year round in the foundling hospice for infants, annexed to the maternity hospital. For fluid we have to intend not only the one preserved in tubes (minimal amount) but the one kept constantly in the hospice with regular grafts from one child to another.

The children were the true deposit of the fluid, the small amount preserved in glass tubes was only a reserve in case of failure of engraftment of the vaccinations, thus interrupting the human chain.

Today, such a method would certainly be considered unethical and a serious violation of human rights and of children in particular, but for those times was a normal and completely lawful thing (5).

4. The *promoters* were lay persons selected among the outstanding people of the community in each city and village of the Duchy where a vaccination hall was instituted. They had the task of promoting vaccinations by going *to the domicile of the inhabitants of their municipalities for the exact review of the candidates to the immunization, and to encourage and persuade the shy and ignorant people*. They had also the duty to control that all the candidates underwent vaccination and the doctors did the verification of the right reaction ten days after, moreover, that the unwilling subjects presented themselves to the next sessions, as well as those who did not have the proper reaction to the inoculation.

5. The part on the *organization* concerned the start and end times of the sessions, the roles of government authorities, vaccinators and parish priests, underlining their part in promoting the campaign and persuading people. The local government authorities and officers had to book in advance the vaccine fluid from the deposit, i.e the child of the foundling hospice who will be used for the vaccination which the vaccinators had to perform "*constantly from arm to arm*"

and subsequently register the names of the vaccinees in a special book. Furthermore, this part of the decree concerned also many other aspects as controls for the proper reaction, reports of physicians and authorities, certificates, registrations of private vaccinations and other bureaucratic aspects.

The sixth and seventh section, *Awards and incentives*, and *Fines and punishments* regarded the aspects finalized to incentive vaccinations, the first ones for vaccinators and promoters, the second ones for people who had missed the duty. While the awards were usually money, the punishments were more various and consisted in the impossibility to be admitted, if not vaccinated, to hospices or colleges, boarding-schools, public and private schools including the university. No one could ask for help and relief from the Government and from the various beneficial entities if not immunized, and similar punishments regarded also parents who had not vaccinated their children.

8. The decree concluded with some *general dispositions*, such as the obligation of doctors, surgeons and midwifes to report the private vaccinations performed and their outcome, otherwise suspended. Finally, *"the presidents of interior, finance and military departments were responsible for the implementation of the Regulation in each case."* In appendix were reported the facsimiles of the vaccination book pages and of certificates that could be requested (8).

The immediate following decree, N. 26, regarded the *instructions for vaccinators* and completed the previous (9). The Instructions were compiled by four members of the Protomedicato Council: Giuseppe Basili, Giovanni Rossi, Andrea Rasori and F. Lorenzini. The first and the third had already been quoted in the Moreau de Saint-Mery invitation, respectively one as the secretary of the council and the other as a vaccine doctor.

It was mandatory to use inoculation subdermal needles, while the scarification lancet was prohibited. Vaccinations had to be done by *"from arm to arm graft"*, so the doctors had to make sure that they had just foundling children recently vaccinated with the active pustules they could use (usually towards the third to fourth day of inoculation). Then followed the instructions to distinguish the good pustules to use, which should be pointed horizontally to the surface, in order to release the fluid without squeezing, so as not to pollute them with blood drops and not to cause pain to the child.

At the moment of control of the reactions the decree explained how to distinguish the proper pustules from the other due to infections or traumas, and how to draw up vaccine books, relations to the authorities and certificates. Moreover was a duty of the vaccinator to control both the children used to vaccinate the population and the people who had to be inoculated in order to avoid transmissions of infectious diseases or inappropriate reactions.

Throughout the following decade (1821-1830) the vaccinations were numerous and, as mentioned above, avoided the spread of major epidemics as it did in neighboring regions (Tuscany, Liguria, Lombardy and Veneto). In the decade of 1830-40 vaccinations decreased, but the large number of immunes from the previous period protected the Duchy from major epidemics, while it was not free from cholera epidemics, as in 1831 and especially in 1836 when it struck 21‰ of the population killing half of them (5).

Causing a likely decline in attention to vaccinations, the Sovereign in June and November 1831 updated the provisions with few variations, urging the population and, above all, the doctors to perform them. Already in 1817, the chairman of the Parma Health and Rescue Committee had complained to the Duchess of the low level of social and administrative commitment of physicians who did not report and denounce the cases of illness undergoing epidemics:

Do you know, Her Majesty (HM), that the doctors or do not report or do little to the account of the typhus sufferers ... It is a good idea to force them to do their duty .... [otherwise] they will be suspended from employment. ….Those "in private practice" know that HM will never accept requests to obtain public assignments of the art that they are exercising (5).

The following year, on March 16, 1832, from the ducal seat of Piacenza, Maria Luigia issued a new resolution, in which it indicated new dates for public vaccinations: in particular the vaccination was to be done only by "using arm-to-arm graft" and reduced vaccination sessions to once a year (10).

Alfredo Frassi in his 1913 article noted that while in Parma was mandatory the "arm-to-arm graft"

method, and this obligation was repeated in 1832, the literature and the experience of the epidemics of time advised to resume occasionally the material from the cows pustules, because the prolonged passage in humankind reduced its *"preservative force"* i.e. the immunizing ability. In any case, the diatribe on this aspect continued for almost the whole of the eighteenth century, and only in the last twenty years of the century the fluid of bovine origin was spread worldwide, harvested and preserved and packaged in suitable and recognized laboratories, already industrial (4, 11, 12).

Despite the general enthusiasm for vaccination and the compulsory practice in many nations in the early decades of the nineteenth century, mostly for newborns, because of the many who did not meet the obligation, the disease continued to reap victims in both endemic and virulent epidemics. These were numerous and serious even, or above all, after the unification of Italy. In the year 1871 Frassi reported a death toll of more than 200 people in Parma, and Pietro Corsini, the physician (medico condotto) of Pellegrino Parmense's, reported more than 500 cases of the disease in his municipality. The smallpox epidemics continued throughout the century and began to decline only after 1888, when the vaccination became mandatory at national level (5).

In conclusion, Maria Luigia's interest in public health matters of her Duchy was evident since from her first legislative procedures and political and private actions.

We do not think that the acts and the various rules in the field of smallpox vaccination were the result of the Duchess' competence, but of the various experts of the local medicine, however, we must recognize her civil and human interest in these aspects. The effectiveness of her "good" regulation, with decreasing epidemics at that time, has been highlighted by the subsequent increased frequency and virulence of smallpox epidemics in Parma after the Italian national unification (1861), when other problems and especially an economic crisis had lost the attention and concern for these problems.

What is the current situation? The last case of smallpox was recorded in Somalia in 1977 and in 1979 the World Health Organization (WHO) decreed the disappearance of the disease from the world and, consequently, the obligation of vaccinations, which for some years were already been suspended in many industrialized countries, including Italy (13).

The virus, however, is still stored in research laboratories and the danger of terrorist spread is always present to the point that, after the attack on the Twin Towers of September 11th 2001, 350,000 Americans, employed in the armed forces and in the healthcare world, were vaccinated. This late vaccination has highlighted a higher frequency of major side effects, including heart complications such as angina and heart attacks, very rare when vaccinations were mandatory and started in the first year of life (13).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Jenner E. An Enquiry into the Causes and Effects of Variolae Vaccinae, a Disease Discovered in some Western Countries of England, particularly Gloucestershire and known by the Name of Cow Pox. Printed for the Author by Sampson Low, No 7, Berwick Street, Soho (London), 1798.
2. Frassi A. Di una variolazione principesca nel 1764. Aurea Parma 1912; I (1-2): 67-72.
3. Benassi U. Per la storia del Progresso: L'inoculazione del vaiolo, particolarmente nei ducati parmensi. Bollettino Storico Piacentino 1922; XVII (f1): 3-19.
4. Assael BM. Il favoloso innesto. Storia sociale della vaccinazione,. Collana "Storia e Società", Edizioni Laterza, Bari 1995: 1-306.
5. Frassi A. Storia della vaccinazione e delle più recenti ricorrenze di vaiuolo a Parma. Ufficio d'Igiene del Comune di Parma, Tipografia Zerbini & Fresching, Parma 1913: 1-49.
6. Tommaseo N. Nuovo Dizionario dei sinonimi della Lingua Italiana, presso Gio, Pietro Vieusseux editore al suo Gabinetto Scientifico-Letterario, Firenze, 1838.
7. WHO, Measles, Fact sheet, July 2017. http://www.who.int/mediacentre/factsheets/fs286/en/
8. Raccolta generale delle leggi per gli Stati di Parma, Piacenza e Guastalla. I semestre 1820, decreto N° 25 Regolamento per le vaccinazioni, Parma 26 Febbraio 1820, Parma dalla Tipografia Ducale, 1821: 38-61.
9. Raccolta generale delle leggi per gli Stati di Parma, Piacenza e Guastalla. I semestre 1820, decreto N° 25, Istruzione ai Vaccinatori pubblicata a seconda dell'art.4, Tit. II del Regolamento Sovrano intorno alle vaccinazioni, in data del 26 Febbraio 1820, Parma 26 Gennaio 1820, Parma dalla Tipografia Ducale, 1821: 62-67.

10. Raccolta generale delle leggi per gli Stati di Parma, Piacenza e Guastalla. I semestre 1832, decreto N° 69, Risoluzione Sovrana, che alcune nuove discipline introduce nelle pubbliche vaccinazioni, onde agevolarne la pratica, assicurare l'esito, e verificarla. Piacenza 16 marzo 1832, Parma dalla Tipografia Ducale, 1832: 58-61.
11. Consiglio Sanitario Provinciale di Parma: Delibera del Consiglio provinciale Parmense e Comitato di Igiene Parmense, a firma di A. Cugini, Parma 21 Marzo 1889.
12. Pietro Corsini Relazione sulla Vaccinazione animale, Battei Parma, 1887.
13. Istituto Superiore Sanità. Epicentro. Il portale dell'epidemiologia per la sanità pubblica. In Malattie e condizioni di salute: Vaiolo. Aggiornamento estate 2017. http://www.epicentro.iss.it/problemi/vaiolo/vaiolo.asp

Received: 27 November 2017
Accepted: 7 June 2018
Correspondence:
Raffaele Virdis
Former associate Professor of Pediatrics,
Auxology and History of Medicine,
University of Parma, Parma, Italy
E-mail: raffaele.virdis@unipr.it

Acta Biomed 2019; Vol. 90, N. 2: 327-330 DOI: 10.23750/abm.v90i2.8218

Case report

Herpes Simplex Virus 1 encephalitis with normal cerebrospinal fluid after brain radiotherapy in a patient with glioblastoma. A case report and review of literature

Carlotta Mutti[1], Erica Curti[1], Rosario Ciliento[1], Andrea Melpignano[1], Irene Florindo[2], Lucia Zinno[2], Enrico Sasso[1, 2], Liborio Parrino[1, 2], Giovanni Pavesi[1, 2], Anna Elisabetta Vaudano[2]

[1] U.O. di Neurologia, Dipartimento di Medicina e Chirurgia, Università di Parma; [2] U.O. di Neurologia, Dipartimento di Emergenza-Urgenza, Medicina Generale e Specialistica, Azienda Ospedaliero-Universitaria di Parma

Summary. Herpes simplex virus encephalitis (HSE) is the most common cause of letal encephalitis and its prevalence appears higher among oncologic patients who undergo brain radiotherapy (RT). We describe a case of 76-year-old woman with glioblastoma multiforme (GBM) who developed HSE shortly after brain RT. Cerebrospinal fluid analysis (CSF) was normal and the diagnosis was driven by brain MRI and EEG. Prompt introduction of antiviral therapy improved the clinical picture. We highlight the importance of EEG and brain MRI for the diagnosis and suggest the possibility of antiviral profilaxys in oncologic patients who undergo brain RT. (www.actabiomedica.it)

Key words: Herpes Simplex Virus 1 (HSV1), encephalitis, glioblastoma, radiotherapy

Background and aim of the work

GBM is the most common fatal primary brain tumor in adults, representing approximately 50% of brain cancer in patients older than 65 years old (1). The median survival in treated patients is about 12-14 months from diagnosis (2,3). HSE is the most common cause of sporadic letal encephalitis (4) with an incidence of approximately 2-4 cases per million per year (5).

Reports exist of co-occurrence of HSE and GBM, of HSE following GBM, and of GBM mimicking HSE (6).

As far as we know there is no clear association in literature between brain RT and HSE, even if several case reports documented HSE shortly after whole brain radiotherapy (WBRT) in oncologic patients with GBM (5).

In patients affected by GBM, HSE diagnosis is particularly challenging because of absent or mild CSF pleocytosis, atypical clinical features, negative neuroimaging in early phases.

Clinical Vignette

We report a case of 76-year-old woman with a right-sided cerebral GBM (WHO IV stadium), who developed HSE shortly after RT. We discuss the possible association between tumor and opportunistic infections and the potential utility of antiviral profilaxys in HSV1 oncologic patients who undergo RT. In September 2016 an Italian 76-year-old woman received the diagnosis of right frontal post-central GBM by cerebral biopsy. Neurosurgical resection was not possible because of tumor's size and location. Therefore she was treated with RT and subsequently adjuvant chemotherapy.

In December 2016, few weeks after RT, she was admitted to our Emergency Department presenting fever, confusion, lethargy, subacute cognitive decline

and recurrent focal seizures with left motor onset. At neurological examination she was stunned, could be aroused with vigorous stimulation, presenting localizing motor response (Glasgow Coma Scale, GCS 9), without focal deficits.

Laboratory tests including white blood cell (WBC) count and biochemical profile were unremarkable except for hyponatriemia (124 mEq/L). Chest X-ray (CXR) showed a possible lobe infiltrate, suspected for pneumonia. Brain computer tomography (CT) excluded tumor progression.

The EEG demonstrated mild generalized background slowing and 0.5-0.75 Hz lateralized periodic discharges (LPD) in left temporal region, highly suggestive of HSE (Fig. 1).

The patient underwent a lumbar puncture (LP). The CSF analysis was completely normal (WBCs 0/mm3, total protein 43 mg/dL, glucose 84 mg/dL), with a negative Gram stain.

Brain MRI showed FLAIR hyperintensity of left temporo-polar, insular and temporo-mesial lobes, involving the hippocampus and, to a lesser extent, the controlateral hemisphere, without contrast enhancement (Fig. 2).

The patient immediately received empirical antiviral therapy with Acyclovir (10 mg/kg every 8 hours), together with antibiotics for pneumonia. In the following days qualitative CSF polymerase chain reaction (PCR) confirmed positivity for HSV-1 (2857 copies/mL). She continued intravenous antiviral therapy with acyclovir, increasing doses at 15 mg/kg/day and, after 21 days, with chronic oral prophylaxis (800 mg twice a day).

Clinical conditions gradually improved. At discharge, one month after the admission, she was alert but confused (GCS 13), without focal deficits. She died eight months later because of tumor progression.

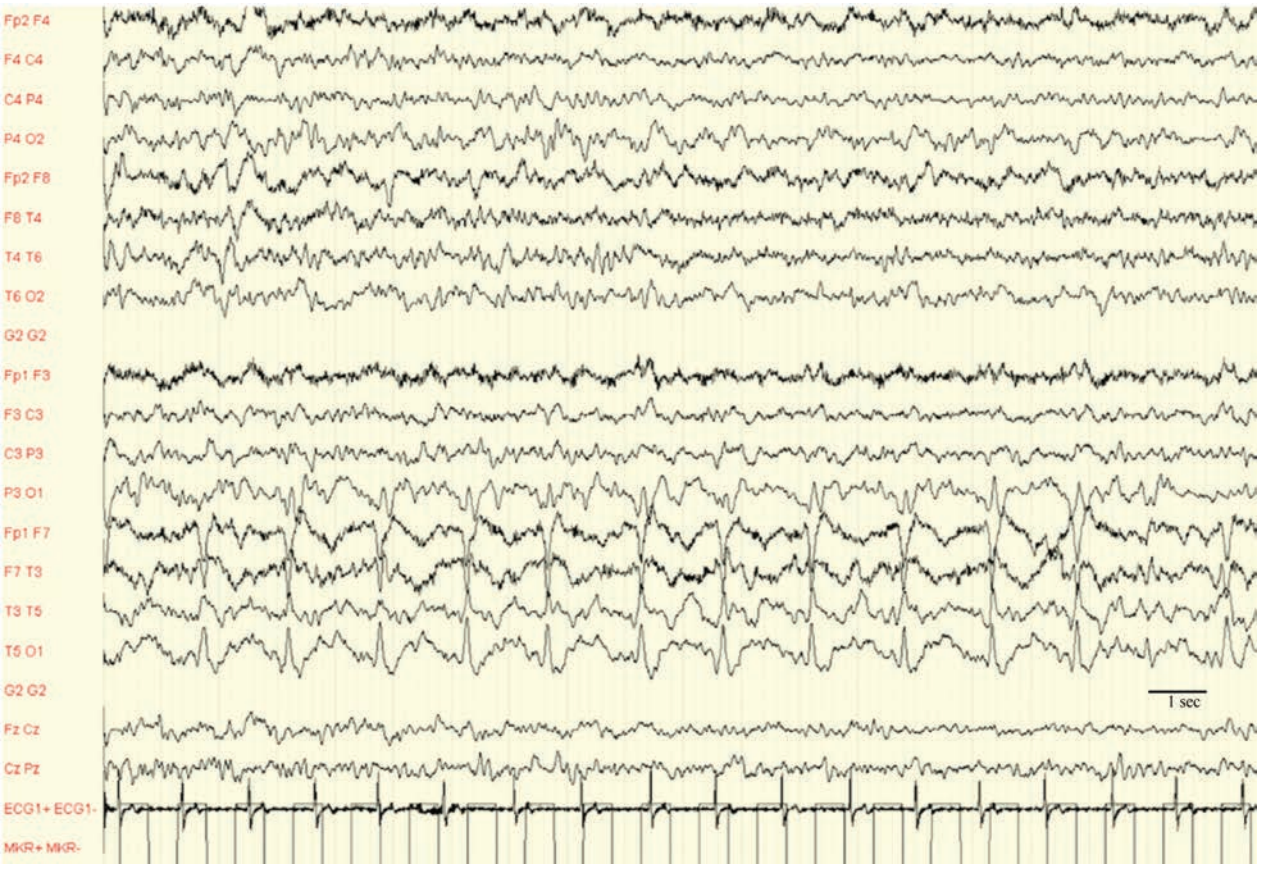


Figure 1. EEG: 0.5-0.75 Hz lateralized periodic discharges (LPD) in left temporal region, sensitivity 70 μV

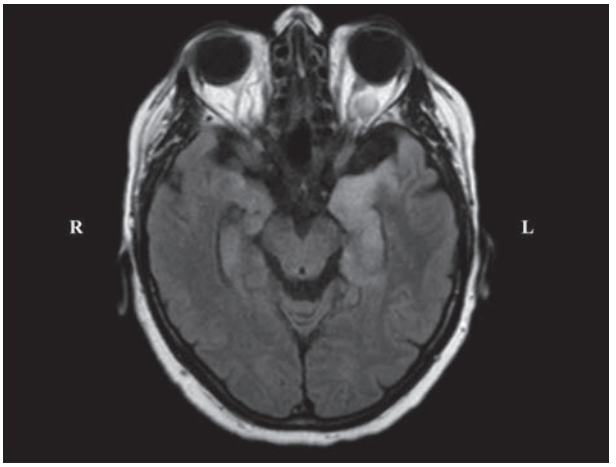


Figure 2. 3T Brain MRI: Axial FLAIR sequence, hyperintensity of left temporo-polar and mesio-temporal lobe. R: right; L: left

Conclusion

We describe the case of a patient with GBM who developed HSE after RT. Even if this eventuality has been already reported (7), the present case is noteworthy as it confirms the association between HSE and GBM and further provides evidence favoring the preventive therapy with antiviral drugs in patient with GBM who undergo RT.

Antiviral prophylaxis with acyclovir has been suggested in a number of haematological malignancies to prevent HSV reactivation during the period of leucopenia (7). Acyclovir is usually well tolerated and major side effects are rare. On these grounds, considering the poor prognosis that HSE has shown in patients with GBM, a benefit/risk discussion for the utility of the introduction of acyclovir prophylaxis during RT, may be pertinent.

GBM is the most common brain tumor in adults, with a median age at diagnosis of 61 years. Histopathologic features are necrosis and endothelial proliferation, resulting in the assignment of grade IV, the highest grade in the WHO classification.

RT is still the cornerstone of GMB treatment and, together with chemotherapy, in particular Temozolomide, it improves the median survival of patients (8), although this remains extremely rare up to 5 years from the diagnosis.

Evidence-based clinical practice guidelines from American Society for Radiation suggest that, following biopsy or resection, GBM patients with reasonable performance status up to 70 years of age should receive conventionally fractionated RT (eg, 60 Gy in 2- Gy fractions) with concurrent and adjuvant Temozolomide (9). However, radiotherapic treatment-induced central nervous system (CNS) toxicity remains an important issue in these patients. In particular patients could develop acute encephalopathy characterized by headache, fever, vomiting, probably linked to cerebral oedema and disruption of blood brain barrier (BBE) (10).

Moreover, even if neurological decline during treatment is more likely due to RT side effects or tumor progression (11), opportunistic infections could represent one of yet underestimated cause of deterioration. Viral infection in immunocompromised patients could be triggered by several factors: immunodepressive environment induced by the tumor 'per se' (2), steroid treatment and RT.

In this contest, precious recognition of concomitant infections is crucial for patients with GBM treated with RT, being the prognosis of even treatable conditions worse in immunocompromised patients (11).

Several case reports of HSE following brain RT are described in literature (5, 12, 13). HSE incidence in patients with cancer undergoing WBRT has been estimated 2000 times higher than in general population (12-13). In addition, anedoctal reports of GBM improvement after treatment with acyclovir raise the question of wheter anti-HSV therapy may play an adjunctive role in GBM treatment (14).

In our case the prompt introduction of acyclovir therapy determine neurological improvement and the patient died eight months later because of tumor progression and not for complication related to HSE.

The HSE presentation in immunocompromised patients might be tricky to be recognize especially as the gold standard for diagnosis (i.e. CSF analysis) might be misleading. In our case, indeed, CSF analysis was completely normal, without pleocytosis or evidence of inflammatory markers.

The normal cell count and poor evidence of CNS inflammatory might be possibly caused by an anergic immune comportmental cellular responce (7). The HSE diagnosis in our patient was driven by EEG and MRI

scan. Bilateral asymmetric mesial temporal lobe hyperintensity in FLAIR was observed that represents a typical finding in HSE (15). EEG is highly informative for HSE, since unilateral LPDs from the temporal lobe are a key diagnostic clue for the disease (16). In our case, the presence of LPD at 0.5-0.75 Hz, immediately address the clinician to the diagnosis of HSE (Fig. 1).

Overall mortality of HSE has decreased from 70% to <20% after the introduction of antiviral treatment. Among survivors, more than 60% have moderate-severe neurological deficits and only 2-3% of patients will survive with fully normal neurological functions. The cognitive domains most frequently impaired are anterograde memory, retrograde memory, executive functions and language. All of these outcomes are worsened if treatment is delayed (4).

Current guidelines recommend treatment of HSE in adults with intravenous acyclovir 10mg/kg given every 8 hours for 2-3 weeks, even if duration of therapy is not clearly defined in the literature. Some authors suggested the need for follow-up CSF analysis prior to discontinuation of antiviral therapy (4).

Considering the challenging diagnosis of HSE in immunocompromised patients affected by GBM, due to atypical clinical findings and potential negativity of laboratory examinations, we highlight the importance of early EEG and brain MRI in supporting diagnosis. Then, considering the favourable risk-benefit balance of acyclovir profilaxys, we suggest the introduction of antiviral treatment in GBM patients at high risk of HSE develoment, such as those who underwent brain RT.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Nam TS, Choi KH, Kim MK, Cho KH. Glioblastoma mimicking herpes sim plex encephalitis. J Korean Neurosurg Soc 2011; 50: 119-22.
2. Mangani D, Weller M, Roth P. The network of immunosuppressive pathways in glioblastoma. Biochem Pharmacol 2017; 15; 130: 1-9.
3. Gately L, McLachlan SA, Dowling A, Philip J. Life beyond a diagnosis of glioblastoma: a systematic review of the literature. J Cancer Surviv 2017; 11: 447-452.
4. Skelly MJ, Burger AA, Adekola O. Herpes simplex virus-1 encephalitis: a review of current disease management with three case reports. Antivir Chem Chemother 2012; 25; 23: 13-8.
5. Sermer DJ, Woodley JL, Thomas CA, Hedlund JA. Herpes simplex encephalitis as a complication of whole-brain radiotherapy: a case report and review of the literature. Case Rep Oncol 2014; 21; 7: 774-9.
6. Cunha BA, Talmasov D, Connolly JJ. Herpes Simplex Virus (HSV-1) Encephalitis Mimicking Glioblastoma: Case Report and Review of the Literature. J Clin Med 2014 12; 3: 1392-401.
7. Berzero G, Di Stefano AL, Dehais C, Sanson M, Gaviani P, Silvani A, et al. Herpes simplex encephalitis in glioma patients: a challenging diagnosis. J Neurol Neurosurg Psychiatry 2015; 86: 374-7.
8. Gzell C, Back M, Wheeler H, Bailey D, Foote M. Radiotherapy in Glioblastoma: the Past, the Present and the Future. Clin Oncol (R Coll Radiol) 2017; 29: 15-25.
9. Cabrera AR, Kirkpatrick JP, Fiveash JB, Shih HA, Koay EJ, Lutz S, et al. Radiation therapy for glioblastoma: Executive summary of an American Society for Radiation Oncology Evidence-Based Clinical Practice Guideline. Pract Radiat Oncol 2016; 6: 217-25.
10. Soussain C, Ricard D, Fike JR, Mazeron JJ, Psimaras D, Delattre JY. CNS complications of radiotherapy and chemotherapy. Lancet 2009; 7; 374: 1639-51.
11. Tan IL, McArthur JC, Venkatesan A, et al. (2012) Soussain C, Ricard D, Fike JR, Mazeron JJ, Psimaras D, Delattre JY. CNS complications of radiotherapy and chemotherapy. Lancet 2009 Nov 7; 374(9701): 1639-51.
12. Graber JJ, Rosenblum MK, DeAngelis LM. Herpes simplex encephalitis in patients with cancer. J Neurooncol 2011; 105: 415-21.
13. Chakraborty S, Donner M, Colan D. Fatal herpes encephalitis in a patient with small cell lung cancer following prophylactic cranial radiation--a case report with review of literature. Anticancer Res 2013; 33: 3263-8.
14. Söderlund J, Erhardt S, Kast RE. Acyclovir inhibition of IDO to decrease Tregs as a glioblastoma treatment adjunct. J Neuroinflammation 2010; 6; 7: 44.
15. Bradshaw MJ, Venkatesan A. Herpes Simplex Virus-1 Encephalitis in Adults: Pathophysiology, Diagnosis, and Management. Neurotherapeutics 2016; 13: 493-508.
16. Schomer DL, Lopes de Silva FH Niedermeyer's Electroencephalography: Basic Principles, Clinical Applications and Related Fields. 2010 Lippincott Williams & Wilkins; 2011-1275.

Received: 14 February 2019
Accepted: 8 March 2019
Correspondence:
Anna Elisabetta Vaudano, MD in Neurology
University of Parma, Neurology Departement,
Via Gramsci 14, 43126 Parma (PR), Italy
Tel. 00390521703512
E-mail: annavaudano@googlemail.com

Acta Biomed 2019; Vol. 90, N. 2: 331-332 DOI: 10.23750/abm.v90i2.6995

Case report

Tetralogy of Fallot whit a "contralateral" ductus arteriosus

Lucio Careddu[1], Francesco D Petridis[1], Emanuela Angeli[1], Giorgio Romano[1], Andrea Donti[2], Gaetano D. Gargiulo[1]

[1] Pediatric Cardiac Surgery and Adult Congenital Unit, S. Orsola-Malpighi Hospital, University of Bologna Medical School, Bologna, Italy; [2] Pediatric Cardiology and Adult Congenital Unit, S. Orsola-Malpighi Hospital, University of Bologna Medical School, Bologna, Italy

Summary. Two neonates were taken shortly after birth to our unit with a prenatal diagnosis of [S,D,S] Tetralogy of Fallot with pulmonary atresia and "unusual" aorta to pulmonary connection. The echocardiogram confirmed the main diagnosis showing: a left aortic arch with a vascular connection between the right innominate artery and the origin of the right pulmonary artery in patient A; and right aortic arch with a vascular connection between the left innominate artery and the origin of the left pulmonary artery in patient B. (www.actabiomedica.it)

Key words: congenital heart disease (CHD), aortic arch, Tetralogy of Fallot

Case Report

According to the Edwards hypothetical double arch model, each embryo has two ducti arteriosi, derived from the right and left sixth pair of aortic arches, respectively. Usually, when a left aortic arch develops the left ductus arteriosus (homolateral) persists, whether when a right aortic arch develops the right ductus persists instead. Nevertheless rarely, the homolateral ductus regresses, and the contralateral may persist as an alternative, thus connecting the base of the subclavian artery (the one branching from the innominate artery) and the pulmonary bifurcation (1).

We present two neonates with prenatal diagnosis of [S,D,S] Tetralogy of Fallot with pulmonary atresia and left aortic arch with right ductus arteriosus: between the right subclavian artery and the origin of the right pulmonary artery in patient A; and right aortic arch with left ductus arteriosus: between the left subclavian artery and the origin of the left pulmonary artery in patient B (Fig. 1).

Both patients successfully underwent central BT-Shunt and ductus ligation and completed the repair successfully after one year and 18 months respectively.

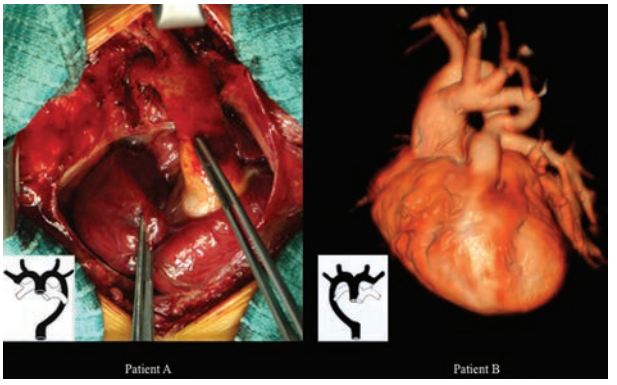


Figure 1. Patient A: Surgical view. Patient B: 3D Computerized tomographic angiography

"Contralateral" ductus arteriosus means: right ductus arteriosus in a left aortic arch (Patient A) and left ductus arteriosus in right aortic arch (Patient B).

Embryologically there are potentially two ducti arteriosi: one "usual" or "homolateral" to the aortic arch, coming off the aortic isthmus; a second one "unusual" or "contralateral" to the aortic arch, coming off the innominate artery.

Therefore patient A has a left aortic arch (to the left of the trachea, riding the left bronchus), with a

right ductus arteriosus from the right subclavian artery to the right pulmonary artery (to the right side of the trachea) (2). Patient B instead has a right aortic arch (to the right of the trachea, riding the right bronchus), with a left ductus arteriosus running from the left subclavian artery to the left pulmonary (to the left side of the trachea) (3).

Discussion

Differential diagnosis in such patients should be performed between a small tubular and very high aortopulmonary window, Major Aorto-pulmonary collateral artery (MAPCA), persistent 5th aortic arch (systemic to pulmonary connection variant), homolateral or controlateral ductus arteriosus. MAPCA is a not PGE1 responsive tortuous vascular structure originating from the descending aorta and with a persistent 5th aortic arch, "systemic-to-pulmonary variant", which runs from the distal ascending aorta to the pulmonary bifurcation, but parallel or homolateral to the aortic arch (same tracheal side) (4).

Persistent 5th aortic arch appears as a vascular structure running inferior and parallel to the "real" aortic arch from the innominate artery to the left subclavian artery (5).

It can be associated with major congenital heart malformations involving the systemic or the pulmonary circuits.It usually has no clinical significance but can be either, beneficial as in systemic outflow tract obstructions or cause hemodynamic compromise when associated with a significant left to right shunt (6).

An early diagnosis may improve outcomes in such kind of anatomy and should be every time checked to avoid complication during staged approach for Tetralogy of Fallot/PA-VSD and could be associated with important multisystem morbidity and mortality (7, 8).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

Reference

1. Freedom RM, Moes CAF, Pelech A, Smallhorn J, Rabinovitch M, Olley PM, et al. Bilateral ductus arteriosus (or remnant): An analysis of 27 patients. The American Journal of Cardiology 1984; 53(7): 884-91.
2. Gul A, Gungorduk K, Yildirim G, Gedikbasi A, Borna H, Omeroglu RE. Prenatal sonographic features of persistent right ductus arteriosis with a left aortic arch. J Obstet Gynaecol 2013; 33(1): 86-7.
3. Steinberg I. Left-Sided Patent Ductus Arteriosus and Right-Sided Aortic Arch. Angiocardiographic Findings in Three Cases. Circulation 1963; 28: 1138-42.
4. Gerlis LM, Ho SY, Anderson RH, Da Costa P. Persistent 5th aortic arch--a great pretender: three new covert cases. Int J Cardiol 1989; 23(2): 239-47.
5. Furtado AD, Manohar SR, Pradhan SK, Pillai V, Karunakaran J. Persistent left fifth aortic arch with pentalogy of Fallot. Thorac Cardiovasc Surg 2012 Apr; 60(3): 233-5. doi: 10.1055/s-0030-1271013. Epub 2011 May 9.
6. Al Akhfash AA, Al Mutairi MB, Al Habshan FM. Persistent fifth aortic arch diagnosed by echocardiography and confirmed by angiography: Case report and literature review. J Saudi Heart Assoc 2009 Oct; 21(4): 245-248.
7. Jedeikin R, Rheuban KS, Carpenter MA, Kron IL. Ductal origin of the left pulmonary artery in severe tetralogy of Fallot: Problems in management. Pediatric Cardiology 1984; 5(4): 323-6.
8. Trivedi KR, Karamlou T, Yoo S-J, Williams WG, Freedom RM, McCrindle BW. Outcomes in 45 Children With Ductal Origin of the Distal Pulmonary Artery. The Annals of Thoracic Surgery 2006; 81(3): 950-7.

Received: 10 January 2018
Accepted: 21 February 2018
Correspondence:
Lucio Careddu, MD
Pediatric Cardiac Surgery and Adult Congenital Unit,
S. Orsola-Malpighi Hospital,
Via Massarenti n. 9 - 40138 Bologna, Italy
Tel. (39)-051-6363218
Fax (39)-051-6363157
E-mail: lucio.careddu3@unibo.it

Acta Biomed 2019; Vol. 90, N. 2: 333-338 DOI: 10.23750/abm.v90i2.6866

Case report

Unusual meningitis caused by non-typhoid Salmonella in an italian infant: a case report

Monica Ficara[1], Valentina Cenciarelli[1], Lisa Montanari[1], Beatrice Righi[1], Simone Fontjin[1], Greta Cingolani[1], Barbara Predieri[3], Alberto Berardi[2], Laura Lucaccioni[2], Lorenzo Iughetti[1,3]

[1] Post-graduate School of Pediatrics, University of Modena and Reggio Emilia, Modena, Italy; [2] Neonatal Intensive Care Unit, Department of Medical and Surgical Sciences of the Mother, Children and Adults, University of Modena and Reggio Emilia, Modena, Italy; [3] Pediatric Unit, Department of Medical and Surgical Sciences for Mothers, Children and Adults, University of Modena and Reggio Emilia, Modena, Italy

Summary. *Background:* Non-typhoid Salmonella (NTS) is an important cause of bacterial meningitis in newborn and infants in developing countries, but rarely in industrialized ones. We describe an unusual presentation of bacterial meningitis in an infant, focusing on his diagnostic and therapeutic management. *Case report:* An Italian two-month old male presented high fever and diarrhea with blood, associated with irritability. Inflammatory markers were high, cerebrospinal fluid analysis was compatible with bacterial meningitides but microbiological investigations were negative. Salmonella enteritidis was isolated from blood. Cerebral ultrasound and MRI showed periencephalic collection of purulent material. Specific antibiotic therapy with cefotaxime was initiated with improvement of clinical conditions and blood tests. Brain MRI follow up improved progressively. *Conclusions:* Most of pediatric patients with NTS infection develop self-limited gastroenteritis, but in 3-8% of the cases complications such as bacteremia and meningitis may occur, especially in weak patients. Cerebral imaging can be useful to identify neurological findings. Although there is no standardized treatment for this condition, specific antibiotic therapy for at least four weeks is recommended. Neuroimaging follow up is required due to high risk of relapse. (www.actabiomedica.it)

Key words: meningitis, non-typhoid Salmonella, Salmonella enteritidis, developing countries, developed countries, antibiotic therapy, neuroimaging

Most of pediatric patients with Non-typhoid Salmonella (NTS) infection develop self-limited gastroenteritis. However, 3-8% of the patients present secondary bacteremia, followed by meningitis, osteomyelitis, endocarditis, arthritis, urinary-tract infection and pneumonia (1, 2). The risk of invasive salmonellosis is higher in case of immunocompromised individuals, patients with hemoglobinopathies and hemolytic anemias or in neonates (3). Salmonella is identified as pathogen in 1% or less of confirmed cases of bacterial meningitis in newborn and infants (4). Salmonella species are a leading cause of Gram-negative bacterial meningitis in the developing countries, although rarely seen in developed ones (5), being associated with high complications and mortality rate (4). We describe a NTS meningitis in a two-month old boy focusing on the clinical management and follow up of these rare and severe cases.

Case presentation

An Italian two-month old male infant was admitted to our Pediatric Emergency Department with high fever (TC 39.3°C) irritability, poor appetite and diarrhea with blood traces. His past medical history was

silent. He was bottle fed. The mother had a history of one-day diarrhea without fever two days before.

He presented with pulsating bregmatic fontanel, no neck stiffness; Kernig's and Brudzinski's signs were negative. Chest, cardiac and abdominal examination did not reveal any abnormality. He was admitted to the medical ward with alert for isolation and rapid investigation. Blood test showed mild increase of inflammatory markers (Platelets 498.000/µl, RCP 2,8 mg/dl). Lumbar puncture was performed. CSF was turbid with predominating polymorphs (950 cells/µl) in association with raised protein (158 mg/dl). Empirical therapy with ampicillin, gentamicin, cefotaxime and acyclovir was started. CSF microbiological investigations (culture and molecular biology) were negative, while Salmonella enteritidis was isolated in blood. Antibiotic treatment was shifted to intravenous cefotaxime at 300 mg/kg/die and performed for six weeks. During the first three days of recovery, the patient presented short episodes of staring, followed by irritable crying. Cerebral ultrasound was performed, and revealed periencefalic purulent suffusion, confirmed by the brain MRI (Fig. 1a). Urgent brain MRI excluded the development of intracranial hypertension. Fever decreased after four days of recovery. Many stool samples were collected, but Salmonella was never found during the hospitalization. A month later, cerebral MRI showed a persistent frontal purulent soffusion (5 mm diameter) although CFS was negative. At the end of the antibiotic therapy, a third cerebral MRI revealed a partial re-absorption of the frontal collection (Fig. 1b). After the discharge (7 weeks from admission), stool samples revealed the presence of Salmonella enteritidis. Blood tests were negative for ongoing or recurring Salmonella infections. Cerebral MRI showed a progressive reduction of the frontal purulent material.

Discussion

Most part of the cases of meningitidis caused by Salmonella in children reported in literature occur in developing countries. NTS invasive infections have often worse-than-expected outcome, despite adequate antimicrobial therapy, because of multiple factors [Table 1. (6-23)]. Developing countries are endemic areas for HIV infection, parasitosis (such as schistosomiasis) and sickle cell anemia, known risk factors able to increase the infectious complications (9). Moreover, the delayed beginning of targeted antibiotic therapies and the inadequate duration associated with poor health awareness status, may play a significant role on prognosis (17, 23). High rate of multi-drug resistant Salmonella strains makes therapeutic choice difficult (22).

The role of imaging findings in Salmonella meningoencephalitis is not clear (24). MRI can be either normal or showing diffuse cerebral vasogenic

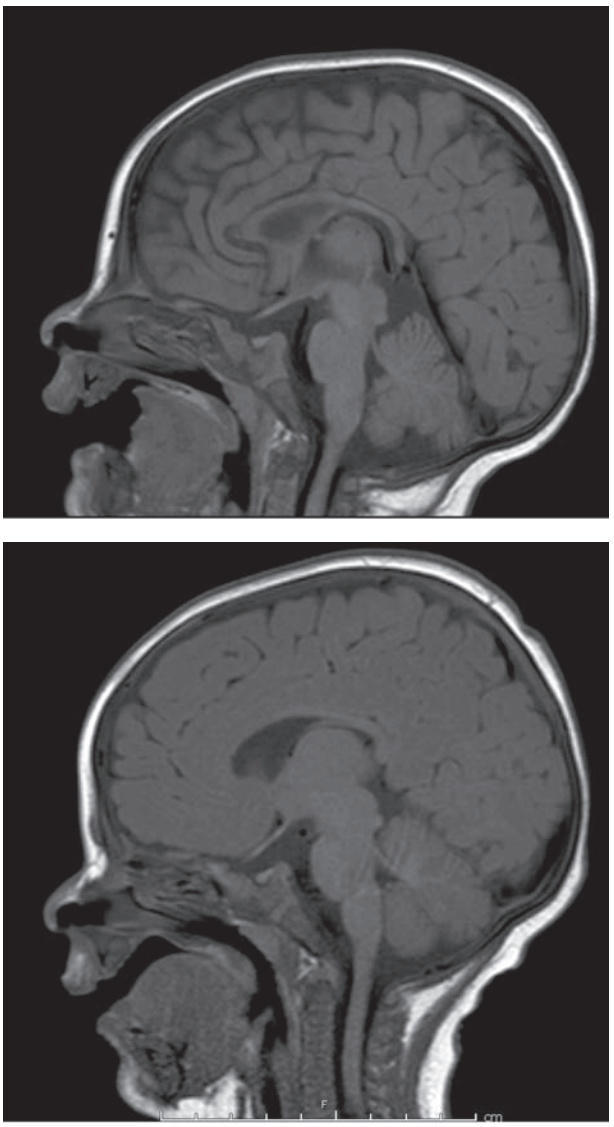


Figure 1. Brain MRI imaging at admission (1a) at the end of the antibiotic treatment (1b).

Table 1. Reports on cases of meningitis caused by Salmonella in the last ten years

Authors	Journal	Year	Antibiotics	Duration	Outcome
Ploton MC et al. (6)	J Paediatr Child Health	2017	Intravenous combination of cefotaxime and ciprofloxacin (for 6 weeks) + ciprofloxacin per os (for 6 weeks)	12 weeks	Good
De Malet et al. (7)	Case Rep Infect Dis	2016	Intravenous cefotaxime (200 mg/Kg/die)	3 weeks	Good
Ricard C et al. (8)	Arch Pediatr	2015	Intravenous ciprofloxacin	15 days	Good
Chacha F et al. (9)	BMC Res Notes	2015	Intravenous ceftriaxone (1 g/die))	2 weeks	Good
Heaton PA et al. (10)	Br J Hosp Med (Lond)	2015	Cefotaxime	6 weeks	Good
Tuan ĐQ et al. (11)	Jpn J Infect Dis.	2015	Case1: ceftriaxone (100 mg/Kg/die) Case2: ceftriaxone (100 mg/Kg/die) + chloramphenicol (100 mg/Kg/die) Case3: imipenem (50 mg/Kg/die) + ciprofloxacin (30 mg/Kg/die) Case4: imipenem + ciprofloxacin, then combination of chloramphenicol and ciprofloxacin	Case 1: 4 weeks Case 2: 7 weeks Case 3: 8 weeks Case 4: 6 weeks	Case 1: recurrence of Salmonella meningitis Case 2: good Case 3: intracranial complications Case 4: good
Bowe AC et al. (12)	J Perinatol.	2014	Cefotaxime	-	Poor (on day 3: poor feeding, lethargy, apnea, bradycardia)
Rai B et al. (13)	BMJ Case Rep	2014	Ceftriaxone	21 days	Good
Adhikary R et al. (14)	Indian J Crit Care Med	2013	Intravenous combination of ceftriaxone, chloramphenicol and ciprofloxacin	After 25 days the patient's therapy was modified because of nosocomial pneumonia	Poor
AJ Johan et al. (15)	Southeast Asian J Trop Med Public Health	2013	Intravenous ceftriaxone, then meropenem because of intracranial complications	Ceftriaxone for 3 weeks Meropenem for 11 weeks	Good
Singhal V et al. (16)	J Clin Diagn Res	2012	Intravenous combination of ceftriaxone and amikacin, then meropenem and netilmycin because of neurological complications	Ceftriaxone plus amikacin for 3 weeks Meropenem plus netilmycin for 14 days	Good
Fomda BA et al. (17)	Indian J Med Microbiol	2012	Intravenous combination of ciprofloxacin (10 mg/Kg twice daily) and ceftriaxone (100 mg/Kg/die)	3 weeks, then other 6 weeks because of recurrent meningitis	Good

(continued)

Table 1 ***(continued)*****.** Reports on cases of meningitis caused by Salmonella in the last ten years

Authors	Journal	Year	Antibiotics	Duration	Outcome
Olariu A et al. (18)	BMJ Case Rep.	2012	Intravenous ceftriaxone (80 mg/Kg/die once a day)	3 weeks	Good
Wu HM et al. (19)	BMC Infect Dis	2011	Most of patients of this study received third-generation cephalosporins, combined with chloramphenicol or ampicillin	-	-
Ghais A et al. (20)	Eur J Pediatr	2009	Intravenous ceftriaxone	4 weeks	Good
Guillaumat C et al. (21)	Arch Pediatr.	2008	Intravenous combination of third-generation cephalosporins and quinolones	At least 3 weeks	-
L. Sangaré et al. (22)	Bull Soc Pathol Exot.	2007	56 cases of meningitis by Salmonella: third-generation cephalosporins and aminoglycosides effective	-	Neurological complications only in one case treated with ceftriaxone and chloramphenicol
Bayraktar MR et al. (23)	Indian J Pediatr.	2007	Meropenem	-	Poor (death on the second day after the initiation of meropenem therapy: diagnostic delay?)

edema, edema of splenium, and focal white matter edema associated with cerebritis (25). MRI can be useful to identify neurological complications associated with Salmonella meningitis such as subdural effusion/empyema, abscesses, ventriculitis, cerebritis, hydrocephalus, venous thrombosis, and infarct (26). In our case, MRI showed a periencefalic collection of purulent material, then resolved. Neurological complications and sequelae (mental retardation, different forms of cerebral palsy, visual and hearing deficit) are very common (27). A retrospective study analyzed the long-term outcomes of the cases of Salmonella meningitis from 1982 to 1994 in Taiwan. Among the twenty-four patients, fifteen presented seizures before their admission to the hospital, and thirteen during the hospitalization. Acute complications included prolonged seizures (100%), hydrocephalus (50%), subdural collection (42%), cerebral infarction (33%), ventriculitis (25%), empyema (13%), intracranial abscess (8%), and cranial nerve palsy (8%). Three patients died. The long-term neurological sequelae consisted of language disorders, motor disability, mental delay, epilepsy, sensorineural hearing loss, visual deficits, abducens nerve palsy, microcephaly, and hydrocephalus (19). In our case, the patient is following periodic clinical controls, but up to now his neurological development results normal. Neuroimaging studies are recommended for every case of Salmonella meningitis even if the patient has presented an apparent clinical resolution and optimal response to antibiotics, due to risk of relapse (5).

There are different recommendations about the need of further CSF examination. Price et al. suggest routine follow-up lumbar punctures after the first negative CSF culture only if clinically indicated (4). According to the normalization of brain MRI after two

months from the treatment ending, we decided not to perform a new lumbar puncture.

Medical treatment of meningitides caused by Salmonella is very difficult and not standardized. In 2003, Owosu-Ofori et al. described two cases of Salmonella meningitis, suggesting that conventional antibiotics (ampicillin, cloramphenicol and cotrimoxazole) had a minimal role in treatment Salmonella meningitis (they had a cure rate of 41.2%, a relapse rate of 11.8%, and an associated mortality of 44.7%). One of the problems with chloramphenicol is that it is bacteriostatic against Salmonella. Optimum management of bacterial meningitis requires antibiotic(s) with bactericidal action (28). Fluoroquinolones (ciprofloxacin) showed a cure rate of 88.9%, while the third-generation cephalosporins (cefotaxime or ceftriaxone) had a cure rate of 84.6%. One of the main concerns in using ciprofloxacin is its potential joint toxicity and cartilage destruction in children. Fluoroquinolones have a lot of positive aspects: high bioavailability (near 100%) following oral administration, excellent penetration into many tissues (including CSF and brain), and good intracellular diffusion. The American Academy of Pediatrics recommends the treatment for Salmonella meningitis with cefotaxime or ceftriaxone with or without fluoroqhinolone for 4 weeks or more. However, cases of relapse following the four-week treatment have been reported. A combination of ciprofloxacin and ceftriaxone or cefotaxime has been suggested especially for the treatment of cerebral abscesses by Salmonella spp (4).

Conclusion

We described a rare case of NTS infection in an immunocompetent patient living in an industrialized country. The infant developed meningitis as complication of systemic infection probably due to his early age. According to our experience, an early diagnosis based on recognition of acute neurological signs and laboratory findings associated to a prompt and appropriated antibiotic therapy for at least four to six weeks can improve the outcome of the patient and reduce the risk of neurological sequelae. Neuroimaging follow up together with accurate neurological clinical examination, is required to prevent and reduce the high risk of relapse.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Shimoni Z, Pitlik S, Leibovici L, et al. Nontyphoid Salmonella bacteremia: age-related differences in clinical presentation, bacteriology, and outcome. Clin Infect Dis 1999; 28: 822-7.
2. Weinberger M, Andorn N, Agmon V, Cohen D, Shohat T, Pitlik SD. Blood invasiveness of Salmonella enterica as a function of age and serotype. Epidemiol Infect 2004; 132: 1023-8.
3. Galanakis E, Bitsori M, Maraki S, Giannakopoulou C, Samonis G, Tselentis Y. Invasive non-typhoidal salmonellosis in immunocompetent infants and children. Int J Infect Dis 2007; 11: 36-9.
4. Price EH, de Louvois J, Workman MR. Antibiotics for Salmonella meningitis in children. J Antimicrob Chemother 2000; 46: 653-5.
5. Owusu-Ofori A, Scheld WM. Treatment of Salmonella meningitis: two case reports and a review of the literature. Int J Infect Dis 2003; 7: 53-60.
6. Ploton MC, Gaschignard J, Lemaitre C, et al. Salmonella Typhimurium bacteraemia complicated bymeningitis and brain abscess in a 3-month-old boy. J Paediatr Child Health 2017; 53: 204-205.
7. De Malet A, Ingerto S, Gañán I. Meningitis Caused by Salmonella Newport in a Five-Year-Old Child. Case Reports in Infectious Diseases, vol. 2016, Article ID 2145805, 4 pages, 2016.
8. Ricard C, Mellentin J, Ben Abdallah Chabchoub R et al. Salmonella meningitis in an infant due to a pet turtle. Arch Pediatr 2015; 22: 605-7.
9. Chacha F, Mshana SE, Mirambo MM et al. Salmonella Typhi meningitis in a 9-year old boy with urinary schistosomiasis: a case report. BMC Res Notes 2015; 3; 8: 64.
10. Heaton PA, Mazhar H, Nabahi A, Fernando AM, Paul SP. Neonatal meningitis and septicaemia caused by Salmonella agama. Br J Hosp Med (Lond) 2015; 76: 484-5.
11. Tuan ĐQ, Hung PH, Mai PX et al. Salmonella meningitis: a report from National Hue Central Hospital, Vietnam. Jpn J Infect Dis 2015; 68:30-2.
12. Bowe AC, Fischer M, Waggoner-Fountain LA, Heinan KC, Goodkin HP, Zanelli SA. Salmonella berta meningitis in a term neonate. J Perinatol 2014; 34: 798-9.
13. Rai B, Utekar T, Ray R. Preterm delivery and neonatal meningitis due to transplacental acquisition of non-typhoidal Salmonella serovar montevideo. BMJ Case Rep 2014 29; 2014.

14. Adhikary R, Joshi S, Ramakrishnan M. Salmonella typhimurium meningitis in infancy. Indian J Crit Care Med 2013 ;17: 392-3.
15. Johan AJ, Hung LC, Norlijah O. Salmonella enteritidis ventriculitis. Southeast Asian J Trop Med Public Health 2013; 44: 456-9.
16. Singhal V, Ek S, Sm R, Coutinho A. Neonatal salmonella typhi meningitis: a rare entity. J Clin Diagn Res 2012; 6: 1433-4.
17. Fomda BA, Charoo BA, Bhat JA, Reyaz N, Maroof P, Naik MI. Recurrent meningitis due to Salmonella enteritidis: a case report from Kashmir India. Indian J Med Microbiol 2012; 30: 474-6.
18. Olariu A, Jain S, Gupta AK. Salmonella kingabwa meningitis in a neonate. BMJ Case Rep 2012 Feb 25; 2012.
19. Wu HM, Huang WY, Lee ML, Yang AD, Chaou KP, Hsieh LY. Clinical features, acute complications, and outcome of Salmonella meningitis in children under one year of age in Taiwan. BMC Infect Dis 2011; 27; 11: 30.
20. Ghais A, Armano R, Menten R, Mathot M, Zech F, Nassogne MC. Meningitis with subdural empyema due to non-typhoid Salmonella in a 9-month-old girl. Eur J Pediatr 2009; 168: 1537-40.
21. Guillaumat C, Dang-Duy TL, Levy C, Cohen R, Leblanc A; Groupe des pédiatres et microbiologistes de l'Observatoire National des méningites. Salmonella meningitis in newborns and infants. The importance of fluoroquinolones. Arch Pediatr 2008; 15 Suppl 3: S161-6.
22. Sangaré L, Kienou M, Lompo P et al. Salmonella meningitis in Ouagadougou, Burkina Faso, from 2000 to 2004. Bull Soc Pathol Exot 2007; 100: 53-6.
23. Bayraktar MR, Yetkin G, Iseri L. Infantile meningitis due to Salmonella enteritidis. Indian J Pediatr 2007; 74: 206.
24. Chidhara S, Rangasami R, Chandrasekharan A. Magnetic resonance imaging and magnetic resonance spectroscopy in Salmonella meningoencephalitis. J Pediatr Neurosci 2016; 11: 88-90.
25. Ahmed M, Sureka J, Mathew V, Jakkani RK, Abhilash KP. Magnetic resonance imaging findings in a fatal case of Salmonella typhi-associated encephalopathy: a case report and literature review. Neurol India 2011; 59: 270-2.
26. Rodriguez RE, Valero V, Watanakunakorn C. Salmonella focal intracranial infections: review of the world literature (1884-1984) and report of an unusual case. Rev Infect Dis 1986; 8: 31-41
27. Lee WS, Puthucheary SD, Omar A. Salmonella meningitis and its complications in infants. J Paediatr Child Health 1999; 35: 379-82.
28. Scheld WM. Rationale for optimal dosing of beta-lactam antibiotics in the therapy for bacterial meningitis. Eur J Clin Microbial 1984; 3: 579-591.

Received: 6 November 2017
Accepted: 7 June 2018
Correspondence:
Prof. Lorenzo Iughetti, MD, PHD
Department of Medical and Surgical Sciences of the Mother, Children and Adults
University of Modena and Reggio Emilia,
Via del Pozzo, 71 - 41124 Modena, Italy
Tel. +39 059 422 5382
Fax +39 059 422 4583
E-mail: iughetti.lorenzo@unimore.it

Acta Biomed 2019; Vol. 90, N. 2: 339-342 DOI: 10.23750/abm.v90i2.6714

Case report

Endovascular treatment of iliac artery rupture after septic embolization

Silvia Eleonora Gazzani[1], Claudio Bianchini Massoni[2], Carla Marcato[1], Ilaria Paladini[1], Cristina Rossi[1]

[1] Unit of Diagnostic and Interventional Radiology, Department of Surgical Sciences, University of Parma, Parma, Italy; [2] Unit of Vascular Surgery, Department of Surgical Sciences, University of Parma, Parma, Italy

Summary. A 56-year man with multiple comorbidities and recent septic embolization presented claudication intermittens (Rutherford3) at right lower limb and complaint in right lower quadrant at abdominal palpation. Duplex and computed tomography angiogram (CTA) showed a 64mm-pseudo-aneurysm (PA) originating from right common iliac artery, occlusion of external iliac and patency of hypogastric artery. An urgent endovascular approach was preferred. By left brachial percutaneous access, coil embolization (Balt SPI™ and Cook MReye™) of hypogastric and common iliac artery and deployment of Amplatzer Vascular PlugII™ into the common iliac artery were performed. Completion angiography showed exclusion of PA. One-day, 3-day and 1-month CTA proofed no vascularization of PA. No fever, no leukocytosis, no signs of infection occurred during follow-up and 10-month CTA showed the complete resolution of pseudoaneurysm. (www.actabiomedica.it)

Key words: infected pseudoaneurysms, endovascular treatment, interventional radiology

Case report

A 56-year old man with multiple comorbidities (severe obesity, moderate chronic renal failure, chronic obstructive pulmonary disease, smoking, cirrhosis, atrial fibrillation, coronary artery disease) presented to the out-patient clinic complaining intermittent claudication (Rutherford category 3) at right lower limb since 2 weeks. He received mitral valve replacement with coronary artery bypass graft three months before for acute endocarditis (Streptococcus gallolyticus), followed by brain septic embolization.

Physical examination revealed absence of right lower limb pulses with mild hypothermia and abdominal pain at deep palpation of the right lower quadrant without pulsating masses. Duplex ultrasound (DUS) found a perfused abdominal mass with 69mm in diameter in the right lower quadrant with steno-occlusion of right iliac axis. An emergency computer tomography angiography (CTA) revealed a right common iliac artery pseudoaneurysm (62x64 mm) with occlusion of external iliac artery, patency of hypogastric and recanalization of the right common femoral artery.

At admission, the patient had no fever and no leukocytosis (white blood cells=6,76x10^3), with anemia (Hb=9.1 g/dl), mild chronic renal failure (creatinine: 2.1 mg/dl) and high C reactive protein (CRP=97.2 mg/l). No blood culture or positron emission tomography were performed for absence of signs of concurrent systemic infection. No systemic antibiotics was undertaken for the same reason.

The following day, the patient underwent a digital subtraction angiography (DSA) (Fig. 1) through left brachial access (6F-sheath introducer, 90cm in length).

The embolization of the hypogastric artery with coils (Balt SPI™ and Cook Mreye™) and of the com-

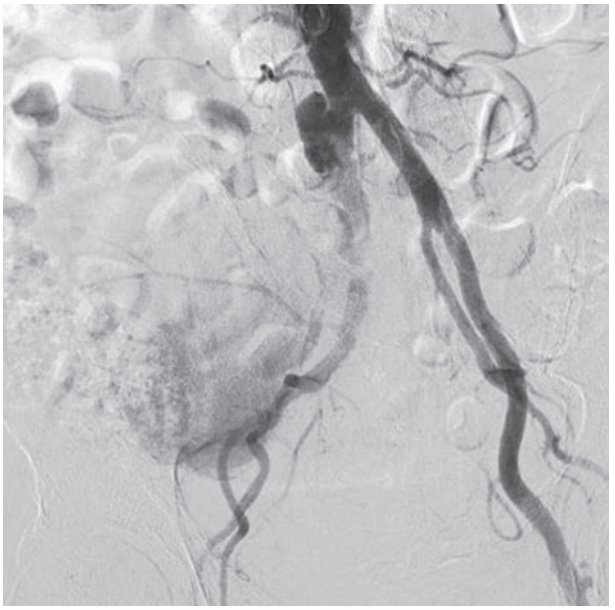


Figure 1. Digital subtraction angiography throughout left humeral access: right common iliac artery pseudoaneurysm with occlusion of external iliac artery, patency of hypogastric and recanalization of the right common femoral artery

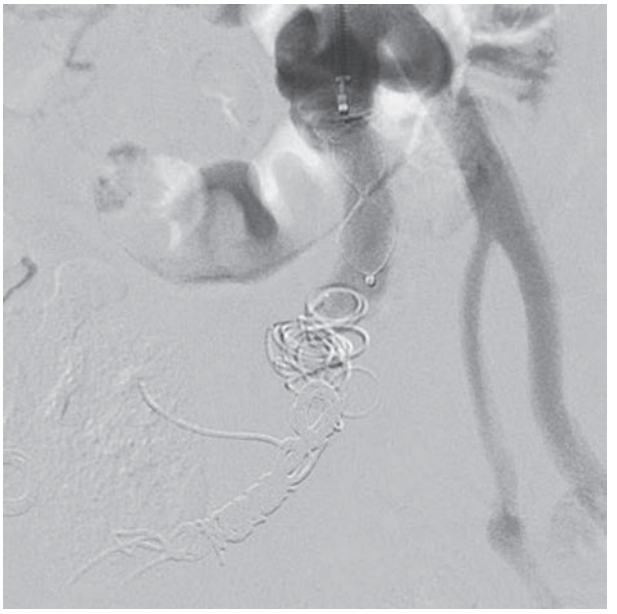


Figure 2. Post-procedural digital subtraction angiography: internal iliac artery embolization with coils (Balt SPI™ and Cook Mreye™) and with Plug at the origin of common iliac artery (6mm-Amplatzer Vascular Plug II ™). Complete exclusion of right common iliac artery, internal iliac artery and pseudoaneurysm.

mon iliac artery with Plug (16 mm-Amplatzer Vascular Plug II™) was performed.

The post-procedural DSA revealed the complete exclusion of the hypogastric artery, and the pseudoaneurysm (Fig. 2). The reperfusion of ipsilateral common femoral artery was maintained by collateral circulation.

During the post-operatory period, no hypotension or ischemia of the right lower limb occurred. A CTA confirmed the exclusion of the pseudoaneurysm. Similar findings were highlighted at CTA performed in the third post-procedural day. At discharge (6th post-procedural day), the patient had no fever, no leukocytosis (WBC=5,66x10^3) with stable anemia (Hb=9.1 g/dl) and renal function (creatinine=1.6 mg/dl). The abdominal pain disappeared, the right leg was normothermic and in hemodynamic compensation without rest pain, acute ischemia or neurological deficit.

After one month, a CTA referred the exclusion of the pseudoaneurysm with a gradual reduction in the size. After 10 months, other CTA showed the complete resolution of the pseudoaneurysm (Fig. 3) and the revascularization of the right common femoral artery by collateral circulation.

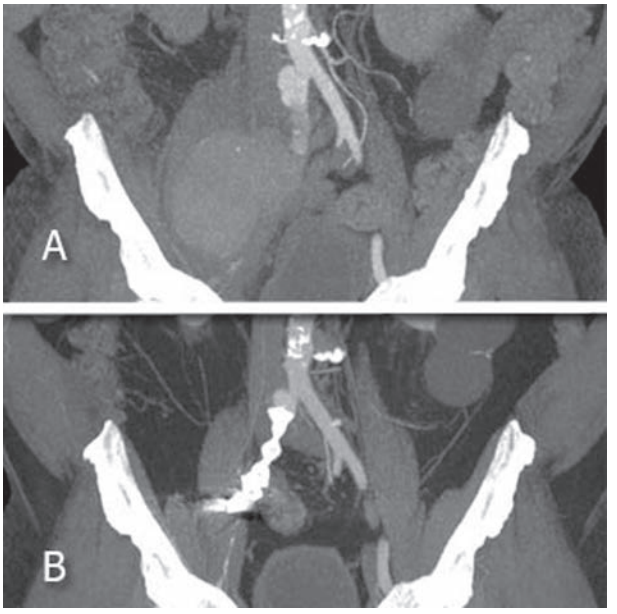


Figure 3. Computed tomography angiography: A-first exam, before the interventional radiological procedure, that shows the pseudoaneurysm.B-control after 10 months, complete resolution of the pseudoaneurysm

Discussion

An arterial pseudoaneurysm is defined as presence of blood flow outside the wall of an artery and contained in surrounding tissues. The pseudoaneurysm is due to an arterial wall rupture, and may occur in any arterial segments. Its formation developed often as complication of a percutaneous access (femoral or brachial) (1), for an aneurysm rupture (cerebral (2), abdominal (3)), for anastomotic structural failure (para-anastomotic aneurysm (4)), infection (5), or trauma (6). The arterial rupture induces a gradual formation of pseudoaneurysm that can rapidly increase, with signs of hypotension and shock, or stabilize remaining slightly symptomatic.

The current case consists in a oligo-symptomatic iliac rupture after an episode of septic embolization (3 months before). However, the pseudoaneurysm was not considered infected for the lack of systemic infection signs and no evidence of infected etiology.

Currently, the CTA is the gold standard for the evaluation of a pseudoaneurysm, especially in aortic, visceral and intracranial districts (7, 8). DUS is reserved for peripheral vessels.

The best approach and treatment depend on the characteristics of pseudoaneurysm (location, morphology, presence and extent of the bleeding), the patient's clinical condition and the experience of operators.

The treatment options are multiple and include conservative medical therapy, open surgery, endovascular treatment or hybrid treatment.

Small intracranial pseudoaneurysms, asymptomatic and without signs of growth or rupture, are generally managed with conservative treatment and follow-up by imaging (ultrasound or CTA) (9). The response to medical therapy can be variable (9): complete thrombosis of the pseudoaneurysm with reduction in size, or increase in volume that needs more aggressive therapy.

Large extracranial or intracranial pseudo-aneurysms, symptomatic or ruptured, require immediate treatment in emergency/urgency setting (7). Pseudoaneurysms secondary to arterial puncture are often treated with ultrasound-guided thrombin injection with high success rate (10). In aortic district, pseudoaneurysms following surgical repair are mainly managed by stentgraft deployment avoiding demanding open reintervention (11, 12). Open surgery remain first option in case of infected pseudoaneurysm in drug abuser (13).

In the current case, considering localization, symptoms and size of pseudoaneurysm, the treatment was mandatory. The right external iliac artery was occluded, but endovascular or in-situ surgical recanalization (stentgraft or bypass graft, respectively) was not considered for high risk of infection.

The therapeutic options were: (I) the surgical ligation of right iliac axis; (II) the right hypogastric and common iliac artery embolization and exclusion with left aorto-uniiliac endograft. A surgical revascularization of right lower limb (aorto-femoral or femoro-femoral crossover bypass) was possible in case of right lower limb ischemia.

The surgical ligation of right axis was excluded for the high surgical risk of the patient, risk of infection and follow-up failure. An endovascular treatment was undertaken deploying less material as possible. Therefore, after pseudoaneurysm exclusion with embolization, aorto-uniiliac endografting was not considered essential. During the immediate post-procedural period, close clinical and radiological monitoring with multiple CTA was needed to assess the necessity of additional procedures (aorto-uniiliac endografting or femoral-femoral crossover bypass). The aorto-uniiliac endograft was not deployed for the complete exclusion of the pseudoaneurysm and the femoral-femoral bypass was not performed for clinical stability of the right lower limb.

The result during follow-up was optimal. After 10 months, complete resolution of pseudoaneurysm and no signs of persistent infection occurred.

Conclusions

The pseudoaneurysm formation is a possible event due to many causes. For abdominal pseudoaneurysms, the symptoms are sneaky and an early diagnosis is infrequent. The CTA is the diagnostic gold standard to characterize the place, the size, morphology, and to drive the therapeutic procedure. Based on the characteristics of the patient, the injury and the availability

of operators, conservative therapy, interventional or surgical procedures are possible.

In our case, the use of endovascular treatment in urgency was effective, avoiding surgical complications and the dissemination of the infectious process. The complete resolution of the pseudoaneurysm after 10 months represents an excellent final result.

Ethical Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee standards. For this type of study, formal consent is not required.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Mlekusch W, Mlekusch I, Sabeti-Sandor S. Vascular puncture site complications - diagnosis, therapy, and prognosis. Vasa 2016 Nov; 45(6): 461-469. Epub 2016 Jun 28.
2. Nomura M, Mori K, Tamase A, Kamide T, Seki S, Iida Y, Nakano T, Kawabata Y, Kitabatake T, Nakajima T, Yasutake K, Egami K, Takahashi T, Takahashi M, Yanagimoto K. Pseudoaneurysm formation due to rupture of intracranial aneurysms: Case series and literature review. Neuroradiol J 2017 Apr; 30(2): 129-137.
3. Lu M, Weiss C, Fishman EK, Johnson PT, Verde F. Review of visceral aneurysms and pseudoaneurysms. J Comput Assist Tomogr 2015 Jan-Feb; 39(1): 1-6. doi: 10.1097/RCT.0000000000000156.
4. Pogorzelski R, Fiszer P, Toutounchi S, Szostek MM, Krajewska E, Jakuczun W, Tworus R, Skórski M. Anastomotic aneurysms after arterial reconstructive operations--literature review. Pol Przegl Chir 2014 Jan; 86(1): 48-56. doi: 10.2478/pjs-2014-0009.
5. Moulakakis KG, Alexiou VG, Sfyroeras GS, Kakisis J, Lazaris A, Vasdekis SN, Brountzos EN, Geroulakos G. Endovascular management of infected iliofemoral pseudoaneurysms - a systematic review. Vasa 2017 Jan; 46(1): 5-9. doi: 10.1024/0301-1526/a000572. Epub 2016 Dec 7.
6. Salsamendi J, Pereira K, Rey J, Narayanan G. Endovascular Coil Embolization in the Treatment of a Rare Case of Post-Traumatic Abdominal Aortic Pseudoaneurysms: Brief Report and Review of Literature. Ann Vasc Surg 2016 Jan; 30: 310.e1-8. doi: 10.1016/j.avsg.2015.07.030. Epub 2015 Oct 31.
7. Macedo TA, Stanson AW, Oderich GS, Johnson CM, Panneton JM, Tie ML. Infected aortic aneurysms: imaging findings. Radiology 2004; 231(1): 250-257.
8. Sueyoshi E, Sakamoto I, Nakashima K, Minami K, Hayashi K. Visceral and peripheral arterial pseudoaneurysms. AJR Am J Roentgenol 2005; 185(3): 741-749.
9. Lee W, Mossop P, Little A, Fitt G, Vrazas J, Hoang J, Hennessy O. Infected (Mycotic) Aneurysms: Spectrum of Imaging Appearances and Management. RadioGraphics 2008; 28: 1853-1868.
10. Esterson YB, Pellerito JS. Recurrence of Thrombin-Injected Pseudoaneurysms Under Ultrasound Guidance: A 10-Year Retrospective Analysis. J Ultrasound Med 2017 Apr 13. doi: 10.7863/ultra.16.09063. [Epub ahead of print].
11. Wu Z, Xu L, Raithel D, Qu L. Endovascular repair of proximal para-anastomotic aneurysms after previous open abdominal aortic aneurysm reconstruction. Vascular 2016 Jun; 24(3): 227-32. doi: 10.1177/1708538115593194. Epub 2015 Jun 25.
12. Gallitto E, Gargiulo M, Freyrie A, Bianchini Massoni C, Mascoli C, Pini R, Faggioli GL, Ancetti S, Stella A. Fenestrated and Branched Endograft after Previous Aortic Repair. Ann Vasc Surg 2016 Apr; 32: 119-27. doi: 10.1016/j.avsg.2015.10.018. Epub 2016 Jan 22.
13. Jayaraman S, Richardson D, Conrad M, Eichler C, Schecter W. Mycotic pseudoaneurysms due to injection drug use: a ten-year experience. Ann Vasc Surg 2012 Aug; 26(6): 819-24. doi: 10.1016/j.avsg.2011.11.031. Epub 2012 Apr 24.

Received: 24 Augusto 2017
Accepted: 20 July 2018
Correspondence:
Silvia Eleonora Gazzani
via Gramsci 14, 43126 Parma
Tel. 0039 0521 704930
Fax 00390521704838
E-mail: eleonora.gazzani@gmail.com

Acta Biomed 2019; Vol. 90, N. 2: 343-347 DOI: 10.23750/abm.v90i2.6661

Case report

A remarkable pattern of a tibial plateau fracture. Use of a safe technique with practical advantages in the surgical field

Georgios Touloupakis, Stefano Ghirardelli, Elena Biancardi, Luigi Bibiano, Guido Antonini, Cornelio Crippa

Department of Orthopedics and Traumatology, San Carlo Borromeo Hospital, Milan, Italy

Summary. Bicondylar plateau fractures are complex injuries often requiring a challenging treatment. We report a case of a 68 year-old-male patient with a complicated tibial plateau bicondylar fracture. The fracture of the tibial plateau involved all columns (lateral, medial, posterior). The fracture pattern of the proximal tibia managed by triple plating through dual posteromedial and anterolateral incisions. Posterior-medial and the medial plating result in increased stability. The posteromedial approach to the knee that we used in our case, offers various advantages. We recommend the option of the posteromedial access, as an approach that allows excellent control of the posterior involvement of this pattern of tibial plateau fracture. (www.actabiomedica.it)

Key words: tibial plateau fixation, posteromedial knee approach, bone plates

Introduction

Bicondylar tibial plateau fractures have always been a challenge to treat. Stable fixation is mandatory and the degree of complication in surgical management increases with a posterior involvement of the tibial plateau. Direct posterior access became popular for the excellent operative visualization in the surgical field, but despite being very practical, it still remains quite demanding during orthopedic procedures (1).

In the past, the most used classification for the tibial plateau fractures, the Schatzker one, has never described a posterior tibial involvement.

Nowadays, the extensive use of classification systems that take into consideration posterior fragments of the tibial plateau, such as the Luo classification, has point out the importance of the posterior fragmentation in the orthopedic surgical society (2).

Here, we present a case of a complicated tibial plateau bicondylar fracture with posterior involvement underlying the management possibilities of posterior fragmentation when using a posteromedial approach.

Case report

A 68 year-old-male patient was involved in a motorcycle accident. He presented to our emergency department with diffuse swelling and severe pain over the left knee and over the left ankle. No evidence of other skeletal injury was reported at the clinical examination. Both a radiographic examination and a CT scan revealed a left tibial fracture (Schatzker type VI) with a depression of the lateral-posterolateral side. A medial and posteromedial involvement of the tibial plateau was found, associated with a non-displaced fracture of the peroneal malleolus (Fig. 1a, 1b, 2d, 2e). The fracture of the tibial plateau involved all columns (lateral, medial, posterior), as previously described by Luo. A long leg partial splint - was applied and limb elevation was recommended until surgery. A 3D reconstruction of the knee was done for a more accurate investigation of the fracture and an in-depth preoperative planning (Fig. 2a, 2b, 2c).

The fracture of the proximal tibia consisted in a split depressed lateral condyle with a posterior-lateral

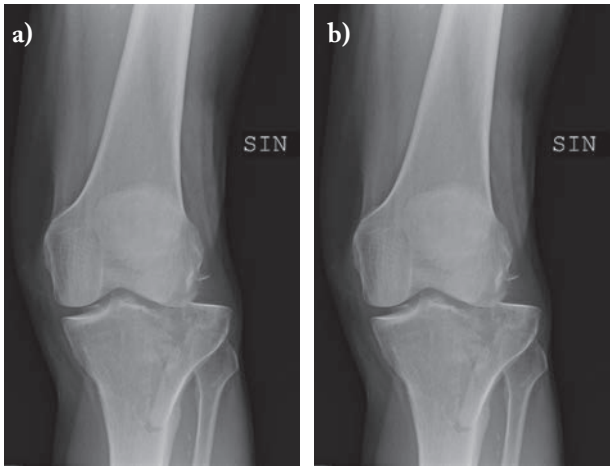


Figure 1. a, b) Radiographs of a 68 year-old-male patient with a tibial plateau type VI fracture according to the Schatzker classification

involvement. Medial plateau fracture was more complex, as the posterior-medial involvement was more extended in the medial side. The patient was operated seven days later to the admission, as a detailed cardiologic examination was requested by the anesthesiologist before surgery.

The patient was placed onto an orthopedic transparent bed in supine position to facilitate fluoroscopy and without using a pneumatic tourniquet. Cephazoline (2 gr iv bolus) was administered 30 minutes prior to skin incision as antibiotic prophylaxis.

The knee was externally rotated and flexed about 30 degrees and a posteromedial approach was firstly made. Incision was made posterior to the superficial medial collateral ligament. The fascia was identified and incised, the space between pes anserinus and the medial gastrocnemius was developed. Under direct visualization, the posterior fragment of the posterior medial column was reduced and stabilized in an anti-glide way with a 3.5 mm T-plate (Synthes, Switzerland). The medial fragment was fixed with an one-third 3.5 mm Tubular Plate (Synthes, Switzerland). Subsequently, a folded pillow was placed under the knee in internal rotation. The lateral fragment of the tibial plateau was exposed with an anterolateral approach. The articular depressed fragment was identified and elevated. Sponge cubes bone grafting was used to support the elevated fragment. Fixation

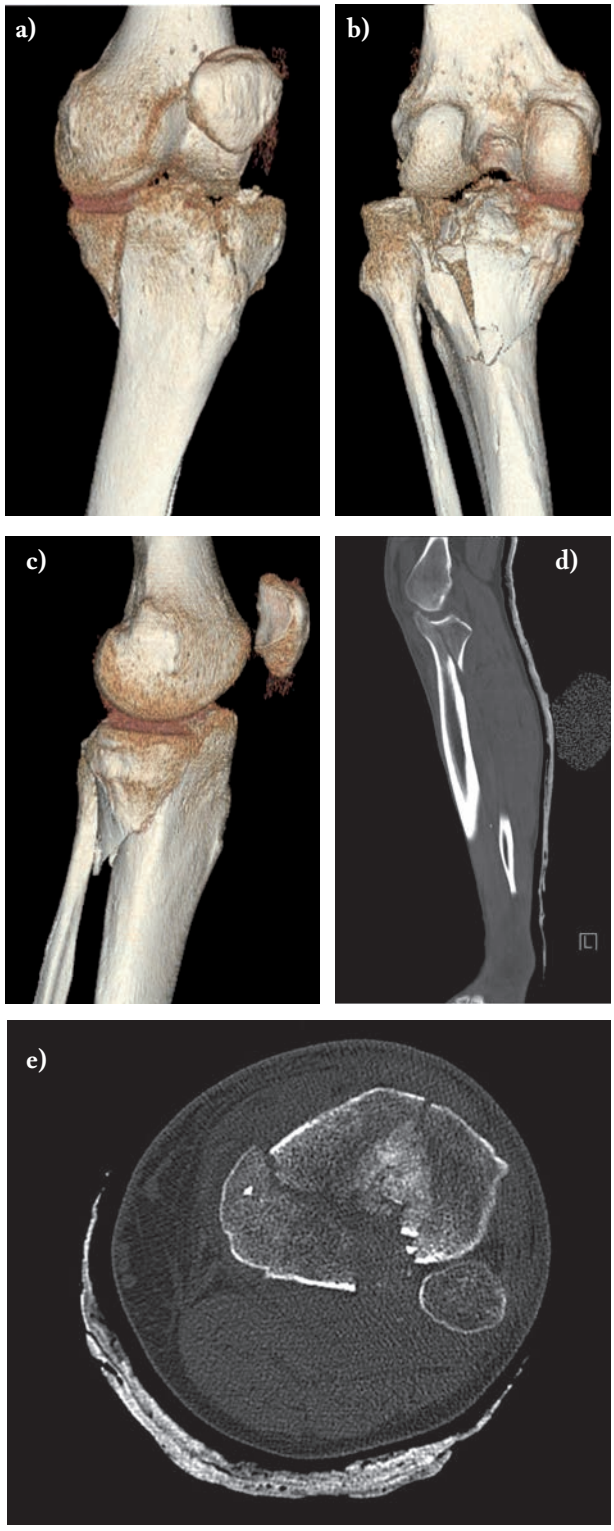


Figure 2. a, b, c, d, e. Ct scans of tibial plateau reveal a complex fracture pattern with involvement of all the four columns (lateral, medial, posterior) of the proximal tibia, as described by Luo

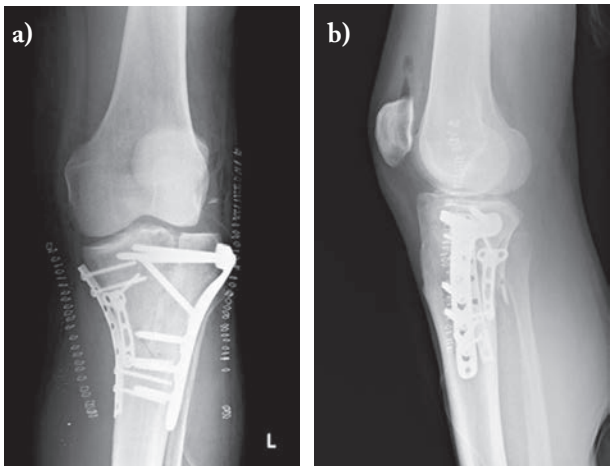


Figure 3. a, b) Post-operative x-rays of the knee

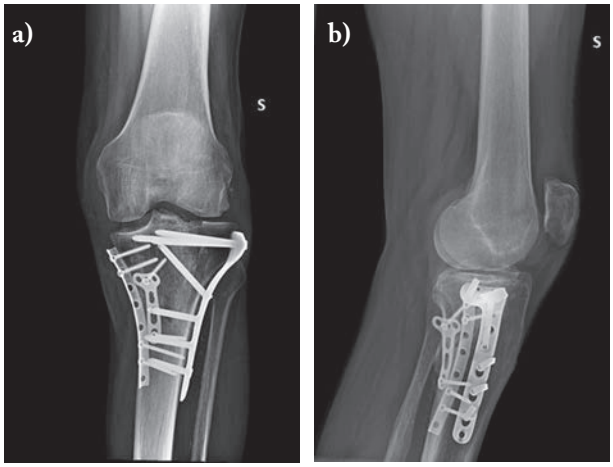


Figure 4. a, b) After a 9-month post-operative follow-up period, the surgical outcome was excellent and the fracture healing was more than satisfactory

was obtained by using an LCP Proximal Tibial Plate 4.5/5.0 (Synthes, Switzerland); the distal screws were placed with a minimally invasive percutaneous plate osteosynthesis (MIPO) technique (Fig. 3a, 3b). A drain was placed and then removed 24 hours later. A knee brace with a full range of motion was applied. The non-displaced lateral malleolus fracture was treated with a tight bandage to be carried for another 20 days.

Motion of the knee with active assisted exercises was started the day after surgery and a non-weight bearing walking was allowed from the third operative day and on. Full-weight bearing was started at week 10 following X-ray exams. For thromboembolic prophylaxis we administered low molecular weight heparin subcutaneously (according to the patient's weight) until the full weight bearing was reached.

X-ray assessments were performed one month after surgery and later after 2, 3, 6 and 9 months in the outpatient clinic (Fig. 4a, 4b). The standard AP and lateral X-ray views were used to assess callus formation of the proximal tibia. On the third month of the follow-up period, the patient reached full range of painless motion. No complications were occurred after surgery.

The informed consent of the patient was obtained, regarding the use of clinical data for scientific publication.

Discussion

Bicondylar plateau fractures are complex injuries often requiring a challenging treatment. Some fracture patterns are not adapted in the Schatzker classification and, due to this fact, several classification systems have been proposed the last years, including some unusual tibial plateau variations.

These fractures frequently present soft tissue lesions and the best treatment option depend on the local health status of the skin around the knee and the actual experience of the surgeon. Several surgical options are valid such as plating or external fixation. The goal of the treatment is the anatomical correction of the tibial plateau and a stable fixation, so as to prevent secondary displacement and to decrease the rate of complications as well (3).

In our case, we explained that it was crucial to obtain the lateral view and the 3d CT scan for a complete analysis of the fracture. The Schatzker classification only considers the anteroposterior view. Identification of posterior displacement is better stressed with lateral view and CT scans.

High-energy traumas often result to a posterior involvement and it is imperative to immediately discuss the possibility of either a posteromedial or a posterolateral fragmentation (4).

Preoperative planning is mandatory to obtain satisfactory results. In our case all the four columns were

interested. The main topic of our discussion regarding the planning preoperatively was the choice of surgical approach for the medial and posterior columns. We decided to proceed with a posteromedial access instead of the posterior one. For the lateral and posterolateral columns, the anterolateral approach was proposed with common agreement of all the surgical members that took place in our pre-operative meeting.

The posterior access of the knee joint requires a deep knowledge of the anatomy of the popliteal fossa, with regards to the neurovascular structures like the tibial nerve, the common peroneal nerve, the popliteal artery and the popliteal vein (5). A long S-shaped incision has to be made and it is required to lift a large skin flap, which can cause dehiscence and skin necrosis, especially if an acute incision is made. This risk is much increased in case of a high-energy trauma. In order to get the posterior surface of the knee joint better exposed, the medial head of the gastrocnemius muscle needs to be cut, which comes up against a fast recovery of the normal range of motion of the knee joint (6). A posterior approach is often combined with another access to the knee in order to treat complex fractures of the tibial plateau, and the prone position is another pitfall. In such cases, the surgeon has to change surgical field during the operation if a combined approach is necessary (7).

The posterior-medial approach that we used in our case offers various advantages. The supine position of the patient does not lead to any complications related to anesthesia. It is a relative safe approach because the saphenous nerve and the saphenous vein could be retracted either anteriorly or posteriorly, and also because the popliteal neurovascular bundle is located medially to the medial head of the gastrocnemius muscle which works as a shield. No muscle dissection is needed; the medial head of the gastrocnemius muscle, which is relaxed by knee flexion, is separated from the tibia by blunt dissection (8). The posterior-medial skin incision allows exploration of the structures anteriorly and posteriorly to the medial collateral ligament. Implant placement could be performed in both the medial and posterior-medial side of the tibial plateau.

Posterior tibial plateau has a complex structure and it is challenging to restore the joint anatomy when posterior multi-fragmentation occurs. In such cases, reduction could be more difficult if it is to perform a posterior medial approach when compared with a direct posterior one. Moreover, the persistent soft tissue suffering, typically seen in high energy tibial plateau fractures, is the condition that will ultimately direct the choice of the approach. We emphasize that the combination of anterolateral approach and posteromedial approach allows a wider skin bridge between the two skin incisions.

In our case all the columns were affected; nevertheless, we decided to face the fracture by choosing an approach which was closer to multifragmentation. The posteromedial side of the tibial plateau seemed more complicated on the CT scan, so, we preferred to deal with the fracture by using an approach the offers better visualization of the more complicated posteromedial fragmentation.

Before attempting a reduction of the fracture, through the posteromedial approach, we mobilized the fragment to allow the extraction of the hematoma and of the osteochondral fragments which often disturb the reduction. This is facilitated by placing the knee in a flexed position. On the other hand, we noticed that positioning the knee in extension facilitates the reduction of the posteromedial tibial plateau. The technique of Kirschner wire joysticks is also helpful in facilitating reduction of small articular fragments.

Accurate reduction of the posterior medial side is mandatory and temporary stabilization with K-wires also allows an intra-operative check with a C-Arm image intensifier, before a definitive stabilization is performed. A small sub-meniscal release at the fracture site at the medial side is helpful, whenever oblique fluoroscopy images do not clearly illustrate the articular surface.

The double posteromedial and medial plating resulted in increased stability. A “T” plate was initially inserted to restore anatomical slope of the posteriormedial plateau. Low profile slim 3,5mm “T” plates have been countered and adapted to the metaphyseal posterior segment of the tibial plateau, in order to facilitate the laborious reconstruction. Subsequently, the one-third tubular plate stabilized the medial column to prevent secondary varus collapse and to increase stability of the metaphyseal zone of the proximal tibia (9). Attention was paid non to insert long screws that

could have interacted with the lateral plating. After medial and posteromedial plating, this apparently complex pattern was transformed to a simple Schatzker III fracture variation that needed to be stabilized and supported by grafting. C-arm controls confirmed the anatomical fragment reduction of the tibial plateau surface.

In this tibial fracture pattern, we strongly suggest and recommend the option of the posteromedial access, as a better alternative approach that allows excellent control of the posterior involvement of the fracture.

Acknowledges

The authors would like to thank Dr. Xenos Dionysios for helpful comments and fruitful discussions.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Alpert JM, McCarty LP, Bach BR Jr. The direct posterior approach to the knee: surgical and anatomic approach. J Knee Surg 2008 Jan; 21(1): 44-9.
2. Zhu Y, Yang G, Luo CF, Smith WR, Hu CF, Gao H, Zhong B, Zeng BF. Computed tomography-based Three-Column Classification in tibial plateau fractures: introduction of its utility and assessment of its reproducibility. J Trauma Acute Care Surg 2012 Sep; 73(3): 731-7.
3. McNamara IR, Smith TO, Shepherd KL, Clark AB, Nielsen DM, Donell S, Hing CB. Surgical fixation methods for tibial plateau fractures. Cochrane Database Syst Rev 2015 Sep 15;(9): CD009679.
4. Prat-Fabregat S, Camacho-Carrasco P. Treatment strategy for tibial plateau fractures: an update. EFORT Open Rev 2017 Mar 13; 1(5): 225-32.
5. Chen H, Chang S, Pan J. Recent progress in the diagnosis and treatment of posterior tibial plateau fractures. Int J Clin Exp Med 2015 Apr 15; 8(4): 5640-8.
6. Bhattacharyya T, McCarty LP 3rd, Harris MB, Morrison SM, Wixted JJ, Vrahas MS, Smith RM. The posterior shearing tibial plateau fracture: treatment and results via a posterior approach. J Orthop Trauma 2005 May-Jun; 19(5): 305-10.
7. Lin KC, Tarng YW, Lin GY, Yang SW, Hsu CJ, Renn JH. Prone and direct posterior approach for management of posterior column tibial plateau fractures. Orthop Traumatol Surg Res 2015 Jun; 101(4): 477-82.
8. Weil YA, Gardner MJ, Boraiah S, Helfet DL, Lorich DG. Posteromedial supine approach for reduction and fixation of medial and bicondylar tibial plateau fractures. J Orthop Trauma 2008 May-Jun; 22(5): 357-62.
9. Adams JD Jr, Della Rocca GJ. Management of Posterior Articular Depression in Tibial Plateau Fractures. J Knee Surg 2016 Jan; 29(1): 28-33.

Received: 31 July 2017
Accepted: 11 November 2017
Correspondence:
Georgios Touloupakis
Via Don Bartolomeo Grazioli 24
20161 Milano
Tel. (+39)3495106291
E-mail: yorgostoulou@gmail.com

Acta Biomed 2019; Vol. 90, N. 2: 348-352 DOI: 10.23750/abm.v90i2.6886

Case report

Severe metabolic alkalosis due to diuretic treatment in a patient with distal renal tubular acidosis: a rare association

Laura Lucaccioni[1], Elena Coccolini[2], Alessandra Dozza[3], Sante Lucio Cantatore[3], Alberto Berardi[1], Barbara Predieri[3], Lorenzo Iughetti[2,3]

[1] Neonatal Intensive Care Unit, Department of Clinical and Surgical Sciences of the Mother, Children and Adult, University of Modena and Reggio Emilia, Modena, Italy; [2] Pediatric Unit, Department Post-graduate School of Pediatrics, University of Modena and Reggio Emilia, Modena, Italy; [3] Department of Medical and Surgical Sciences for Mothers, Children and Adults, University of Modena and Reggio Emilia, Modena, Italy

Summary. *Introduction:* Distal renal tubular acidosis is a rare genetic disease, characterised by deficit in renal tubular transport. Clinical features are metabolic acidosis with hypercloraemia and hypokalemia, and inability in urine acidification. Hypercalciuria may also be present, often treated with the use of a diuretic therapy with thiazides. *Case Presentation:* We present a severe disease onset in a neonate with consanguineous parents, both autosomal-recessive for an ATP6VOA4 gene mutation, and a nevertheless severe episode of metabolic alkalosis, occurred in the same patient after few months, during the diuretic therapy. *Conclusion:* Biochemical results lead us to hypothesize a susceptibility to the treatment that need further investigations. (www.actabiomedica.it)

Key words: distal renal tubular acidosis, metabolic alkalosis, hydrochlorothiazide

Introduction

Distal renal tubular acidosis (dRTA), called "Classic distal" or "Type 1", is a rare genetic disease grouping in the renal tubular acidosis (RTA) syndromes. These diseases are characterized by different tubular transport defects that lead to the inability to secrete hydrogen ions (H^+) with development of metabolic acidosis (1). In children, dRTA is most of the times observed as primary entity often in families with autosomal-dominant (AD) or autosomal-recessive (AR) pattern of inheritance (2). Type 1 presents an inefficient H^+ secretion, with inadequate hydrogen ion gradient between the blood and tubule fluid (3, 4). This leads to low plasma $HCO3^-$ levels, metabolic acidosis, electrolytes alterations, as hypercloraemia and hypokalemia, and inability in urine acidification. Hypercalciuria may also be present as result of calcium phosphate mobilization from bones to compensate systemic acidosis, and hypocitraturia, as consequence of increased citrate excretion to buffer systemic acidosis. High urine pH, high calcium (Ca^{++}) and low citrate urine levels promote nephrocalcinosis, often associated with nephrolithiasis. Chronic renal failure could develop as long-term effect. Clinical manifestations may also be failure to growth, anorexia, vomiting, dehydration and hypotonia (1, 2).

RTA main treatment consists in continuous administration of the appropriate amount of alkali in the form of either bicarbonate or citrate (1, 5, 6). This leads to correct metabolic acidosis and electrolytes alterations, improves growth and prevents renal and bone diseases (2).

Thiazide diuretics (TDs) have also been used to treat renal hypercalciuria, reducing the risk of nephrolithiasis. In particular, the most used is the hydrochlorothiazide (HCT) (7, 8).

The appropriate dosage and duration of treatment is controversial (9) since literature documents several cases of pseudo-Bartter's syndrome (10-12).

We present an early diagnosis of dRTA characterized by the homozygotic mutation of the ATP6VOA4 gene, showing two severe electrolytes dysfunctional episodes: the first at the disease onset and the second during the TDs treatment, leading to speculate a possible susceptibility to the diuretic treatment.

Case presentation

XY, 29-days-old, was admitted to the Emergency Department for severe weight loss and critical dehydration (estimated loss of 520 gr in 15 days). Medical history was uneventful except for parental consanguinity (first-degree cousins).

Physical examination showed impairment of general conditions, dry skin, cold extremities, refill time of 5". Biochemical evaluation revealed critic metabolic acidosis with hypernatremia, hyperchloraemia and ipokaliemia, hyperammoniemia and acute renal insufficiency (Table 1).

Urine analysis showed an alkaline pH (6.5) with presence of leucocytes, proteins and blood. The anion gap was normal.

He was rehydrated with intravenous fluids to support the circle and electrolyte replacement (K^+ and HCO^-_3) to correct the severe electrolyte imbalance. He was rapidly transferred to Neonatal Intensive Care Unit (NICU) because of his critical conditions. Suspecting a sepsis, microbiologic samples were collected (cerebrospinal fluid, blood, urine) and empirical antibiotics therapy was started (ampicillin and gentamicin). Cultures resulted negative. Cerebral ultrasound was normal whereas the abdominal scan showed bilateral medullary nephrocalcinosis. Despite the infusive treatment with K^+ and HCO^-_3, XY continued to show a trend to maintain hypokaliemic metabolic acidosis and hypercalciuria: this led to the dTRA hypothesis.

XY was transferred to the Pediatric Unit four days later, presenting improved clinical conditions and plasma electrolyte concentrations. Continuous vital parameters monitoring, fluids balance, blood gas analysis and electrolytes status were performed. Infusive treatment was adjusted until oral administration of $NaHCO_3$ and Potassium Citrate (Kcit) was achieved.

The detection of a homozygotic mutation in ATP6VOA4 gene located on cr. 7q33-34 confirmed the suspect of dTRA. Parents and brother are heterozygotic carriers of the same mutation.

At the discharge, the oral therapy with $NaHCO_3$ and Kcit was integrated with administration of HCT (about 2 mg/kg twice a day) to reduce kidney stones formation.

One a month later, the patient was admitted again to the emergency department presenting vomiting, decreased urine output and poor appetite. He presented lost of weight and dehydrated appearance (dry skin and furred tongue).

Blood analysis revealed a severe hypocloraemic and hypokaliemic alkalosis (Table 1) and electrocardiogram showed a prolongation in QT interval (QTc >0, 50 sec). He was immediately rehydrated with intravenous fluids and electrolyte replacement, and the diuretic treatment was stopped, with good resolution. A month later the treatment with HCT was started

Table 1. Gas analysis, plasmatic electrolytes concentration and renal function at first evaluation with severe metabolic acidosis (first row) and gas analysis and plasmatic electrolytes concentration at second evaluation with severe metabolic alkalosis (during treatment with hydrochlorothiazide – second row)

	PH	pCO2 (mmHg)	pO2 (mmHg)	BE (mmol/L)	HCO3- (mmol/L)	Na++ (mEq/L)	K+ (mEq/L)	Ca++ (mEq/L)	Cl- (mEq/L)	Urea (mg/dl)	Creatinine (mg/dl)	Ammonium (mmol/l)
First episode	7,01	24,2	43,3	-23,6	6,0	157	2,44	7,40	109	231	1,54	103
Second episode	7,61	72,9	29,8	-43,3	71,9	130	1,49	--	54	--	--	--

again during a recovery at the hospital: periodic blood analysis revealed the same trend to hypocloraemic and hypokaliemic severe alkalosis, leading the doctors to stop the diuretic treatment.

No other episodes were referred and XY is still periodically evaluated by paediatric nephrologists: he now presents regular weight and height growth, no neurological deafness and no hearing loss; full blood count, renal and liver function, acid-base blood status and urines are normal; last abdominal ultrasound was unchanged. Current oral therapy of the child involves $NaHCO_3$ four times/day and Kcit.

Discussion

Clinical and biochemical features of dTRA onset can be easily confused with neonatal sepsis.

XY presented poor general conditions, with very severe hypokalemia, hypernatremia and metabolic acidosis. However, sepsis was excluded by negative microbiologic cultures, not improving with antibiotic therapy and persistent metabolic acidosis resistant to bicarbonate treatment.

The alkaline urinary pH associated to normal values of the anion gap could lead to dRTA diagnosis. In particular, in case of hyperchloraemic metabolic acidosis with normal anion gap, the presence of urine PH >5.5 and normal or low plasmatic K levels are strongly suggestive of dRTA. Moreover, the presence of nephrocalcinosis found during the first abdominal ultrasound is another sign strongly indicative of dRTA (1, 2).

Our hypothesis was confirmed by genetic investigations: in particular mutations of the ATP6V0A4 gene configuring the AR dTRA variant without sensorineural deafness (1, 4).

A family history of consanguinity (Figure 1) is the hallmark of patients with AR dTRA (2): they are more severely affected and require aggressive administration of intravenous fluids and $NaHCO_3$ and Kcit replacement.

On the second admission, XY presented a severe hypocloraemic (Cl 54 mEq/L) and hypokaliemic (K

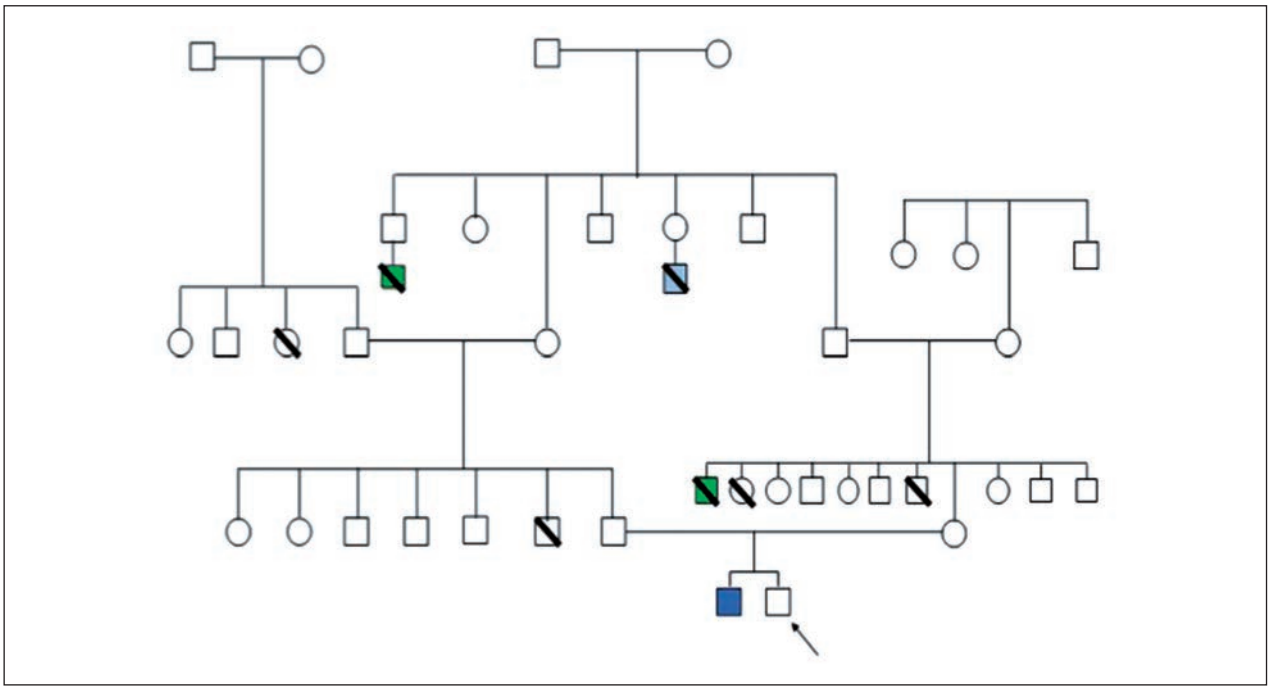


Figure 1. Family tree. In green: newborns dead after a few days of life for unknown reasons. In light blue: a 16 years old boy dead for neoplasm. In dark blue: a brother with thyroglossal duct cyst. The remnants deaths in the family were in adulthood, for unknown reasons

1,49 mEq/L) alkalosis, with life-threatening value of pH (7,61), $HCO3^-$ (71,9 mmol/L) and BE (43,3 mmol/L). The hypokaliemia caused an important prolongation in QT interval (QTc >0, 50 sec), justifying an aggressive therapeutic approach.

TDs therapy, stopped after this episode, is normally used for renal hypercalciuria treatment even in children, decreasing the risk of nephrolithiasis. In fact, TDs reduce NaCl reabsorption and renal excretion of Ca^{++}, favouring diuresis with an anti-hypertensive effect; it also increases K^+, $HCO3^-$, Mg^{++} and phosphates excretion (13).

It is controversial what dosage is appropriate and how long the treatment should be performed. It is reported that low-dose TDs (0,5-0,75 mg/kg/day) in children with idiopathic renal hypercalciuria are safe and effective in long-term control of hypercalciuria (9), however sometimes it is required to increase dosages to 1-2 mg/kg/day in children and 2-4 mg/kg/day in neonates/infants to obtain a long-lasting correction (14).

Our patient received HCT at the dosage of about 2 mg/kg twice a day, because of his age and the presence of bilateral medullary kidney stones, to prevent kidney functional impairment. In literature there are few cases of pseudo-Bartter's syndrome described from surreptitious diuretic (10-12). It is a condition characterized by hypokaliemia, hypochloremia, metabolic alkalosis and hyperreninemia with normal blood pressure and positive TDs urine excretion.

It is indeed likely that our patient developed a pseudo-Bartter episode (with severe alkalosis) although his strong predisposition to acidosis. Excluding parental difficulties on treatment administration (both HCT and bicarbonate) and errors in galenic preparation of HCT consigned to the family, and seen the biochemical trend during HCT treatment checked during a recovery, we can speculate a particular susceptibility of our dRTA patient to the thiazidic treatment that may need further investigations for understanding the pathophysiological pathway.

During the first year of life XY was evaluated every three months adjusting the treatment dosage. He did not develop hearing loss and he always maintained $HCO3^-$ level in the normality range (23-24 mmol/L). Unmodified nephrocalcinosis still persists also considering that XY may not benefit of the diuretic treatment.

Conclusion

To the best of our knowledge this is the first case of distal tubular acidosis type 1 developing a severe metabolic alkalosis due to thiazidic diuretic treatment, although appropriate regular dose administration. It is possible that our patient presents an increased susceptibility to hydrochlorothiazide that may need further investigation.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Rodriguez Soriano J. Renal tubular acidosis: the clinical entity. J Am Soc Nephrol 2002; 13(8): 2160-2170.
2. Caldas A, Broyewr M, Dechaux M, Kleinknecht C. Primary distal tubular acidosis in childhood: Clinical study and long-term follow-up of 28 patients. J Pediatr 1992; 121(2): 233-241.
3. Batlle D, Ghanekar H, Jain S, Mitra A. Hereditary distal renal tubular acidosis: new understandings. Annu Rev Med 2001; 52:471-484.
4. Fry AC, Karet FE. Inherited renal acidoses. Physiology (Bethesda) 2007; 22: 202-211.
5. Domrongkitchaiporn S, Khositseth S, Stitchantrakul W, Tapaneya-olarn W, Radinahamed P. Dosage of potassium citrate in the correction of urinary abnormalities in pediatric distal renal tubular acidosis patients. Am J Kidney Dis 2002; 39(2): 383-391.
6. Tapaneya-Olarn W, Khositseth S, Tapaneya-Olarn C et al. The optimal dose of potassium citrate in the treatment of children with distal renal tubular acidosis. J Med Assoc Thai 2002; 85 Suppl 4: S1143-1149.
7. Lamberg BA, Kuhlback B. Effect of chlorothiazide and hydrochlorothiazide on the excretion of calcium in urine. Scand J Clin Lab Invest 1959; 11: 351-357.
8. Voskaki I, al Qadreh A, Mengreli C, Sbyrakis S. Effect of hydrochlorothiazide on renal hypercalciuria. Child Nephrol Urol 1992; 12(1): 6-9.
9. Choi JN, Lee JS, Shin JI. Low-dose thiazide diuretics in children with idiopathic renal hypercalciuria. Acta Paediatr 2011; 100(8): e71-74.
10. Colussi G, Rombolà G, Airaghi C, De Ferrari ME, Minetti L. Pseudo-Bartter's syndrome from surreptitious diuretic

intake: differential diagnosis with true Bartter's syndrome. Nephrol Dial Transplant 1992; 7(9): 896-901.
11. Unuma K, Tojo A, Harada K et al. Autopsy report on pseudo-Bartter syndrome with renal calcification induced by diuretics and diet pills. BMJ Case Rep 2009; 2009.
12. Olveira Fuster G, Mancha Doblas I, Vázquez San Miguel F, Esteva de Antonio I, C-Soriaguer Escofet F. Surreptitious intake of diuretics as the cause of pseudo-Bartter's syndrome: apropos of a case and differential diagnosis. An Med Interna 1996; 13(10): 496-499.
13. Wile D. Diuretics: a review. Ann Clin Biochem 2012; 49(Pt 5): 419-431.
14. Naseri M, Sadeghi R. Role of high-dose hydrochlorothiazide in idiopathic hypercalciuric urolithiasis of childhood. Iran J Kidney Dis 2011; 5(3): 162-168.

Received: 9 November 2017
Accepted: 20 July 2018
Correspondence:
Professor Lorenzo Iughetti,
Departments for Medical and Surgical Sciences of Mothers, Children and Adults, University of Modena & Reggio Emilia, Paediatric Unit, Modena, Italy
Tel: +39-594225382; Fax: +39-594223895
E-mail: lorenzo.iughetti@unimore.it

Acta Biomed 2019; Vol. 90, N. 2: 353-354 DOI: 10.23750/abm.v90i2.6886

Letter to the editor

Osteoarchaeology and the History of Medicine in our experience

Silvia Iorio[1], Paola Badino[2], Ilaria Gorini[2], Massimo Aliverti[3]

[1] Department of Molecular Medicine, Unit of History of Medicine, Sapienza University of Rome; [2] Centre of Research in Osteoarchaeology and Paleopathology, Department of Biotechnology and Life Sciences, University of Insubria; [3] Sant'Anna di Como U.O. Psichiatria, Cantù

The acquisition of scientific methods and instruments has become fundamental for archaeological research (1). The concept of *New Archaeology* is essential to this convergence towards other sciences and the creation of a close alliance between the humanities and science. Archaeology and biology meet along the path of research, albeit for the free and respectful coexistence, perhaps a certain amount of distance should be kept. Through mediated listening and interaction, we can create the basis for a harmonious growth of archaeology within science and science within archaeology. Constant communication and the interconnection between humanistic, biological and technological research has revealed the need to delineate bioarchaeology as an autonomous science. Therefore, this underlines the need to clarify the professional identity. We are living in a time of constant scientific progress that enriches the face of archaeology and professionals working in this field. Moreover, "osteoarcheology" is also a field with its own specialized skill set. The image of archaeological work is changing shape, while the contents are redefined in these widening boundaries and limits. We should draw on archaeology's strong traditions, and at the same time confide in a culture that stems from monological thinking, and in our Research Centre where we often discuss how to handle the autonomy of the individual professional skills involved in the study of antiquity. Currently, we are focusing on the precise meaning behind training tomorrow's leading researchers in managing data capture and the application of concepts and models that can influence a scientist's way of thinking in a difficult balance that finds itself at the crossroads of new and rapidly changing trends (2).

When studying the bone remains, another important question is their destination.

Important osteological finding are often stored in anthropology laboratories or superintendence warehouses. However, it is not uncommon, to find some of these artefacts in museums in different cities (3).

For this reason, museology must now deal with the reality of special funding, and biology should not fail to oversee the task of carrying out training for these specialized skills (4). Museums need vast and wide-reaching scientific knowledge, alongside historical and humanistic traditions, and therefore doctors and biologists should also have a role in guiding and managing these collections due to their complexity. In human remains from antiquity, there is a high and irreplaceable value of pathogenic factors over time (5, 6, 7).

This is a complex operation that, by fragmenting skills and general operations into various activities defined as segments or parts of the work process, appears to have succeeded in breaking down and explaining the complexity of an aspect of archaeology that requires more resources and expensive commitments in order to be enriched and properly developed.

Archaeological finds become truly important based on our awareness of historical facts, yet also due to the study of the variation of pathogenic factors over time. Should strong limitations continue to prevent the fundamental understanding of these numerous illnesses, museums would remain alien to the growth process of modern health and paleopathology, an aspect that stands out in these studies. However,

proper use of museums and their resources would allow today's scientists, as well as tomorrow's scientific and historical studies, to offer important information and approaches that perhaps had been previously overlooked or ignored.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Ubelaker SH. Human skeletal remains: excavation, analysis, interpretation. Washington DC: Taraxacum, 1989.
2. Licata M, Monza F. Ethical issues in paleopathological and anthropological research experiences. Acta Biomedica 88(3): 315-318.
3. Monza F, Licata M. I preparati anatomici nei musei, Una particolare categoria di beni culturali. Medicina nei secoli 2015; 27(2): 615-28.
4. Monza F. Esporre i resti umani: un problema tra ricerca, etica e comunicazione. Il caso britannico. Museologia Scientifica Memorie 2014; 11: 214-44.
5. Nicosia L, Licata M, Armocida G, Vecchio I, Iorio S, Rossetti C. The case of an osteochondroma in a skeleton from the seventeen century crypt of Northern Lombardy, Acta Medica. Mediterranea, 2016, 32: 1567.
6. Licata M, Borgo M, Armocida G, Nicosia L. New paleoradiological investigations of ancient human remains from North Wets Lombardy archaeological excavations. Skeletal Radiol 2015; 45(3): 323-31.
7. Caldarini C, Catalano P, Gazzaniga V, Marinozzi S, Zavaroni F. The study of ancient bone remains. In Bones: Orthopaedic Pathologies in Roman Imperial Age 2015: 3-38.

Received: 9 November 2017
Accepted: 28 November 2017
Correspondence:
Silvia Iorio
Department of Molecular Medicine,
Unit of History of Medicine,
Sapienza University of Rome
E-mail: silvia.iorio@uniroma1.it

Acta Biomed 2019; Vol. 90, N. 2: 355-356 DOI: 10.23750/abm.v90i2.8371

Metformin, arterial contrast and acute kidney injury

Amos Lal[1], *Nirmal J. Kaur*[2], *Nitin Trivedi*[3]

[1] Department of Internal Medicine, Saint Vincent Hospital, Worcester, Massachusetts, USA; [2] Division of Cardiovascular Medicine, Saint Vincent Hospital, Worcester, Massachusetts, USA; [3] Division of Endocrinology, Department of Medicine, Saint Vincent Hospital, University of Massachusetts Medical School, Saint Vincent Hospital, Worcester, Massachusetts, USA

To the Editor:

We read with great pleasure the article by Namazi et al. in your esteemed journal regarding metformin use in patients with diabetes undergoing coronary angiography (1). We were specially intrigued by the discussion in results section where it was noted that M (+) and M (-) groups had no difference in the terms of contrast induced nephropathy (CIN) or Acute kidney injury (AKI) after contrast exposure. We wish to share from our data of critically ill patients on metformin treatment who underwent arterial contrast exposure for urgent coronary angiography. We did a retrospective chart review of 154 patients who underwent emergency coronary angiography (CAG) with arterial contrast exposure. The study was approved by the institution review board (Metrowest Medical Center IRB, Framingham Massachusetts, USA). 154 patients admitted with acute coronary syndrome during months of January 2014 - December 2014; 67 patients used metformin (100% had diabetes mellitus) whereas 87 were not on metformin (31% had diabetes mellitus). Baseline demographics and results are explained in Table 1. M (+) group of patients were continued on metformin after arterial contrast exposure; M (-) group was compared with the first group in terms of CIN (change in serum creatinine or GFR).

CIN or AKI after contrast exposure was defined as more than 25% rise in serum creatinine levels from baseline at 48 hours after contrast exposure absolute rise in creatinine by 0.5 mg/dL (2). Our study revealed no difference in contrast induced nephropathy (CIN) between the two groups (p=0.29), when compared at 48 hours after arterial contrast exposure. Higher serum creatinine may be have precluded the use of metformin in the control group. The American College of Radiology (ACR) guidelines recommend discontinuing metformin before or after exposure to iodinated contrast or checking kidney function after the procedure for patients with eGFR ≥ 30 mL/min/1.73 m^2. Patients undergoing arterial catheter studies or with eGFR below 30 mL/min/1.73 m^2, recommendations are to discontinue metformin before and 48 hours after the procedure and should not be restarted until the renal function is normalized (3).

Our single center, small observational study showed no difference in the incidence of CIN in patients who continued to be on metformin after arterial contrast exposure compared to the control group. Oktay et al. published similar findings in their study of patients who received arterial contrast for CAG (4, 5). Another group from the Netherlands also showed no difference in CIN episodes despite continuation of metformin in patients undergoing emergency CAG. Another recent study from Auti et al. in a recent study further risk stratified the Indian population with chronic kidney disease who are at higher risk for sustaining CIN (2). Study from Namazi et al. sheds light on the ever propagating issue of continuation versus discontinuation of metformin to avoid metformin associated lactic acidosis in patients undergoing coronary angiography, and attempted to prove the safety of metformin use. We intend to extend that spectrum associated with this issue by providing evidence against

Table 1

	Metformin (+) (67)	Metformin (-) (87)	P
Age, Years	65.81 ± 1.163	70.07 ± 1.199	**0.0135**
Gender, Female	30 (44.7%)	40 (45.9%)	
Diabetes	100%	31%	
HbA1C	7.569 ± 0.2234	7.192 ± 0.1777	0.1836
Baseline Creatinine	0.9834 ± 0.05473	1.585 ± 0.1792	**0.0046**
% change in Creatinine at 24 hours	-3.610 ± 3.397	-6.728 ± 2.224	**0.4252**
% change in Creatinine at 48 hours	22.36 ± 29.44	44.82 ± 31.24	**0.6076**
Incidence of AKI/CIN (>25% increase in Creatinine)	9 (67)	7 (87)	**0.2988**
Baseline eGFR mL/min/1.73m^2	76.48 ± 2.708	59.30 ± 3.085	**<0.0001**
% change in eGFR at 24 hours	-0.3281 ± 2.754	6.058 ± 2.299	**0.0768**
% change in eGFR at 48 hours	-12.21 ± 5.973	-3.883 ± 4.040	**0.2366**
Amount of arterial contrast received (mL)	180.4 ± 11.50	177.4 ± 8.837	0.8298
Average daily amount of Metformin (mg)	1525 ± 581.6	NA	

CIN associated with metformin and arterial contrast exposure from our center. Bigger and appropriately powered randomized controlled trials would definitely provide a concrete evidence, but emerging data shows chronic metformin treatment prior to primary PCI has no significant impact on CIN.

Amos Lal, and Nirmal J. Kaur contributed equally to the manuscript.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Namazi MH, AlipourParsa S, Roohigilani K, Safi M, Vakili H, Khaheshi I, et al. Is it necessary to discontinue metformin in diabetic patients with GFR > 60 ml/min per 1.73 m2 undergoing coronary angiography: A controversy still exists? Acta Biomed 2018; 89(2): 227-32.
2. Auti O, Manoj K, Annapandian V, Santosh Kumar D, Murugan K, Karthik G, et al. Routine screening of renal function before intravenous contrast examination: Is this required in the Indian scenario? Journal of Natural Science, Biology and Medicine 2019; 10(1): 87-90.
3. Makin V, Lansang MC. Should metformin be used in every patient with type 2 diabetes? Cleve Clin J Med 2019; 86(1): 17-20.
4. Oktay V, Calpar Çıralı İ, Sinan ÜY, Yıldız A, Ersanlı MK. Impact of continuation of metformin prior to elective coronary angiography on acute contrast nephropathy in patients with normal or mildly impaired renal functions. Anatolian journal of cardiology 2017; 18(5): 334-9.
5. Zeller M, Labalette-Bart M, Juliard JM, Potier L, Feldman LJ, Steg PG, et al. Metformin and contrast-induced acute kidney injury in diabetic patients treated with primary percutaneous coronary intervention for ST segment elevation myocardial infarction: Amulticenter study. Int J Cardiol. 2016;220:137-42.

Received: 10 April 2019
Accepted: 14 May 2019
Correspondence:
Amos Lal, MD
Department of Internal Medicine
123 Summer Street
Saint Vincent Hospital, Worcester,
Massachusetts. USA 01608
Tel. 5083635000
E-mail: manavamos@gmail.com;
Amos.Lal@stvincenthospital.com

Acta Biomed 2019; Vol. 90, N. 2: 357-358 DOI: 10.23750/abm.v90i2.8459

Editorial

The Science of Choosing Wisely: should it be applicable to any intervention for healthy and active longevity?

Domenico Cucinotta

Master of Geriatric Medicine in San Marino and Ferrara Universities; past chairman department of internal medicine and aging, University Hospital, Bologna, Italy

We do experience a longevity revolution

The number of persons aged 80 or over is projected to more than triple by 2050 and to increase more than seven-fold by 2100. In most developed countries, a universal health coverage provides financial protection against the cost of illness and promotes the care for the whole population, but at a cost/health spending that now accounts about 9% of GDP on average in OECD, and exceeds 10% in many other countries (1). This could be reasonable if the benefits exceed the costs, but there is an ample evidence of inequities and inefficiencies and inappropriate usage of tests and of harmful treatments which need to be addressed. Because the consequence could be the lack of financing of care of longeve persons, particularly when dependent.

Today scenario for healthy longevity is scientifically based on prevention, timely diagnosis and treatment of intercurrent illnesses, good diet, regular physical activities, brain training, drugs that should delay many diseases of old age and food supplements.

Are these interventions evidence based? Sometimes we have controversies concerning the efficacy of some treatment (2). And ***"Primum non nocere"*** (first, do not harm) remains a basic tenet of medical practice for older persons too. It suggests the necessity to convince population to adopting a good lifestyle as soon as possible, and to avoiding any intervention of any type, which could damage the person and particularly intrinsic capacities lifelong.

The WHO program (3-5) identified five intrinsic capacities for healthy aging: mobility, cognition, vitality, psycho-social-neurosensorials: vision, hearing. Since healthy ageing depends on an individual's intrinsic capacity (IC), the environment and interactions between them, a focus on IC has the potential to design interventions for improving the health of individuals.

According to WHO, vital functions maintain with aging by 3 different steps:

a. Increase intrinsic capacity reserves in early aging (45-70 yrs.);
b. Preserve cognitive functions in late aging (70 yrs. +);
c. Restore cognitive functions when needed *(and possible!)*.

Using this program the WHO aims to decrease the number of dependent older adults by 15 million by 2025.

The need for action with a focus on evidence-based policies and novel strategies ensuring healthy and successful longevity maintaining IC at maximum level is a priority, avoiding to support expensive interventions, either diagnostic or therapeutics, of no demonstrated benefit, or potentially harmful (6).

In recent years, the United States, and later many other countries, increased efforts to reduce inappropriate use of treatments and tests, either because of costs or of negative clinical results. Perhaps the most visible has been the Choosing Wisely Campaign (CWC) (7), a remarkable physician-led campaign to reduce the provision of unnecessary or harmful services in healthcare. CWC helps physicians and patients discuss the necessity of tests and treatments. CWC addressed this problem by asking specialist societies to generate a list

of the most prevalent low-value services in their field and more recently has spread worldwide, also into surgical contest and in prevention. CWC started in 2009, expanded in 2012 and most scientific societies of many countries in all part of the world produced their own list of interventions not to be done.

The mission of CWC is to promote conversations between clinicians and patients/subjects by helping these to choose care that is supported by evidence, not duplicative of other tests or procedures already received, free from harm, truly necessary, questioned and discussed. The recommendations should not be used to establish coverage decisions or exclusions. They are meant specifically to look for appropriate and necessary treatment.

Is it time to apply CWC methodology also to antiaging-healthy aging medicine? And to any approach to longeve persons? And to limit the tendency of human beings to overestimate the effects of any action?

These questions have been addressed, using a questionnaire, to scientists attending at a meeting of the Academy of Healthy Aging (Stockolm 2019). They were asked to list the five things, according to personal expertise, that should be avoided during life to promote and/or maintain healthy longevity.

The results demonstrated that the knowledge of the problem that inappropriate care could harm instead of protect is not today part of the basic culture of many researchers/clinicians and of common medical practice regarding healthy longevity. The message "do not harm" was not understood and most part of scientists provided suggestions to increase level of exams and care, independently by efficacy or danger of negative outcomes.

Some questions could be of help to curb any therapeutic illusion, the first might be formulated as "Before you conclude that a treatment was effective, look for other explanations." The second heuristic might be "If you see evidence of success, look for evidence of failure" (8).

Physicians and medical professionals of any discipline and all scientists interested into active and healthy longevity should apply CWC methodology, providing statements concerning also what should not to be done during life for maintenance of intrinsic capacity lifelong. A tailored approach for old people with multiple long term morbidities and how put them at the hearth of the decision about their care is a necessity, to reduce treatment burden, minimize unwanted side effects from taking multiple medicines, cutting treatments of limited benefits, avoiding fatal medical errors and inappropriate prescription.

References

1. European Commission. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions: Europe 2020 flagship initiative Innovation Union. Official Journal of the European Union C 121, 19 April 2011.
2. Cucinotta D. Pros and Cons in General Medicine and Geriatrics-2018. Acta Biomedica 2019; 90(1): 168-173.
3. World population prospects - The 2015 revision. United Nations NEW YORK 2015.
4. WHO Clinical Consortium on Healthy Ageing 2017 – report of consortium meeting 21 and 22 November 2017 in Geneva, Switzerland. Geneva: World Health Organization; 2018. Licence: CC BY-NC-SA 3.0 IGO.
5. Cesari M, Araujo de Carvalho I, Amuthavalli Thiyagarajan J, Cooper C Martin FC, Reginster JY, Vellas B, Beard JR. Evidence for The Domains Supporting The Construct of Intrinsic Capacity. J Gerontol A Biol Sci Med Sci 2018 Feb 2. doi: 10.1093/gerona/gly01.
6. Bellantuono I. Find drugs that delay many diseases of old age, Comment, Nature 2018; 554: 293-295.
7. Rashid A. The importance of choosing wisely. J R Soc Med 2016; 109: 36-7.
8. Casarett D. The science of choosing wisely-Overcoming the Therapeutic Illusion. N Engl J Med 2016; 374: 1203-1205.

Acta Biomed 2019; Vol. 90, N. 2: 359-363 DOI: 10.23750/abm.v90i2.8376

Review

Definitions of successful ageing: A brief review of a multidimensional concept

Annele Urtamo[1], Satu K. Jyväkorpi[1], Timo E. Strandberg[2]

[1] University of Helsinki, Department of General Practice and Primary Health Care, and Helsinki University Central Hospital, Unit of Primary Health Care, Helsinki, Finland; [2] University of Helsinki, Clinicum, and Helsinki University Hospital, Helsinki, Finland, and University of Oulu, Center for Life Course Health Research, Oulu, Finland

Summary. Successful ageing has become an important concept to describe the quality of ageing. It is a multidimensional concept, and the main focus is how to expand functional years in a later life span. The concept has developed from a biomedical approach to a wider understanding of social and psychological adaptation processes in later life. However, a standard definition of successful ageing remains unclear and various operational definitions of concept have been used in various studies. In this review we will describe some definitions and operational indicators of successful ageing with a multidimensional approach. (www.actabiomedica.it)

Key words: healthy ageing, quality of life, functionality

Introduction

In recent years, the concept of successful ageing has induced much debate (1-3), and various definitions of the concept have been introduced in various studies (4). According to the classic concept of Rowe and Kahn, successful ageing is defined as high physical, psychological, and social functioning in old age without major diseases (5, 6). In this brief review we use the classic name, although several terms of this concept, such as healthy ageing, active ageing, productive ageing, and ageing well, etc. have been used in the field (7). The relationship of these terms, and the dimensions of successful ageing are presented in the Figure 1.

The main focus in the concept of successful ageing is how to expand healthy and functional years in the life span (8, 9). The phenomenon of successful ageing can be viewed from a population or an individual perspective (7). At the population level, definition includes determinants of health and participation for the purpose of promoting policies, whereas at the individual level it is defined by outcomes of health, physical, and cognitive function, and life involvement (7). Because, successful ageing is a multidimensional concept encompassing domains of physical, functional, social, and psychological health, all of these dimensions should be taken into account, both with objective and subjective conditions, when studying the phenomenon (4, 8, 10, 11).

Kim and Park (12) conducted a meta-analysis of the correlates of successful ageing and they identified that four domains describing successful ageing were; avoiding disease and disability, having high cognitive, mental and physical function, being actively engage in life, and being psychologically well adapted in later life. Similarly, in the model of "Aging well" by Fernandez-Ballesteros et al. (13, 14), successful ageing is defined by the domains of health and activities of daily living (ADL), physical and cognitive functioning, social participation and engagement, and also positive affect and control, when the definition by Baltes et colleagues (15, 16) is also considered. Kok et al. (18) found in

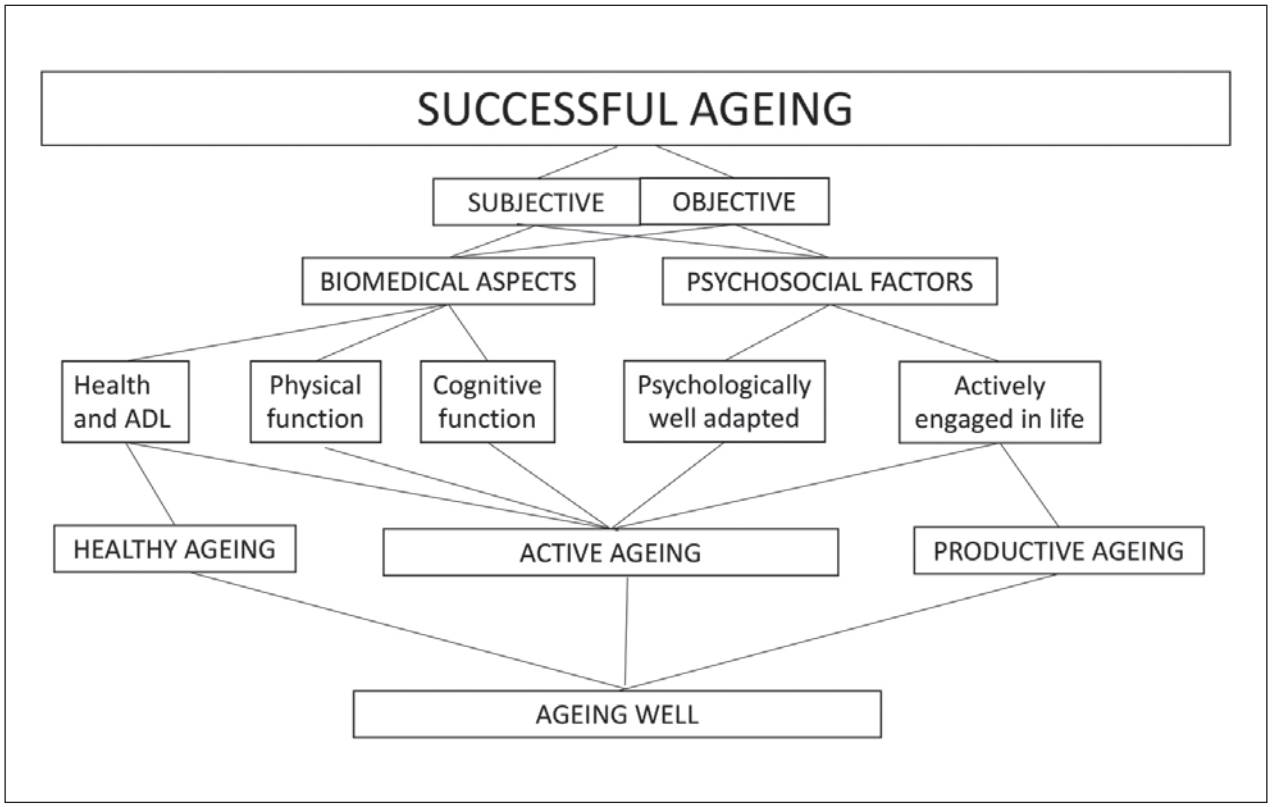


Figure 1. The dimensions of successful ageing. Modified from Fernandez-Ballesteros 2019, (7).

their study that many older adults were ageing relatively successfully, but there was a variation between indicators of characters of successful ageing, and the combinations of successful indicators varied also between individuals.

Most definitions of successful ageing include also outcomes which can be described as the operational definitions of the concept (7). The operational definitions are generally based on objective measurements of health and functionality and do not necessarily take into account individual's perceptions of their own health and wellbeing which would give more comprehensive view of ageing (4). Kleinedam and colleagues (19) have suggested that well-constructed operationalisation of successful ageing includes measurements of physiological health, well-being and social engagement, with subjective and objective aspects.

The aim of this brief review is to describe and discuss about conceptual and operational definitions of successful ageing with the multidimensional approach.

Biomedical aspects

Physiological function

Over the last decades, life expectancy has increased substantially. The increasing number of individuals reach over 80 years of age which has led to growing prone of multimorbidity, frailty and disability in older population (20). The cohort studies have shown that morbidity and functional limitations are associated with lower quality of life in old age (21, 22). Recent study showed that a good SRH and low levels of cardiovascular risk factors in midlife are associated with active and healthy ageing (23).

The concept of successful and healthy ageing has been generally associated with longevity, and the absence of disease and disability, which is based on the definition of successful ageing by Rowe and Kahn (5, 6).

Many studies have focused on longevity research to define successful ageing, highlighting the impor-

tance of having lived a very long and healthy life (10). The study of Andersen-Ranberg and colleagues suggested that "healthy centenarians do not exist, but autonomous centenarians do," which shows that longevity may have a price (10, 24). In addition, very few of the centenarians would be classified as "successful" according to Rowe and Kahn's criteria (10).

Avoiding disease and disability is common criterion also in the studies of successful ageing (12). However, recent studies have suggested that absence of disease and disability is not the most important element in the concept of successful ageing, and people with chronic disease can also age successfully (15, 25, 26). Young and colleagues' model of successful ageing (17), and also the model of selective optimisation with compensation proposed by Baltes and Baltes (15, 16), takes into account adaptive psychological and social mechanisms which can compensate limitations of physiological health. Manierre (27) has demonstrated that Young and colleagues' model provides a holistic perspective of successful ageing among people with chronic diseases.

Cognitive function

Maintaining cognitive abilities and preventing memory disorders are key aims in old age (28). Hartley et al. (28) have suggested that successful cognitive functioning should be a central component of successful ageing. Cognitive development in old age differs individually (28). Longitudinal studies have shown that midlife is a critical period for the beginning of the pathology of cognitive disorders, although indicators of the disease process remain still poorly understood (29). A compensation for age-related changes, a reliance on memory, and a cognitive reserve are themes that might explain higher cognitive functioning in old age among some individuals (28). According to this, relatively higher function may reflect relatively more successful ageing (28).

The cognitive functioning comprises perception, attention, memory, and higher functions, but indicators of successful cognitive functioning are often chosen to tap particular dimensions of functioning (28). Depp and Jeste (11) found that 13 of 29 operational definitions of successful ageing consisted indicators of cognitive functioning, and eight of those used a clinical assessment tool as an indicator. They also found that standards for successful cognitive ageing have large differences in studies (11, 28). Hartley and colleagues suggest that the clinical cognitive measurements may not be optimal for reflecting of current thinking in cognitive psychology (28).

Physical function

The furthest developed domain of successful ageing is physical functioning (30). Maintaining physical function is an important component of successful ageing (31). Regular physical activity during the life span is a strong predictor of healthy ageing (30-33). Decrease of muscle mass and muscle strength are related to ageing processes, but also to chronic diseases and lifestyle (nutrition, physical inactivity) (31). Sarcopenia is characterised by low muscle strength and low muscle mass and quantity, and it is associated with the development of functional disability (34). Sarcopenia is also a component of frailty, which is a syndrome that refers to vulnerability to stressors, loss of reserves, and an increased risk to functional disability and mortality (31).

The indicators of mobility performance and physical function are well known, and there is a consensus of measures and evaluation, for example, walking speed is an excellent marker of overall health and predicts the maintenance of physical function (30). The indicators of mobility performance and physical function can include both objective and subjective measures, for example ability to perform ADL and physical performance tests (30, 31). Chronic pain is a common condition in older adults and contributes to functional decline and limitation of activity (35).

Psychosocial factors

Psychosocial conditions contribute to ageing processes (15). Baltes and Baltes (15) have proposed the model of selective optimisation with compensation (SOC) which explains adaptation to deficits of ageing with successful psychological and behavioural processes. The SOC model consists both objective and sub-

jective criteria and reflects people's capacity to make choices that suit best to individual resources. In addition, Young and colleagues have proposed an alternative model which captures the possibility to compensate physiological limitations with psychological and social dimensions (17). According to the study of Kim and Park (12), older adults can age successfully, if they are socially active and psychologically well adapted, even though they encounter decline of physical and cognitive function.

Actively engaged in life

Good social functioning is often determined as an important factor in successful ageing, especially by older adults themselves (36). It reflects a wish to retain a role in society and being involved with people (36). Social functioning includes indicators of loneliness, social activity, and emotional and instrumental support given to others. For example, the participants could be defined as being actively engaged, if they have reported involvement in voluntary work, or participating in a sport, social or other kind of club (36).

Psychologically well adapted in later life

Recent studies have shown that life satisfaction, purpose in life, and perception of the ageing process contributed to ageing successfully, and therefore psychological domain of adaptation in later life is an important part of successful ageing (9). Emotional functioning could be assessed by depressive symptoms and satisfaction with life (15), and subjective feeling could be assessed with questions, e.g. "describe how successfully you have aged" (21).

Conclusions

Definition of successful ageing has shifted from biomedical to more holistic view, and towards more subjective aspects of the ageing process (1). The multidimensional approach of successful ageing could be more informative than focus on single health outcomes, such as chronic diseases or functioning (1), and therefore it can be used for understanding and promoting the concept in the populations of ageing societies. There still remains need for universal description and consensus of successful ageing which incorporate broad scientific evidence, and also need for operational definitions of indicators for this phenomenon.

Funding: This study was supported by Helsinki University Hospital VTR (EVO) –funding and Academy of Finland (311492).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

Reference

1. Calasanti T. Combating ageism: How successful is successful aging? The Gerontologist. 2016; 56: 1093-1101. doi:10.1093/ geront/gnv076.
2. Katz S, Calasanti T. Critical perspectives on successful aging: Does it 'appeal more than it illuminates'? The Gerontologist 2015; 55: 26-33. doi:10.1093/geront/gnu027.
3. Bülow MH, Söderqvist T. Successful ageing: A historical overview and critical analysis of a successful concept. Journal of Aging Studies 2014; 31: 139-149. http://dx.doi.org/10.1016/j.jaging.2014.08.009 0890-4065.
4. Cosco TD, Prina AM, Perales J, Stephan BC, Brayne C. Operational definitions of successful aging: A systematic review. International Psychogeriatrics 2014; 26: 373-381. doi:10.1017/S1041610213002287.
5. Rowe JW, Kahn RL. Human aging: usual and successful. Science (New York, NY) 1987; 237: 143-149.
6. Rowe JW, Kahn RL. Successful aging. The Gerontologist 1997; 37: 433-440.
7. Fernandez-Ballesteros R. The Concept of successful aging and related terms. In Fernandez-Ballesteros R, Benetos A, Robine J-M (Eds.). The Cambridge handbook of successful aging. (pp. 6–22). Cambridge University Press 2019.
8. Fries JF. Aging, natural death, and the compression of morbidity. New England Journal of Medicine 1980; 303: 130-135.
9. Kivimäki M, Ferrie JE. Epidemiology of healthy aging and the idea of more refined outcome measures. International Journal of Epidemiology 2011; 40: 845-847.
10. Martin P, Kelly N, Kahana B, et al. Defining successful aging: A tangible or elusive concept? The Gerontologist 2014; 55(1): 14-25. doi:10.1093/geront/gnu044
11. Depp CA & Jeste DV. Definitions and predictors of successful aging: A comprehensive review of larger quantitative studies. The American Journal of Geriatric Psychiatry 2006; 14: 6-20. doi:10.1097/01.JGP.0000192501.03069.bc.
12. Kim S-H, Park S. A Meta-analysis of the correlates of successful aging in older adults. Research on Aging 2016; 39(5): 657-677. doi: 10.1177/0164027516656040.

13. Fernández-Ballesteros R, García LF, Abarca D, et al. Lay concept of aging well: Cross-cultural comparions. Journal of the American Geriatrics Society 2008; 56: 950-952. doi:10.1111/j.1532-5415.2008.01654.x.
14. Fernández-Ballesteros R, Robine JM, Walker A, Kalache A. Active Aging: A Global Goal. Current Gerontology and Geriatrics Research 2013; Article ID 298012. doi.org/10.1155/2013/298012.
15. Baltes PB, Baltes MM. Psychological perspectives on successful aging: The model of selective optimization with compensation. In P. B. Baltes, M.M. Baltes (Eds.), Successful aging: Perspectives from the behavioral sciences. (pp. 1–34). New York: Cambridge University Press 1990.
16. Baltes PB. On the incomplete architecture of human ontogeny. Selection, optimization, and compensation as foundation of developmental theory. The American Psychologist 1997; 52: 366-380. doi:10.1037//0003-066X.52.4.366.
17. Young Y, Frick KD, Phelan EA. Can successful aging and chronic illness coexist in the same individual? A multidimensional concept of successful aging. JAMDA 2009; 10: 87-92. doi:10.1016/j. jamda.2008.11.003.
18. Kok AA, Aartsen MJ, Deeg DJ, Huisman M. Capturing the diversity of successful aging: An Operational definition based on 16-year trajectories of functioning. The Gerontologist 2015; 57(2): 240-251. doi:10.1093/geront/gnv127.
19. Kleinedam L, Thoma M, Maercker A et al. What is successful aging? A Psychometric validation study of different construct definitions. The Gerontologist 2018; 20: 1-11. doi:10.1093/geront/gny083.
20. Newman AB, Murabito JM. The epidemiology of longevity and exceptional survival. Epidemiol Rev 2013; 35(1): 181-97. doi: 10.1093/epirev/mxs013.
21. Strandberg AY, Strandberg TE, Stenholm S, Salomaa VV, Pitkälä KH, Tilvis RS. Low midlife blood pressure, survival, comorbidity, and health-related quality of life in old age: the Helsinki Businessmen study. Journal of Hypertension 2014; 32: 1797-1804. doi:10.1097/HJH.0000000000000265.
22. Davis JC, Bryan S, Best JR, et al. Mobility predicts change in older adults' health-related quality of life: evidence from a Vancouver falls prevention prospective cohort study. Health Qual Life Outcomes 2015; 13: 101. doi: 10.1186/s12955-015-0299-0.
23. Urtamo A, Huohvanainen E, Pitkälä KH, Strandberg TE. Midlife predictors of active and healthy aging (AHA) among older businessmen. Aging Clin Exp Res 2019; 31: 225. doi.org/10.1007/s40520-018-1100-0.
24. Andersen-Ranberg K, Schroll M, Jeune B. Healthy centenarians do not exist, but autonomous do: A population-based study of morbidity among Danish centenarians. Journal of the American Geriatrics Society. 2001; 49: 900-908. doi:10.1046/j.1532-5415.2001.49180.x.
25. Nosraty L, Jylhä M, Raittila T, Lumme-Sandt K. Perceptions by the oldest old of successful aging, Vitality 90+ Study. Journal of Aging Studies 2015; 32: 50-58. doi:10.1016/j.jaging.2015.01.002.
26. Pruchno RA, Wilson-Genderson M. A Longitudinal examination of the effects of early influences and midlife characteristics on successful aging. J Gerontol B Psychol Sci Soc Sci 2015; 70(6): 850-859. doi:10.1093/geronb/gbu046.
27. Manierre M. Successful present, successful future? Assessment of a nonbinary model of successful aging. The Gerontologist 2018; 20: 1-11. doi:10.1093/geront/gnx198.
28. Hartley A, Angel L, Castel A, et al. Successful aging: The role of cognitive gerontology. Experimental Aging Research 2018; 44(1): 82-93, doi: 10.1080/0361073X.2017.1398849.
29. Irwin K, Sexton C, Daniel T, Lawlor B, Naci L. Healthy Aging and Dementia: Two Roads Diverging in Midlife? Front Aging Neurosci 2018; 10: 275. doi:10.3389/fnagi.2018.00275.
30. Anton SD, Woods AJ, Ashizawa T, et al. Successful aging: Advancing the science of physical independence in older adults. Ageing Research Reviews 2015; 24: B304-327. doi.org/10.1016/j.arr.2015.09.005.
31. Strandberg TE. Preventive effects of physical activity in older people. In Fernandez-Ballesteros R, Benetos A, Robine J-M (Eds.). The Cambridge handbook of successful aging. (pp. 169–178). Cambridge University Press 2019.
32. WHO. World Report on Ageing and Health. 2015. https://apps.who.int/iris/bitstream/handle/10665/186463/9789240694811_eng.pdf.
33. Sabia S, Singh-Manoux A, Hagger-Johnson G, Cambois EJ, Kivimäki M. Influence of individual and combined healthy behaviours on successful aging. Canadian Medical Association Journal 2012; 18: 1985-1992. doi: 10.1503/cmaj.121080.
34. Cruz-Jentoft AJ, Bahat G, Bauer J, et al. Writing Group for the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), and the Extended Group for EWGSOP2.Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 2019; 48(1): 16-31. doi: 10.1093/ageing/afy169.
35. Karttunen NM, Turunen J, Ahonen R, Hartikainen S. More attention to pain management in community-dwelling older persons with chronic musculoskeletal pain. Age Ageing 2014; 43: 845-850.
36. Jopp DS, Wozniak D, Damarin AK, De Feo M, Jung S, Jeswani S. How could lay perspectives on successful aging complement scientific theory? Findings from a U.S. and a German life-span sample. Gerontologist 2014; 55(1): 91-106. doi:10.1093/geront/gnu059

Received: 10 April 2019
Accepted: 14 May 2019
Correspondence:
Annele Urtamo,
University of Helsinki, Department of General Practice and Primary Health Care, and Helsinki University Central Hospital, Unit of Primary Health Care, Helsinki, Finland
Tel. +358 50 316 8049
E-mail: Annele.Urtamo@helsinki.fi

Acta Biomed 2019; Vol. 90, N. 2: 364-374 DOI: 10.23750/abm.v90i2.8419

Original article

Proactive interception and care of Frailty and Multimorbidity in older persons: the experience of the European Innovation Partnership on Active and Healthy Ageing and the response of Parma Local Health Trust and Lab through European Projects

Yari Longobucco[1], Chiara Benedetti[1], Sara Tagliaferri[1], Vincenza Valentina Angileri[1], Elisa Adorni[1], Michele Pessina[1], Luna Zerbinati[1], Lorenzo Cicala[1], Giovanna Pelà[1], Vittoria Giacomini[1], Mirca Barbolini[2], Fulvio Lauretani[1, 3], Marcello Giuseppe Maggio[1, 3]

[1]Department of Medicine and Surgery, University of Parma, Parma, Italy; [2]Public Health and European Commission Senior Expert; [3]Medicine-Geriatric-Rehabilitation Department, University-Hospital of Parma, Parma, Italy

Summary. According to the 2018 European Union Ageing Report, the demographic profile of the European population is projected to be older. Aging cannot be considered a homogeneous process, and only in certain cases is "successful", with maintained functional ability, which is determined by intrinsic capacity, the environment, and their interaction. When intrinsic capacity is lost, elders with chronic diseases develop frailty, a condition with high-risk of disability. Old-age dependency-ratio is projected to increase from 29.6% to 51.2% in the EU in 2070: thus, the need of new approaches targeting the prevention of disability. Numerous studies are conducted in the European Innovation Partnership on Active and Healthy Ageing and addressing identification, treatment, coordination and integration of care in frail older subjects. SUNFRAIL is aimed at developing a model, good practices and tools to improve the identification, prevention and care of frailty and management of multimorbidity. SPRINTT is testing the effectiveness of a multi-component treatment able to treat frailty and sarcopenia. VIGOUR, a project aimed at strengthening integrated-care in different contexts of European Countries, verifies enablers and obstacles encountered in the real world by these good practices. Through the creation of Parma-Lab and Frailty-Team in the Academic-Hospital of Parma combined with the contribution of Parma Health-Trust in the "Community Health-Centers", the Projects were translated into Health Services Arena. This response bridging European Studies and clinical practice, aims to early detecting and caring 75-year older citizens with frailty and multimorbidity, living in the community, not institutionalized and at risk of hospitalization and mobility ADL-disability. (www.actabiomedica.it)

Key words: ageing, frailty, sarcopenia, multimorbidity, integrated care

Epidemiology of aging in Europe and Italy

According to the 2018 European Union Ageing Report the demographic profile of the European population is projected to change dramatically over the coming decades, with older people accounting for an increasing proportion. The percentage of citizens in the EU aged over 65 is predicted to rise from 18% to 28% by 2060; the percentage of over-80s will increase from 5% to 12% during the same time-period (European Commission, 2015). The proportion of older people aged 65 and over will become a much larger share, rising from 19% to 29% of the population, while the share of those aged 80 and over will increase from

5% to 13%, becoming almost as large as the young population in 2070. As a result, the demographic old-age dependency ratio (people aged 65 or above relative to those aged 15-64) is projected to increase from 29.6% to 51.2% in the EU as a whole over the projection period (1).

The comparison between 2015 and the estimates of 2080 shows how the European population will continue to age (2). The progressive aging of the elderly population is particularly interesting, with the exponential increase of the cohorts of the over 80s and the doubling of the dependency index of the elderly (that is, the ratio between the elderly population and the working age population [15-64 years]) which will increase from 28.8% in 2015 to 51.0% by 2080 (2). The interval between the overall life expectancy and the healthy life expectancy represent the time during which the living conditions of the population are characterized by higher levels of frailty and disability (3). Furthermore, epidemiological data (ISTAT) referring to Italian population support the European trend showing that aged 65 years or more will increase from 22.3% in 2017 to 33.3% in 2065 (4).

The population progressive ageing trend and the increased demand for health and social care, coupled with countries reduced resources for health and social services, hampers further the access to care for individuals with frailty and multimorbidity conditions, especially in people with lower economic status. It is therefore important to address these conditions taking into consideration overall individual's biomedical and socio-economic factors by considering equity, prevention and sustainability as main key principles.

Heterogeneity of ageing Process: toward healthy ageing and intrinsic capacity

Aging cannot be considered a homogeneous process, as it includes different potential trajectories. Aging can be "successful" or healthy, characterized both by the absence of significant chronic diseases, by a level of health perceived as satisfactory, and by the presence of self-sufficiency with satisfactory temporo-spatial orientation. Defining healthy aging is a prerequisite to promoting it (5). A definition provides a common language that facilitates person-centered care and care planning. The World Health Organization (WHO) defines health as "complete physical, mental and social well-being, not merely the absence of disease or infirmity" (6). Definition of healthy aging with multiple implications that recognises the importance of concepts and central to geriatrics, such as culture, function, engagement, resilience, meaning, dignity, and autonomy, in addition to diminishing chronic disease (7).

To address these principles, in 2012, the European Commission has launched the European Innovation Partnership on Active and Healthy Ageing (EIP on AHA), in order to tackle the potential and challenges of ageing in the EU (8).

Specific objective of EIP on AHA is to promote the clinical best practices that combine the biopsychosocial model of function, disability, and health, emphasising abilities and participation of the older adults in their families, work, and communities (9). In addition to disease-based cure, treatment, and comfort, a primary endpoint for healthy aging is optimising functional status. Then healthy ageing can be defined as "the process of developing and maintaining the functional ability that enables well-being in older age" (10). Functional ability is determined by intrinsic capacity (the composite of all the physical and mental capacities of an individual), the environment, and the interactions between the two (11).

Intrinsic capacity is the composite of all the physical and mental capacities of an individual. Five different domains can be proposed as of primary interest for better defining the intrinsic capacity framework: (i) cognition, (ii) psychological (including mood and sociality), (iii) sensory function (including vision and hearing), (iv) vitality, and (v) locomotion (including muscular and strength function) (12).

In a perspective where aging must be taken into consideration in good health, the concept of resilience is also fundamental because of its ability to capture the adaptation and responsiveness to adverse events.

Traditional models of healthy ageing suggest that having a high level of functioning across a number of domains is a required capacity; resilience is present when a high level of functioning is present after a negative event, or some form of adversity (13). Among the factors that may make the subject more vulnerable there are: genetic predisposition, familial heritage, so-

cio-environmental factors, early life stress, and chronic illness or treatment largely determining vulnerability to psychiatric disorders such as Major Depression or anxiety disorders. Moreover, other factors that make the subject more resistant to stressor attacks are positive emotions socio-environmental factors, cognitive flexibility, and exercise.

Animal studies support the role of an enriched environment for coping different tasks related to stress and depression measures as examples of stressful conditions. The environment concurs to better aging trajectories (14).

Models of Functional decline

Where and when disruption of resilience mechanisms occurs, stressors take precedence over adaptation mechanisms, leading to the development of functional decline reaching in certain cases the loss of self-sufficiency. In these cases, severe reduction in cognitive abilities and the presence of numerous chronic-degenerative diseases, result in mobility impairment and quickly leading to the condition highly associated with elevated risk of institutionalization of the elderly population, the mobility and ADL-disability, where coexistence of both cognitive and physical frailty could contribute to the developing of disability (15). Therefore, the concept of loss of autonomy does not include only the physical domain defined as the ability to carry out an activity in different areas, but also affect psychological (cognitive, emotional and behavioural), and finally social-economic context as the ability to maintain interpersonal relationships within the family and the community (16, 17).

Frailty

Definition and Projects

In the framework of functional decline, halfway between the normal state of functional independence and the pathological one of disability, is the condition of frailty, which affects not only the physical domain, but also the psychological and social one (18).

The term frailty refers to condition of increased vulnerability and poor resolution of homeostasis when facing a stressor event. This situation, which is dynamic and multidimensional, leads to an increased risk of adverse health outcomes. The occurrence of factors such as acute illnesses, chronic diseases and one's own genetic heritage, have negative impacts on the development of frailty (19, 20). Frailty is correlated to age-related, dynamic, stochastic, non-linear and multidimensional depletion of the systems which leads to a loss of physiological reserve and redundancy in which even minor stress factors can lead to negative outcomes and complications due to the lower capacity of the system to recover homeostasis (21-26).

The consequences of frailty include increased morbidity, risk of falls, social isolation, institutionalization and ultimately mortality and reduced quality of life and independence.

In this way, frailty and multimorbidity defined as the co-occurrence of two or more chronic diseases in the same individual, increase over time together (27).

Frailty is configured as a dynamic process (28) that needs to be readily recognized and treated promptly in order to prevent worsening of functional abilities and the appearance of disability.

In this regard, Figure 1 shows similarities and differences between oncology and geriatric medicine. The new challenge of geriatric medicine is to be identified in the need of capturing symptoms and signs requiring a combined physical and cognitive approach and ranging from "hyperplasia" stage to "cancer development".

Frailty is also frequently associated with sarcopenia, defined as "muscle loss together with a loss of function" as measured by grip strength and muscle mass" and where severity is identified by low physical performance assessed by 4-meter gait speed, Short Physical Performance Battery (SPPB), Timed up and go test (TUG), and 400 meter (29).

Numerous studies are currently conducted on frailty in the European context (Figure 2). The SUN-FRAIL Project, coordinated by Emilia Romagna Region with the scientific support of Parma Health Trust, is aimed at developing and experimenting a model, good practices and tools to improve the identification, prevention and care of frailty and management of multimorbidity, while the Sarcopenia and Physical fRailty IN older people: multi-componenT Treatment strategies (SPRINTT) is testing the effectiveness of

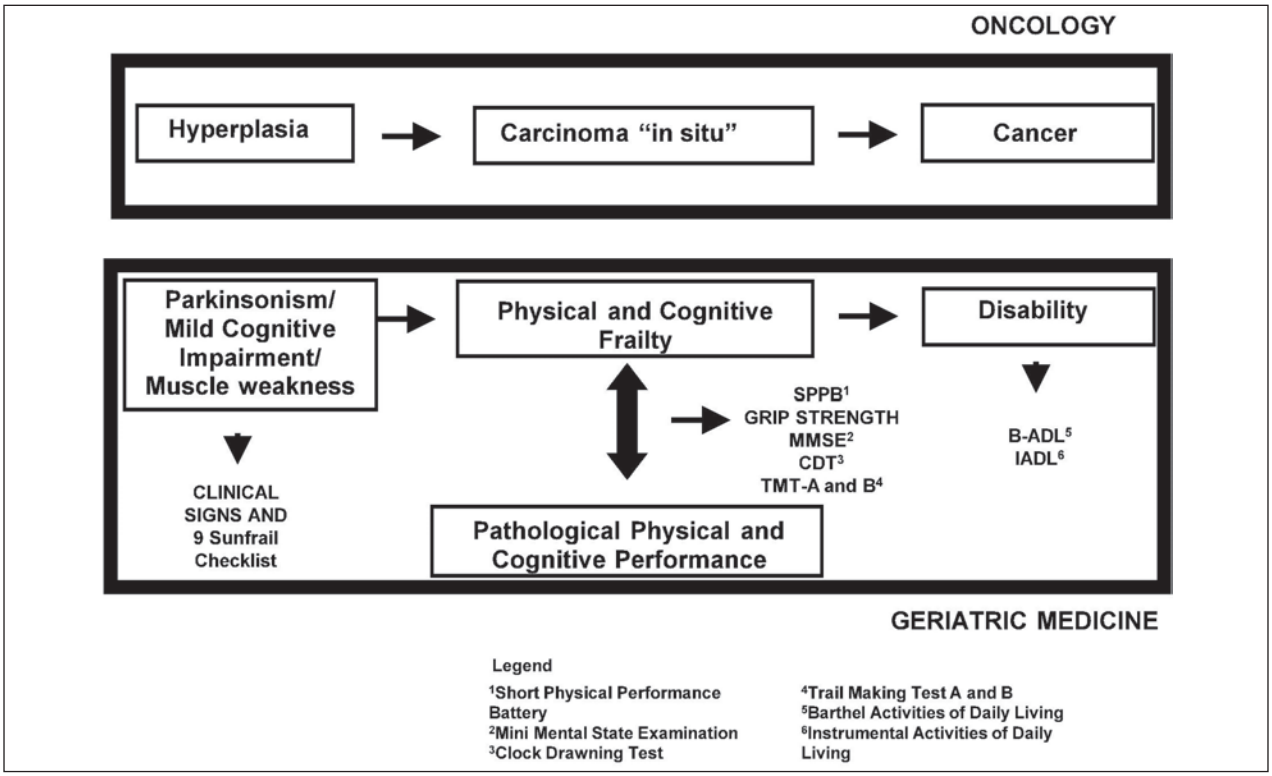


Figure 1. "New era" of the geriatric medicine: standardization of the physical and cognitive frailty in elderly patient: similarities with cancer development in oncology

a multi-component treatment able to treat frailty and sarcopenia (30).

The potential enablers and obstacles encountered in the real world by these promising good practices are presently addressed through the VIGOUR, a European project aimed at strengthening integrated care in different organizational contexts of European Countries.

Screening and Identification of frailty: Sunfrail

There is increasing number of researchers supporting the multidimensional nature of frailty (31). Authors suggest that frailty cannot be limited to physical domain, but necessarily involve psychological, cognitive, emotional, social and spiritual aspects (32). The SUNFRAIL project has the general objective to improve the early identification process of the frail subjects, having as target population the people aged over 65 who live in the community, through initiatives managed by local health and social services providers in European countries.

By addressing the common aspects and peculiarities of different European health and social care systems and services, the SUNFRAIL project developed common quality standards for the management of frailty and multimorbidity.

Particularly, the Sunfrail Tool is a nine-question, easy to use tool designed to identify frailty risk factors and multimorbidity according to the bio (physical), psycho (cognitive and psychological) and social domains. It can be administered by professionals and community actors, generating an initial "alert" for further investigations or activation of pathways within the health, social and community systems. The Sunfrail Tool was developed by an international multidisciplinary team (composed of geriatricians, sociologists and public health experts) following the methodology indicated for the creation of questionnaires (33-35). The team identified a minimum set of items starting from

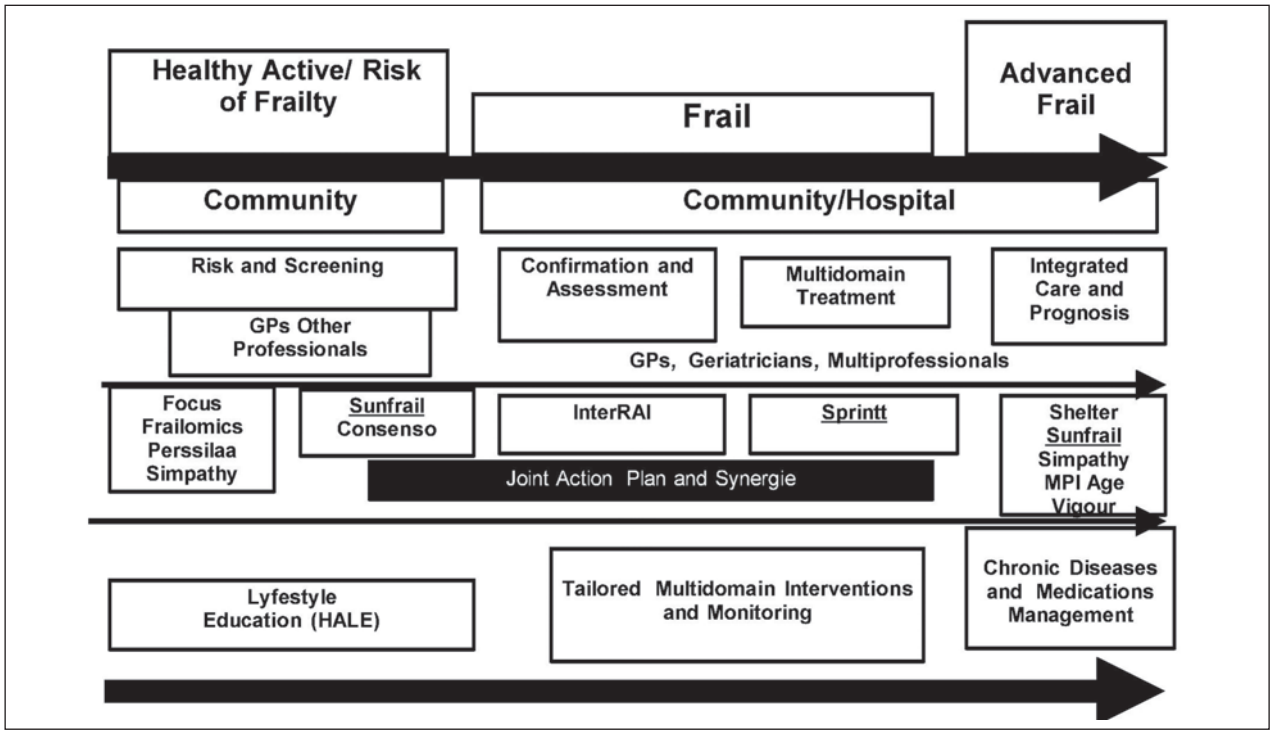


Figure 2. Frailty as continuum from initial assessment to integrated care according to frailty status. In the first line, we describe categories of older individuals, in the second the settings, in the third the European Projects targeting different stage of frailty, and in the bottom line proposed interventions according to different European Projects and Initiatives. Finally, underlined, the Projects described in the Manuscript

already existing tools in the literature, in particular the Edmonton Frailty Scale (36), the Tilburg Frailty Indicator (37) and the Gerontopole Frailty Screening Tool (38); the reference model is the bio-psycho-social model. After a debate, 9 items were selected, 5 of which in the physical domain, 1 in the cognitive domain and 3 in the socio-economic domain. The 9-item Sunfrail Tool, does not produce a numerical score. Based on the alerts generated by the administration of the tool, frailty and related risk factors need to be confirmed through Comprehensive geriatric assessment (CGA) in order to orient care.

The project has also designed the Sunfrail Tool for Human Resources Development; a short, multidisciplinary training program on frailty and multimorbidity according to the bio-psychosocial model. The experimentation of the Sunfrail Tool in various European settings involved 651 older adults over 65 years of age. The main results confirmed the capacity of the Tool to identify frailty and related risks in the population over 65, especially in primary care and community settings. The Tool appeared particularly suitable to identify frailty risks in a population without clear signs of disability or un-known by services and to orient the selection of preventive care pathways.

An assessment conducted on beneficiaries and professional opinions highlighted that the Sunfrail Tool is understandable, easy to use in every day practice, facilitating access and linking health, social and community services. It proved to be effective in raising older adult awareness on frailty risk factors and on services available, in strengthening professional's knowledge and approach to frailty and facilitating the connections among existent health, social and community services.

The experimentation of the Sunfrail Tool for human resources indicated that an interdisciplinary, intersectoral and multiprofessional approach is essential to promote teamwork, integrated care and coordination among services.

Overall, the Sunfrail project promoted a change of mindset and management approach.

The requests for adoption from professionals (GPs, nurses), local authorities, Italian and European regions, the commitment for further funding, the design of additional pilot studies and the creation of permanent stakeholder groups confirm the achievement of project's expected outcomes.

Assessment and multidomain treatment of physical frailty: SPRINTT

Once the condition of physical frailty is identified (SPPB is one of the most used tests and score ranging from 3 to 9 suggest the presence of frailty), together with its one of the main biological substrates, sarcopenia, various interventions have been proposed to maintain older individuals in good health and active status as long as possible (Figure 3) (39). In particular, through the European SPRINTT project (Sarcopenia and physical frailty in older people: multi-component treatment strategies), a randomized control trial conducted in frail sarcopenic older subjects 70 yr and older, to compare and demonstrate the effectiveness of a multi-component intervention composed of physical activity, nutritional and technological intervention, versus educational intervention (40). The primary endpoint of the study is to reduce the incidence of motoric-disability, defined as the participant's inability to complete a 400-meter walk in 15 minutes without sitting, without the help of another person or using a walker and without stopping for more than 1 minute at a time.

The secondary endpoints of the study are:

a) Changes in physical performance parameters
   - SPPB;
   - Dynamometric analysis of the upper limb (handgrip strength);
   - Activities of Daily Living (ADL);
   - Instrumental ADL (IADL);
   - 4 meters walking speed;
   - Pepper Assessment Tool for Disability (PAT-D).

b) Changes in body composition (measured by DXA), anthropometric parameters (body mass index, circumference of half arm, calf circumference) and nutritional status (Mini Nutritional Assessment-Short Form, MNA-SF).

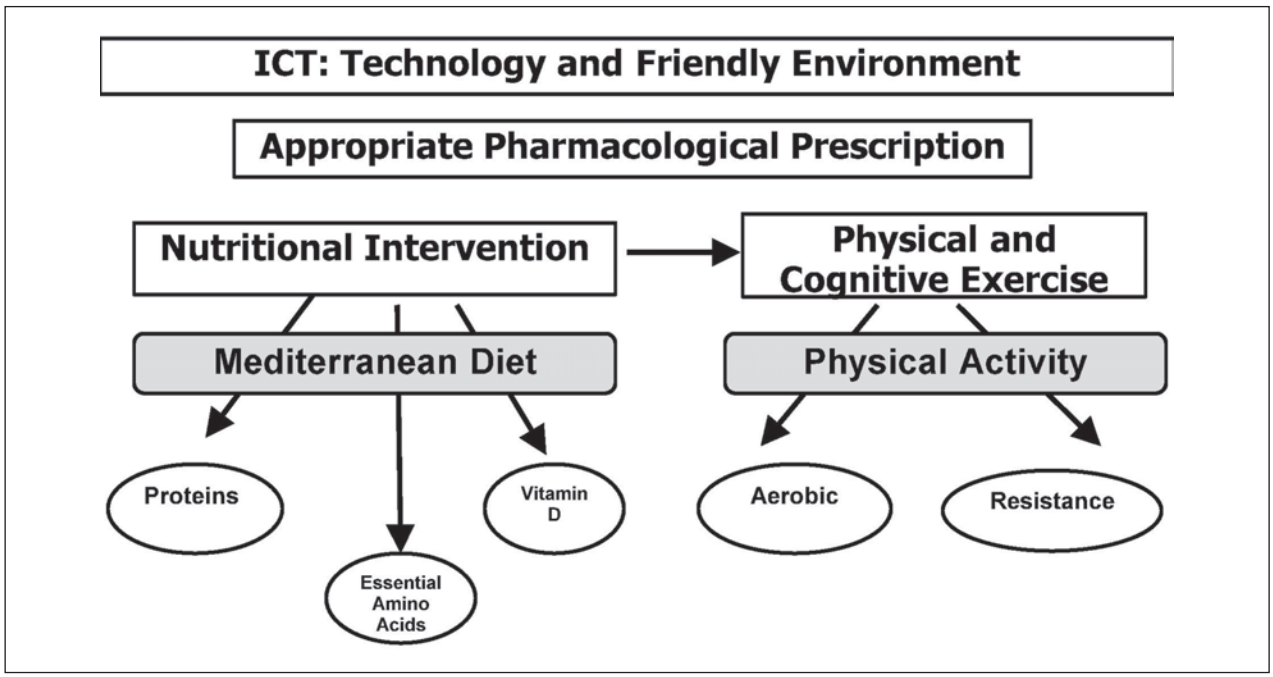


Figure 3. Multiple approach to Physical and Cognitive Frailty

c) Changes in cognitive function (assessed by MMSE) and mood (via CES-D scale).
d) Incidence of falls (assessed through questionnaires filled in by the participants and through the use of a dedicated technological device).
e) Changes in the quality of life (assessed through the EuroQol-5D tool).
f) Differences in the use of health resources (including day hospital, first aid, hospitalization, use of other care facilities).
g) Mortality rate.

This five-year project is the result of a rich collaboration between 18 major research institutes in the geriatric field in 11 European countries, including the University of Parma.

SPRINTT project represents the opportunity to agree on a therapeutic indication, the end-points and the clinical trial methodology that will allow developing innovative treatments for this geriatric syndrome that is currently under-diagnosed.

This is the first European clinical trial of an interventional and non-competitive nature, on elderly patients affected by sarcopenia, which will allow the use of physical activity as a reference point for future studies with experimental drugs.

SPRINTT is based on the LIFE study (41, 42), a multicentric randomized controlled trial conducted in the United States, who studied the effectiveness of physical activity in more than 1600 elderly sedentary people at risk of disability. The study showed that a moderate-intensity physical activity program reduces the incidence of major motor disabilities of more than 2.6 years (18). The SPRINTT protocol is oriented to propose on a large scale a protocol of activities to improve the lives of older people.

Other goals of SPRINTT study are to identify the biomarkers of anabolism and muscle catabolism in this population and to develop an economic health model concerning physical frailty and sarcopenia in a real-life context.

In detail, the treatment envisaged in the SPRINTT protocol foresees the division into two groups: the experimental group follows a multi-component intervention (MCI) while the control group follows a program of education lessons to a healthy lifestyle (HALE). A part of the activity carried out in the MCI concerns physical activity, with walking as the first exercise to prevent or postpone the primary disability outcome, the inability to perform the 400 m walking test; the program of physical activity is completed by exercises of balance, strength and flexibility.

Another section of the protocol comprises nutritional intervention, based on a nutritional counselling, aiming at two main targets:

- a total energy intake of 25-30 kcal/kg of body weight;
- an average daily protein intake of 1.0-1.2 g/kg of body weight.

At last, the treatment includes a technological monitoring, in order to evaluate changes in levels of physical activity.

The Modernization of health care systems: the VIGOUR Project

VIGOUR aims to effectively support health authorities in the process of transformation and modernization of their health and care systems towards integrated and sustainable care models. The project will allow these authorities to customize the design of services aimed at patients with multidimensional needs, using innovative approaches to risk stratification, monitoring and provision of services. It will also support authorities in defining strategies - sensitive to the local context - for integrating good practices and consolidating existing knowledge and tools within integrated assistance.

The Real World of Frailty: the response of Parma Health Trust

Parma Health Trust of Emilia Romagna Region, is responsible for the delivery of primary care, hospital care, outpatient specialist care, public health care, and health care related to social care. The AUSL operates through the districts, at which level local councils and health services determine requirements, plan health and social services, and assess results.

Primary health and social care services are provided through the "Community Health Centers" (Case della Salute), acting as the main pillars for the integrated care through multidimensional evaluation of people with complex health and social care needs,

design and coordinate the integrated care from and to the hospital. Primary and secondary prevention, case detection, management and support to self-management (through telemedicine) are essential components of care delivered with the active participation of patients and voluntary associations.

Emilia Romagna Region Risk Stratification model using longitudinal administrative databases (health and social care), estimates the risk of hospitalization and death for the resident adult population and creates 'patient risk profiles', allowing proactive case management within Primary Health and Social Care services network.

The Azienda Ospedaliero-Universitaria of Parma acts as a secondary care focal point for the integrated care of patients with complex needs, through multidimensional evaluation, providing the indications for the individualized care plans (PAI), collaborating to identify the patient's care pathway and to monitor the interventions.

Furthermore, the University Hospital of Parma is a highly specialized polyspecialistic hospital offering a complete range of diagnostic, therapeutic and rehabilitative services. Since 2015 the Geriatric Clinic Unit, in close collaboration with the Emilia-Romagna Region, provides scientific support to the activities of the European Partnership for Innovation on Healthy and Active Aging (EIP-AHA), particularly to the Working Group A3 on Frailty and of the European Reference Sites Network. It also provides scientific support to relevant EU projects and initiatives in the field of functional and cognitive decline in older persons (Sunfrail, the EU Joint Action on Frailty Advantage, Sprintt and Vigour). Particularly, the Geriatric Clinical Lab of Parma has acquired a unique experience in developing a patient-centered approach to frailty and multimorbidity through the step approach described in the next paragraph.

The Real World of Frailty: the response of Parma Lab and Team of Academic Hospital

The daily activity in the proactive care of frail and sarcopenic older individuals and application /participation at the above-mentioned European Projects allowed the stepwise process with different phases:

1. Setting up of a multiprofessional team composed of two geriatricians, one cardiologist, two young physicians, one nutritionist biologist, two community nurses, one physiotherapist and two motoric scientists.
2. Creation of a lab located in pavilion 27 of the Parma Hospital, where a multidisciplinary team uses equipment such as ultrasound scanners (for chest, abdominal and muscle ultrasound), BIA, GAITRite system and an equipped gym.
3. The activities performed by the Geriatric Clinical Lab of Parma in screening and confirmation of frailty and sarcopenia, in close collaboration with Community Health Centers (Case della Salute), included and are still including:
   a) Enrollment of subjects in the SPRINTT study; these subjects have a high clinical-assistance complexity requiring the constant presence of a multi-professional team (43).
   b) Screening of frailty in primary care setting, mainly by nursing staff, using the SUN-FRAIL tool. Potentially frail subjects were invited to perform a 2nd level multidimensional visit (by geriatricians, physicians and nurses). More than 900 subjects in the Parma Health Trust were screened in seven Community Health Centers (Case della Salute), with the participation of associated general practitioners.
   c) Confirmation of frailty by the Geriatric Clinical Lab of University-Hospital of Parma, two Community Health Centers and a General Practitioner Unit. The tests used were the Mini Mental State Examination and the Short Physical Performance Battery, combined with a pharmacological survey. The frail subjects eligible for the SPRINTT study underwent a complete geriatric visit with a Dual Energy X-Ray Absorptiometry (DEXA), in order to assess the condition of Sarcopenia. The frail and sarcopenic subjects also underwent a complete cardiology visit, to evaluate the safety to perform the physical activity required by the study protocol safely.
   d) Individualised care plans and proactive responses identified for individuals with mul-

timorbidity, focusing on prevention and tailored care. People with multimorbidity define good health and well-being as enjoyment of life, maintenance of independence, having social relationships and participating in society (43). The study of multimorbidity does not disregard the study of the subject's function. This complete evaluation allows the application of various targeted treatments based on the complexity of the subject, its functional reserve, its nutritional status and polypharmacy. Regarding the treatment, the nutritional intervention is managed by the nutritionist biologist, while physical activity is administered by the physiotherapist or by motoric scientists according to the participant's needs, with the clinical supervision of a physician and a nurse. Each patient condition and performance is then monitored and discussed in the framework of a staff meeting in which the various possible multicomponent interventions can be taken into consideration. The staff meets twice a week and promotes health with a multi-domain approach.

e) Overall, educational activities on the concept of healthy and active ageing are performed in order to increase local awareness on this topic in the oldest population. The campaign was realized from October 2016 to November 2017 for the specific goal of SPRINTT recruitment and is still ongoing through the collaboration and support of various stakeholders including patients and citizen organizations (University of the Third Age and AUSER), Municipalities, and trade unions (Coldiretti and CUPLA).

Furthermore, through the newly funded VIGOUR project, integrated care will be enhanced by strengthening professional and functional integration among the available primary care and hospital services network, in order to avoid inappropriate hospitalization and to facilitate hospital discharge. Emilia Romagna Region plans to participate by working on identifying frailty and multimorbidity in older persons over 75 (especially in community-primary care settings), through the combined application of the Risk stratification and the Sunfrail tool.

Conclusions

If successful, the approach described and the translation of these European Projects into Health Services arena, could provide a suitable response, in terms of organization and strategies, to frailty and multimorbidity in 75 year older citizens living in the community, not institutionalized and at potential risk of hospitalization and mobility and ADL-disability. All these timely interventions could slow down and reverse the functional decline in these subjects. Not less, the involvement of the stakeholders including patients and citizen organization can represent a response to the social needs of this particular population. In this way, the aging process can be reported on the tracks of healthy and active aging according to all aspects of the biopsychosocial model.

Acknowledgements

We thank Sprintt partecipants and their families, Maurizio Conca, Pasquale Rosanova, Maria Teresa Zanelli, Franco Ferrari, and Pietro Schianchi from Department of Medicine and Surgery of University of Parma, University-Hospital of Parma, AUSER Parma and Coldiretti Senior for their enthusiastic support.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Eurostat. Population by age group. By Eurostat Data 2017. http://ec.europa.eu/eurostat/tgm/refreshTableAction.do?tab=table&plugin=1&pcode=tps00010&language=en
2. Eurostat. Population structure and ageing. Eurostat yearbook. Statistics explained.2017.http://ec.europa.eu/eurostat/statisticsxplained/index.php/Population_structure_and_ageing.
3. Beltrán-Sánchez H, Soneji S, Crimmins EM. Past, Present, and Future of Healthy Life Expectancy. Cold Spring Harb Perspect Med 2015 Nov 2; 5(11). pii: a025957.
4. Istat. Il futuro demografico del paese. By Istat Data 2018. https://www.istat.it/it/files/2018/05/previsioni_demografiche.pdf

5. Benziger CP, Roth GA, Moran AE. The Global Burden of Disease Study and the Preventable Burden of NCD. Glob Heart 2016; 11: 393-7.
6. Grad FP. The preamble of the constitution of the World Health Organiza-tion. Bull World Health Organ 2002; 80: 981-984.
7. Phelan EA, Anderson LA, LaCroix AZ et al. Older adults' views of "success-ful aging"—how do they compare with researchers' definitions? J Am Geriatr Soc 2004; 52: 211-216.
8. Bousquet J, Michel J, Standberg T, Crooks G, Iakovidis I, Gomez M. The European Innovation Partnership on Active and Healthy Ageing: the European Geriatric Medicine introduces the EIP on AHA Column. Eur Geriatr Med 2014; 5: 361-2.
9. Friedman SM, Mulhausen P, Cleveland ML, et al. Healthy Aging: American Geriatrics Society White Paper Executive Summary. J Am Geriatr Soc 2019 Jan; 67(1): 17-20.
10. Beard JR, Officer A, de Carvalho IA, et al. The World report on ageing and health: a policy framework for healthy ageing. Lancet 2016; 387: 2145-2154.
11. Beard JR, Bloom DE. Towards a comprehensive public health response to population ageing. Lancet 2015; 385: 658-661.
12. Cesari M, Araujo de Carvalho I, Amuthavalli Thiyagarajan J, et al. Evidence for the Domains Supporting the Construct of Intrinsic Capacity. J Gerontol A Biol Sci Med Sci 2018 Nov 10; 73(12): 1653-1660.
13. Cosco TD, Howse K, Brayne C. Healthy ageing, resilience and wellbeing. Epidemiol Psychiatr Sci 2017 Dec; 26(6): 579-583.
14. Sampedro-Piquero P, Alvarez-Suarez P, Begega A. Coping with Stress During Aging: The Importance of a Resilient Brain. Curr Neuropharmacol 2018 Mar 5; 16(3): 284-296.
15. Lauretani F, Meschi T, Ticinesi A, Maggio M. "Brain-muscle loop" in the fragility of older persons: from pathophysiology to new organizing models. Aging Clin Exp Res 2017 Dec; 29(6): 1305-1311.
16. Stuck AE, Walthert JM, Nicolaus T, Bula CJ, Hohmann C, Beck JC. Risk factors for functional status decline in community-living elderly people: a systematic literature review. Soc Sci Med 1999; 48: 445-69.
17. Guralnik JM, Ferrucci L, Pieper CF, et al. Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery. J Gerontol A Biol Sci Med Sci 2000; 55(4): M221-31
18. Walston J, Hadley EC, Ferrucci L, et al. Research agenda for frailty in older adults: toward a better understanding of physiology and etiology: summary from the American Geriatrics Society/National Institute on Aging Research Conference on Frailty in Older Adults. J Am Geriatr Soc 2006; 54(6): 991-1001
19. Clegg A, Young J, Iliffe S, Rikkert MO, Rockwood K, Frailty in elderly people. Lancet 2013; 752-762.
20. Xue QL, The frailty syndrome: definition and natural history. Clin Geriatr Med 2011; 1-15.
21. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A: Biol Sci Med Sci 2001; M146-M157.
22. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly people, Can Med Assoc J 2005; 489-495.
23. Searle SD, Mitnitski A, Gahbauer EA, Gill TM, Rockwood K. A standard procedure for creating a frailty index. BMC Geriatr 2008; 24.
24. Rockwood K, Mitnitski A. How might deficit accumulation give rise to frailty? J Frailty Aging 1 2012; 8-12.
25. Rockwood K, Mitnitski A. Frailty defined by deficit accumulation and geriatric medicine defined by frailty. Clin Geriatr Med 2011; 17-26.
26. Fried LP., Xue QL, Cappola AR, et al. Nonlinear multisystem physiological dysregulation associated with frailty in older women: implications for etiology and treatment. J Gerontol A: Biol Sci Med Sci 2009; 1049-1057.
27. Villacampa-Fernández P, Navarro-Pardo E, Tarín JJ, Cano A. Frailty and multimorbidity: Two related yet different concepts. Maturitas 2016; 10.008.
28. Lang PO, Michel JP, Zekry D. Frailty syndrome: a transitional state in a dynamic process. Gerontology 2009; 55(5): 539-49.
29. Morley JE. Frailty and Sarcopenia: The New Geriatric Giants. Rev invest clin 2016; 68(2): 59-67.
30. Landi F, Cesari M, Calvani R, et al. The "Sarcopenia and Physical fRailty IN older people: multi-componenT Treatment strategies" (SPRINTT) randomized controlled trial: design and methods. Aging Clin Exp Res 2017 Feb; 29(1): 89-100.
31. Gobbens RJ, Luijkx, KG, Wijnen-Sponselee MT, Schols JM. Toward a conceptual definition of frail community dwelling older people. Nursing Outlook 2010; 58: 76-86.
32. Cesari, M, Pahor M, Lauretani F, et al. Frailty syndrome and skeletal muscle:result from Invecchiare in Chianti study. Am J Clin Nutr 2006 May; 83(5): 1142-8.
33. Kelley K, Clark B, Brown V, Sitzia J. Good practice in the conduct and reporting of survey research. Int J Qual Health Care 2003; 15(3): 261-6.
34. Burns KE, Duffett M, Kho ME, et al. A guide for the design and conduct of self-administered surveys of clinicians. CMAJ 2008; 179(3): 245-52.
35. Bennett C, Khangura S, Brehaut JC, et al. Reporting guidelines for survey research: an analysis of published guidance and reporting practices. PLoS Med 2010; 8(8): e1001069.
36. Rolfson DB, Majumdar SR, Tsuyuki RT, Tahir A, Rockwood K. Validity and reliability of the Edmonton Frail Scale. Age Ageing 2006; 35(5): 526-9.
37. Gobbens RJ, Van Assen MA, Luijkx KG, Wijnen-Sponselee MT, Schols JM. The Tilburg Frailty Indicator: psychometric properties. J Am Med Dir Assoc 2010; 11(5): 344-55.
38. Tavassoli N, Guyonnet S, Abellan Van Kan G, et al; Geriatric Frailty Clinic (G.F.C.) for Assessment of Frailty and Prevention of Disability Team. Description of 1,108 older patients referred by their physician to the "Geriatric Frailty

Clinic (G.F.C.) for Assessment of Frailty and Prevention of Disability" at the gerontopole. J Nutr Health Aging 2014; 18(5): 457-64.
39. Lauretani F, Ticinesi A, Gionti L, et al. Short-Physical Performance Battery (SPPB) score is associated with falls in older outpatients. Aging Clin Exp Res 2018 Dec 4.
40. Cesari M, Landi F, Calvani R, et al. Rationale for a preliminary operational definition of physical frailty and sarcopenia in the SPRINTT trial. Aging Clin Exp Res 2017 Feb; 29(1): 81-88.
41. Fielding RA, Rejeski WJ, Blair S et al. The Lifestyle Interventions and Independence for Elders Study: Design and Methods. J Gerontol A Biol Sci Med Sci 2011; 66(11): 1226-1237.
42. Pahor M, Guralnik JM, Ambrosius WT, et al. Effect of Structured Physical Activity on Prevention of Major Mobility Disability in Older Adults: The LIFE Study Randomized Clinical Trial. JAMA 2014; 311(23): 2387-2396.
43. Marzetti E, Cesari M, Calvani R, et al. The "Sarcopenia and Physical fRailty IN older people: multi-componenT Treatment strategies" (SPRINTT) randomized controlled trial: Case finding, screening and characteristics of eligible participants. Exp Gerontol 2018 Nov; 113: 48-57.

Received: 2 May 2019
Accepted: 16 May 2019
Correspondence:
Yari Longobucco
Department of Medicine and Surgery
University of Parma, Parma, Italy
Tel. +39 0521 906425
E-mail: yari.longobucco@unipr.it