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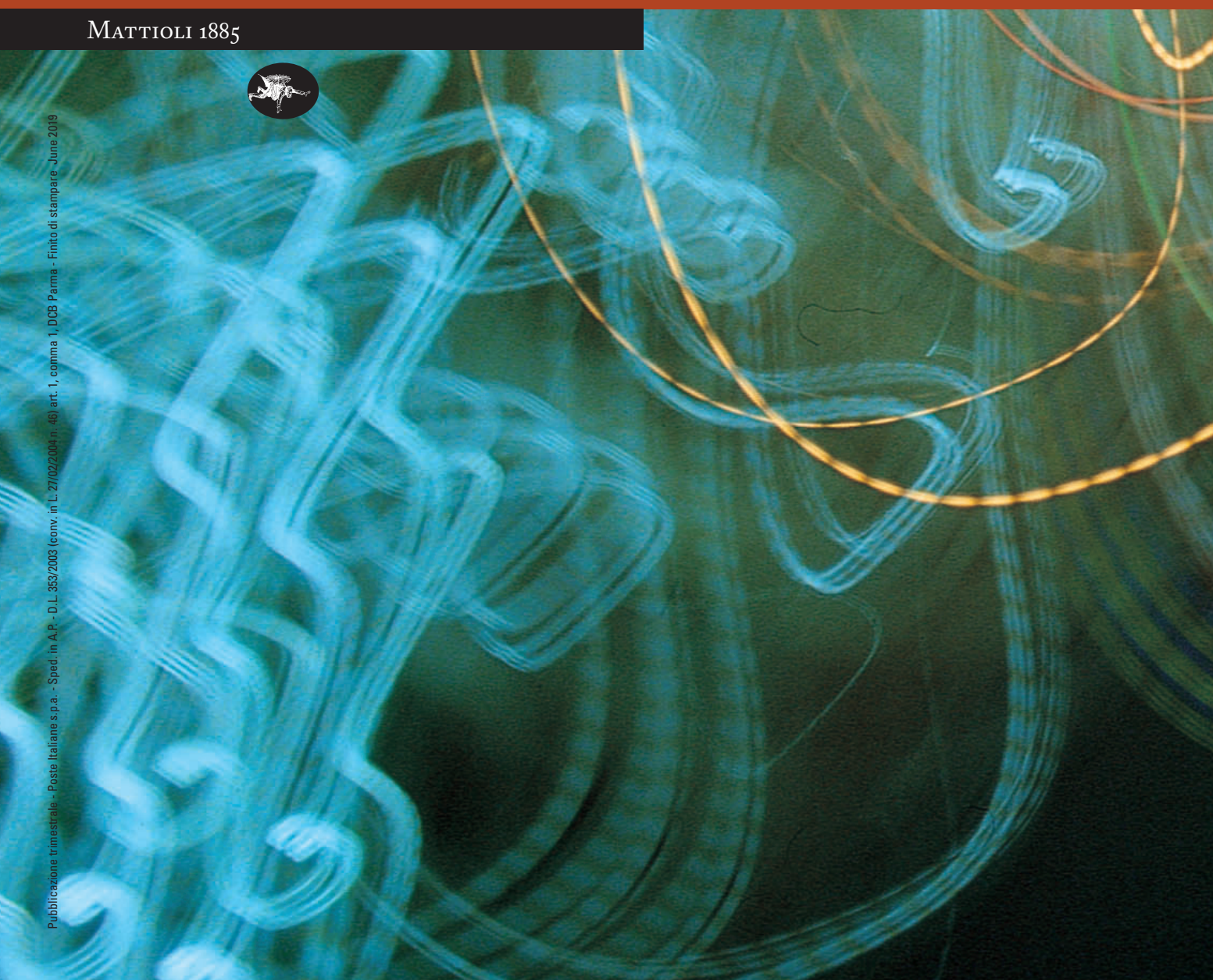

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Review

Status dystonicus: management and prevention in children at high risk

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Summary. *Background:* Status dystonicus (SD) is a movement disorder emergency associated with significant morbidity and life-threatening events that requires immediate and effective treatment. Nevertheless, SD is currently an under-recognized and undertreated condition, partly due to the lack of a standard definition and because it can be the acute complicated course of both primary and secondary dystonias. In subjects with SD, due to the delay of identification and lacking prevention of trigger and precipitant factors, intensive care management is consistently required. *Objectives:* We performed a critical review of this topic, outlining clinical features and linked genetic disorders to recognize subject at higher risk of SD, describing precipitant and trigger factors and proposing potential pharmacological treatment strategies in order to prevent hospitalization. *Results:* Genetic predisposition included: primary dystonias particularly in the case of TOR1A mutation; epileptic encephalopathy such as ARX and GNAO1 genetic variants and neurodegenerative disorders as PANK2. Early recognition of SD should be oriented by the following sign and symptoms: fever, tachycardia, respiratory change, hypertension, sweating and autonomic instability, elevated serum CK. Pain, fever and dehydration are main trigger factors that have to be prevented or quickly controlled. Achieving sleep could be the first therapeutic option in those with high risk of developing SD. Recently, enteral or transdermal clonidine as safety and efficacy therapeutic alternative was proposed. *Conclusion:* Recognizing high risk children for Status dystonicus from the onset of subtle signs and avoiding trigger factors could drive towards better management avoiding intensive treatments. (www.actabiomedica.it)

Key words: childhood, treatment, dystonia

1. Introduction

Status dystonicus (SD), is currently defined as "increasingly frequent and severe episodes of generalized dystonia, which necessitate urgent hospital admission (1). Many other terms have been used to refer to this condition, such as dystonic storm (2), life-threatening dystonia (3), desperate dystonics, and dystonic state (4).

SD commonly arise from both primary and secondary dystonias and rarely it is a complication of acute symptomatic dystonia related to infections, brain injuries, drowning or drugs (4).

Inside primary and secondary dystonias, many authors reported different genetic disorders at high risk of developing SD, although a systematic review has not been performed yet (4, 5). Underlying pathophysiology is not completely understood even because different precipitant factors could be the reasons of arising SD (6).

Up to now, there are no epidemiological studies on the prevalence of SD. Even if SD could not be referred as an age-dependent disorder, it is shown to be more frequent in childhood, probably due to the major risk of trigger factors and vulnerability of the developmental brain (1).

Furthermore, there is lack of high quality evidenced base medicine guidelines to inform management strategies. Dopaminergic modulation operated by antidyskinetic drugs could be a transient or ineffective therapeutic option (4, 5, 7), moreover usually introduced too late. Different drugs are commonly reached out in the way to allow muscular relaxation, sleep sedation or handle pain due to sustained muscle contraction. Ideally, such treatments should be performed in paediatric intensive care settings (6).

Our work propose the most recent update on SD in children based on a systematic review of the literature until the end of year 2017 and on our personal experience. The search was limited to articles published in english language and it was performed on PubMed database using the following terms: [Status dystonicus; dystonic storm; dystonia; life-threatening dystonia]. Seventy-five articles have been identified and from these we selected reviews and studies reporting SD in childhood. Single case reports were excluded. Our aims are: outlining clinical features and linked genetic disorders to recognize subject at higher risk of SD; describing precipitant and trigger factors that could be easily treated and proposing potential pharmacological strategies in order to prevent hospitalization in Pediatric Intensive Care Unit (PICU). In table 1 we reported strengths and limits of the principal reviews on this topic.

2. Status Dystonicus: clinical features and genetic predisposition

Dystonia is characterized by involuntary sustained or intermittent muscle contractions causing repetitive twisting movements, abnormal postures, or both (7). Dystonia is usually a fluctuating state, where clinical severity grows up over minutes, hours or days and there is paucity of available diagnostic biomarkers. Several conditions may mimic SD (e.g. status epilepticus, neuroleptic malignant syndrome, serotonine syndrome, acute parkinsonism or other dyskinesias): the phenomenology of the underlying movement disorder, associated neurological symptoms and signs, age of the patient and history of triggers are helpful clues to differentiate SD from other hyperkinetic movement disorder (6). Some patients are prone to SD, due to acquired or genetic underlying condition. The acquired dystonias (e.g. dystonic cerebral palsy) are the most common underlying dystonias leading to SD (8, 9). Another important information comes from genetic background other than acquired individual vulnerability in the pediatric age. In the largest series, describing 68 patients with isolated or recurring SD, 26% of subjects had primary dystonias (82% DYT1-TROR1A genetic mutation) and 35% heredodegenerative disorders, particularly pantothenate-kinase–associated neurodegeneration (PANK2 genetic mutations) and Wilson diseases (ATP7B genetic mutations), typically after initiation of D-penicillamine (8). An increasing number of reports documented that different epileptic encephalopathies of genetic origin could present SD. A high risk of SD in patients with ARX gene mutation, infantile spasm and expansion of the trinucleotide repeat that codes for the first PolyA tract has been highlighted (10). Missense mutations in GNAO1 were described in patients with epileptic encephalopathy and even in subjects with distinctive and severe movement disorder marked by episodes of severe, refractory ballismus requiring intensive care unit admissions that could lead to necessary deep brain stimulation (11, 12). Another important clue in this group of patient is the high recurrence of SD in almost 20% (8).

3. Precipitating factors

SD appears more commonly in children due to the vulnerability of the homeostatic systems and it could develops often as a triggered event. In that way many factors could potentially act as a trigger and generate a SD. Even if in about one-third of SD appears suddenly and without apparent causing factors, avoiding or treating the potential triggers as soon as possible must be considered as the first therapeutic choice. The main triggers are infections (particularly gastroenteritis with dehydration) and therapeutic adjustments (e.g. dopamine-receptor blockers such as pimozide and haloperidol, metoclopramide and clonazepam) (6, 8). In specific metabolic disorders such as Wilson disease, chelation therapy with penicillamine, zinc sulphate or trientine have also been linked to the development of SD (6). Other potential triggers are: trauma with head

Table 1.

Reviews (year)	Strengths	Limits of the study	Practical utility
Mariotti P. et al. Movement Disorders 2007	First critical review Accurate description of two personal cases with one year of follow-up	No clear information about dosage of drugs No information about trigger factors and warning sign	Not available
Grosso S. et al. European Journal of Paediatric Neurology 2012	Detailed description of personal cases with dosage of drugs and clinical course Flow chart management	Limited number of subjects No clear information about dystonia-targeted therapy No description about how to prevent SD	Therapy in PICU
Fasano A. et al. Movement Disorders 2012	Comprehensive systematic analysis of SD Large case series Information about successful treatment strategies Outcome description	No information about dosage of drugs and timing of pharmacological intervention No specific for pediatric population	Diagnosis
Allen ML. et al Developmental Medicine & Child Neurology 2014	First description about how to prevent SD in hospitalized child Screening for dystonia severity (grade) and action plan with overview of the management of SD and how to treat complications	No distinction in management depending on underlying etiology	Diagnosis and Therapy
Lopez MR and Fasano A. Movement Disorders 2017	Update on. phenomenology, progression and outcome Underlying etiology and pathophysiology Differential diagnosis	No description about how to prevent SD No information about dosage of drugs and timing of pharmacological intervention	Diagnosis
Lumsden DE et al. Curr Opinion Neurology 2017	Whole update on SD Screening for dystonia severity and action plan	No information about treatment outside PICU and dosage of dystonia-targeted therapy or calibrating sedation	Multilevel intervention and pharmacological management in PICU

injury, surgical procedures, anesthesia, 'metabolic disorder' decompensation, pain, gastro-esophageal reflux disease and constipation. Puberty-related deterioration in CP is less commonly reported but this condition as well as discomfort of whatsoever origin should be considered (9). Among all, pain, fever and dehydration are the most frequent trigger factors (4, 8), that could be prevented and quickly controlled. However, in 32,6% of SD an apparent precipitating factor remains unrevealed (8).

4. Therapeutic options outside PICU

Recent phenomenologic categorization divides episodes of SD into either tonic (mainly sustained contractions and abnormal postures) or phasic (rapid and repetitive dystonic contractions) phenotypes (8). In different studies it was underlined the importance to recognize and treat as soon as possible the life-threatening aspect and the development of one or more of the following signs: bulbar weakness, respiratory failure, metabolic derangements, exhaustion and pain (1, 4). In these well-defined life-threating events an admission to PICU is otherwise unavoidable.

Frequently the first-line pharmacological treatment is able to achieve a well recovery in only 10% of the patients.

The other patients need sedation (benzodiazepines and propofol were the most used, followed by barbiturate anesthesia), neurosurgery (either DBS or ablations), and more rarely intrathecal baclofen (ITB) (8).

In order to control an episode of SD as safely as possible, treatment should take place either in the intensive care unit or in a high dependency unit (13).

A practical therapeutic multiphasic approach in PICU could be summarized as follows (4, 6):

- Address precipitants (particularly accurate pain control loop)

- Begin supportive care
- Calibrate sedation
- Dystonia specific medications.

In that way intravenous fluid, antibiotics, nutritional requirements (nasogastric or parenteral) and antipyretics should be provided as early as possible but opioid analgesia might be also required (5, 6, 8).

In SD the sustained active muscle contraction leads to exhaustion and rhabdomyolysis: an important initial measure is to help the child to sleep without compromising respiration (13).

In some cases early recognition of SD and its prompt treatment could prevent serious complications and intensive care might be not necessary (14).

Our purpose is to identify early the subjects at high risk in order to prevent SD;

Basically, our strategy act on four items:

1) Periodic clinical check-up focused on patients at high risk due to brain damage, genetic or environmental factors. Particular attention should be given to the first three years of age and during pubertal spurt. Clinical evaluation has to consider: developmental abilities, functional skills and night-time sleeping pattern.
2) Prevention of precipitant factors through the early recognition of trigger (e.g. fever and dehydration). This aspect must be managed especially by the parents and for this reason specific 'parent training' could be helpful.
3) Sustained muscular contraction should be avoided and use of oral benzodiazepine could be helpful. Oral baclofen was reported to improve gait and lower-limb dystonia in children with primary dystonia (15). Pain control could be obtained not only with oral paracetamol or others nonsteroidal anti-inflammatory agents but also promoting correct sleep-wake pattern [e.g. administration of melatonine (16)].
4) Early administration of anti-dyskinetic drugs. Different aspects must be taken into account before the administration of anti-dyskinetic drug: mixed motor disorders are frequent (e.g., dystonia associated with spasticity); the course of dystonia might be influenced by ongoing brain maturation and by the remarkable plasticity of young brain; drug tolerability and effectiveness can be different in children; the therapeutic strategy must be discussed with both patient (according to their cognitve/intellectual ability) and parents (17, 18). Clinical evaluation is the starting point to define the therapeutic strategy, to delineate the topography, type (hyperkinetic/fixed dystonia) and severity of the dystonic manifestations, and degree of functional impairment in daily life (16). Trial with L-dopa should be always tried in children with dystonia of unknown or as-yet undiagnosed etiology due to the clinical variability of the dopa-responsive dystonias. In children with mixed movement disorders trihexiphenidyl is more effective on speech and upper-limbs function than on lower-limbs function. Different studies documented the utility of tetrabenazine for mobile dystonia (but not fixed) and particularly with facial involvement or deleyed-onset dystonia (19). D2 dopamine receptor blockers (e.g. Pimozide, aloperidol) could be of some benefits in patients with acute exacerbations and/or painful dystonic spasms (5, 6); and botulinum toxin injection for patients with focal dystonia (20). Intrathecal baclofen pump can be helpful in children with secondary dystonia, especially when associated with spasticity (8).

For children with severe drug-resistant primary generalized dystonia, early pallidal DBS is recommended before the onset of fixed skeletal deformities and major educational or social setbacks (8, 13).

Recently high dose of clonidine in the acute management of severe exacerbations of childhood dystonia or in SD, administered via different routes, has been proposed. This therapeutic option allowed a good response without bearing significant cardio-respiratory depression (21, 22).

Pediatric drug doses and side effects are shown in Table 2.

5. Conclusion

Our review gives an updated information about how to recognize children at high risk of SD and to

Table 2.

Drugs	*Initial and Target daily dosage*	*side effect*	*Precautions for use*
Melatonine	• If used as chronobiotic administer melatonin 3 or 4 h before actual sleep onset time. Start with a low dose of 0.2-0.5mg fast release melatonin 3 - 4 h before bedtime; increase by 0.2-0.5 mg every week as needed (maximum 3 mg; adolescents: 5 mg) until effect If no response after 1 week: increase dose by 1 mg every week until effect appears When 1 mg is effective: try lower dose Maximum dose: <40 Kg: 3 mg; >40 Kg: 5 mg • If used as sleep inductor start with 1-3 mg 30 min before bedtime	*morning drowsiness;* slight transient headache and gastrointestinal symptoms during the first days of the treatment. Rarely reported dizziness, rash, and hypothermia	• *6 months*
L-dopa	• 1 mg/kg/day of L-dopa (for classical DOPA responsive disorder) mg/kg/day up to 3–5 mg/kg/day, or even 8–10 mg/kg/ proceeding by very small increments and six intakes/day	Gastrointestinal disorders	• Could be toxic in mithocondrial disorders
Trihexiphenidyl	• Start 0.03–0.06 mg/kg/day up to 0.05–0.7 mg/kg/day by increments of 0.03–0.05 mg/kg/week in two to three daily intakes	Anthicolinergic side effects: Drowsiness, Memory impairment, Blurred vision, Dry mouth, Urinary retention, Constipation	• Efficacious if administered early at onset of movement disorders
Baclofen	• 0.3 mg/kg/day 0.5 to 1.5 mg/kg/day, by increments of 0.1 mg/kg/week, in two to three intakes	Drowsiness, Gastrointestinal Disorders.	• Worsening of axial hypotonia,
Tetrabenazine	• Start 0.5 mg/kg/day 4–5 mg/kg/day (without exceeding150–200 mg/day) by increments of 0.5 mg/kg/week, beginning with one intake then two	Drowsiness, Asthenia, Parkinsonian syndrome, Depression	• Caution in patients with akinetic-rigid syndome
Pimozide	• Start 0.5 to 1 mg/day 2–8 mg/day in two intakes	Drowsiness, Weight Gain Long QT syndrome	• Suspected susceptibility to malignant hyperthermia

identify early the subtle clinical signs before the onset of a SD. Moreover, specific therapeutic strategies could prevent the need of intensive therapy.

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

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Review

Mechanisms of pathophysiology of blood vessels in patients with multiple sclerosis treated with ozone therapy: a systematic review

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Summary. Multiple sclerosis (MS) defines as an intricate disease with numerous pathophysiological processes, including: inflammation, demyelination, oxidative stress, axonal damage, and repair mechanisms that interfere in this disease and highly related to the pathogenesis of MS. In parallel, recent studies have shown that the ozone administration could be very useful in treating neurological disorders and inflammatory and degenerative neurological diseases. In this review, we examine the recent literature on the pathophysiology of blood vessels in patients with multiple sclerosis treated with ozone therapy. (www.actabiomedica.it)

Key words: ozone, multiple sclerosis, pathophysiological, treatment

Introduction

Ozone (O3) as one of the most significant air pollutants, is a gas or a triatomic molecule that including the three atoms of oxygen, also, O3 in the mid-nineteenth century was discovered as well as it has a cyclic structure or a dynamically instable structure due to its mesomeric structure or states. In nature, O3 is created during storms due to the electrical evacuation of the beams that react with atmospheric oxygen to yield ozone. Moreover, it is as a clinical tool to characterization of secure or safe, cheap, and effective with an extensive area of therapeutic applications. Pain management of various diseases is a region where ozone excels, and further studies have been performed to indicate its analgesic attributes (1-5).

In recent years, set of applications on ozone or oxygen-ozone therapy has evident an increasing. O3-therapy is utilized in medicine to remedy different conditions of diseases and also, currently is one of the diverse minimally invasive treatments available in medicine science for physicians and researchers, plus, for example, O3-therapy can be delivered by injection of rectal insufflation, which organizes a simple and minimally invasive pathway, with slight toxicity, automatically replaces to the classical mayor autohemotherapy and a large bibliography is based on the exploitation of ozone chemical properties, an unstable allotropic form of oxygen with the symbol O3 and a molecular weight of 48 kDa. Nevertheless, there are a lot of literatures about the positive effects of the oxygen and ozone therapy on diverse pathophysiological process, tissues, and organs. Furthermore, the biological effects of the rectal insufflation of ozone have been revealed extensively either experimentally or clinically. However, there is requiring of strong materials for the in vivo researches and real-time monitoring of the ozone effects during treatment (6-13).

According to studies and research reports, treatment with O3 can elevates blood oxygen impregna-

tion, progresses blood circulation, activates erythrocyte metabolism, improves tissue oxygenation and oxygen secure and restores cell function, effectively increasing oxygen metabolism. In addition, O3 can be provided by major ozone autohemotherapy, which includes in drawing a given amount of venous blood and after in reinfusing it after it has been added to a mix of O2/O3.More biologic effects have been ascribed to ozone: elevated glycolysis, effects on red blood cells, rheology; fungicide, bactericidal, and virustatic immunomodulating action, analgesic and anti-inflammatory effects. Furthermore, O3 can also reportedly improve arterial and venous blood flow, increase the elasticity of erythrocytes, increase the capability of blood to pass through vessels such as the capillaries and consequently increase oxygen supply to whole organ systems. Moreover, also decreases platelet aggregation, and promotes formation of hydrogen peroxide at the site of thrombus, which impairs thrombosis and induces thrombus decomposition as well as O3 also activates platelets inducing the subsequent liberation to the blood of growth factors that can comfort wound regeneration. In truth, labroratory studies have been suggested that treatment with O3 is effective in protecting organs from reperfusion losses. Hence, ozone indicates the therapeutic properties of vascular and metabolic treatment. This broad spectrum of practice describes the multitude marks for medical ozone administration (2, 11, 12, 14-25). On the other hand, previous studies demonstrated the ozone capabilities of raising peripheral tissue oxygenation as well as the impact of ozone autohemotheraphy on vessels diseases, injuries, macular degeneration and prevention of limb ischemia (15, 17, 26, 27). In a study by Percorelli et al. reported upregulation of the heme oxygenase-l expression in endothelial cells by ozonated serum (28).

O3 therapy can be produced a number of messengers that attain to total cells in the body and act to reverse chronic oxidative stress by readjusting the modified cellular redox balance. Plus, these cellular messengers could motivate the emancipation of stem cells from the bone marrow for regenerating distressed or degenerated organs. Nevertheless; this treatment method has been engaged for decades as a supplementary medical approach in a spacious range of pathologies including resistant infections, orthopedic pathologies, degenerative eye disease, various pain syndromes, ischemic vascular abnormalities and neurodegenerative diseases. However, the use of O3 treatment on the neurodegeneration related to normal aging remains a rigorous range for investigation (3, 29-32).

Neurodegenerative disorders have numerous various etiologies and are a group of heterogeneous diseases of the nervous system such as the brain, spinal cord, and peripheral nerves. The outbreak of them increases with extended life hope, illustrating a serious health problem global. In parallel, recent studies have shown that the ozone administration could be very useful in treating neurological disorders and inflammatory and degenerative neurological diseases, via a signed effect on the activity of the cytochrome-c-oxidase. Neuroprotective features of O3 therapy effects have been predicted in vivo, although, little is known on its clinical and therapeutic impact in neurodegeneration, but O3 rapidly by generating of two messengers such as H2O2 and a mixture of lipid ozonated products acts as pro-medication, which it due to from the response of O3 with the cell membrane and lipoproteins-bound polyunsaturated fatty acids currently the researchers are distinguishing the clinical efficacy of ozone major autohemotherapy in the treatment of multiple sclerosis (MS). As a perspective, the goal is to apperceive whether MS patients underwent ozone autohemotherapy demonstrate a reduced number of annual relapses or reducing the symptoms of patient, an improvement in the functional scores, and stabilization of the number of white matter lesions. But its effects on brain are remains not clear or unknown (33-36).

Ozone autohemotherapy (OA) as an emerging remedial technique is another procedure of administration for ozone therapy that is achieving increasing in the treatment of neurodegenerative disorders (37-39). OA involves the collection of venous blood from the patient (from100 to 240 g), and then the blood is blended with an oxygen/ozone (O2-O3) mixture, and it via the same vein is reinfused (40, 41). In a study, Molinari et al., 2014 in a long-term monitoring reported that OA had a clear effect on progress of the reduction of chronic oxidative stress and enhance of the mitochondrial functionality of neural cells in MS patients (38). Furthermore, Larini et al., 2003 have revealed that OA can ameliorate blood circulation, activate antioxidant enzymes and scavenge free radicals

(42), as well as in recent years, several studies demonstrated that OA has been already utilized to treat vascular disease (such as peripheral artery disease), advanced ischemic diseases and neurological disease (E.g. spontaneous spinal epidural hematoma, multiple sclerosis), but after OA, no scientific evidence of the oxygen concentration in the brain cortex, which this result is in accordance with published studies (43-47).

MS is a degenerative neurological pathology or a chronic inflammatory autoimmune disease of the central nervous system (CNS) characterized by both vascular and metabolic impairments that leads to demyelination and axonal damage or leads to a deficiency or complete loss in the transmission of nerve impulses for the exact etiology is not yet understood. On the other define, Multiple sclerosis (MS) defines as an intricate disease with numerous pathophysiological processes, including: inflammation, demyelination, oxidative stress, axonal damage, and repair mechanisms that interfere in this disease and highly related to the pathogenesis of MS. In addition, high levels of lipid peroxidation and decreased antioxidants have been found in blood and cerebrospinal fluid of patients at active phases of MS as well as increased oxidative stress levels seen in MS patients has been clearly showed. Moreover, reactive oxygen species (ROS) meliorate transendothelial leukocyte migration and helps to oligodendrocyte injury and axonal degeneration. Since ROS plays an axial role in the primary stage as well as the chronic stage of MS, thus, antioxidant therapy might be an attractive approach to limit disease progression. So, O3 therapy has a rationale of application as an adjuvant remedy for MS patients. In accordance with past studies, the O3 therapy elevated the total level of oxygen in the tissue. Increasing oxygen in control is more obvious than MS patients (48-62). Araneda et al. have shown that vascular endothelial growth factor (VEGF)-immunoreactive glial cells are in contact with blood vessel walls during post-ozone improvement showing revascularization and regeneration of the BBB (63). In another study by Broadwater et al., and Molinari et al., have reported that in MS patients the O3 therapy elevated the overall level of oxygen in the tissue. Findings them suggested that might be explained by increased ozone-induced metabolism. Also it have been shown, the little levels of mitochondrial activity in MS patients probably due to oxidative damage to DNA. They also observed that the ozone ameliorates mitochondrial activity level, it can be that the enhancement in the neurons metabolic function effected an gain in the level of oxygen expenditure (9,64) as well as Molinari et al., revealed that in a clinical trial study with ozone autohemotherapy on MS patients an increment of cytochrome-coxidase level together with reduction of the chronic oxidative stress level typical of MS cases (9). Other report by Delgado-Roche et al., 2017 have indicated a considerable depletion of oxidative damage on lipids and proteins in patients treated with ozone. Likewise, the pro-inflammatory cytokines levels were lesser after o3 therapy. Findings can supplies novel insights on the into ozone-induced molecular events, and remonstrate o3 therapy as a potential therapeutic alternative for MS patients (65).Furthermore, Lu et al., have proposed that ozone autohemotherapy could be useful to treat MS patients, especially because of its raising effect on mitochondria activation (66).

Conclusions

To the best of our knowledge, our findings of this review article suggest that Ozone-therapy is a new therapeutic technique that is achieving elevating importance in treating on MS patient. Therefore, it should be noted that the therapeutic potential of ozone needs to be much attention through its strong capacity with a reduction of toxicity of MS patients undergoing remedy with other drugs and side effects, and it promotes a reduction of cellular oxidative stress, oxidative damage on lipid and proteins, decrease of the pro-inflammatory cytokines levels and an improvement of oxygen blood transportation and delivery. These results will provide many insights to propose the potential neuroprotective mechanism of medical ozone in MS. Finally, O3 therapy approach could be considered as an affirmative supplement to the actual pharmacological remedies addressed to neurodegenerative disorders such as MS as well as it should also be considered for the clinical efficacy of OA in the treatment of MS.

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

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Update

What still prevents to acknowledge a major role for pulmonary rehabilitation in COPD treatment?

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Summary. Chronic obstructive pulmonary disease (COPD) is a major health issue, particularly in aging people. Despite an increasing availability of drugs to treat COPD, recent data indicate that an actual control of the disease is achieved in a minority of patients. This makes apparent that additional treatments of COPD should be taken into account, such as pulmonary rehabilitation (PR), which was introduced in the 1960s and has large evidence of clinical effectiveness. PR is a non-pharmacologic therapy based on a comprehensive, multidisciplinary, patient-centered intervention comprising exercise training, self-management education and psychosocial support. PR treated patients develop an increased exercise tolerance and quality of life, reduced dyspnea and anxiety, and are concerned by less hospital admissions for disease exacerbations. Notwithstanding, the use of PR in COPD patients is negligible, being globally estimated in 2-5%. Here we update the evidence in favor of PR and the actual need to consider it as a treatment to be considered for COPD patients with significant impairment in daily living activities. (www.actabiomedica.it)

Key words: COPD, drug treatment, pulmonary rehabilitation, effectiveness, exercise tolerance, dyspnea, quality of life

Introduction

Chronic obstructive pulmonary disease (COPD), as defined by the updated Global initiative for chronic Obstructive Lung Disease (GOLD) guidelines "is a common, preventable, and treatable disease that is characterized by persistent respiratory symptoms and airflow limitations that are due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases" (1). COPD is a significant burden to patients, particularly aging people, and society, that is especially associated to dyspnea, with an increasing impairment on daily living activities and quality of live resulting from the disease progress, and a mortality estimated in more than 3 million people worldwide per year (2). In front of an increasing availability of drugs to treat COPD, according to a recent review "Current therapies provide only limited short-term benefit and fail to halt progression" (3). Actually, the two pivotal studies Toward a Revolution in COPD Health (TORCH) (4) and Understanding Potential Long-term Impacts on Function and Tiotropium (UPLIFT) (5) had a limited success. Namely, though both treatments decreased exacerbations and hospitalization rates, the salmeterol and fluticasone combination reduced over 3 years, compared to placebo, the decline in lung function but not the all cause mortality, while the treatment with tiotropium over 4 years did not significantly slow the decline in lung function, while reducing mortality (6). In a recent survey on 3672 COPD patients from US and Europe under a standard of care drug treatment, more than 80% reported to experience at least one symptom "often", and 70% of patients reported some

level of non-adherence (7). Thus, it is apparent that additional treatments of COPD should be taken into account. Among these, pulmonary rehabilitation (PR), which was introduced in the 1960s, has large evidence of clinical benefit (8). In presenting PR to family physicians, Nici et al. defined it as a "nonpharmacologic therapy that has emerged as a standard of care for patients with COPD" that is based on a comprehensive, multidisciplinary, patient-centered intervention comprising exercise training, self-management education and psychosocial support, and resulting in an increased exercise tolerance, reduced dyspnea and anxiety, reduced hospital admissions for disease exacerbations, and improvement in health-related quality of life (HRQoL (9). Despite such background, the number of COPD patients undergoing PR is negligible, being globally estimated in 2-5% (10). For example, in US the analysis of data for Medicare beneficiaries with COPD who received PR from January 1, 2003, to December 31, 2012, showed that patients receiving PR increased from 2.6% in 2003 to 3.7% in 2012, but the improved use of PR was attributed to prior users more than new users of PR (11). It is not rare that less than 1% of COPD patients undergo PR each year, as reported for example from New Zealand, where this low rate of participation occurred even though PR was provided in 19 of 21 District Health Boards regions (12).

Here we update the evidence in favor of PR and the actual need to consider it as a treatment to be performed in COPD patients with impairment in daily living activities

Evidence of PR effectiveness as assessed by meta-analyses

The highest grade of scientific evidence for medical treatments is provided by positive systematic reviews and meta-analyses. The first meta-analysis on randomized controlled trials of PR (with control groups receiving no rehabilitation), in patients with COPD was performed in 1996. Significant improvements were found for all the outcomes, though dyspnea and better control over COPD were mentioned as clinically important, while the value of the improvement in exercise capacity was not clear (13). From 2002 to 2015 three Cochrane meta-analyses were published. Their main data are summarized in table 1. Actually, the first meta-analysis reanalyzed by the Cochrane database systematic review methods the same trials previously assessed, with similar conclusion but a final statement mentioning that "Rehabilitation forms an important component of the management of COPD" (14). In the 2006 meta-analysis, adding 8 further trials, the conclusion were that " Rehabilitation relieves dyspnea and fatigue, improves emotional function and enhances patients' sense of control over their condition", with the same final statement reported above (15). The 2015 meta-analysis, based on 65 trials, due the further confirmation of the positive outcomes, has lead the authors to write "It is our opinion that additional RCTs comparing pulmonary rehabilitation and conventional care in COPD are not warranted". Indeed, because some studies included in the analysis addressed community-based programs, the authors

Table 1. Main data from Cochrane meta-analyses on pulmonary rehabilitation in COPD

Author, year [ref]	Number of trials included in the analysis	Weighted mean difference
Lacasse et al, 2002 [13]	23	Dyspnea score: 0.98 units, 95% Confidence Interval (95% CI) 0.74 - 1.22 units; n=9 trials. 6- minute walking distance: 49 m, 95% CI: 26 - 72 m; n=10 trials.
Lacasse et al. 2006 [14]	31	Dyspnea score: 1.0 units; 95% confidence interval: 0.8 to 1.3 units; n = 12 trials). 6-minute walking distance: 48 meters; 95% CI: 32 to 65; n = 16 trials).
McCarthy et al. 2015 [15]	65	Dyspnea: 0.79, 95% confidence interval (CI) 0.56 to 1.03; n = 19 trials 6-minute walking distance 43.93, 95% CI 32.64 to 55.21; n = 38 trials

suggested "Future research studies should focus on identifying which components of pulmonary rehabilitation are essential, its ideal length and location, the degree of supervision and intensity of training required and how long treatment effects persist (16). In fact, in 2014 a meta-analysis of 18 trials on home-based pulmonary rehabilitation programs was published, with data comparison between treated and untreated patients suggesting this kind of PR as an effective therapeutic intervention to relieve COPD-associated respiratory symptoms and improving HRQoL and exercise capacity (17). However, a recent meta-analysis compared hospital (outpatients) and home-based exercise training rehabilitation programs for COPD. Ten trials were analyzed, with low to moderate evidence that outpatient and home-based exercise training programs are equally effective (18). Therefore, the suitability to apply home-based PR with an expected outcome comparable to outpatients programs needs to be investigated by large-scale controlled trials to identify the most favorable standard program (17).

Another issue in search of elucidation is the effectiveness of PR in patients with mild COPD. Two meta-analyses are available. The authors of the first meta-analysis, including 3 studies with different designs (retrospective, one group pretest-posttest, and randomized controlled trial) concluded that most of the PR programs had positive effects on exercise capacity and HRQoL in patients with mild COPD, but the evidence was insufficient and studies with robust designs and with longer follow-up should be conducted (19). The second meta-analysis, including 4 randomized controlled trials, found a clinically and statistically significant improvement in short-term HRQoL, but not at the follow-up. There was an improvement in the 6-minute walk test with PR, though not considered clinically relevant. The data for muscle strength and maximal exercise capacity were insufficient for meta-analysis (20).

Also the capacity of PR to reduce COPD exacerbations was evaluated in three Cochrane meta-analyses. The first included 6 trials and showed that PR significantly decreased hospital admissions (odds ratio 0.13 (95% CI 0.04 to 0.35)), number needed to treat (NNT) 3 (95% CI 2 to 4) over 34 weeks, and mortality (odds ratio 0.29 (95% CI 0.10 to 0.84), NNT 6 (95% CI 5 to 30) over 107 weeks) (21). In the 2011 update, 9 trials were analyzed, the figures confirming the significant reduction of hospital admissions (odds ratio 0.22 (95% CI 0.08 to 0.58)), NNT 4 (95% CI 3 to 8) over 25 weeks) and mortality (OR 0.28; 95% CI 0.10 to 0.84), NNT 6 (95% CI 5 to 30) over 107 weeks) (22). In the latest meta-analysis the number of selected studies was more than doubled, but the PR programs used in the 20 studies showed large variety in terms of exercise training (type, intensity and supervision), patient education (from none to extensive self-management programs) and kind of organization (one setting or various settings, e.g. hospital, outpatient centre and home). Such heterogeneity prevented to reach firm conclusions on the PR effects on hospital readmissions and mortality, and the authors claimed future research on the influence of PR programs in terms of exercise sessions, self-management education and other components affecting the outcomes (23).

Issues to be highlighted

Effects of PR on pulmonary function

Most studies on effectiveness of PR in COPD focused the interest on the improvement of HRQoL and physical performance and reduction of dyspnea, while pulmonary function, although obviously related to bronchial obstruction, was rarely included as a measure of efficacy. Stav et al. assessed the efficacy of a 3 year outpatient PR program in 80 patients with moderate to severe COPD, measuring pulmonary function and exercise capability, at 6, 12, 18, 24, 30, and 36 months. The control group received standard care only. The decline in forced expiratory volume in 1 second (FEV1) after the 3 years was significantly lower in the PR group compared to control, corresponding to 74 ml versus 149 ml ($p<0.001$), and maximal sustained work and endurance time improved early and was maintained all over the study with PR, but not in the control group (24). In the study named FEV1 as an Index of Rehabilitation Success over Time (FIRST), the effects of PR on lung function were evaluated in 257 COPD patients treated with inhaled corticosteroids or long-acting β2-agonists and/or tiotropium during a 3-year duration of

PR, compared with 67 patients treated only with drugs. Lung function was measured at baseline and at one-year intervals. In the PR group, FEV1 increased from 1240 mL (57.3% of predicted value) to 1252.4 mL (60.8%) after 3 years, whereas in the controls the values were 1367 mL (55% of predicted) at baseline and 1150 mL (51%) after 3 years ($p<0.001$) (25). The authors claimed for confirmation of such outcome from randomized trials. However, thus far only in the meta-analysis on randomized controlled trials of home-based PR for COPD patients pulmonary function (measured by FEV1/forced vital capacity) was found to be significantly better ($p< 0.0001$) after 12 weeks of intervention compared with the nonintervention control group (17). Another pulmonary function index is tidal volume, that in an analysis of three studies, which were based on training at high intensity (70%-80% maximum workload) in PR treated patients with moderate to severe stable COPD, was significantly improved in patients with reduced dyspnea (26).

Outcome of PR according to the severity of COPD

An impact of COPD severity on the clinical success of PR would be of obvious importance. In a study on 167 COPD patients undergoing PR, each subject was classified into one of 4 categories A, B, C, and D, according to exercise capacity, respiratory symptoms and health status. The groups were homogeneous in age, body mass index, smoking pack-years, and comorbidities. Significant improvements in all outcomes were detected, with categories A and C showing a more pronounced improvement in exercise capacity and symptoms. However, despite these differences, the likelihood to have a minimum clinically important difference in each outcome was similar for all categories when compared (27). Another study prospectively compared the benefit obtained by PR in 229 COPD patients according to baseline disease burden. Subjects were divided into 4 quartiles by their baseline level of dyspnea, FEV1 percent predicted and 6-minute-walk distance, with change in HRQoL (as assessed by the SF-36 questionnaire) being the primary outcome. After PR, clinically significant improvements were observed in most components of SF-36, particularly concerning physical function, health perception, physical role, emotional role, social function, mental health, pain, vitality, and depression. The authors concluded that PR results in significant improvement in quality of life, dyspnea, and functional capacity irrespective of baseline disease burden (28). A similar improvement was shown using the SF-36 questionnaire in 440 patients treated with PR, of whom 229 completed the program. Of interest, patients completing PR had greater pain and depression score to the SF-36, and lower FEV1, and included a lower proportion of current smokers, while cigarette smoking was the sole independent predictor of PR dropout (29).

Cost-effectiveness of PR

Early economic evaluations of PR were limited to the cost. For example, in 1997 a study considered 46 stable COPD patients undergoing a PR program in 10 sessions including education, training, group therapy, and an individualized regimen of home-based extremity and inspiratory muscle exercise. The program resulted in significant decrease of dyspnea and significant increases of exercise capacity and forced vital capacity, with a cost of the 10 outpatient sessions of $650 (30). Golmohammadi et al. calculated that the total direct cost per 100 person-years of follow-up before the program was $122,071 while after the program it was $87,704, with an average reduction of total costs of $34,367 per 100 person-years, corresponding to $344 per person per year ($p=0.02$). Such reduction resulted from decreased health service utilization, reduced direct costs and improved health status of COPD patients treated with PR (31). Subsequent studies analyzed the economic benefit produced by the reduced hospital utilization and health cost (32, 33), while Griffiths et al. were the first to use the modern tool for cost-effectiveness analysis, specifically the quality adjusted life years (QALYs), that is a measure of disease burden, including both the quality and the quantity of life lived (34). The cost/utility analysis was performed along a randomized controlled clinical trial of PR vs. standard care in 200 patients randomly assigned to either an 18 visit, 6 week rehabilitation program or standard medical care. The difference between the mean cost of 12 months of care for patients in PR and control group was calculated. The results showed that each PR program for up to 20

patients cost £12,120, with a mean incremental cost of adding PR to standard care of -£152 (95% CI -881 to 577) per patient (p=NS). The incremental utility of adding PR was 0.030 (95% CI 0.002 to 0.058) QALYs per patient (p=0.03). Thus, the outpatient PR program produced cost per QALY ratios within limits considered to be cost effective and likely to result in financial benefits to the health service (35). In another study from UK, also the cost effectiveness of maintenance schedules following initial PR was investigated in COPD patients who completed at least 60% of a standard 8-week PR program and were randomized to a 2-h maintenance session at 3, 6 and 9 months (73 subjects) or treatment as usual (75 subjects). QALYs and incremental cost-effectiveness ratio (ICER) were used. At 12 months, incremental cost to the NHS and social services was -£204.04 (95 % CI -£1522 to £1114), and QALY gains were -0.007 (-0.461 to 0.447) and +0.015 (-0.050 to 0.079). Based on point estimates, PR maintenance therefore was dominant over usual treatment from the perspective of the NHS and social services. A need of future research to evaluate whether also more intensive PR maintenance regimens offer benefit to patients at reasonable cost was suggested (36). Recently, Atsou et al. estimated the effectiveness and cost-effectiveness of PR in a hypothetical cohort of COPD patients in France using a multi-state Markov model adopting society's perspective. Simulated cohorts of COPD patients in GOLD stage 2 to 4 with and without PR were compared in terms of life expectancy, QALYs, disease-related costs, and ICER. At the horizon of a COPD patient's remaining lifetime, PR would result in mean gain of 0.8 QALY, with an over disease-related costs of 14,102 € per patient. The ICER was 17 583 €/QALY. Sensitivity analysis showed that PR was cost-effective in every scenario (ICER <50 000 €/QALY). According to authors, these outcomes should provide a useful basis for COPD PR programs (37).

Conclusions

In 2006, the American Thoracic Society/European Respiratory Society statement on pulmonary rehabilitation concluded the analysis of the available literature with the assertion "A considerable body of theoretical and practical knowledge has already been developed, resulting in the establishment of pulmonary rehabilitation as a science. We look forward to refining its process, improving its efficiency, optimizing its benefits, and expanding its scope" (38). In fact, in the 2013 updated document the conclusion was "The considerable growth in the science and application of pulmonary rehabilitation since 2006 adds further support for its efficacy in a wide range of individuals with chronic respiratory disease" (39). This highlights that also respiratory chronic disorders different from COPD should be treated with PR. In front of this large evidence, PR remains greatly underestimated and underused. According to Troosters et al., "the future of pulmonary rehabilitation is bright", but requires engaging more patients in better tailored programmes, that need to be widely advertised, with healthcare professionals well trained to deal with the individual needs and preferences of patients. Also, patients need to develop self-management skills enabling them to maintain the benefits of the programme activity (40).

The latest document from the American Thoracic Society/European Respiratory Society, based on the demonstration of physiological, symptom-reducing, psychosocial, and health economic benefits achieved by PR, and of insufficient funding, resources and reimbursement, lack of healthcare professional, payer, and patient awareness and knowledge, was aimed at enhancing implementation, use, and delivery of PR to suitable individuals worldwide. This document contains policy recommendations to evolve healthcare professional, payer, and patient awareness and knowledge of PR, to increase patient access to PR and to ensure quality of PR programs. The ATS and ERS will undertake actions to improve access to and delivery of PR services for suitable patients, and call on their members and other health professional societies, payers, patients, and patient advocacy groups to join in this commitment (41).

We must hope that this initiative can finally succeed in making acknowledged the actual role of PR in COPD treatment.

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

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Original article

Marital status and paternity in patients with Transfusion-Dependent Thalassemia (TDT) and Non Transfusion-Dependent Thalassemia (NTDT): an ICET - A survey in different countries

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Summary. *Background*: More than five decades ago, thalassemia major (TDT) was fatal in the first decade of life. Survival and quality of life have improved progressively thanks to the implementation of a significant advance in diagnostic and therapeutic methods, consisting mainly of a frequent transfusion program combined with intensive chelation therapy. Improvement also includes imaging methods used to measure liver and cardiac iron overload. Improved survival has led to a growing number of adults requiring specialised care and counselling for specific life events, such as sexual maturity and acquisition of a family. *Aims of the study:* The main aim is to present the results of a survey on the marital and paternity status in a large population of adult males with TDT and NTDT living in countries with a high prevalence of thalassemia and a review of current literature using a systematic search for published studies. *Results:* Ten out of 16 Thalassemia Centres (62.5%) of the ICET-A Network, treating a total of 966 male patients, aged above 18 years with β- thalassemias (738 TDT and 228 NTDT), participated in the study. Of the 966 patients, 240 (24.8%) were married or lived with partners, and 726 (75.2%) unmarried. The mean age at marriage was 29.7 ± 0.3 years. Of 240 patients, 184 (76.6%) had children within the first two years of marriage (2.1 ± 0.1 years, median 2 years, range 1.8 - 2.3 years). The average number of children was 1.32 ± 0.06 (1.27 ± 0.07 in TDT patients and

1.47 ± 0.15 in NTDT patients; p: >0.05). Whatever the modality of conception, 184 patients (76.6%) had one or two children and 1 NTDT patient had 6 children. Nine (4.8%) births were twins. Of 184 patients, 150 (81.5%) had natural conception, 23 (12.5%) required induction of spermatogenesis with gonadotropins (hCG and hMG), 8 (4.3%) needed intracytoplasmic sperm injection (ICSI) and 3 adopted a child. 39 patients with TDT and NTDT asked for medical help as they were unable to father naturally: 7 TDT patients (17.9%) were azoospermic, 17 (37.7%) [13 with TDT and 4 with NTDT] had dysspermia and 15 (33.3%) [13 with TDT and 2 with NTDT] had other "general medical and non-medical conditions". *Conclusions:* Our study provides detailed information in a novel area where there are few contemporary data. Understanding the aspects of male reproductive health is important for physicians involved in the care of men with thalassemias to convey the message that prospects for fatherhood are potentially good due to progressive improvements in treatment regimens and supportive care. (www.actabiomedica.it)

Key words: thalassemia, marital status, paternity, comorbidities, endocrine complications, iron overload, chelation therapy

Introduction

Thalassemias are the most common monogenic hematologic disorders with a worldwide distribution (1). Based on clinical and haematological features and molecular characterization, β-thalassemia is classified into 3 distinct categories: thalassemia major, also known as transfusion dependent thalassemia (TDT), thalassemia intermedia [characterized usually as non-transfusion dependent thalassemia (NTDT)], and thalassemia minor (2-4). It is estimated that more than 60,000 babies are born annually with thalassemia major and more than 80 million are carriers of β-thalassemia (1). The severity of the disease depends on the degree of imbalance between a- and β-globin chain synthesis leading to ineffective erythropoiesis (IE), bone marrow expansion and a chronic hemolytic anemia (2-4). Anemia is treated with frequent packed red blood cell (PRBC) transfusions which result in the accumulation of iron, released by the breakdown products of hemoglobin (heme and iron) and increased absorption of iron from the intestine related to anemia (5-7).

Between 1949 and 1957, in Ferrara, only 9% of patients reached the age of 6 years, and by the end of the 1970s, half of Italian thalassemic patients had died before the age of 12 years. Since the 1980s, due to treatment with a combination of regular transfusions and chelation, and/or bone marrow transplantation, survival improved significantly, but still remains suboptimal at national levels (5).

Today, in developed countries, survival of patients on conventional treatment has increased to 40-50 and more years, and keeps improving (6-8). Improved patient care has now expanded to encourage patients to aspire to the vocational, social, sexual, and reproductive goals of their healthy peers (9).

Overall, hundreds of uneventful pregnancies have occurred in women with TDT and NTDT (10,11); apart from infertility (9), only a few studies have addressed the sexual and reproductive health of men with thalassemias (12-14).

The main aim of the present study was to investigate the marital and paternity status in a large population of male patients over the age of 18 years with TDT and NTDT living in countries with a high prevalence of β- thalassemia.

Survey Design and Participants

Questionnaire development

A. First step

In April 2018, the Coordinator (VDS) of the International Network of Clinicians for Endocrinopa-

thies in Thalassemia and Adolescence Medicine (ICET-A) (15,16) designed and promoted a survey questionnaire to collect data on "Marital and paternity status in patients with TDT and NTDT, aged over 18 years".

The criteria for patients' inclusion in the survey were: 1) Male patients with TDT or NTDT who were over the age of 18 yrs at the time of data collection. The term TDT was based on clinical (regular transfusion with packed red cells, every 2-3 weeks, since the first years of life), haematological and biochemical findings, and the term NTDT was applied to patients with mild to moderate anemia, splenomegaly, mild degree of growth impairment, requiring red blood transfusions in certain circumstances, such as: delayed puberty, infections, surgery, pregnancy or falling Hb in adult life (1-4).

Exclusion criteria were:1) TDT and NTDT patients with incomplete records, 2) bone marrow transplanted patients, 3) eating disorders, and 4) renal insufficiency.

B. Second step

All ICET-A members were requested, by mail, to comment on the data included in the preliminary questionnaire draft. The study was planned to fulfil the following information: personal doctors' data (place of work, specialization), patients' demographic characteristics including age, marital status and paternity, patients' transfusion and iron chelation regime, serum ferritin level and associated complications.

Patients were classified as 'married' or 'lived with partners'. For these individuals, the duration of marriage, the spouse's health status (healthy, β-thalassemia carrier, TDT or NTDT), number of children born after natural conception, induction with gonadotrophins or artificial insemination with a sperm donor, intracytoplasmic spermatozoan injection (ICSI), or adoption were requested.

C. Third step

After final approval, the questionnaire was sent to the 16 Thalassemia Centers of the ICET-A Network with an official invitation to participate in the survey. The deadline to return the completed questionnaire in an Excel format was fixed for 4 months.

For uniform collection of data, the diagnosis of organ dysfunction was based on the following definitions supported by laboratory results, as well as confirmatory clinical evidence: a) cardiac complications were defined as the presence of any of the following: history of heart failure, left and/or right mild or overt ventricular dysfunction, arrhythmia with or without myocardial magnetic resonance imaging siderosis (MRI T2* <20 msec) (17-19); b) liver dysfunction was defined by the presence of organ enlargement associated with significant and persistent increase of alanine aminotransferases (ALT > 41 IU/L), with or without positive blood tests for hepatitis C virus antibodies (HCV ab) and HCV-RNA; c) presence of gallstones in the gallbladder assessed by ultrasonography (USG); d) extramedullary hematopoiesis (EMH) diagnosed by USG, computed tomography scan (CT scan) or MRI for detection of extramedullary hematopoietic foci, with or without symptoms; e) renal complications were based on the presence of functional abnormalities, such as: abnormal creatinine clearance, hypercalciuria, proteinuria or in presence of USG renal cyst or renal lithiasis; f) bone abnormalities were defined as presence of facial bone deformities; g) others: any additional significant patient pathology.

Assessment of iron overload was mainly based on the serum ferritin levels. A value of < 1,000 ng/ml indicated mild grade of iron load, of 1,000-2,500 ng/ml moderate and of > 2,500 ng/mL severe grade of iron load (20,21).

The associated endocrine complications were classified as follows: a) primary hypothyroidism (subclinical and overt) were defined by normal or low free thyroxine and abnormally high levels of thyroid-stimulating hormone: >10 μIU/mL); b) secondary or central hypothyroidism was defined by low free thyroxine and normal or decreased TSH (22); c) the diagnosis of thyroid cancer was based on histopathology/cytopathology; d) hypogonadism was based on the criteria reported in our previous publication (9); e) diabetes, both insulin and non-insulin dependent, were defined according to the standards of American Diabetes Association (23); f) latent hypocortisolism was diagnosed in the presence of basal cortisol < 4.2 μg/dl (98 nmol/l)

(24), and g) for the diagnosis of growth hormone deficiency (GHD) in adults the recommendations of American Association of Clinical Endocrinologists were used (25).

The diagnosis of osteopenia or osteoporosis was based on the World Health Organization (WHO) criteria, assessed by Dual Energy X-ray absorptiometry (DXA) (26).

Infertility was defined as failure to achieve pregnancy after ≥ 12 months of regular unprotected sexual intercourse (27).

The term dysspermia was used to encompass different conditions related to sperm quality and function (low sperm concentration, oligospermia, poor sperm motility, asthenospermia and abnormal sperm morphology, teratospermia). Semen analysis was performed according to the World Health Organization (WHO) guidelines (28). Patients were considered to be normozoospermic when sperm concentration exceeded 20 x 10^6/mL, oligozoospermic between 5 and 20 x 10^6/mL, severely oligozoospermic below 5 x 10^6/mL, and cryptozoospermic when spermatozoa were detected only after careful analysis of the concentrated sample (29). When no spermatozoa were detected in any field, both before and after centrifugation, patients were considered to be azoospermic.

Ethical approval

Ethical approval for our study was obtained in accordance with local institutional requirements and with the Declaration of Helsinki (http://www.wma.net).

Statistical analysis

Data entry and analysis were done using SPSS software package for windows version 13. Descriptive statistics included frequency and percentage for qualitative variables; mean, median, standard deviation (SD), standard error (SE), lower bound and upper bound, and interquartile range for quantitative variables. Statistical significance of the differences between variables was assessed using the unpaired two-tailed Student's t test. Chi square and Fisher's Exact tests were used to calculate the probability value for the relationship between two dichotomous variables. A P value less than 0.05 was considered statistically significant.

Results

a. Participating Centres and Patients' marital status

Ten of 16 (62.5%) ICET-A Network Thalassemia centres participated in the study: Bulgaria, Cyprus, Greece, India, Iran, Italy (2 centres), Oman, Qatar and Turkey. A total of 966 patients with β-thalassemia (738 TDT and 228 NTDT) with a minimum age of 18 years by the end of April 2018 were included in the study**.** The countries' distribution of patients is illustrated in table 1.

The total patients' median age at last observation was 42 years; 95% confidence interval for mean: lower bound 40.3 and upper bound 42.6; age range 18- 66 years. The age (mean ± SE) in 185 TDT patients was 40.0 ± 0.59 and in 55 NTDT patients was 46.4 ± 1.42 years, respectively (P <0.001).

b. Age at marriage or at starting a live-in relationship

Of 966 patients with TDT or NTDT, 240 (24.8%) were married or lived with a partner. Of the 240 patients, 185 (77.1%) had TDT and 55 (22.9%) NTDT (Figure 1).

19 patients (7.9%; 18 TDT and 1 NTDT) were married to a woman with TDT or NTDT, and 10 patients (4.1%; 5 TDT and 5 NTDT) married a woman with β-thalassemia trait (Table 2). All patients received genetic counselling before marriage or taking a partner. The minimum and maximum age at marriage or living with a partner, in both group of patients, was 29 and 30.4 years. The mean age (± SE) was not different in the two groups of patients (P > 0.05).

c. Fertility rate and assisted reproduction

184 out of 240 patients with TDT and NTDT (76.6%) had children. The mean age at birth of the first child was 31.84 years. The interval between the age at

Table 1. Demographic characteristics of male patients over age 18yrs with TDT and NTDT

Country	Number of patients with TDT	Number of patients with NTDT	Total number of patients	Married or living with partners N. (%)	Unmarried patients N. (%)
Bulgaria	14	0	14	1 (7.1)	13 (92.8%)
Cyprus	106	16	122	70 (57.3)	52 (42.6%)
Greece	156	41	197	60 (30.4)	137 (69.5%)
India	32	3	35	6 (17.1)	29 (82.8%)
Iran	272	87	359	45 (12.5)	314 (87.4%)
Italy (1)	19	15	34	8 (23.5)	26 (76.4%)
Italy (2)	30	17	47	16 (34)	31 (65.9%)
Oman	38	15	53	20 (37.7)	33 (62.3%)
Qatar	38	8	46	8 (17.3)	38 (82.6%)
Turkey	33	26	59	6 (10.1)	53 (89.8%)
Total	738	228	966	240 (24.8%)	726 (75.2%)

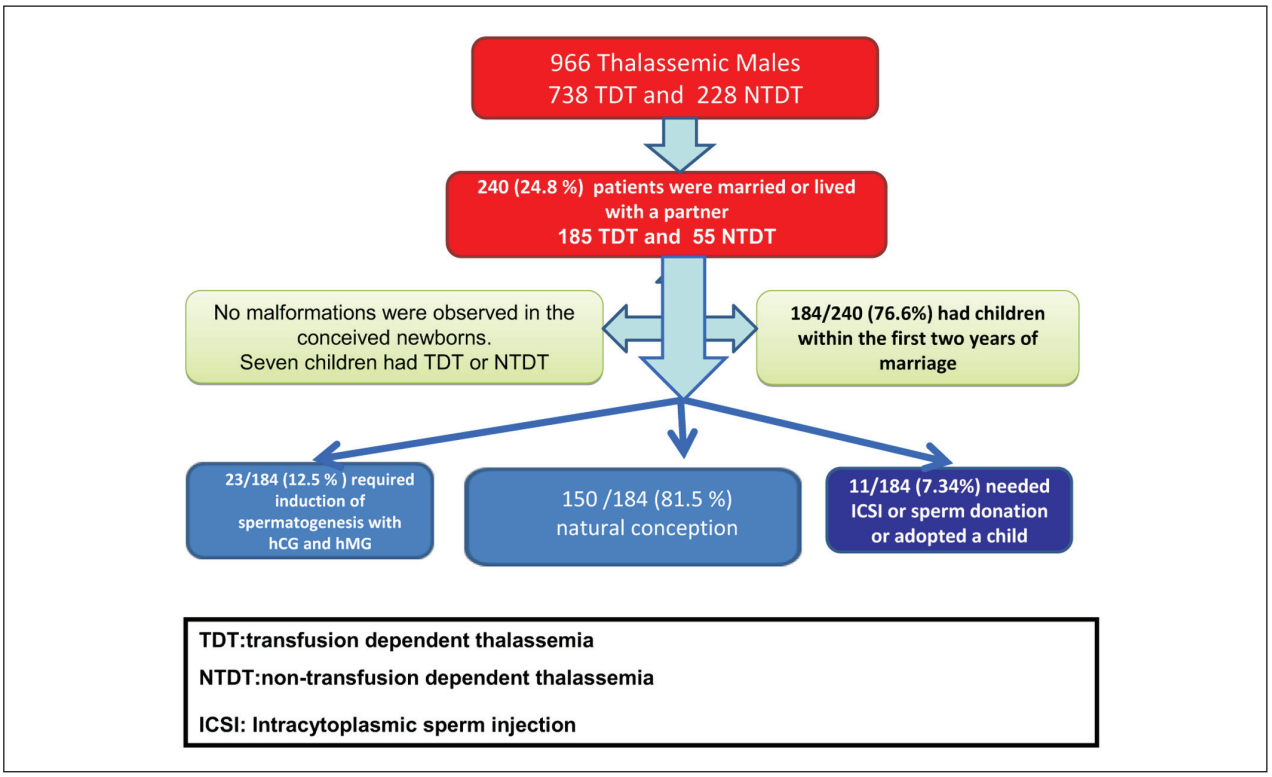

Figure 1. Marital status, fertility rate and assisted reproduction in thalassemia patients enrolled in our study

marriage (or at start of a live-in relationship) and the birth of the first and last child, expressed in interval years (mean ± SE) were: total group 2.1 ± 0.12 years, TDT: 2.1± 0.15 years, NTDT: 2.1 ± 0.22 years (P:NS); last child interval: total group 4.5 ± 0.26 years, TDT: 4.3 ± 0.30 years, NTDT: 5.1 ± 0.53 years (P > 0.05).

The average number of children was 1.32 ± 0.06 (1.27 ± 0.07 in TDT patients and 1.47 ± 0.15 in

Table 2. Mean age at marriage or at starting a relationship with a woman with TDT, NTDT or carrier for β-thalassemia

Age at marriage or at starting a live-in relationship	TDT and NTDT (240)	TDT (185)	NTDT (55)
Mean (SE)	29.73 ± 0.37	29.72 ± 0.42	29.76 ± 0.79
Lower Bound	29.00	28.90	28.18
Upper Bound	30.46	30.55	31.35
Median	30.00	30.00	30.00
Std. Deviation	5.73	5.70	5.86
Wife with TDT (N)	19	18	1
Wife with NTDT (N)	4	3	1
Wife with β-thalassemia minor (N)	10	5	5

Table 3. Number of TDT and NTDT registered as having children whatever the modality of procreation

	TDT and NTDT (240)	TDT (185)	NTDT (55)	P value
Number of patients with children	184 (76.6%)	139 (75.1%)	45 (81.8%)	0.3
Number of children:				
Mean (SE)	1.32 ± 0.06	1.27 ± 0.07	1.47 ± 0.15	> 0.05
Lower Bound	1.19	1.13	1.17	
Upper Bound	1.45	1.41	1.78	
Median	1.00	1.00	1.00	
Std. Deviation	1.014	0.979	1.120	
Number of children	TDT and NTDT	TDT	NTDT	
1	82	63	19	> 0.05
2	80	60	20	
3	16	12	4	
4	5	4	1	
6	1	0	1	
Total number of children	184	139	45	

NTDT patients). Whatever the modality of conception, 184 patients (76.6%) had one or more than one child and 1 NTDT patient had 6 children, at the age of 21, 24, 25, 40, 41 and 42 years (Table 3). 4.8% of births (n =9) were twins.

Thirty patients divorced and one patient married 3 times (at the age of 22, 41 and 42 years). No malformations were observed in the newborns. Seven children had TDT or NTDT.

150 out of 184 patients (81.5%) reported a natural conception, 23 (12.5%) with hypogonadotropic hypogonadism requiring induction of spermatogenesis with gonadotropins (hCG and hMG), and 2 (1.4%) needed intracytoplasmic sperm injection (ICSI) and 3 (1.6%) adopted a child (Figure 1 and Table 4).

Of 45 patients with TDT and NTDT who were unable or unwilling to father a child naturally, 7 patients with TDT (17.9%) had azoospermia; 17 (37.7%; 13 with TDT and 4 with NTDT) dysspermia, and 15 (33.3%; 13 with TDT and 2 with NTDT) had "medical and non-medical conditions" (e.g. associated comorbidities, no response to gonadotrophins after 2 years of treatment, presence of hemoglobinopathy in their wives) (Table 4).

d. Comorbidities and Endocrine complications

128 (53.3%) out of 240 patients had been splenectomised. HCV antibodies were present in 56 out of 231 patients (23.3%; missing data in 9) and HCV-

Table 4. Clinical description of modality of procreation in TDT and NTDT patients and reported causes of infertility

	Total number of patients: TDT and NTDT N. (%)	TDT patients with children N. (%)	NTDT patients with children N. (%)	P value: TDT vs. NTDT
Modalities of conception or paternity in patients requiring to be father				
Natural conception (NC)	150 (81.5%)	109 (78.5%)	41 (91.1%)	0.16
Induced by gonadotrophins	23 (12.5%)	21 (15.1%)	2 (4.5%)	
Sperm donation (AID)	6 (3.3%)	6 (4.3%)	0 (0%)	
ICSI conception	2 (1.1%)	1 (0.7%)	1 (2.2%)	
Adoption (AD)	3 (1.6%)	2 (1.4%)	1 (2.2%)	
Total	184	139	45	
Causes of infertility in patients asking for fathering a child				
Azoospermia	7 (15.6%)	7 (17.9%)	0 (0%)	0.31
Dysspermia	17 (37.8%)	13 (33.3%)	4 (66.7%)	
Medical conditions	6 (13.3%)	6 (15.4%)	0 (0%)	
Others	15 (33.3%)	13 (33.3%)	2 (33.3%)	
Total	45	39	6	

Legend: ICSI: Intracytoplasmic sperm injection

Table 5. Reported comorbidities in 240 TDT and NTDT married male patients or living-in relationship with a woman

Comorbidities	Total (240 patients)	TDT (185 patients)	NTDT (55 patients)	P value: TDT vs. NTDT
Splenectomy	128 (53.3%)	91 (49.1%)	37 (67.2%)	0.02
Osteopenia/Osteoporosis	120 (50%)	95 (51.3%)	25 (45.4%)	0.44
Cholelithiasis	108 (45.0%)	76 (41.0%)	32 (58.1%)	0.025
Cardiac complications	42 (17.5%)	33 (17.8%)	9 (16.3%)	0.8
Liver dysfunction	24 (10%)	21 (11.3%)	3 (5.4%)	0.2
Extramedullary hematopoiesis	20 (8.3%)	10 (5.4%)	10 (18.1%)	0.002
Kidney stones	12 (5%)	11 (5.9%)	1 (1.8%)	0.22
Pulmonary hypertension	11 (4.6%)	7 (3.7%)	4 (7.2%)	0.28
Renal complications	10 (4.2%)	10 (5.4%)	0 (0%)	0.08
Adrenal mass	3 (1.3%)	3 (1.6%)	0 (0%)	0.34

RNA positivity in 17 out of 223 patients (7.1%, missing data in 17).

There was no statistical difference in HCV antibodies and HCV-RNA positivity in TDT vs NTDT= p:0.08 and 0.6, respectively.

The most common reported comorbidities were: osteopenia/osteoporosis (50%) and cholelithiasis (45.0%), followed by cardiac complications (17.5%). No cases of heart and liver failure or malignancies were reported.

A detailed presentation of both groups of patients is given in table 5.

Comparison between those with and without children was not statistically significant for all reported comorbidities ($P > 0.05$).

The commonest endocrine complication was hypogonadotropic hypogonadism (51/240 patients; 21.3%). In the whole group of patients, non-insulin dependent diabetes, primary hypothyroidism, central hypothyroidism and hypoparathroidism were re-

Table 6. Endocrine complications in 240 TDT and NTDT married male patients or living-in relationship with a woman

Endocrine complications	Total TDT and NTDT (240 patients)	TDT (185 patients)	NTDT (55 patients)	P value: TDT vs. NTDT
HH	51 (21.3%)	47 (25.4%)	4 (7.2%)	0.004
With children / Without children	31/20			
Non- insulin dependent diabetes	28 (11.7%)	26 (14.0%)	2 (3,6%)	0.03
With children/Without children	24/4			
Primary hypothyroidism	24 (10.0%)	22 (11.8%)	2 (3.6%)	0.07
With children/Without children		18/6		
Central hypothyroidism	20 (8.3%)	19 (10.2%)	1 (1.8%)	0.04
With children/Without children	13/7			
Hypoparathyroidism	18 (7.5%)	15 (8.1%)	3 (5.4%)	0.51
With children/Without children	14/4			
Insulin dependent diabetes	14 (5.8%)	13 (7.0%)	1 (1.8%)	0.15
With children/Without children	8/6			
Growth hormone deficiency	8 (3.3%)	6 (3.2%)	2 (3.6%)	0.88
With children/Without children	4/4			
Latent hypocortisolism	4 (1.7%)	3 (1.6%)	1 (1.8%)	0.92
With children/Without children	4/0			
Thyroid cancer	3 (1.3%)	3 (1.8%)	0 (0%)	0.34
With children/Without children	2/1			

Legend: HH = Hypogonadotropic hypogonadism

ported in 11.7%, 10.0%, 8.3% and 7.5%, respectively. The less commonly reported endocrine complications were GHD, latent hypocortisolism and thyroid cancer (3.3%, 1.7% and 1.3%, respectively).

The percentages in TDT and NTDT are reported in table 6.

Almost all endocrine complications were more prevalent in TDT patients compared to NTDT patients.

Comparison between those with and without children was not statistically significant for all reported variables ($P > 0.05$) except for hypogonadism ($P < 0.05$).

e. Chelation therapy and serum ferritin

The majority of TDT and NTDT patients received iron chelation therapy for at least 2 years before paternity: with desferioxamine (DFO) 91 (37.9%), deferiprone (DFP): 33 (13.8%) and a combined therapy with both chelating agents 54 (22.5%) patients.

Substantially, in the year of paternity there were no significant changes in the regime of drugs of chelation treatment, although this information was missing in 62 patients (25.8%).

At the last observation, the most common chelating regime was the combination of DFO plus DFP (75 patients; 31.2%), followed by deferasirox (DFX: 53 patients, 22.0%). DFO or DFP monotherapy was given to 76 patients (31.6%). In 36 patients, this information was missing (15%).

The total average serum ferritin (SF) level in both groups of patients before paternity was 2,281 ± 162 ng/ml, with a range of 100 -13,085 ng/ml. No substantial changes of SF levels were observed in the year of paternity (2,042 ± 161 ng/ml; $P > 0.05$). The highest registered level was 9,500 ng/ml. A detailed description of SF levels in TDT and NTDT is reported in table 7.

At the last observation, the SF level was on average 1,680 ± 149 ng/ml (1,825 ± 179.9 ng/ml in TDT patients and 1,165 ± 222.9 ng/ml in NTDT). In both groups the highest registered level was 15,484 ng/ml and 9,500 ng/ml, respectively (Table 7).

Table 7. Iron chelation therapy and serum ferritin levels before, during and after paternity

TDT patients	Serum ferritin at least 2 years before paternity (A)	Serum ferritin in the year of the first paternity (B)	Last serum ferritin level (C)	P value: A vs B	P value: B vs C
Mean	2581.0 ± 191.5	2211.8 ± 181.8	1825.7 ± 179.9	0.115	<0.001
Lower Bound	2202.7	1852.0	1470.7		
Upper Bound	2959.2	2571.6	2180.7		
Median	2000.0	1500.0	949.5		
Standard Deviation (SD)	2475.6	2089.5	2413.7		
Minimum	105	105	91		
Maximum	13085	9495	15484		
Interquartile Range	2700	2508	1626		
NTDT patients	**(A)**	**(B)**	**(C)**		
Mean	1059.9 ± 162.73	1387.1 ± 328.31	1165.5 ± 222.9	0.48	0.083
Lower Bound	731.0	719.1	717.7		
Upper Bound	1388.8	2055.0	1613.4		
Median	620.0	600.0	559.0		
Standard Deviation (SD)	1041.9	1914.3	1592.3		
Minimum	100	100	105		
Maximum	5000	9500	9500		
Interquartile Range	1210	1430	943		

f. Relationship between serum ferritin level and some comorbidities

The relationship between serum ferritin levels, ALT, and the most common registered comorbidities and endocrine complications, assessed with Pearson Chi-Square, at first paternity, was statistically significant only for ferritin versus cholelithiasis (chi square = 6.2; p: 0.04) and for serum ferritin. At last observation, a statistically significant relationship was found both for cholelithiasis (chi square = 16.2; P <0.001) and hypogonadotropic hypogonadism (chi square: 7.7; P: 0.02).

Discussion

More than five decades ago, TDT was a fatal disease in the first decade of life. This poor prognosis has progressively improved and survival increased considerably, due to the implementation of significantly advanced diagnostic and therapeutic methods, consisting mainly of a frequent transfusion program combined with intensive chelation therapy, and improved hematological, biochemical, molecular and imaging methods (1-3). Today, the expectation for having a family is a key component of quality of life and an important aspiration for many patients with thalassemias (7-10).

Therefore, fertility-related issues are important in the management of patients with thalassemias.

Up to now, attention has been mainly focused on issues of fertility in women with thalassemias, with relatively low interest in the reproductive issues faced by male TDT and NTDT patients (11-14). To investigate the effects of thalassemias, its treatment and complications on male fertility, we reviewed the current literature using a systematic search for published studies and promoted a multicentre survey through the ICET-A network in different countries with high prevalence of β-thalassemia.

Reviewing the literature, we found only two studies, both from Iran, reporting data on the marital status, with very limited data on paternity of patients with TDT and NTDT (12,13).

The first study on the marital status of 228 TDT patients over 15 years of age, was done at the Department of Pediatrics, Children and Adolescent Health

Research Centre of Zahedan University of Medical Sciences (Iran). Of the whole group of 228 patients only 32 (14%) were married, 24 (75%) of whom were males. Of the married male patients, only 7 had children. The mean ferritin levels for married patients (both males and females) was 4,419 ± 2,727 ng/ml (13).

The second paper reviewed 74 patients with NTDT. Among them, 50 (67.7%) were female (mean age: 29.6±8.1 years), 21 of whom (42%) were married. Out of 24 male patients, 14 (56.0%) were married. Their age at marriage was 25.3± 4.2 years. Among the married male patients, 11 (78.5%) had children within the first two years of marriage (12). Common reported complications were facial disfigurements, HCV related hepatitis, mellitus and cardiac diseases (12).

In our survey, 240 (24.8%) out of 966 male TDT and NTDT were married or lived with partners, and 726 (75.2%) unmarried. The mean age at marriage or living with partner was 29.7 ± 0.3 years. Out of 240 patients 184 (76.6%) had children within the first two years of marriage (2.1± 0.1 years, median 2 years, range 1.8 - 2.3 years). The total average number of children per family was 1.32 ± 0.065 (1.27 ± 0.072 in TDT patients and 1.47 ± 0.151 in NTDT patients; p >0.05). Whatever the modality of conception, 184 patients had one or two children and 1 NTDT patient had 6 children. Nine births (4.8%) were twins.

In the general population, infertility is a common clinical problem affecting 13 to 15% of couples worldwide. Male infertility is the singular cause of infertility in nearly 20% of infertile couples (30,31).

Extreme transfusional iron input in thalassemia patients due to regular blood transfusions and hemolysis as well as increased intestinal iron absorption, lead to iron overload, facilitating the production of reactive oxygen species (ROS) (32). ROS can negatively affect fertility via a number of pathways, including interference with capacitation and possible damage to sperm membrane and DNA, which may impair the sperm's potential to fertilize an egg and develop into a healthy embryo (33-35).

A higher degree of DNA damage in spermatozoa of β-thalassaemia patients was found in one of our studies (36). In addition, patients with low sperm concentrations were more likely to have a higher degree of defective chromatin packaging. The positive association between low serum ferritin levels and abnormal sperm morphology suggested a potential detrimental effect on spermatogenesis by the iron chelator desferrioxamine, which is used to reduce iron overload (37). Furthermore, the increase in sperm DNA damage and the negative correlation between sperm motility and DNA damage, found by other researchers, suggest that iron overload in β-thalassaemia patients predisposes sperm to oxidative injury (38,39).

Other potential negative prognostic factors that should be considered in thalassemias are the chronic hypoxia due to anemia (40), the alteration of trace elements and antioxidant enzymes (35), the folate deficiency (41) and the concomitant presence of other comorbidities (42).

At least 2 years before paternity, the majority of our TDT and NTDT patients received iron chelation therapy with desferioxamine (DFO) 91 (37.9%), deferiprone (DFP) 33 (13.8%) or combined therapy with both chelating agents 54 (22.5%) patients.

It is interesting to note that our married patients maintained an efficient chelation regime therapy around the time of paternity, mainly consisting of DFO in 75.5% of cases and of DFO or DFP either as monotherapy or in combination with DFO. At the last observation, the commonest iron chelation regimes were the combination of DFO and DFP in 75 (33.3%) patients, followed by DFX (53 patients; 23,5%) and DFO (41 patients; 18,2%) patients. The mean ferritin levels at last observation indicates that patients were on a more efficient chelation regime probably because they became more compliant to treatment after paternity.

Overall, comorbidities and endocrine complications were observed in both TDT and NTDT groups of patients. Osteopenia/osteoporosis represents a common cause of morbidity (51.3% of TDT and 45.4% of NTDT patients). The mechanism of pathogenesis of reduced bone mass is multifactorial and complex. Progressive bone marrow expansion, hypogonadism, a defective GH-IGF-1 axis, and imbalanced cytokine profiles play major roles in the development of osteopenia/osteoporosis. Iron overload, iron chelation therapy, liver disease and other endocrine dysfunctions could be additional factors (43-45). Some studies suggest that there is a gender difference not only in

the prevalence but also in the severity of osteoporosis syndrome in thalassemias (male patients are more frequently and more severely osteopenic/osteoporotic than females), although some other studies reported no gender variation (46).

Our large multicentre study confirms the high prevalence of cholelithiasis in patients with thalassemias, with significantly higher prevalence of 58.1% in NTDT versus 41.0% in TDT in male patients over the age of 18 years. Given the usual benign course of asymptomatic patients, preventive cholecystectomy usually is not considered mandatory, but careful follow-up is suggested because cholelithiasis predisposes patients to complications such as pancreatitis, cholangitis, and acute bile tract obstruction (47,48).

Overall, disease-related endocrine complications were more prevalent among patients with TDT than in patients with NTDT, although in the latter group the prevalence of some endocrine complications (e.g. central hypothyroidism, latent hypocortisolism, GHD) was higher compared to other reports (49-51).

The originality of our study is that it provides detailed information in an area where the contemporary data are few. Understanding the aspects of male reproductive health is important for physicians involved in the care of men with thalassemias to convey the message that prospects for fatherhood are potentially promising due to improvements in treatment regimens and supportive care.

However, some limitations should be considered. In spite of good knowledge about the genetic transmission of the disease, 19 patients (7.9%; 18 TDT and 1 NTDT) married a woman with TDT or NTDT, and 10 patients (4.1%; 5 TDT and 5 NTDT) married a woman with β-thalassemia minor. Due to the paucity of information stated in the medical records this aspect was not fully explored. Their decision may have been related to the high quality of care received in their place of residency and the hope that gene therapy could soon be available to cure the genetic disease.

Although our study provides some insights into the reproductive health experience of persons with TDT and NTDT, further work is required. Areas of concern include patients' quality of life in married and unmarried patients.

In conclusion

More than five decades ago, thalassemia major was fatal in the first decade of life. This poor prognosis has changed since survival started to improve progressively due to the implementation of significantly improved diagnostic and therapeutic methods. One of the main objectives of medical teams caring for patients with thalassemias is to offer the best achievable quality of life. In the current social frame, marriage and reproduction are considered to be important among the standards of normal social behavior. This leads to a growing number of adults in need of specialised care and counselling during specific life events such as reproductive health issues and the establishment of a family. The comprehensive data presented in this article could serve as a reliable reference for physicians counselling thalassemia patients for whom fertility is a major concern. With recent advances in assisted fertility techniques, more male thalassemic patients may be helped to father children. However, because infertility cannot be predicted on an individual basis, it is important to continue the policy of offering sperm preservation in patients with spontaneous puberty and in those treated with gonadotropins. Since fertility preservation is becoming more and more important, practical materials and development of professional practice guidelines should be a high priority aspect for thalassemia associations and medical societies.

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

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Original article

Evaluation of muscle tears in professional athletes using diffusion-weighted imaging and apparent diffusion coefficient: preliminary results

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Summary. *Purpose:* Many studies have evaluated the role of DWI in musculoskeletal diseases but less is known on muscle tears. Especially for professional athletes, muscle injuries are responsible for large time lost. The aim of this study was to investigate on potential relationship between the muscle tear degree and the diffusion characteristics. *Methods:* In this retrospective study, patients signed a comprehensive consent form according to Good Clinical Practice guidelines before proceeding with all examinations. It satisfied all the requirements of the Declaration of Helsinki and the Italian national law for the protection of personal data. We have analyzed 38 professional athletes (36 males; mean age±standard deviation 27±8 years) with a muscle tear. They were 26 football and 12 athletics players, with clinically suspected injuries of the lower limbs muscles. All of patients underwent a 1.5-T MRI with standard protocol (STIR, TSE T2, SE T1, PD T2, PD fat sat T2) plus the DWI sequences with 0, 400 and 800 B-values (s/mm^2). Per each B value, an experienced radiologist measured the signal intensity (SI, in arbitrary units [au]) using a region of interest (ROI) placed within the tear on DWI images. SI drop off at the third B value was calculated referred to the first B value. Similarly, ADC was measured using the ADC map in a small ROI within the tear. Bivariate associations were evaluated using the Student t test. Logistic regression was performed using the tear degree as dependent variable. Data were given as mean±standard deviation. *Results:* According the Muller-Wohlfarth classification, the 38 muscle tears were classified in type 3a in 22/38 cases and 3b in 16/38 cases. At bivariate analysis, 3a-tears had a SI at the third B value (24±9 au) lower (P=0.003) than that of 3b-tears (34±9 au). Similarly, 3a-tears had a SI drop off (73±10%) lower (P=0.008) than that of 3b-tears (82±9%). ADC was not significantly associated to tear degree (P=0.093). At regression analysis, SI at the third B value was the only independent predictor of the tear degree (P=0.032), while the SI drop off was borderline significant (P=0.070). *Conclusion:* This preliminary data showed a positive correlation between the degree of muscle tears and the SI at the third B-value. Compared to 3a- tears, 3b-tears tend to show higher SI and a higher SI drop off. (www.actabiomedica.it)

Key words: muscle tear, DWI, professional athlete

Introduction

Magnetic resonance (MR) diffusion-weighted imaging (DWI) is highly sensitive to tissue water diffusivity. This MR sequence has been successfully used in several neurological and oncological conditions (1-3).

In the field of musculoskeletal, many studies have evaluated the role of DWI mainly in oncological diseases (4). In particular, it was used to differentiate be-

tween benign and malignant bone tumors through a threshold of the apparent diffusion coefficient (ADC). Apart from the general evidence on a trend for a lower ADC in malignant lesions, data are still conflicting (5). Similarly, this trend appears to be true for soft-tissue tumors (6). Moreover, DWI appears to perform well in the assessment of the response to chemotherapy of bone and soft-tissue tumors, with an increasing ADC from baseline in responders (7).

From a technical point of view, the DWI sequence is characterized by the so-called *b* values, that is a technical parameter to be managed during acquisition. When b=0 s/mm^2 (or close to), DWI provides mostly a T2-weighted image. Conversely, when b is as high as 800 s/mm^2 a genuine diffusion-weighted image is obtained, generally with poorer spatial resolution and signal to noise ratio. Diffusion-weighted MR images must therefore be assessed visually, comparing images obtained with a low b value to those obtained with a high b value, with the difference in signal between these two images being related to water diffusion. Conversely, ADC quantitative analysis is the first line assessment method for diffusion data.

According to the recent guidelines (8) for muscle injuries, muscle tears may be evaluated using a fat-suppressed fluid-sensitive (e.g. short tau inversion recovery [STIR]) sequence, which allow for the detection of edematous changes around the myotendinous and myofascial junctions; and a T1-weighted spin-echo sequence, that are less sensitive to edematous changes within the muscle in acute injury but may be useful in the assessment of subacute hemorrhage or hematoma. This imaging protocol allows to defining the site, extension, and muscle retraction. Moreover, it allows to define a prognosis and a correct patient management (9).

Muscle tears are specific issues for professional athletes, representing a relevant source of time lost from competition, with strong external pressure for a rapid return to play (RTP). ISMuLT guidelines suggest a prognosis depending on the muscle tear degree. In particular, for the structural 3a and 3b muscle injuries it is recomanded 15-18 and 25-35 days off of competition, respectively (10). Impotantly, an inaccurate estimation of the tear degree may prompt an inappropriate prognosis with the risk of an early RTP and an increase risk of recurrent tear (11).

To our knowledge, the role of DWI in the evaluation of muscle tears has never been assessed. In our clinical practice, DWI is added to the standard imaging protocol thanks to its capability in the evaluation of soft tissues. In fact, high degree tears are expected to involve a large part of muscle fibers and, consequently, a higher diffusivity of water molecules.

The aim of this preliminary study was to retrospectively investigate on potential relationship between the muscle tear degree and the diffusion characteristics.

Materials and methods

Study design and population

In this retrospective study, patients signed a comprehensive consent form according to Good Clinical Practice guidelines before proceeding with all examinations. It satisfied all the requirements of the Declaration of Helsinki and the Italian national law for the protection of personal data.

We analyzed consecutive 38 elite professional athletes (36 males; mean age±standard deviation 27±8 years). They were 26 football and 12 athletics players with a clinically suspected injury of the lower limb muscles without a direct trauma (10).

Imaging protocol

All patients underwent a 1.5-T MR imaging between 48 and 72 hours from the indirect trauma (10) with the standard protocol (Avanto, Siemens Medical Solution, Erlangen, Germany; or Achieva, Philips Medical System, Eindhoven, Netherlands) and a body matrix coil phased array 16 channels. The imaging protocol included a coronal STIR, an axial T2-weighted turbo spin-echo (Achieva) or a proton density (Avanto), an axial proton density fat saturated, and an axial T1-weighted spin echo. Moreover, for each patient, an axial DWI sequence with parallel imaging (acceleration factor 2) was acquired using b_1=0 s/mm^2, b_2=400 s/mm^2, and b_3=800 s/mm^2. Further details are provided in Table 1 and 2.

Table 1. Achieva, Philips Medical System, Eindhoven, Netherlands, MRI characteristics

Sequence	Plane	TR (ms)	TE (ms)	Matrix (mm)	GAP (mm)	FOV (mm)	Thinkness (mm)
STIR[1]	Coronal	3000-6000	50	272x220	0.8	400-500	4
T2 TSE[2]	Axial	3500-6000	100	448x327	0.8	400-500	4
PD Fat Sat[3]	Axial	3500-6000	80	324x247	0.8	400-500	4
SE T1[4]	Axial	450-500	18	380x219	0.8	400-500	4
DWI[5]	Axial	7000-13000	55	140x138	0.8	400-500	4

1. short tau inversion recovery; 2. T2 turbo spin-echo; 3. proton density fat saturation; 4. spin echo T1; 5. diffusion-weighted imaging

Table 2. Avanto, Siemens Medical Solution, Erlangen, Germany, MRI characteristics

Sequence	Plane	TR (ms)	TE (ms)	Matrix (mm)	GAP (mm)	FOV (mm)	Thinkness (mm)
STIR[1]	Coronal	5000-6000	118	320x320	0.6	400-500	3
PD[6]	Axial	3000-6000	12-123	238x384	0.6	400-500	4
PD Fat Sat[3]	Axial	3000-6000	12-123	238x384	0.8	400-500	4
SE T1[4]	Axial	636	10	224x320	0.8	400-500	4
DWI[5]	Axial	4000-8000	70	160x160	0.8	400-500	4

1. short tau inversion recovery; 2. T2 turbo spin-echo; 3. proton density fat saturation; 4. spin echo T1; 5. diffusion-weighted imaging; 6. proton density

Image analysis

Muscle tears were evaluated according to the Muller-Wolhlfahrt classification (12). In this classification muscle injuries are classified as non-structural and structural as reported in Table 3.

Per each *b* value, an experienced radiologist (more than 15 years of experience in musculoskeletal radiology) measured the signal intensity (SI, in arbitrary units [au]) on DWI images in a point region of interest (ROI) placed within the tear using T2 images to accurately place the ROI (Figure 1). ADC was measured using the ADC map in a ROI within the tear.

Statistical analysis

The Shapiro-Wilks test was used to ascertain whether the distribution of the continuous variables was normal. The SI drop off at b_3 was calculated respect to b_1.

The association between the muscle tear degree and ADC, SI drop off, and the SI at b_3 was calculated using the ANOVA. Bivariate correlations were estimated using the Pearson correlation coefficient. Multivariate regression analysis was performed using the SI drop off as dependent variable and with both the muscle tear degree and ADC as predictors. Finally, a

Table 3. Muller-Wolhlfahrt classification (12)

Non-structural Injury	Type 1: Overexertion-related muscle disorder	Type 1a: Fatigue-induced muscle disorder Type 1b: Delayed-onset muscle soreness (DOMS)
	Type 2: Neuromuscular muscle disorder	Type 2a: Spine-related neuromuscular Muscle disorder Type 2b: Muscle-related neuromuscular Muscle disorder
Structural Injury	Type 3: Partial muscle tear	Type 3a: Minor partial muscle tear Type 3b: Moderate partial muscle tear
	Type 4: (Sub)total tear	Subtotal or complete muscle tear Tendinous avulsion

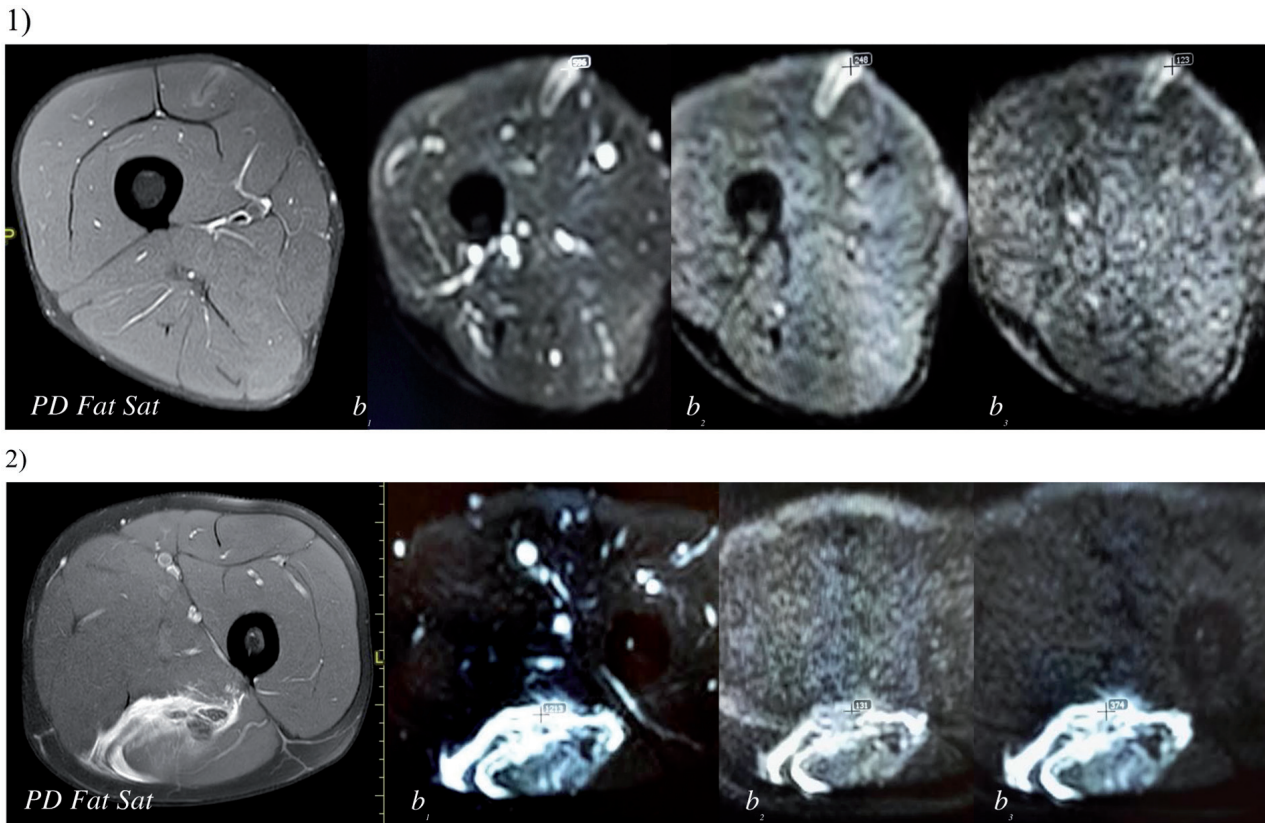

Figure 1. ROI placement on DWI sequences (b_1 , b_2 , b_3) in 3a type (1) and 3b type (2)

receiver operator characteristic (ROC) curve was built for the SI drop off and the SI at b_3.

Continuous data were given as mean±standard deviation or median and interquartile range, as appropriate; categorical data were given as counts and proportions. A p-value <0.05 was considered as statistically significant. SPSS software (SPSS v20, IBM Inc., Chicago, IL) was used for calculation.

Results

According the Muller-Wohlfarth classification (12), the 38 muscle tears were classified as type 3a in 22 cases (58%) and 3b in 16 cases (42%). No avulsion injuries and no complete tendon ruptures were reported. The most commonly affected muscle was the femoral biceps (18/38 cases), followed by the rectus femoris (7/38 cases), the soleus (4/38 cases), the medial gastrocnemius (2/38 cases), vastus intermedius (2/38 cases), gluteus (1/38), medius (1/38), adductur longus (1/38), pectineus (1/38), vastus lateralis (1/38), and obturator internus (1/38). The musculotendinous junction was involved in 33/38 cases (87%); in four cases, the injury was located in the myofascial site, and in one other case, the tear was intramuscular.

All of the 38 patients showed a focal intramuscular T2 hyperintensity and this was considered to reflect a muscle injury (9). In 18/38 cases (47%) MRI findings were found as suggestive of blood products in evolution.

The mean ADC was $(2.03\pm0.55)\cdot10^{-3}$ mm²/s while the SI drop off at b_3 was 77±10%. The 3a tears had a mean SI at b_3 (24±9 au) significantly lower (p=0.003) than that of 3b-tears (34±9 au). In addition, the 3a-tears had a SI drop off (73±10%) lower (p=0.008) than that of 3b tears (82±9%) (Figure 2). The ADC was borderline significantly associated to the tear degree (p=0.093). In particular, 3a tears had a mean ADC

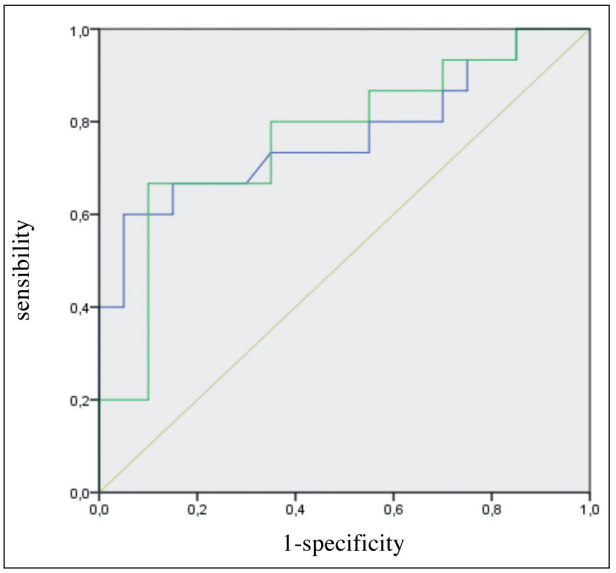

Figure 2. ROC curve; line green: SI drop off, line blu: SI at b_3

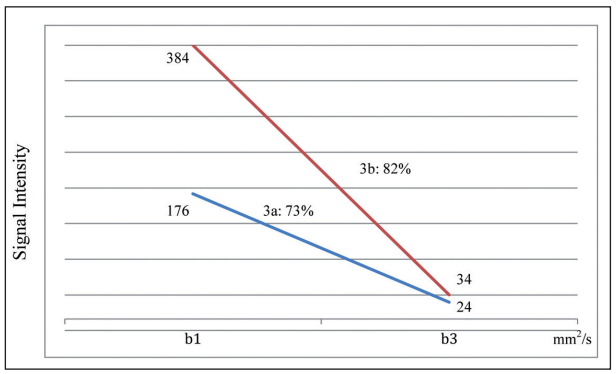

Figure 3. SI drop-off

(1.91±0.49)·10^{-3} mm^2/s lower than that of 3b tears (2.21±0.59)·10^{-3} mm^2/s.

At multivariate regression analysis, both the muscle tear degree (p=0.033) and the ADC (p=0.006) were independently associated to the SI drop off at b_3. At ROC analysis with the muscle tear degree as dependent variable, the area under the curve was 0.768 for the SI drop off and 0.767 for the SI at b_3 .

Discussion

Muscle injuries are a common issue in elite athletes often occurring during competition or training. More than 90% of them are caused by excessive strain or contusion (13), causing prolonged absence from competition (14).

In these clinical setting, MR imaging is the preferred method of evaluation thanks to its high contrast resolution, reproducibility, and anatomic depiction (15). Interestingly, DWI is not routinely exploited for the assessment of the muscle injuries, being mainly applied for the characterization of soft tissue tumors (diagnosis and follow-up) and for the assessment of vertebral collapse and bone marrow cellularity (16). Indeed, symptoms and timing of the muscle injury are generally enough to make an accurate diagnosis and the role of imaging is to better evaluate the site of the injury and to determine the grading (17). One only study, to our acknowledge, included DWI in the standard MR protocol for the evaluation of the rotator cuff tears in order to improve the diagnostic accuracy of MR imaging (18).

In this study, we exploited the DWI sequence so to measure the ADC of the muscles injuries in relation to its degree. Although only with borderline significance, we demonstrated that 3a tears had a mean ADC lower than that of 3b tears. This reflects the fact that 3a tears are characterized by a lower amount of ruptured fibers compared to 3b tears, with a lower amount of free water (thus, lower ADC). This is also in line with the data reported by Agten et al. (19), being 1.81·10^{-3} mm^2/s in non-structural type-1 injuries that are characterized by an even lower amount of free water. In practice, the higher the muscle degree, the higher the ADC and we may speculate on an even higher ADC in type-4 tears. This proportionality opens a future perspective where DWI-derived ADC may be used as a predictor of the muscle injury degree. Moreover, being ADC a continuous variable, it could be used as an adjunct to the Muller-Wohlfarth classification.

In the clinical practice, the DWI sequence is typically evaluated qualitatively as a subjective radiologist's judgment, looking for SI modifications. Indeed, the ADC map is rarely calculated. As such, we have also assessed the role of the SI drop off and that of the SI at b_3 itself as predictor of the muscle tear degree, showing an area under the curve at ROC analysis of 0.768 and 0.767, respectively.

A limitation of this study is that the intra-observer evaluation was not evaluated. Another additional limit

is represented by the study conducted retrospectly.

However, these results are still preliminary and further studies are required to validate the present data and to better elucidate the clinical application of DWI and ADC.

Conclusion

This preliminary study showed a positive association between the degree of muscle tears and the SI at b_3 in DWI. Together to the SI drop off, it may be used to quantify the degree of muscle tear and could be useful in the better characterize the muscle injuries.

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

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Original article

Mobilization of the contralateral limb in Slump position: effects on knee extension in healthy adult subjects

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Summary. *Background and aim:* In the acute phase of neuropathic pain due to nerve root disorders, the neurodynamic approach proposes the mobilization of the contralateral limb to decrease the pain and increase the range of motion in the affected limb. The aim of this study was to evaluate the effect of the contralateral knee mobilization on the ipsilateral knee extension range of motion in Slump position in healthy adult subjects. *Methods:* Thirty-eight healthy subjects underwent a placebo, control and experimental manoeuvres that included a passive contralateral knee mobilization into extension. The knee, hip and cervical angles and distance between glabella and femoral condyle achieved in Slump test position were measured with an optoelectronic motion analysis system before and after each manoeuvre. *Results:* Experimental manoeuvre produced a statistically significant increase of the knee extension ROM when compared to the control (p=.017) and placebo (p=.007) manoeuvres. A significant increase of the hip angle and distance between glabella and femoral condyle was detected after the experimental manoeuvres (p<.001), but not after the placebo and control manoeuvres. *Conclusions:* The contralateral mobilization in Slump position increases the ipsilateral knee extension ROM. Further research is required to confirm that the knee increment ROM was due to the neural component. (www.actabiomedica.it)

Key words: contralateral mobilisation, neural mobilization, neurodynamics, neurodynamic test, Slump test

Introduction

Neurodynamics studies the relationship between the mechanical and physiological properties of the nervous system (1). Accordingly, it postulates that the exposure of the nerve tissue to different mechanical stresses, like tensile or compressive loads, impairs its extra- and intra-neural blood flows and may cause neural ischemia. Furthermore, it has been demonstrated that inflammation of the neural tissue alters its histological structure (2). The clinical sign of these alterations is an increased mechanosensitivity of the nerve itself, therefore the nerve is more sensitive to mechanical stimuli, like pressure or tension (1). The neural mechanosensitivity is usually assessed with the provocation (i.e. Tinel's) and neurodynamic test, in which movement of a neural mechanically sensitive structure can reproduce the symptoms reported by the patient. In neurodynamic testing, the nerve tissue is assumed to be involved when the symptoms reported by the patient change after moving a segment distant from the symptomatic region, otherwise the symptoms are believed to originate in non-neural tissues (i.e. soft tissues) (3).

The Slump test is an example of a neurodynamic test, used for the assessment of the increased mecha-

nosensitivity of the meningeal and neural tissues (4) in patients with low back pain (5, 6) and with lumbar disc herniation (7). Despite the Slump test is described in several ways and there is not a universally accepted procedure (8); the Slump position is achieved with a maximal passive thoraco-lumbar and cervical flexion; afterwards, a passive or active knee extension and ankle dorsiflexion are performed in order to increase tension to the neural and meningeal structures (9). Irrespective of the procedure used to perform the test, if patient complaints of symptoms in the back of the thigh in Slump position that change after a cervical extension, the sciatic nerve is assumed to be involved. As passive knee extension range of motion measurements in Slump position have shown to be reliable in healthy subjects, (10) in the case of neural impairment, the degree of knee extension in Slump position may be considered a direct indicator of the nerve mechanosensitivity, since this movement causes considerable strain on the sciatic nerve. Therefore, a reduced knee extension would indicate a higher mechanosensitivity.

Even though neural techniques have been shown to be useful in the management of neural symptoms (11-13) and limited evidences support their use in the treatment of low back pain, (5, 6, 14), the research in this field needs to gather further insights into the underlying mechanisms (15). The effects of neural mobilization of the lower limb, (i.e. a knee extension mobilization in Slump position) on the knee range of motion (ROM) have been studied by several authors. Ten passive knee extension have been reported to produce a significant increase of knee ROM in Slump position in asymptomatic subjects (16). A similar ROM increase was also observed after an active knee extension protocol lasting 6 weeks (17). Moreover, Herrington (18) found a positive effect of either slider or tensioner neural mobilizations aimed at improving knee extension ROM.

Although these studies may support the use of knee mobilization in Slump position to decrease the mechanosensitivity in the symptomatic lower limb, this technique may be considered too irritating in patients with a neuropathic pain due to an impairment of the peripheral nervous system. Therefore, the mobilization into extension of the knee contralateral to the affected limb has been suggested to decrease neuropathic pain (19). The lumbar roots' anatomy supports the rationale of contralateral knee mobilization. As the lumbar roots arise from the spinal cord with an angle that can be divided into horizontal and axial components, it is postulated that tensioning the contralateral root will cause a movement of the spinal cord in the caudal direction, which will reduce the tension of the ipsilateral nerve root through the axial component (19).

Recently a randomized clinical trial (20) assessed the effects of the contralateral mobilization in Slump position compared with a sham mobilization and no mobilization on asymptomatic subjects. The effects were measured using a Numerical Rating Scale that scored the tension sensation reported by the subjects in the posterior thigh region. The authors found that the contralateral mobilization reduced the tensile sensation compared to no movement, whereas the sham mobilization did not. The purpose of this study was to further investigate the effects of contralateral knee mobilization using a different outcome measure, i.e. the knee extension in the Slump position. Our experimental hypothesis was that the mobilization into extension of the left knee will decrease the tension in the right nerve roots and, accordingly, increase the right knee extension in the Slump position.

Methods

Participants

A repeated measure design was performed at the Motion Analysis Laboratory of the Piero Palagi Hospital, Florence, Italy. Voluntary participants, recruited through a sample of convenience, were included if they met the following inclusion criteria: being healthy, aged>18 years, naive to manual therapy and neurodynamics, and having a limited right knee extension in Slump position that increased after an active cervical extension (3, 21). Participants were excluded if they had neck or back pain in the previous 3 months, a history of major trauma to the lumbar, hip or knee regions or if they suffered from neurological conditions. Subjects suffering from diabetes were also excluded, since a reduced mechanosensitivity has been found in this patients (22).

Prior to participating in any study-related procedures, participants read and signed an informed written consent form and were informed about the procedure but not about the aim of the study. The study protocol was approved by the local Institutional Ethical Committee.

Procedure

In all participants the right knee extension ROM was measured in the Slump position before and after an experimental, a placebo and a control manoeuvre, as described later. In the present study we used the positioning proposed by Maitland (4). The subject sat on a table with the pelvis fixed against a support to maintain the sacrum in vertical position. The distance between the support and the anterior edge of the seat was adjustable in order to fit the subject's thigh length. The popliteal fossae were put against the edge of the table and the thighs were parallel. The subject was asked to "slump" the trunk while keeping the cervical spine in neutral position. Then, the examiner passively flexed the cervical spine at the end of movement and applied a slight pressure over the shoulders in order to further increase thoracic and lumbar spine flexion. Finally, a belt was fastened across the thighs to ensure that no hip flexion took place, and across the shoulders just below the seventh cervical vertebra to keep the subject's position stable during each test (Figure 1).

Kinematic measurements were performed by using an optoelectronic motion analysis system (SMART-E 600; BTS, Milan, Italy) with five infrared cameras and five passive markers, applied on the following anatomic landmarks: right lateral malleolus (M1), lateral condyle of the right femur (M2), greater trochanter of the right femur (M3), spinous process of the seventh cervical vertebra (M4) and glabella (M5). In order to avoid any discrepancy in the markers placement among the three tests, the markers were attached on each subject at the beginning of the experimental session and were removed only after the completion of the three manoeuvres. The error of the system in the recognition of the markers position within the calibrated volume was less than .3 millimetres for all acquisitions.

In the Slump position three manoeuvres (experimental, placebo and control), each lasting for 2 minutes, were performed with a 10 minutes interval between one another. The use of a rest period of 10 minutes between each manoeuvre was based on a cadaveric study on the median nerve (23). However, the same time span has been demonstrated adequate also for restoring the viscoelastic properties of the soft tissues (24) and neural tissues (25) in living humans. The order of the three manoeuvres was randomized among participants.

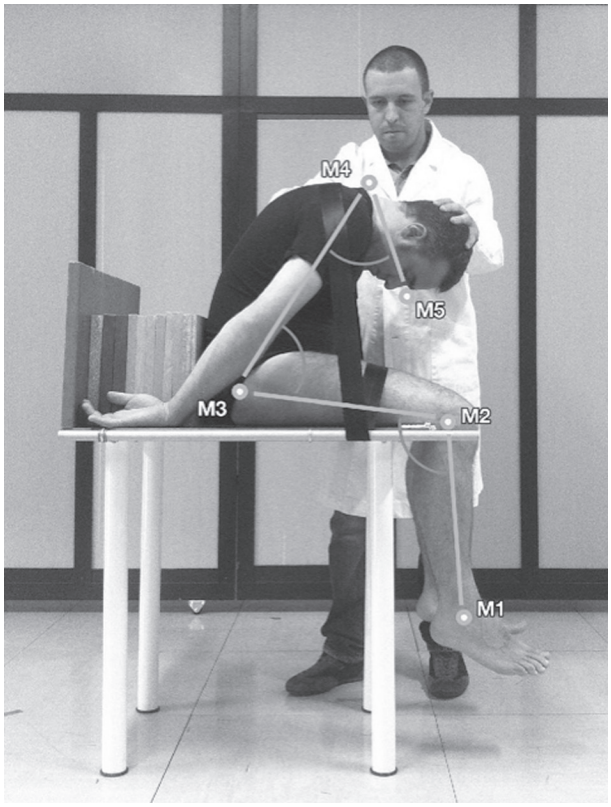

Figure 1. Markers and subject positioning

The experimental manoeuvre (Figure 2) consisted of 12 passive left knee mobilization into extension (while maintaining the ankle in maximum dorsiflexion) until the achievement of the second resistance (R2), i.e. the range where the maximum resistance to movement is perceived (26). To standardize the mobilization speed, the operator reached R2 every 10 seconds following the rhythm paced by auditory cues. The neural tissue R2 has been found to be reliable in healthy subjects (27).

The placebo manoeuvre (Figure 3) consisted in mobilising the left ankle joint into dorsiflexion with

Figure 2. Experimental manoeuvre. The researcher carried out a passive mobilization of the left knee into extension (while maintaining the ankle in dorsiflexion maximum) from the Slump position (A) until the achievement of the second resistance (B)

Figure 3. Placebo manoeuvre. The researcher carried out a passive mobilization of the left ankle joint into dorsiflexion in Slump position with the knee flexed at 90° (A) until second resistance was perceived (B)

the flexed knee until R2 was perceived. Although the dorsiflexion of the ankle is considered a neurodynamic movement, the mobilization of the ankle to end-range with the knee flexed to 90° should not be able to transmit tension along the sciatic nerve and reach the nerve roots lumbar (19, 20). The ankle mobilization was performed with the same amount and frequency described above for the experimental condition. In the control manoeuvre, the subject did not receive any mobilization but kept the Slump position for 2 minutes.

A physiotherapist with a postgraduate degree in manual therapy (TG) performed both the subject positioning and the different manoeuvres in all participants. Before and after each manoeuvre, a different examiner, (17) blinded to the performed manoeuvre, passively moved the subject's right knee into extension until perceiving R2. When the examiner perceived the maximum resistance to knee extension movement, he ordered the start of kinematic acquisition, that lasted 5 seconds. All tests were performed in a single session lasting approximately 60 minutes.

Statistical analysis

From kinematic data, three angles in the sagittal plane were computed: the knee angle (KE), defined as the angle between the thigh segment (identified by M2 and M3), and the leg segment (identified by M1 and M2), the hip angle (HA), defined as the angle between the trunk segment (identified by M3 and M4) and the thigh segment, and the cervical angle (CA), defined as the angle between the head segment (identified by M4 and M5) and the trunk segment. Moreover, the distance between M2 and M5 (head-knee distance, HKD) was measured (Figure 1). It is noteworthy that the HA, as defined in this study, includes hip, pelvis and trunk movement. For each angle, data from the first and the last 1000 milliseconds of recording were ignored and the mean value during the middle 3 seconds was calculated and used for all analyses.

As the Shapiro-Wilk test found that all variables were normally distributed, analyses were conducted using parametric tests. An ANOVA for repeated measures with manoeuvre as within-subjects factor (3 levels: experimental, placebo, control) was used to assess the stability of the initial position among the three manoeuvres. In order to verify whether the interval between each manoeuvre (10 minutes) was sufficient to restore the viscoelastic properties of the soft and neural tissues that had been elongated, the analysis was repeated considering the temporal sequence of the different manoeuvres, i.e. comparing the knee extension ROM measured in the Slump position before the first, the second and the third manoeuvre. Paired t-tests were also used for pairwise comparisons. Mauchly's sphericity test was used prior the ANOVA; whenever

the sphericity test was not met, Greenhouse–Geisser correction was made.

The effect of the three manoeuvres was assessed by an ANOVA 3x2 for repeated measures with two within-subjects factors, time (2 levels: pre and post) and manoeuvre (3 levels: experimental, placebo, control). When a significant Time x Manoeuvre was found, a post-hoc analysis was conducted to compare the three manoeuvres to one another.

For all statistical analyses, the α value was set at p<.05 and the software used was SPSS, Version 17.0 (SPSS Inc., Chicago, IL, USA).

Table 1. Subjects characteristics (N=38)

Gender	
Male	28 (73.7%)
Female	10 (26.3%)
Age (years)	41.5±16.9
Height (centimeters)	172,9±8.8
Weight (kilograms)	73.5±12.8
Dominant limb	
Right	33 (86.8%)
Left	3 (7.9%)
Ambidextrous	8 (5.3%)

Data are expressed as mean ± standard deviation or frequency with percentage

Results

Thirty-eight asymptomatic subjects (10 females, 28 males) participated in this study. The characteristics of the sample are reported in Table 1.

No significant differences were found among the experimental, placebo and control manoeuvres for the four variables (KA, HA, CA, HKD) measured at the initial assessment. Conversely, the analysis showed that the KA measured at the initial assessment differed among the first, the second and the third manoeuvre performed (F=3.775, p=.028). Indeed, the KA measured before the first manoeuvre was lower than the value measured before the second (t=-2.657; p=.012) and the third manoeuvre (t=-2.034; p=.049), whereas no differences was found between the second and the third manoeuvre (t=-0.158; p=.875).

The manoeuvres produced different effects on the KA, as shown by the significant Time x Manoeuvre interaction (F=6.365, p=.003). The post-hoc analysis showed that the experimental manoeuvre increased significantly the knee extension ROM compared to both the placebo (F=8.222, p=.007) and control (F=6.283, p=.017). Conversely, no differences were found among control and placebo (F=0.593, p=.446). However, a significant Time x Manoeuvre interaction was found also for the HA (F=47.387, p<.001) and for the HKD (F=18.167, p<.001), but not for the CA (F=1.155, p=.322). For both variables, differences were found when comparing the experimental manoeuvre with the placebo (HA: F=69.365, p<.001; HKD: F=23.892, p<.001) and with control (HA: F=51.675, p<.001; HKD: F=21.497, p<.001) manoeuvre, but not between placebo and control (HA: F=3.820, p=.058; HKD: F=.988, p=.327) (Table 2).

All the subjects in the study did not experience adverse events during any manoeuvre.

Discussion

The purpose was to investigate the effect of the contralateral neural mobilization on the knee exten-

Table 2. Mean and standard deviation of kinematic measures before and after each manoeuvre in the 38 participants

	Experimental		Placebo		Control		p value		
	pre	post	pre	post	pre	post	T	M	TxM
KA	160.8±7.1	163.3±7.2	160.8±7.2	161.6±.5	161.3±6.9	162.3±7.2	<.001	NS	<.005
HA	72.7±5.8	75.4±6.1	72±6.3	72.4±6.8	72.8±4.8	72.9±5.3	<.01	NS	<.001
CA	58.3±5.7	57.7±5	58.6±6.2	57.2±6.1	57.5±6.3	55.7±6.0	<.005	<.01	NS
HKD	.35±.0	.37±.0	.35±.0	.35±.0	.35±.0	.35±.0	NS	<.05	<.001

KA=knee angle; HA=hip angle; CA=cervical angle; HKD=head-knee distance; T=time factor; M=manoeuvre factor; TxM=time x manoeuvre interaction; NS=non significant

sion ROM in the Slump position. We observed a significant increase of the knee extension ROM after the neural mobilization of the contralateral knee, but not after a control and a placebo mobilization. It is reasonable to assume that the contralateral mobilization provoked a displacement of the ipsilateral nerve root because several studies have shown a displacement of the sciatic nerve after a neural mobilization in vivo through ultrasound imaging. In healthy subjects, the full knee extension in a modified Straight Leg Raise position produced a sciatic nerve mean longitudinal distal excursion of 12.4 millimetres (SD=4.4) (28). In fifteen asymptomatic participants, tensioning and sliding techniques provoked a sciatic nerve excursion of 3.2 (SD=2.1) and 17.0 millimetres (SD=5.2), respectively (29). Moreover, in Slump position knee extension produced distal longitudinal excursion of the sciatic nerve ranging from 2.6 (SD=1.5) to 3.2 (SD=2.0) millimetres (30). Such excursion, though minimal, might suffice to explain the increased knee extension ROM observed in the present study, that was also very limited and might be therefore attributed to the neural component.

On the other hand, we observed that the HA and the HKD were also increased after the experimental manoeuvre. Even though the increased KA was observed uniquely after the experimental manoeuvre, it was associated with a similar amount of hip extension. This finding raises doubts about the hypothesis that the increased knee extension ROM observed after contralateral knee mobilization into extension might be attributed to the reduced neural tension. Indeed, the hamstrings stretching induced by the repeated, passive contralateral knee extension might have caused a posterior pelvic rotation, which in turn might have decreased the hamstrings tension in the right limb. Therefore, as we placed no markers on the pelvis landmarks, it is not possible to know whether the increase of the knee extension ROM was due to the neural or muscular component. Considering that the average increase of the knee and hip extension was quite similar (2.5° and 2.7°, respectively) the improvement of the knee extension ROM seems most likely attributable to the ischial tuberosities' posterior rotation induced by the contralateral hamstring tension. This distal pelvis motion might have therefore produced a distal shift of the hamstring length, explaining the association found in the experimental condition between the increased knee extension and the HA. This argument raises doubts about the neural displacement that is produced with the contralateral mobilization, because the experimental manoeuvre induced a repetitive stretching of the hamstrings, which caused a posterior pelvic rotation. In light of this consideration, the results of previous studies (16, 17) claiming an increase of knee extension following an ipsilateral neural mobilization are questionable, as the posterior pelvic rotation was not considered a potential confounding factor.

Recently, Shacklock, et al. (20) studied the contralateral mobilization in Slump position in asymptomatic subjects. They found a reduction of stretch sensation, assessed with a Numerical Rating Scale, in the posterior thigh in the intervention group ($p \leq .001$), but no changes in the control and sham groups ($p=.996$). The experimental manoeuvre consisted in one contralateral knee extension mobilization to end-range, maintaining that position for 30 seconds. The authors concluded that the reduced perception reported by the subjects of the experimental group was attributable to the neural component rather than to the relaxation of the posterior thigh soft tissues, since the magnitude of the response reduction in the control and sham groups was not statistically significant. However, our data suggest that a different explanation for this finding is also possible, i.e. that the mobilization into extension of the contralateral knee could produce a posterior pelvic rotation which causes an ipsilateral hamstring relaxation and consequently a reduction of the stretch sensation in the back of the thigh; therefore, the conclusions of the authors raise questions.

A second finding of the present study is that the time interval between each manoeuvre (=10 minutes) seems to be not adequate to allow the complete restoration of the baseline viscoelastic properties of the tissues. Our results, in fact, showed the knee extension ROM of the pre-assessment of the first manoeuvre was significantly less when compared to the pre-assessment of the other two subsequent manoeuvres, whatever was the order established through the randomization. This result conflicts with other published data (24, 25). However, though all the structures elongated during the first knee extension did not

recover their full original resting length after the first pre-assessment, this unlikely biased increased ROM after the first pre-assessment, because it means that all the structures elongated during the first knee extension did not recover their full original length (viscoelastic properties) after the first pre-assessment. Actually, thanks to the randomized order of the three manoeuvres (experimental, control and placebo), no difference emerged among the pre-assessment measures. However, since the three conditions were randomized and differences among the pre-assessments were absent and this potential systematic error was minimized.

The present study has several limitations that should be discussed. Firstly, as mentioned, we did not study the pelvis motion. The finding of a slight increase of the HA associated with the increased knee extension strongly indicate that future studies should control for the posterior pelvic rotation by using radiographic imaging, as suggested by McHugh et al. (31) or by recording pelvic kinematics with additional markers on pelvic landmarks. Secondly, the present study included only healthy subjects, so the results cannot be generalized to a symptomatic population.

In conclusion, the results of this study demonstrated that mobilising the contralateral knee towards extension in Slump position increases the ROM of the extension if the ipsilateral knee. However, we cannot state with certainty that the increase of the knee extension ROM in Slump position is due to the effect of mobilization of neural tissue. In light of our findings and those of previous literature, we conclude that the underlying theory underpinning the contralateral neurodynamic mobilization has not been demonstrated in healthy subjects. Therefore, future research is needed to fill this grey area.

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Original article

Unplanned migration flow: the acceptance system response, Perugia

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Summary. *Background and aim of the work:* Since several years, Italy is facing an important flow of immigration. Umbria is not a region of disembarkation of refugees, nevertheless is one of the Italian regions with the highest rate of foreign population compared to the total resident population. Aim of this paper is to collect data on migrants and refugees' health care services access, focusing on migrants' characteristics arrived and living in Umbria. *Methods:* We conducted a retrospective cohort study at the Local Health Unit Umbria of Perugia, Italy. Descriptive analysis was performed in order to identify the characteristics of the migrants living in Perugia area. Data analysis was performed using Excel® software. All frequencies are expressed as percentage. *Results:* Between 2015-2017, 2,688 migrants came to Umbria Region. The mean age is 23.6 years, almost all are male, and however the majority of female comes from Nigeria. Only 25.5% of migrants durably sojourned in Umbria Region, and only half of them are residents and received the assignment of general practitioner. *Conclusions:* Unplanned migration may cause an abrupt rupture of the social-cultural supports that sustain both psychological and physical well-being. Regional Health Service should collect data on migrants and refugees' health and their health care services access, in order to offer an efficient and appropriate regional health system. (www.actabiomedica.it)

Key words: unplanned migration, Italy, migrant health, refugees, Regional health service

1. Introduction

Due to the presence of wars, high social inequalities and low grade of physical and mental health in several areas of the world, more and more people are forced to leave their own country. World Health Organization (WHO) estimates an amount of 1 billion of migrants all over the world, with important public health impacts for both people and health services (1). Actually, the right to health was established in the WHO Constitution and confirmed, without racial differences, in the Sixty-first World Health Assembly in 2008, through the approval of Health of migrants resolution (2). Even though, the Universal Declaration of Humans rights imagines all health rights equally distributed to all people, including migrants, refugees and vulnerable people, there are still differences in health services access and health care (3).

Since several years, Italy is facing an important flow of immigration. Actually, only in 2016, arrived 181,436 migrants (4). Data similar also in 2014 and 2015 (4). Umbria is not a region of disembarkation of refugees, nevertheless is one of the Italian regions with the highest rate of foreign population compared to the total resident population (10.87% vs 11.99% of Emilia-Romagna, first in the ranking). Actually, according to the State-Regions Conference, due to the large amount of new migrants arrival during the 2014-2015, Italy had to reorganize the immigration policies and acceptance system (5). Migrants had to be reas-

signed among the Italian regions taking into account the population, gross domestic product (GDP) and the number of migrants already hosted by each region. This important issue had challenged Regions in order to rearrange the health services. Actually, Umbria Region has been fit out, since 2000, important rules on migrants' access to health services, promotion and protection of immigrants' health (6).

In fact, it is one of the Italian region that have promoted the use of all health territorial services such as General Practitioners and Paediatricians. In particular, the Regional Resolution 106/2015, followed by operating protocol, figures out the operating procedure for migrants' health care. According to this protocol, the first medical examination provided to the migrants should take in account migrants' health status in relation to potential risks for migrants themselves and for the community. During the first medical examination, doctors should actively notify, to the surveillance system, potential notifiable infectious diseases. Moreover, if there are some critical conditions, such as risk of health, the hospital transfer is urgently provided. Nevertheless, first medical examination is also important to guarantee the communication of migrants' name list to the health districts and successively registration in Regional Health Service. The aim of this paper is in line with the conclusions of sixty-first WHO assembly, which emphasized the importance to recognize migrants' health risks and needs, and to collect additional data on migrants and refugees' health and their health care services access. In particular, we want to focus on migrants' characteristics arrived and living in Umbria.

2. Methods

We conducted a retrospective cohort study during the period February 2015-November 2017 at the department of Prevention at the Local Health Unit Umbria of Perugia, Italy. Data on migrants arrived were electronically recorded in an *ad hoc* registry. Later, we cross-checked data from this electronic database with data from health-service registry. We survey the health-service registry in order to identify information related to migrants staying in Perugia area.

According to legal classification, migrants' status was defined as follow: Foreigner Temporary Present on the Italian territory (STP, Straniero Temporaneamente Presente) and migrant with residence permit. STP are illegal immigrants who can access the Italian National Health System in case of emergency care, without risk of being charged by police (7).

Migrants waiting for residence permit are foreign non-EU citizens with valid passport and/or entry visa. Resident permit should be apply as soon as possible at the entry time and if the stay exceed 3 months (8).

Descriptive analysis was performed in order to describe the characteristics of the migrants living in Perugia area. Data analysis was performed using Excel® software. All frequencies are expressed as percentage.

3. Results

During the period February 2015-November 2017, 2,688 migrants came in Umbria Region. In the majority of the case people come from Nigeria (n=595), Eritrea (n=245), Guinea (n=230), Gambia (n=223) and Senegal (n=202) (Figure 1). The mean age is 23.6 years, between 19.6 years among Syrians and 28.1 years among Pakistanis. Almost all are male, with 2,240 man vs 357 women (in 91 of the cases gender is not known). Figure 2 shows the distribution of genders according to country of origins. Even though, the majority of female comes from Nigeria (n=185) the highest proportion of Male/Female is among Palestinians (50%), followed by Nigeria (31.1%), Morocco (31.1%) and Cameroon (29.5%). Among the 2,688 individuals arrived in our Region, only 685 subjects (25.5%) are tracked for long time (more than 6 months) in our health-service registry and plausibly they durably sojourn in Umbria Region. Actually, only 7/685 left Umbria before than 6 months. Whilst, 194/685 persisted for a mean of 387.4 days, and they are no-more active in health-service registry. However, 484/685 migrants are still living in the area. Actually, 251 people are residents and received the assignment of general practitioner, 13/484 individuals had STP code, 45/484 migrants waiting for residence permit renewal and lastly 145/484 non-European migrants with residence permit is close to its expiry date. Figure 3 presents the

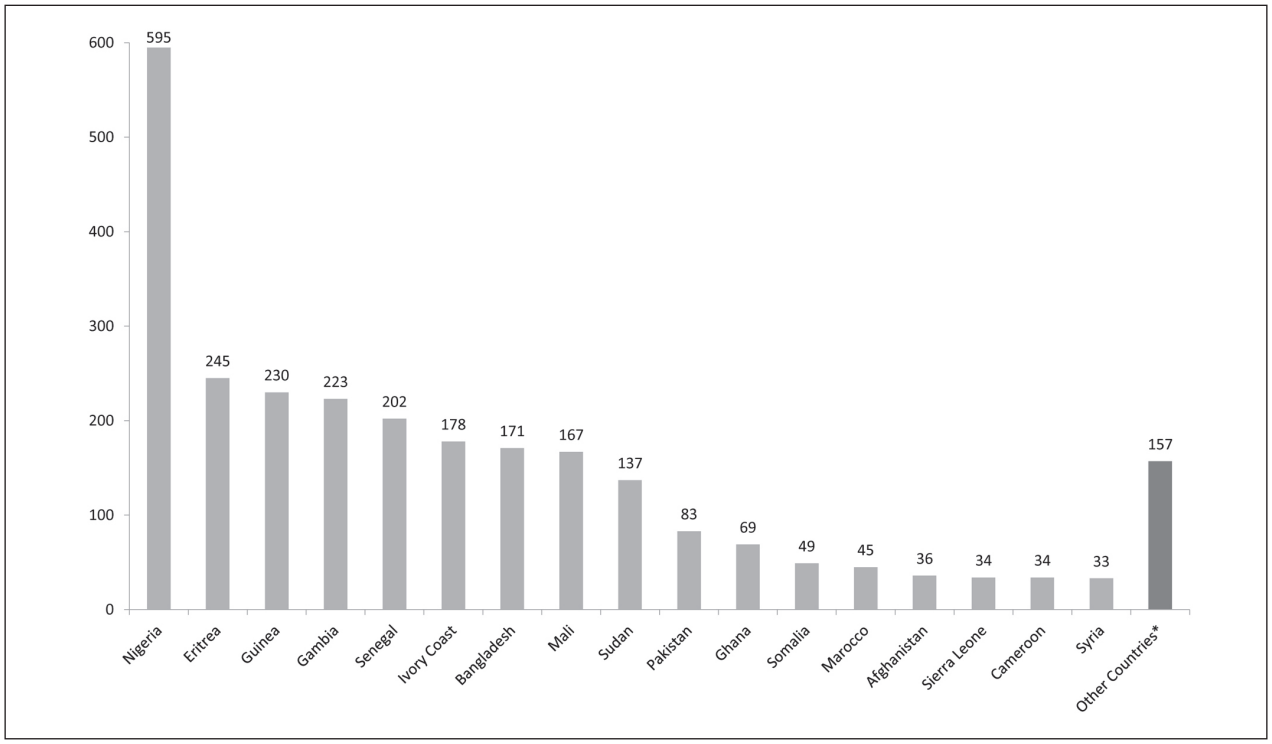

Figure 1. Total amount of migrants arrived in Umbria Region in February 2015- November 2017, according to country of origin.
* Ethiopia, Liberia, Niger, Togo, Palestine, Iraq, Burkina Faso, Ciad, Guinea Bissau, Iran, Benin, Libya, Egypt, Congo, Nepal, Sri Lanka, Zimbabwe, South Africa, Angola, Comoros Islands, India

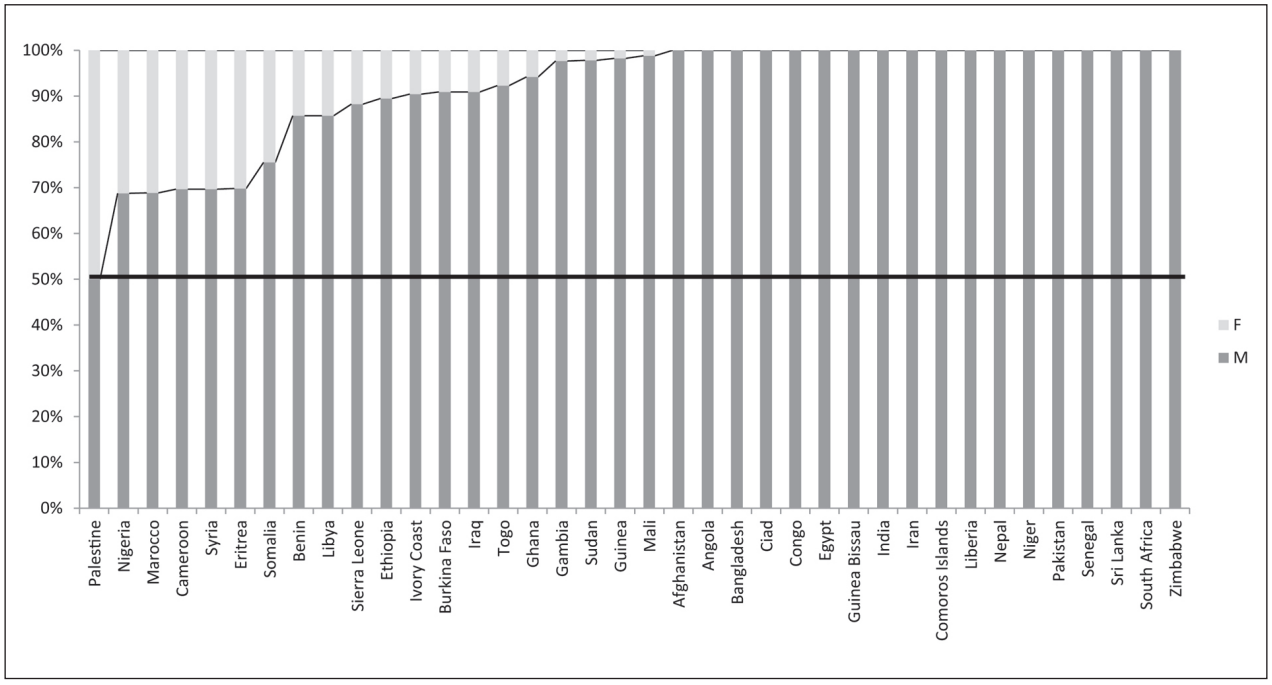

Figure 2. Distribution of genders according to country of origins

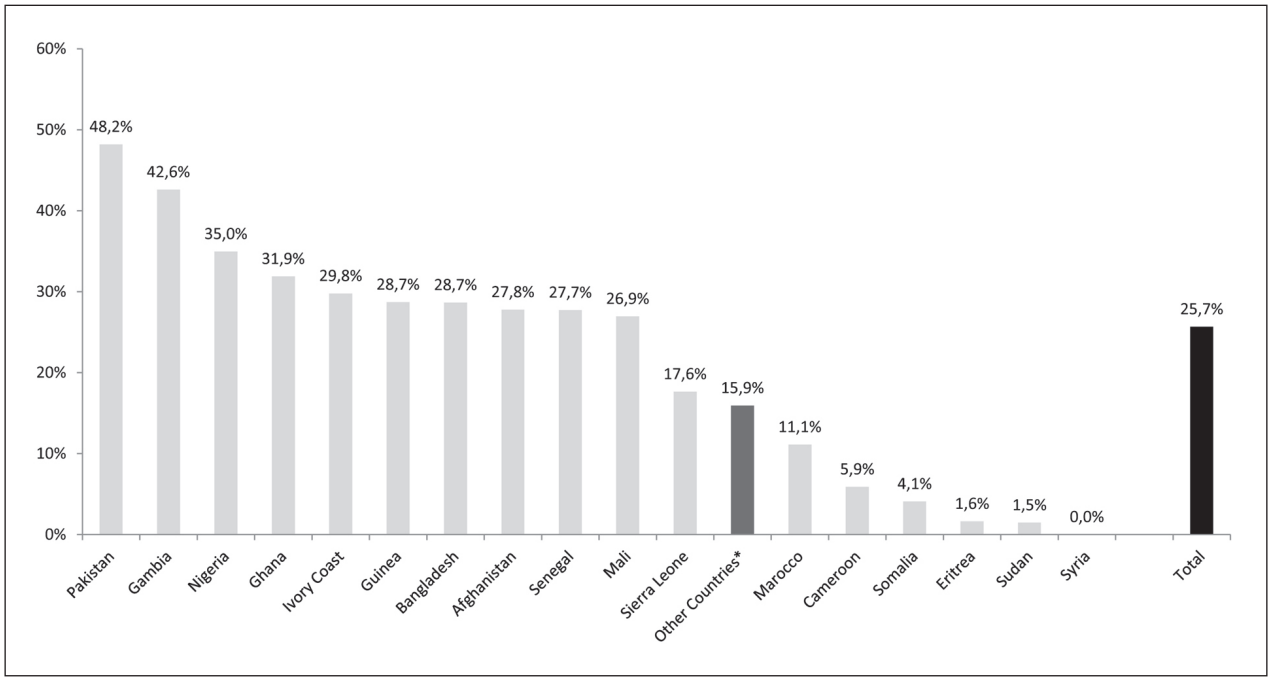

Figure 3. Percentage of migrants arrived and durably sojourning in Umbria Region, according to country of origin.
* Ethiopia, Liberia, Niger, Togo, Palestine, Iraq, Burkina Faso, Ciad, Guinea Bissau, Iran, Benin, Libya, Egypt, Congo, Nepal, Sri Lanka, Zimbabwe, South Africa, Angola, Comoros Islands, India

distribution of this subpopulation, in percentage on the total arrivals, according to the country of origin.

4. Discussion

According to Italian National data, citizenship of non-EU citizens are still increasing year by year, +16% compared to previous period (9). In particular, the number of asylum seekers is growing much more compared to other reasons such as work or family reunification. Asylum seekers are hosted with a higher hostile sentiment in many European Countries, however immigration is necessary for many European Countries economies. Actually, even though, growing evidences suggest that health systems are perceived inaccessible for the majority of migrants (10). Moreover, migrants are the cornerstones of health sector through the domestic care provided to sick and elderly people. Our study, according to literature evidence, found a young migrant population with a mean age of 23.6 years (11). This aspect generates several important considerations: first, migrants who were able to survive to the trip and after the self-selection in their own countries, constitutes a pool of young and healthy people (12). This phenomenon is known as "healthy immigrant effect", that means a no overuse of health care system. Moreover, young people are much more prone to work, in particular in low-skilled and hard jobs. Young migrants are also important to increase the birth rates, that is very low in the majority of developed countries, such as migration is able to contrast the aging of the western societies (13).

Moreover, migrant population faced different health needs, principally infectious diseases, dehydration, malnutrition and post-traumatic stress disorder. Regarding infectious diseases, in the majority of the cases they are infection acquired in the host countries, mainly due to poor living conditions, low educational level on preventive measures, or absence of information on health service access (14, 15). Dehydration and malnutrition are typical of both poor travel conditions and poverty faced also in the host countries; while post-traumatic stress disorder is the most frequent mental

health problem identified in particular among asylum seekers and refugees (16). Even though these symptoms could be common across different cultural background, growing evidence also supports the hypothesis that some symptoms might be culture specific (17). Cultural integration actions, such as migrant-specific information materials and screening programmes, are fundamental to reduce burden of diseases among migrants and to improve health management (17).

These considerations need to be taken into account in order to re-organize the health service systems, especially in a "health for all" prospective, where health service had to promote health, quality of life and human dignity (18).

In Italy there is an intense political debate, with some anti-immigrant parties focalizing on unsafety both regards health and social security. Nevertheless, according to most studies migrants are, at least initially, healthier compared to non-migrants, and the diseases appear after a lag of time principally due to low socio-economic status and low access to preventive health system. An important limit of our study is the impossibility to evaluate health status of migrants, however these data provide important information regarding migrants characteristics and are able to estimate the volume of migrants really presents in our area.

5. Conclusion

Unplanned migration may cause an abrupt rupture of the social-cultural supports that sustain both psychological and physical well-being (19, 20). Even though, Umbria is not a region of disembarkation of refugees, it has the highest rate of foreign population compared to the total resident population, among Italian regions. This is particularly true after the 2015, when the number of international migrants worldwide were the highest ever recorded. Actually, in order to face this new circumstance, Umbria region emitted the Regional Resolution 106/2015, followed by operating protocol, aimed to figure out migrants' health risks and needs, and to collect additional data on migrants and refugees' health and their health care services access, in order to improve and to offer an more and more efficient and appropriate regional health system.

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

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Original article

Peripheral neuropathies after bariatric surgery. Preliminary results from a single-centre prospective study in Northern Italy

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Summary. *Introduction:* Bariatric surgery (BS) has gained popularity in order to treat morbid obesity. However, post-operative (PO) neurologic complications have become increasingly recognized. Our aim was to examine incidence, clinical presentation, and outcomes of neurologic disorders secondary to BS. *Methods:* Patients who underwent BS between the years 2012 and 2015 at Parma University were included in this survey, and assessed before (T0) and 1 year after surgery (T1). Baseline characteristics and medical comorbidities, type of surgery, and PO complications were retrieved. Patients with a previous history of peripheral neuropathic disease were excluded from the analysis. If a patient presented with a new onset neurologic symptom including extremity numbness, paresthesia, muscle weakness, the status was considered "positive" for PO-neuropathy. *Results:* Overall, we retrieved data from 61 patients (n=30 Roux-en-Y Gastric bypasses, n=31 Gastric banding; 81.0% females). Of them, 7 (11.4%) developed some signs of PO-neuropathy, that eventually disappeared at T+24 months. The most common manifestations were paresthesia (n=6) and muscle weakness (n=4), similarly distributed in Gastric Bypass (n=4) and Gastric Banding (n=3) groups. Although patients affected by PO-neuropathy exhibited higher SF-36 score at T0 (p=0.018), no significant differences were found regarding BMI (T0, T1), percentual weight loss, serological data (i.e. vitamin B1, B2, B6, B12: in all cases p>0.05). *Conclusion:* PO-BS neuropathy is usually associated with lower levels of vitamin B1, B2, B12. However, no differences in PO-BMI, excess weight loss, and metabolic data levels were found. Larger data and more extended follow-up are required to validate our results. (www.actabiomedica.it)

Key words: peripheral nervous system diseases, bariatric surgery, malnutrition, obesity, morbid, obesity

Introduction

During the past five decades, prevalence of overweight (body mass index, BMI ≥25 kg/m^2) and obesity (BMI ≥30 kg/m^2) has globally risen, ultimately becoming of international public health concern (1-3). Morbid obesity (MOb, i.e. BMI >40 kg/m^2) has been recognized by the World Health Organization (WHO) as a systemic disease, being associated with an increased risk of hypertension, diabetes mellitus, obstructive sleep apnea, osteoarthritis, and infertility, as well as with increased risk for certain types of cancer and musculoskeletal disorders (MSD), collectively causing a considerable increase in direct and indirect costs (1-10).

Even though lifestyle changes are essential long-lasting results (4), bariatric surgery (BaS) had been recognized as the most efficient long-term treatment for both MO and complicated obesity (COb, i.e. BMI > 35 kg/m^2, with obesity-related complications such as hypertension, obstructive sleep apnea, diabetes mellitus, dyslipidemia, and disabling MSD) who have failed conservative treatment (5-7).

Even though BaS significantly improves the overall quality of life (QoL) of patients (8-11), acute and chronic complications have been diffusely reported, including a broad spectrum of neurologic disorders (7, 12, 13). For instance, available reports suggest that 1.3% up to 16% of all patients who have undergone BaS for MOb eventually complain some kinds of neurological complications months or years following the procedure, and no part of the neuraxis seems exempt (5-7, 14, 15).

Peripheral neuropathies (PN; i.e. acute, subacute, and chronic polyneuropathies, as well as focal entrapment neuropathies; radiculoplexopathy; and burning feet) following BaS seemly represent a significant issue. Not only half of the neurological complications of BaS usually affects peripheral nervous system, but complications of the peripheral nervous system have been described in around a tenth to a third of all BaS patients (5-7, 14), with an average time of 3.7 years following surgery to develop neuropathy (12, 13, 15).

Multiple etiologies have been proposed for the development of PN after BaS (15). Mechanisms of neural injury may include mechanical compression and entrapment in mononeuropathies. Still, as such complications are more frequently seen in patients who have rapid and significant early weight loss, with and without diarrhea, dumping syndrome or nausea and vomiting, the most important factors in their pathogenesis are usually identified in nutritional deficiencies due to malabsorpion or prolonged emesis (12, 13, 15).

The purpose of this study was therefore to determine the incidence rate of PN in a cohort of severe obese individuals treated at our institution and beneficing from a standardized follow up including pre- and post-operative nutritional assessment, in order to evaluate its potential risk factors.

Materials and Methods

1. Study Sample. This was a retrospective review of patients who underwent Roux-en-Y gastric bypass (RYB) or gastric banding (GB) at a specialized bariatric center between March 2010 and March 2015 at the Department of Medicine and Surgery, Section of General Surgery and Surgical Therapy, University of Parma. All the patients met the international criteria for bariatric surgery (16): age at surgery 18 to 60, BMI ≥40 kg/m^2 or BMI 35-40 kg/m^2 with comorbidities in which surgically-induced weight loss is expected to improve the disorder (i.e. metabolic disorders, cardiorespiratory disease, severe joint disease, obesity-related severe psychological problems, etc.). All patients underwent an interdisciplinary assessment prior to BaS (T1) and during the follow ups (T+24 months, T2): this specific protocol has been described in previous studies (8-11, 17-19), and included the collection of following data: general patient demographic characteristics (sex, and age), medical comorbidities (i.e. diabetes, hypertension, hyperlipidemia, psychiatric disorders, and neurologic diseases), preoperative BMI, postoperative course, BMI at follow-up (T2), re-hospitalization, neurologic signs/symptoms identified at follow-up (i.e. paresthesia, dysesthesia, abolition of deep tendon reflexes, ataxia, and Wernicke's encephalopathy), treatment received, and progression of the neuropathy. Weight loss results were expressed as the change in BMI, percentage of excess weight loss (%EWL) and percentage of excess BMI loss (%EBMIL). Patients' quality of life in physical and mental domains was measured using The Medical Outcomes Study Short-Form 36 Health Status Survey (SF-36) (20), which was submitted to the patients at T1 and T2. The SF-36 has been previously used in BaS and has good construct validity, high internal consistency and high test-retest reliability (10, 21-23). Moreover, an official Italian translation was previously validated (10, 22, 24).

2. Clinical assessment. PN was defined by clinical criteria encompassing all new onset neurologic symptom including extremity numbness, paresthesia, muscle weakness, neuropathy, imbalance, dizziness, or memory deficit that were either complained or clini-

cally identified at follow-up. PN cases were included in the analyses if the patient had received one or more gastrointestinal operations for morbid obesity (i.e. BaS) and afterwards he developed symptomatic, clinically defined PN. On the other hand, PN cases excluded from the analyses if:

1) PN developed after operation but from another known cause such as alcohol abuse, heavy metal intoxication, monoclonal gammopathy, associated necrotizing vasculitis, or toxic exposure, or the patient had pre-existing PN preoperatively;
2) essential data were missing from medical records;
3) conditions that affect weight, such as metastatic cancer, untreated hyperthyroidism, pregnancy etc. had been previously identified;
4) the patient had received either before or after BaS the diagnosis of neurologic conditions that would interfere with assessment of PN symptoms, such as multiple sclerosis, severe cervical or lumbosacral radiculopathy, stroke etc.

3. Laboratory assessment. Routine lab work were assessed at T1, during follow up and included: blood count, metabolic profile, iron studies, lipid panel, folate level, vitamin B1, vitamin B2, vitamin B6, vitamin B12 level, and 25-hydroxy vitamin D levels.

4. Statistical analysis. Descriptive analyses were performed for all variables being examined. Patients who had neurological complications after BaS were compared to those who did not present any complications. Univariate analysis of continuous variables was performed through Student's t test for paired or unpaired data, when appropriate, whereas association between discrete variables was analysed using Chi-squared tests or Fisher's exact test. All tests were two-tailed and statistical significance was set at $p<0.05$. All statistical analyses were performed using IBM SPSS Statistics 25.0 for Macintosh (IBM Corp. Armonk, NY).

5. Ethical considerations. This paper details a part of a larger study that was carried out in accordance with the principles of the Declaration of Helsinki, and was specifically reviewed by an institutional board. Details are provided elsewhere (8,9-11). All patients received both written and oral information regarding the procedure, and all provided informed consent before undergoing the surgical procedure. Subjects refusing their consent were excluded from the study population.

Results

Among 76 patients scheduled for BaS at our institution during the study period, 74 (97.4%) signed their consent to the study and underwent the preoperative assessment. Six patients were excluded from the analyses (7.9%): for complications unrelated to BaS during the follow-up (n=1, 1.3%), because of a prior diagnosis of neurologic disease (n =1, 1.3%), or were lost to follow-up (n=4, 5.3%). A total of 61 patients attended post-surgery appointments and were included in the final analyses (83.3% of the original sample), including n=30 RYB (49.2%), and n=31 GB (50.8%).

The characteristics of the study population are shown in Table 1. Briefly, the sample consisted mostly of females (n=50, 82.0%), with a mean age at T0 of 41.8±11.4 years, and a preoperative BMI of 44.4±7.2 kg/m^2. No cases of nutritional deficiencies regarding micronutrients (Vitamin B1, B2, B6, B12) were reported. Mean total weight loss was 59.8%±22.3 at T2.

Overall, 7 patients (11.4%) developed some signs of PO-neuropathy during follow up. In six cases, symptoms eventually disappeared within 6 months after T2 (Table 2). In the only case without spontaneous resolution, paresthesia was associated with significant forearm muscle weakness, and a subsequent medical assessment identified a case of previously not reported Carpal Tunnel Syndrome (CTS).

The most common manifestations were paresthesia/dysesthesia (n=5, 8.2%) and localized muscle weakness (n=3, 4.9%). As shown in Figure 1, all cases of muscle involvement were diagnosed at the upper limbs, and involved shoulder region (n=2, both cases affecting trapezius muscle), arm (n=1, muscular venter of the biceps) and ventral forearm (n=1, brachioradialis and flexor carpi ulnaris muscles). Regarding the laterality, right upper limb was involved in 3 out 4 cases, and all cases were right dominant. Conversely, paresthesia and dysesthesia predominantly affected

Table 1. Characteristics of the 61 Bariatric Surgery (BS) cases included in the analysis. Confrontations were performed through Student's t test for unpaired data for continuous variables and by means of Chi squared test or Fisher's test (= *) for dichotomous variables

	All cases (n=61, 100%)	NP (n=7, 11.5%)	Non-NP (n=54, 88.5%)	p value
Age (years; mean±SD)	41.8±11.4	42.7±13.9	41.7±11.2	0.831
Female Sex (n, %)	50, 82.0%	6, 85.7%	44, 81.5%	1.000
Smoking history (n, %)	8, 26.7%	0, -	8, 30.8%	0.550*
Diabetes (n, %)	10, 16.4%	2, 28.6%	8, 14.8%	0.702
Surgical procedure				0.963
Gastric banding	31, 50.8%	3, 42.9%	51.9%	
Gastric bypass	30, 49.2%	4, 57.1%	48.1%	
BMI T0 (Kg/m^2; mean±SD)	44.4±7.2	44.3±3.8	44.4±7.6	0.969
BMI T1(Kg/m^2; mean±SD)	32.7±4.9	32.0±5.1	32.8±4.9	0.719
T2 data				
EWL (%)	59.8±22.3	61.9±30.8	59.5±21.4	0.843
Vitamin B1 (nmol/L)	113.3±36.3	111.5±37.1	126.8±28.3	0.297
Vitamin B2 (µg/L)	258.1±76.7	213.7±72.2	263.9.±76.0	0.103
Vitamin B6 (µg/L)	19.6±5.1	17.2±3.3	19.9±5.2	0.185
Vitamin B12 (pg/mL)	249.1±101.3	251.3±99.3	260.3±106.4	0.829
SF-36 (T0)	478.3±160.2	344.5±146.5	495.6±154.8	0.018
SF-36 (T+24)	686.8±152.4	589.6±167.5	699.4±147.3	0.141

Notes: NP=neuropathic pain; Non-NP=non neuropathic pain; BMI=Body Mass Index (Kg/m^2); T0=Before Surgery; T1=at follow up (12 months after surgery); SF-36=Short Form (36) Health Survey; EWL=Percent excess body weight lost

Table 2. Characteristics of patients affected by peripheral neuropathies during the follow up

Sex	Age	Operation	Upper limb	Lower Limb
F	28	RYGB		Paresthesia, Tibial region (left)
F	54	RYGB	Muscle weakness, shoulder (right) Muscle weakness, arm (left)	Paresthesia, Feet (bilateral) Dysesthesia (thermal perception; tibial region)
F	47	RYGB	Paresthesia, hand and wrist (right) (CTS)	
M	53	RYGB	Muscle weakness, shoulder (right)	
M	43	GB		Paresthesia, Foot (right)
F	44	GB		Dysesthesia (thermal perception; left foot)
F	55	GB	Muscle weakness, ventral forearm (right) Parestesia, hand and wrist (right)	

Notes: RYGB=Roux-en-Y Gastric Bypass; GB=Gastric Banding; CTS=Carpal Tunnel Syndrome

lower limbs, as paresthesia of the feet was identified in 2 cases (one, of them bilaterally), while involvement of the tibial region was identified in 2 other cases. Simultaneous involvement of upper and lower limb was identified in one case.

Cases of PN were similarly distributed among RYB (n=4) and GB (n=3, p=0.963) groups. Although patients affected by PN exhibited lower cumulative SF-36 score, both at T1 (344.5±146.5 vs. 495.6±154.8), and at T2 (589.6±167.5 vs. 699.4±147.3), the difference was significant only at T1 (p=0.018 and p=0.141, respectively).

Also regarding assessed serological data for micronutrients (i.e. vitamin B1, B2, B6, B12), lower val-

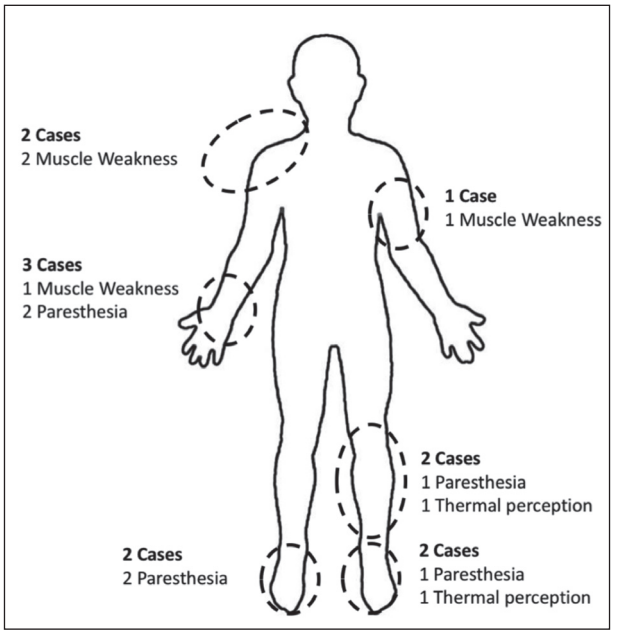

Figure 1. Graphical representation of neuropathic disorders reported by patients. Overall, 6 out of 7 patients reported paresthesia, 4 muscle weakness (all in the upper limbs), and 2 further cases had thermal perception disorders. On the contrary, no cases of neuropathic pain were referred

ues were reported for PN patients at T2, but the difference was not significant (all cases $p>0.05$).

Discussion

Peripheral neuropathies following BaS are a relatively common complication (12, 13, 15, 25, 26). BaS-associacted peripheral polyneuropathies typically develop as predominantly sensory disorders, characterized by painful paresthesias with stocking-glove distribution, i.e. affecting lower and upper extremities. An increased risk for mononeuropathies has been similarly described: median mononeuropathy at wrist, or CTS, develops in up to 7% of all gastric bypass patients, followed by a lesser risk reported for peroneal, ulnar and radial isolated mononeuropathies (15).

Even though multiple etiologies have been proposed for the development of peripheral neuropathies following BaS (15), the association with rapid and significant early weight loss, diarrhea, dumping syndrome or nausea and vomiting has suggested that the main effector may be found in nutritional deficiencies generally due to malabsorption or prolonged emesis (12, 13, 15). Despite their obesity, 20 to 30% patients usually have micronutrient deficiencies prior to BaS (i.e. low thiamine, vitamin C, iron, zinc, vitamin B12, and 25-hydroxyvitamin D3 deficiency), and such deficiencies may get worse after surgery because of prolonged vomiting, loss of absorptive surface, altered dietary patterns, loss of gastric acid, loss of intrinsic factor, encompassing calcium, zinc, selenium, vitamin A, 25-hydroxyvitamin D, and thiamine (5, 6, 12, 13). In a meta-analysis of nearly 1,000 patients, 25% of them were B12-deficient, 20% folate-deficient, and 1% thiamine deficient (5, 6, 12-14).

Although previous reports have suggested that the risk for PN after BaS may eventually depend on the extent of weight loss, and surgical procedure used, growing from gastric banding, vertical banded gastroplasty, sleeve gastrectomy, Roux-en-Y gastric bypass, partial biliopancreatic bypass (5, 6), in our sample patients with and without PN had significant differences regarding EWL, nutritional indices (i.e. vitamin B1, B2, B6, B12), surgical procedures. Moreover, nearly all cases of PN we identified had a spontaneous resolution during the follow up. Only one case requested subsequent treatment because of a diagnosis of CTS: even though CTS is the most commonly reported among patients receiving BaS, and rapid weight loss may make the nerves more susceptible to compression through loss of subcutaneous tissue, loss of protective far pads, or structural changes, accurate evaluation of the case suggested that CTS pre-existed BaS (5, 6, 27-29). It is therefore reasonable that BaS *per se* had no role in the pathogenesis of the mononeuropathy.

In our sample, no cases of nutritional deficiencies were identified prior to BaS. Moreover, although PN cases exhibited relatively lower concentrations of vitamin B1, B2, B6 at T2, not only none of them had significant deficiencies, but the difference between PN cases and non-PN cases was not significant. As a consequence, we can speculate that a surgical treatment performed before the patient eventually develops micronutrient deficiency as well as an accurate follow-up course, maintaining a good nutritional status, may be associated with a low risk for long-lasting PN and severe complications such as Wernicke's encephalopathy.

Several other factors suggest that a good preoperative assessment of the patient may be useful in averting "classical" PN associated with nutritional deficiencies. First at all, PN are usually classified among "late" complications, i.e. developing between 6 weeks and 2 years after surgery BaS, whereas all cases we identified eventually resolved within less than three years from surgery. The transient nature of PN we identified in our sample may therefore be found in the transient inflammatory status that usually characterizes the early post-operative period (25, 26).

Moreover, the typical pattern of post-BaS peripheral neuropathy is represented by symmetric disorders (13), whereas in nearly all PN cases we reported a monolateral pattern was identified (5, 6, 13, 25, 26).

Our study is affected by several limits. First at all, it should be stressed that our study, as well as the majority of available reports, had a retrospective design, without pre-operative electrophysiology studies that may lead to an early, preoperative identification of borderline neurological disorders (5, 6). In other words, we are unable to assess whether PN cases had a previous, border-line disorder, similarly to the CTS we identified, that post-operative status may have enlighten rather than elicited.

Second, our study lacks an anatomopathological assessment. As a consequence, we can only speculate that the disorders we identified had an inflammatory nature. In this regard, nerve biopsies from BaS patients who had neurological complications (polineuropathy or radiculoplexus neuropathy with acute or subacute onset) suggest that a cachexia-like state with nutritional deficiencies may induce inflammatory changes and immune mechanisms of neuropathy (25, 26). Unfortunately, without specific biopsies we are unable to assess whether PN cases had a specific anatomopathological pattern of inflammation or not.

Third, we focused only on Vitamin B1, B2, B6, B12. As potential involvement of several other nutritional factors in post-operative PN has been previously suggested, our study is unable tu rule out that the specific pattern we identified as associated with low vitamin C, iron, zinc, and 25-hydroxyvitamin D3 deficiency (5, 6, 12, 13).

In conclusion, PN is a relatively common complication of BaS. In cases with a good pre-operative nutritional status and without significant deficiencies of micronutrients, it may be characterized by a transient nature, with no long-lasting consequences.

Conflict of interest: The facts, conclusions, and opinions stated in the article represent the authors' research, conclusions, and opinions and are believed to be substantiated, accurate, valid, and reliable. However, as this article includes the results of personal researches of the Authors, presenting correspondent, personal conclusions and opinions, parent employers are not forced in any way to endorse or share its content and its potential implications. 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

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Original article

Co-existence of maternal overweight and obesity with childhood undernutrition in rural and urban communities of Lagos State, Nigeria

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Summary. *Background:* The coexistence of over-nutrition and under-nutrition is emerging as a public health problem in many low and middle income countries. This study aimed at determining prevalence of coexisting maternal overweight and obesity with childhood stunting (MOCS) and the associated socio-demographic factors in rural and urban communities of Lagos State, Nigeria. *Methods:* This was a cross sectional survey conducted using the multistage random sampling technique. A total of 300 mother-child pairs were studied, consisting of 150 each from rural and urban communities. Maternal overweight and obesity and undernutrition in children were determined using standard criteria. *Results:* The prevalence of overweight and obesity among mothers was significantly higher in urban than rural areas (50.7% vs. 41.3%; p=0.022) while the prevalence of childhood stunting was significantly higher in rural than urban areas (43.3% 12.6%; p<0.001). Coexisting maternal overweight and obesity with childhood stunting was observed in 31 (10.3%) mother-child pair with a significantly higher prevalence in rural than urban areas (14.7% vs. 6.0%, p=0.014). In multivariate logistic regression, maternal short stature (OR 3.3, 95% CI=1.2-9.0, p=0.02) and living in rural area (OR 0.2, 95% CI=0.1-0.5, p=0.001) were the identified risk factors for coexisting maternal overweight and obesity with childhood stunting. *Conclusion:* The prevalence of coexisting MOCS is high especially in rural areas. Effort at reducing childhood malnutrition should focus on appropriate interventional measures aimed at improving maternal nutritional status. (www.actabiomedica.it)

Key words: coexistence, overweight; obesity, stunting, mothers, child, Lagos, Nigeria

Introduction

Under-nutrition persist as a significant health burden particularly in low and middle income countries such as those in Sub-Saharan Africa (1). However, as a result of urbanization and accompanying nutritional transition in many of these low and middle income countries, there are new incursions of increasing prevalence of overweight and obesity coexisting with under nutrition, a phenomenon referred to as "double burden" malnutrition (2, 3). Double burden malnutrition represents complex situation where under-nutrition, over-nutrition and their consequences such as micronutrient deficiencies, infectious diseases and non-communicable diseases coexist. Reports indicate that double burden malnutrition can occur at household or community levels or even within the same individual (4). In an international survey from 42 developing countries, about 16% of households were with underweight and overweight members. This is

worrisome in developing countries where health facilities to manage these conditions and its consequences are scarce.

In Nigeria, the prevalence of childhood stunting is one of the highest in the world while the prevalence of overweight and obesity in the general population is on the increase. According to the National Demographic and Health Survey (NDHS) conducted in 2013, the prevalence of stunting, underweight and wasting among the under-fives are 37%, 29% and 18% respectively (5). From the same survey, 17% of Nigerian women are overweight and 8% are obese. The prevalence of these nutritional indices varies according to the background characteristics and place of residence in Nigeria.

The persistence of child under nutrition in low income countries has been associated with coexistence of maternal obesity and childhood under-nutrition (MOCS) in the same household. It is hypothesized that adoption of energy-dense but nutrient-poor foods for household results in increased overweight in adults, and failure to meet linear growth potential in children (6). It is therefore important to explore and estimate magnitude of double burden malnutrition in populations and also determine associated contributory factors with a view of providing appropriate interventions aimed at reducing the burden of malnutrition.

Information on coexistence of childhood stunting and maternal obesity in Nigeria is limited. Therefore, the aim of this study was to determine the current nutritional status of mothers and their children, prevalence of coexisting maternal overweight and obesity with childhood stunting (MOCS) and the associated socio-demographic factors in rural and urban communities of Lagos State, Nigeria

Methods

Study area

This study was part of as larger study on maternal and child nutritional status in rural and urban communities in Lagos state, Nigeria.

The study was carried out in the Epe Local Government Area and the Alimosho Local Government Area in Lagos State, Nigeria, which were randomly selected from the 20 local governments in Lagos state officially recognized by the Federal government of Nigeria.

Lagos state is Nigeria's former capital city. The vegetation is that of the tropical rain forest. It has an annual rainfall of 180 centimeters and a mean temperature of 26°C. It is 200-500 meters above sea level. Lagos has a very diverse and fast-growing population, resulting from heavy and ongoing migration to the city from all parts of Nigeria as well as neighbouring countries. Though the Yoruba, an African people inhabiting southwest Nigeria, constitute the city's principal ethnic group, there are a significant proportion of other ethnic groups, particularly the Ibos, Ibibio and Hausa.

Study design

The study was a cross sectional survey of children aged zero to fifty-nine months in rural and urban communities located in randomly selected Local Government Areas of Lagos State, Nigeria.

Ethical statement

Ethical approval was obtained from the Lagos State University Teaching Hospital Research/ Ethics Committee with approval file number LREC/10/06/297; verbal informed consent was also obtained from the parents that were involved.

Method of sampling

A multistage random sampling technique was used to select subjects. Epe and Agbowa were randomly selected from the Epe LGA, representing urban and rural communities, respectively, while Akowonjo and Orisunbare were selected from the Alimosho LGA, representing urban and rural communities, respectively. From each communities, houses were selected randomly using the table of random numbers.

Complete data was collected from 300 subjects comprising 150 each for rural and urban communities.

In each household, the parents of children aged zero to fifty-nine months were identified, and informed consent was obtained prior to the beginning

of the study. The parents were interviewed according to the proforma specifically designed for the study. Information was obtained on demographic, socio-economic characteristics, breastfeeding practices and type of solid, semi-solid or soft foods taken by children. The families were assigned a socio-economic class using the method recommended by Ogunlesi et al (7). The parents' occupations and highest education attained were scored from 1 (highest) to 5 (lowest). The mean score for both parents gave the social class, falling within the 1 – 5 range. Those with mean scores of 1 and 2 were further classified as upper class, those with mean score of 3 were classified as middle class, while those with mean scores of 4 and 5 were classified as falling in the lower social class.

Anthropometry

The children were weighed using an electronic weighing scale calibrated in 100 g units (SECA/ UNICEF, Australia). Children who were too scared to stand on the scale were weighed together with the mother, and the mother's weight automatically deducted to obtain the weight of the child. All children were weighed naked and to the nearest 0.1 kg.

Length of children aged less than twenty-four months was measured using an infantiometer. This was done on a firm surface with assistance, usually by the mother. The knees were held down and the head held firmly against the headboard. These measurements were done to the nearest 0.1 cm. Height was measured using a height board for children aged twenty-four to fifty-nine months. This was done with the children standing erect without shoes, with eyes facing forward and the feet together on the horizontal plane.

Mother's weight was measured using the same weighing scale used for children.

The height of mothers was also measured using height board. This was done with the mothers standing erect without shoes and with the eyes looking horizontally and the feet together on a horizontal level.

Waist circumference was measured with a flexible inextensible tape (Butterfly, China) midway between the iliac crest and the lower most margins of the ribs with bare belly and at the end of normal expiration according to the WHO guidelines (8). Standardization checks on the on the tools for anthropometric measurements were done periodically.

Malnutrition in children was calculated from the degree of stunting (height-for-age), underweight (weight-for-age) and wasting (weight-for-height) following World Health Organization (8) guidelines and cut off points. In this study, a child was said to be underweight, wasted and stunted if the Weight-for-Age, Weight-for-Height and Height-for-Age were below minus two Standard Deviation (-2 SD) from the median of each international reference standard, respectively.

Maternal overweight and obesity was determined by calculating Body Mass Index (BMI) as follows; weight (kg)/height^2 (m) and based on WHO standard, mothers were classified into underweight, normal, overweight and obese (<18.5, 18.5-24.9, 25-29.9, ≥ 30 kg/m^2 respectively). Waist circumference was used as measure of central body fat distribution. According to international reference standard, those with cut off point >88 cm were considered centrally obese (8).

Statistical analysis

WHO Anthro 2007 was used to generate z-score values for weight-for-age, height-for-age and weight-for-height. All data were entered into and analysed using SPSS for windows software version 13. The means and standard deviations (SD) were calculated for continuous variables, while ratios and proportions were calculated for categorical variables. Independent t-test was used to calculate mean differences for continuous variables between urban and rural communities. Categorical variables were compared using the Pearson Chi square (χ^2) test. 'P' values less than 0.05 were accepted as statistically significant.

Results

Socio-demographic characteristics

A total of 300 mother-child pairs were studied, consisting of 150 each from rural and urban communities, and the results obtained were analysed. Table 1 shows the socio-demographic characteristics and

Table 1. Socio-demographic characteristics of study population according to place of residence

Parameters	Total (n = 300)	Rural (n = 150)	Urban (n = 150)	P Value
Mothers characteristics				
Age (years)*	30.0±5.6	29.6±6.1	30.4±5.6	0.186
Family setting				0.001
Monogamy	265 (88.3)	119 (79.3)	146 (97.3)	
Polygamy	35 (11.7)	31 20.7)	4 (2.7)	
Parity				0.001
1	83 (27.7)	24 (16.0)	59 (39.3)	
2	183 (61.0)	101 (67.3)	82 (54.7)	
5-7	34 (11.3)	25 (16.7)	9 (6.0)	
Age at first birth				0.001
15-19	29 (9.7)	22 (14.7)	7 (4.7)	
20-24	127 (42.3)	77 (51.3)	50 (33.6)	
25-29	96 (32.0)	41 (27.3)	55 (36.9)	
30-34	37 (12.3)	5 (3.3)	32 (21.3)	
35-39	8 (2.7)	3 (2.0)	5 (3.4)	
Educational level of mother				0.001
No formal education	4 (1.3)	3 (2.0)	1 (0.7)	
Primary	72 (24.0)	55 (36.7)	17 (11.3)	
Secondary	155 (51.7)	79 (52.7)	76 (50.7)	
Postsecondary	69 (23.0)	13 (8.7)	56 (37.3)	
Social class[#]				0.001
Upper	19 (6.3)	0 (0.0)	19 (12.7)	
Middle	50 (16.7)	15 (10.0)	35 (23.3)	
Lower	231 (77.0)	135 (90.0)	96 (64.0)	
Children characteristics				
Age (months)*	25.3±16.4	31.3±16.4	19.3±4.0	0.001
Sex				0.248
Male	142 (47.3)	66 (44.0)	76 (50.7)	
Female	158 (52.7)	84 (56.0)	74 (49.3)	
Birth order				0.000
1	118 (39.3)	39 (26.0)	79 (52.7)	
2-4	161 (53.6)	87 (64.7)	64 (42.7)	
5-7	21 (7.0)	14 (9.3)	7 (4.7)	

Values are number (%) unless otherwise stated; *values are mean ± SD. [#]Upper social class includes parents such as senior government employee, high scale traders, and professionals, middle class include junior government employee, teachers and technicians while lower social class are peasant farmers, artisans, security agents, messengers, apprentice, laborers and the unemployed.

nutritional status of the study population according to their place of residence.

Polygamous homes was siginificantly common in rural than uban communities (p<0.001) while the parity of mothers was higher in rural than urban communities (p<0.001). The educational level of mothers was significantly better in urban as opposed to rural areas (p<0.001) and a significant proportion of the mothers in rural compared with urban communities (90.0% vs. 60.4%, p<0.001) were from the lower social class.

The mean age of the children in the rural communities was significantly higher than that of children from urban communities (31.3 vs. 19.3 months; p<0.001). Gender distributions of the children were not statistically different, with a male: female ratio of 1:1.3 in rural areas and 1:1 in urban areas. Seventy-nine (52.7%) children in urban areas were the first born compared with 39 (26%) children in rural areas. This difference was statistically significant (p<0.001).

Anthropometrics, Nutritional Status of Mothers and their children

The anthropometric characteristics of mothers according to their place of residence is as shown in Table 2. The weight of mothers in urban areas was significantly higher than those from rural areas (p=0.042). The prevalence of overweight in rural and urban areas was 27.3% and 32% respectively while the prevalence of obesity in rural and urban areas was 14.0%% and 18.7% respectively. This difference was statistically significantly (p=0.022).

Using waist circumference, there was no significant difference in the prevalence of central obesity between mothers from rural and urban areas (p>0.05).

The mean weight and height were significantly higher among children from rural than urban areas (Table 3). Both weight and height increase with age in both sexes in children from rural and urban areas. However, the degree of association of weight and height with age were higher in urban children (r=0,894 and 0.946 respectively) than rural children (r=0.736 and 0.857 respectively).

The prevalences of underweight and stunting were significantly higher among children from rural than urban areas (19.4% vs. 9.3%, OR 3.8, 95% CI=1.8-8.1, p<0.001) and (43.3% vs. 12.6%, OR 7.4, 95% CI=3.8-14.1, p<0.001) respectively.

Prevalence of maternal overweight/obesity with child stunting

There were 31 (10.3%) mother-child pair with maternal overweight/obesity and child stunting with

Table 2. Anthropometrics and nutritional status of mothers and children according to place of residence

Anthropometry/Body composition	Total (n =300)	Rural (n = 150)	Urban (n = 150)	P value
Mothers				
Weight, kg	63.9 (13.5)	62.3 (13.2)	65.5 (13.7)	0.042
Height, cm	160.3 (6.6)	159.9 (6.5)	160.6 (6.7)	0.337
BMI				0.022
<18.5	20 (6.7)	16 (10.7)	4 (2.7)	
18.5-24.9	142 (47.3)	72 (48.0)	70 (46.7)	
25-29.9	89 (29.7)	41 (27.3)	48 (32.0)	
30-34.9	42 (14.0)	20 (13.3)	22 (14.7)	
35-39.9	7 (2.3)	1 (0.7)	6 (4.0)	
WC, cm				1.000
≤88	184 (61.3)	92 (61.3)	92 (61.3)	
Greater than 88	116 (38.7)	58 (38.7)	58 (38.7)	
Children				
Weight-for-age z score, < -2 SD	43 (14.3)	39 (26.0)	14 (9.3)	0.001
Height-for-Age z score, < -2 SD	84 (28.0)	87 (64.7)	19 (12.6)	0.001
Weight-for-height z score, <-2 SD	31 (8.3)	14 (9.3)	13 (8.7)	0.118
Coexisting MOCS	31 (10.3)	22 (14.7)	9 (6.0)	0.014
Coexisting centrally obese mother with childhood stunting	28 (9.4)	24 (16.2)	4 (2.7)	0.000

BMI=body mass index; WC=waist circumference; MOCS=maternal overweight and obesity with childhood stunting, cm=centimetre, kg = kilogram

Table 3. Factors associated with overweight/obese mother and stunted child

Parameters	Overweight/obese mother and stunted child		χ^2	P value
	Yes No (%)	No No (%)		
Mothers characteristics				
Age (years)			2.4	0.124
≤30	14 (8.0)	160 (92.0)		
>30	17 (13.5)	109 (86.5)		
Family setting			0.07	0.788
Monogamy	27 (10.3)	236 (89.7)		
Polygamy	4 (11.8)	30 (88.2)		
Parity			2.2	0.137
≤4	26 (9.8)	240 (91.2)		
>4	6 (2.0)	289 (92.0)		
Number of children in the family			8.3	0.004
≤4	21 (8.3)	232 (91,7)		
>4	10 (22.7)	34 (77.3)		
Level of education			4.3	0.227
No formal education	0 (0.0)	4 (100.0)		
Primary	11 (15.3)	61 (84.7)		
Secondary	16 (10.3)	139 (89.7)		
Tertiary	4 (6.2)	65 (94.2)		
Residence			6.1	0.014
Rural	22 (14.7)	128 (85.3)		
Urban	9 (6.0)	141 (94.0)		
Height (cm)			7.8	0.005
<152	7 (25.9)	20 (74.1)		
≥152	24 (8.8)	249 (91.2)		
Social class			1.03	0.597
Upper	1 (5.3)	18 (94.7)		
Middle	4 (8.0)	46 (92.0)		
Lower	26 (11.3)	205 (88.7)		
Children characteristics				
Age (months)			6.5	0.167
0-11	3 (4.9)	58 (95.1)		
12-23	13 (14.4)	77 (85.5)		
24-35	4 (6.6)	57 (93.4)		
36-47	4 (8.7)	42 (91.3)		
48-59	7 (16.7)	35 (83.3)		
Gender			0.781	0.377
Male	17 (12.0)	125 (88.0)		
Female	14 (8.9)	144 (91.1)		
Birth order			0.38	0.537
1-4	28 (10.0)	251 (90.0)		
≥5	3 (14.3)	18 (85.7)		

Table 4. Logistic regression analysis for determinants of coexistence of maternal overweight and obesity with childhood stunting

Independent variables	Odds ratio	95% CI	p value
Overweight/obese mother and childhood stunting*			
Parity	0.95	0.63-1.43	0.803
Number of children in the family	1.15	0.86-1.54	0.334
Area of residence	3.29	1.21-8.98	0.02
Mothers height	0.16	0.06-0.48	0.001

*Adjusted for age of mother, age of child and social class

a significantly higher prevalence in rural than urban areas. (14.7% vs. 6.0%, p=0.014). Twenty-eight (9.3%) mother-child pair had coexisting maternal central obesity and child stunting with a significantly higher prevalence in rural areas (16.2% vs. 2.7%, p<0.001).

Determinants of co-existence of maternal overweight/obesity with child stunting

Tables 4 shows the influence of various socio-demographic factors on co-existence of maternal overweight/obesity with child stunting. There was significant association between co-existing MOCS and increase number of children in the family, rural residence and low height of mothers. In a multiple regression analysis, after adjusting for potential confounders, only maternal short stature and rural residence were the significant determinant of coexisting MOCS.

Discussion

This study provided further evidence of the occurrence of coexisting MOCS both in rural and urban settings of a resource poor nation where inadequate energy intake is certainly the major macro-nutritional problem.

In this study, the prevalence of overweight (29.7%), obesity (16.3%) and central obesity (38.7%) observed in rural and urban women were high compared to the prevalence of 6.7% for under-nutrition among this same group of women. This same trend was reported in the country since early 2000s. In a study of three rural communities in southwest Nigeria, 20.8% and 8.4% were overweight and obese respectively (9). In Abeokuta, Southwest Nigeria, 58.6% of market women were overweight (10), and in Jos, Central Nigeria, 29.4% and 25.9% of women were overweight and obese respectively (11) while in Sokoto, Northern Nigeria, the prevalence of central obesity was 28% (12). These obviously attest to high prevalence of overweight and obesity even among those living in rural areas and of low socio-economic class. This is in contrast to the usual finding in developing countries where overweight and obesity are more common among the affluent and in people of high socio-economic class. This has a direct consequence of increase in the prevalence of non-communicable diseases such as hypertension diabetes and other metabolic disorders in this population. These could stretch the health system and government budget which are already inadequate to cater for the existing problem of infections and chronic energy deficiency.

Despite various national and state programs and policies aimed at reducing under-nutrition among under-fives, the prevalence of childhood stunting in this study remains high especially in rural areas. Our previous study demonstrated that the risk factors were mostly socio-economic factors which will translate to provision of adequate and appropriate diet and better caring practices for children (13).

Reports indicate that the prevalence of coexisting MOCS is high in Asia, Latin America and in countries that are in the middle range of Gross National product (GNP) while the prevalence is low in African countries (6, 14, 15). However, the prevalence of coexisting MOCS in this study was 10.3%. This is in contrast to less than 5% reported for most sub-Saharan African countries by Jehn and Brewis (16) and 1.81% reported for Nigeria using the year 2008 Demographic Health Statistics survey (15). Our prevalence was similar to 8.4% obtained in Mexico (17), 11% in rural Indonesia (14) and 16.2% in neighbouring Republic of Benin (18). The high prevalence is evidence that the country

is in nutritional transition that is similarly occurring in almost all low and middle income countries, whereby the consumption of energy dense and nutrients poor diet leads to overweight and obesity in mothers and stunting in children.

Similar to finding in other studies (14, 16), our study shows that increasing number of children in the family was significantly associated with coexisting MOCS. This can be ascribed to reduced amount of nutrient rich food reaching the children as the family size increases.

In this study, maternal short stature was a risk factor for high prevalence of coexisting MOCS and this is similar to findings in rural Indonesia and Bangladesh (14). There is a link between early childhood stunting and development of obesity later in life. Therefore, the significant association between maternal short stature and coexisting MOCS could represent an intergenerational effect perpetuating malnutrition from one generation to the next.

Many studies reported that coexisting MOCS is more prevalent in urban than rural areas of developing countries because overweight and obesity are commoner in urban areas (16, 19) while other studies reported higher prevalence in rural areas (14). Our study shows that both coexisting MOCS and coexisting centrally obese mother with childhood stunting were more prevalent in rural areas. Interestingly, the prevalence of maternal central obesity in rural and urban areas was similar. This implies that, coexisting MOCS may represent a defined entity and not statistical artefact as alluded to by Dieffenback and Stein (20).

The limitations of this study include involvement of only 2 local government areas of Lagos State. However, the findings can be generalized to the broader community. We recommend that further cross-sectional research work involving other communities and a larger sample frame is needed to better understand this dual burden of malnutrition and the associated risk factors.

In conclusion, this study has shown that the prevalence of maternal overweight and obesity and childhood stunting is high in Lagos, Nigeria. Moreover, the prevalence central obesity which is a better indicator of adverse consequence of accumulated body fat is also high both in rural and urban areas of Lagos State, Nigeria. In contrast to earlier report, the prevalence of coexisting MOCS is high in Nigeria and is similar to what obtains in Asia and Latin America. Notably, the prevalence of coexisting MOCS is higher in rural than urban areas despite higher prevalence of maternal overweight and obesity in urban areas. In this study, rural residence was also identified as a major risk factor of coexisting MOCS. This might indicate that coexisting MOCS is a distinct entity whose root could be related to the on-going nutritional transition. Therefore, in order to reduce the persistently high prevalence of stunting, efforts must be geared towards educating parents and guardians on appropriate family dietary practices that will support growth and development of their children. This may involve public health enlightenment campaign discouraging consumption of energy dense, nutrient poor food by mothers and their children and also regulation of the food industry such that unhealthy foods do not get advertised or found in stores.

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Author's contribution:
IOS concieved, designed, analyzed the data and wrote the first draft. COS participated in the design, data collection, analysis and interpretation of data. WAO and IOO participated in the design, supervised data collection and guided writing of the manuscript. All authors read and approved the final manuscript.

Supplementary Materials:
Relevant research materials such as raw data are available on request

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

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Original article

Validation of *in vitro* labeling method for human use of heat-damage red blood cells to detect splenic tissue and hemocateretic function

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Summary. *Background and aim:* Selective imaging of the splenic tissue is obtained with heat-damaged, or heat-denatured, red blood cells (RBCs) of the patient labeled with 99mTc in a variety of clinical scenarios. Aim of the study was to validate the process used for labelling heat-damaged red blood cells "totally *in vitro*", after blood sample collection, before re-inject labeled RBCs to the patient. Moreover, we assessed efficacy of the staff training programme in order to guarantee repeatibility and method standardization in the clinical routine. *Methods:* The validation process of the labeling procedure was performed in three different patients during three consecutive days. After collection of a blood sample using a heparinized syringe, we isolated erythrocytes from other blood components by centrifugation and washing steps. Then, we added the stannous pyrophosphate (PYP) to the erythrocytes pellet, after pH control. The 'pretinning' of RBCs was necessary to reduce Tc-99m once pertechnetate was entered them. After the labeling reaction with 130 MBq of 99mTc-pertechnetate, the erythrocytes were denatured in a water bath at a temperature of 49°-50°C, for 10 min. Radioactivity of blood aliquotes was measured with a dose calibrator and labelling efficiency (LE%) was determined. The labelling purity was measured using a gamma counter and calculated using the formula: counts of pellet/counts of pellet+(counts of surnatant)*100.Training program was evaluated using a Learning Questionnaire (LQ). with a grading score from 6 ("") to 1 ("nothing") for each operator (n=3). *Results:* We didn't observed the presence of macroaggregates during the entire process, until the final sample. The labelling efficiency resulted at very high values in the three consecutive measured aliquotes (mean value 73.67%) as well as the labelling purity (>95.22%). In our instituion, we use splenic imaging with labelled heat-damaged RBCs to detect ectopic spleen, splenosis, extramedullary hematopoiesis. We performed 3 procedures with heat-damaged labeled RBCs with a mean labelling efficiency 73.67%.Training and learning programmes were scored by key objective areas with a mean value of 5. *Conclusions:* Our *in vitro* labeling process of heat-damaged RBCs is simple and safe, providing a useful technique easy to implement in clinical routine for splenic imaging Learning outcome of the training programme was scored as effective by all the operators with evidence of high-efficiency-reproducible procedure mantained over time. (www.actabiomedica.it)

Key words: labeled red blood cells, biomedical imaging, quality assessment, splenic imaging

List of abbreviations:
ACD: Acid-citrate-dextrose
CT: Computed Tomography
HAES: Hydroxyethyl starch sodium chloride solution
HGB: Erythrocyte Hemoglobin
HMPAO: Hexamethylpropyleneamine Oxime
ITLC: Istant Thin Layer Chromatography
LE: Labeling efficiency
LQ: Learning Questionnaire
MR: Magnetic Resonance

PET: Positron Emission Tomography
PYP: Stannous pyrophosphate
PI: Propidium Iodide
QA: Quality assessment
QC: Quality Control
RBC: Red Blood Cells
RCP: Radiochemical Purity
SPECT: Single-photon Emission Computed Tomography

Introduction

Red blood cells are found in large numbers in the circulatory system, are easily harvested and are relatively resistant to physical and chemical stimuli that would damage other cells (1).

Red blood cell labelling techniques with Tc-99m evolved from ex vivo labelling methos to in vivo labelling methods aided by the availability of commercial kits (2).

Circulating senescent erythrocyte have an important role in the estimation of splenic reticuloendothelial function, as damaged red blood cells and cellular debris are sequestered by the spleen when phagocytized by the macrophages. Through the process of heating, RBCs undergo fragmentation and spherocytosis, leading to increased stiffness and, consequently, entrapment by the spleen.

This makes the heat-damaged RBC study a sensitive and specific method of identifying splenic tissue and investigating reticulo-endothelial function. One of the most frequent indications for the study is to assess for the presence and location of splenic tissue in patients who have undergone surgical removal of the spleen after trauma or suffering for idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura and hereditary spherocytosis.

Labelling of erythrocyte is one of the extemporaneous preparation of radiopharmaceuticals involving multiple steps in vitro (blood manipulation, labelling and pre-labelling procedures, dispensing) and in vivo (sampling, administering, etc.).

This procedure is performed according to specific rules and recommendations, which require a classified environment and qualified personnel (2, 3), since inadequate quality assurance of the compounding processes, involvement of inexpert personnel for carrying out compounding and inappropriate environmental conditions may all generate an unfavorable impact on the final product.

Some European countries have adopted specific guidelines and regulations for production of extemporaneous radiopharmaceuticals, especially for labeling of autologous cells, since these cannot be efficiently sterilized after the labeling procedure.

EAMN performed a survey demonstrating that cell labelling is a well-established technique in Europe and it is mainly performed by trained personnel under sterile conditions in a laminar flow cabinet or cell isolator (class A), installed according to local regulations (4-6).

The Italian standards of good preparation were approved by the National Healthcare System in 2005 ("Roles of Good Preparation of the radiopharmaceuticals").

According to the specific regulation, labeling procedure and staff training have to be validated in order to guarantee patient safety, diagnostic method efficacy and accuracy (7, 8).

The radiolabelling of blood cells may be occasionally problematical. Failure to label may be due to pharmaceutical factors, such as difficulties with collecting sufficient cells, sedimentation problems or instability of the cell chelator, or problems may be patient-related, such as patient medications or the presence of specific disease. In conclusion, labeled red blood cells require adequate technique and adequate training/experience (9, 10).

In our Nuclear Medicine Department RBCs are heat-damaged and labelled with Tc-99m (^{99m}Tc-RBCs) using a "totally *in vitro*" process after collection of the blood sample. Aim of this study was to validate the process used for labeling the damaged red blood cells with Tc-99m. Moreover, we assessed the staff training programme in order to guarantee repeatibility, high-efficiency results and method standardization in the clinical routine.

Materials and Methods

Our method is based on a standardized multistep procedure: blood sample collection, red blood cells isolation and labeling, quality control of prepared

radiopharmaceutical, re-injection of labeled RBCs to the patient, acquisition and post-processing of images. All phases of this process need appropriate learning and adequate training of the staff, both conducted with a standardized approach.

In this study we propose to score and measure c results of the critical steps of the entire procedure, without any intervention on the clinical routine. Quality assessment of the "*in vitro*" steps was performed measuring effect of Tc-99m on blood cells and erythrocyte viability, amounts of cells efficaciously labeled using 99mTc ate the lowest level of radioactivity and in a volume as small as possible, maintenance of a very high radiolabeling yield and stability of the complex. The validation process of the labeling procedure was performed in three different patients during three consecutive days.

"*In vivo*" quality indicators of the staff training efficacy were considered a) feasibility of a standardized operator training for the whole staff; b) high quality of the obtained diagnostic images; c) high reproducibility of the labeling method over time.

Isolation of erythrocyte

Peripheral venous blood (7 ml) is withdrawn from the patient using a 19 gauge i.v. line into a sterile heparinized syringe. Then, 1ml of acid-citrate-dextrose anticoagulant solution is added to the syringe (ACD; formulation A according to the European Pharmacopoeia, consisting of 0,73 g of anhydrous citric, 2,2 g of sodium citrate dihydrate and 2,45 g of dextrose monohydrate in 100 ml of water for injection). The whole sample of blood-ACD solution is dispensed into a falcon centrifugation tube and centrifuged at 2500x g at room for 5 minute, then we separete the cell-free plasma (CPF) from the pellet via a long lumbar needle or a butterfly needle of at least 20 gauge, by gently pushing the piston of the syringe up, without disturbing the erythrocyte.

The pellet is washed three times with 10 ml 0.9% aqueous solution of sodium chloride (saline), centrifuged at 2500x g for 5 minute; then, the supernatant is removed from the erythrocyte pellet, also removingmore than 99% of plasma proteins electrolytes and antibodies from the starting sample.

Reconstitution of the stannous pyrophosphate (PYP)

Stannous pyrophosphate (PYP) is commercially supplied as a ready-for-labeling kit (TechneScan® PYP; Covidien). The lyophilized drug is reconstituted by adding 10 ml 0.9% aqueous solution of sodium chloride (saline), and shake until complete dissolution of the lyophilized. The pH of the reconstituted drug is between 4.5 and 7.5.

Treatment of Red Blood Cells with PYP

Pretinning of RBCs is necessary to reduce Tc-99m once pertechnetate has entered red blood cells. Therefore, we add 0.3 ml of stannous pyrophosphate (PYP) to the erythrocytes pellet, and incubate for 35 min using a mechanical agitator to facilitate the process of reduction. Then, the pellet is washed two times with 10 ml 0.9% aqueous solution of sodium chloride (saline), centrifuged at 2500x g for 5 minute and the supernatant is removed from the erythrocyte pellet, to physically separate stannous-treated cells from the non-cellular associated stannous ion in the sample.

Labelling of RBCs with $^{99m}TcO_4Na$

Freshly prepared $^{99m}Tc\ O_4Na$ (70-110MBq) in saline solution (1 ml) is added to the erythrocyte suspension and incubated for 10 minutes at room temperature, using a mechanical agitator to facilitate the process of labelling.

The labeling process is stopped by adding at least 10ml NaCl 0,9% (w/v) into the solution; labelled cells and unbound $^{99m}TcO_4Na$ are separated by centrifugation. Then, the supernatant containing unbound is removed via a long lumbar needle or a butterfly needle of at least 20 G, by gently pushing the piston of the syringe up, without disturbing the pellet and the amount of radioactivityin the supernatant is measured to calculate the labelling efficiency (LE).

The pellet containing the labelled mixed erythrocyte is gently resuspended in 3-5 ml of NaCl 0,9%.

Denaturation of red blood cells

The whole sample of the labeling red blood cells solution is dispensed into a falcon, and incubate at a

temperature 49°-50°C for a maximum of 10 min, with gentle agitation in a water bath to allow the denaturation of RBCs.

Immediately after finishing the denaturation, we transfer the ^{99m}Tc-labeled RBCs dose in a syringe for administering to the patient (11).

The sample is visually inspected and reinjected into the patient as soon as possible, and not later than 1 h after completation of the labelling procedure. injection of the labelled RBCs is performed slowly, preferably using a needle of at least 22 g to prevent cell damage due to shear stress.

In vitro quality measures

Regarding the quality control (QC) of ^{99m}Tc-labelled RBCs, although only a few of them are used regularly in clinical practice. In our QC laboratory we check the quality of each $^{99m}TcO_4Na$ preparation, according to the manufacturer's guideline or the specific monograph.

Immediately after the reconstitution of TechneScan® PYP is necessary a visual inspection of the final product searching for aggregates, clumps or clots.

The pH of the stannous pyrophosphate (PYP) preparation is measured by pH test strips and it has to be 4.5-7.5. After each production, LE (%) is determined with a dose calibrator (Capintec CRC-15 R) by measuring the amount of radioactivity in the supernatant (soluble ^{99m}Tc-compounds) and the pellet (cell-associated ^{99m}Tc) of the labeling solution after centrifugation. LE is calculated using the formula:

LE between 50% and 80% is expected.

$$\left(\frac{\text{Activity of cell pellet}}{\text{Activity of cell pellett + Activity of supernatant}}\right) \times 100$$

To determine the purity of the labeling, we withdraw a 10 ul aliquot of the pellet, put it in a test tube A containing 990 ul of NaCl and centrifuge at 2500x g for 5 minutes. Then, we withdraw 500uL of supernatant and put it in a test tube B.

The purity of labeling is expected ≥95 and, it is determined measuring the radioactivity of blood aliquots with a gamma counter (Videogamma-1250 ACN) and calculated using the formula: counts of pellet/counts of pellet+(counts of surnatant)*100.

Quality assessment of operator training

The training process of the local radiopharmacy is scheduled according to the guidelines for safe preparation of radiolabelled blood cells (12-15). This consists of theoretical instructions (local rules and recommendations, available guidelines and pharmacopoeia, guidelines for working in aseptic conditions, including the use of a Class IIa safety cabinet, equipment maintenance), trainee observation (1 wk), supervised practice (2-3 wk) and proficiency assessment (at least three test sets) by personnel certified for cells labelling and performing in vitro quality controls. Training scheduling and competency assessment are standardized, following the Quality Assurance Manual of the local radiopharmacy. Before the personnel is qualified for routine activity without supervision, each trainee undergoes competency assessment. Training program was evaluated using a Learning Questionnaire (LQ). The main objectives of the program were converted into a list of items aimed to capture information about the extent of being comfortable with each of the key objectives (rules, safety cabinet, equipment maintenance). Learning was assessed using a score system from 6 ("a lot") to 1 ("nothing") for each operator (n=3).

Results

Preparation and stability of PYP

TechneScan® PYP was supplied in amounts of 20 mg of sodium pyrophosphate decahydrate for vial. According to manufacturer›s guidelines, the kit was reconstituted whit 10 ml of sodium chloride 0.9%, and the preparation used within 4 hours after resuspension. The pH of the reconstituted drug was 6 in all the samples.

Efficiency and purity of RBC labeling

Labeling efficiency of the samples used to validate the process resulted 73.56%, 72.56% and 73.88%. Mean value of LE (%) during three consecutive days

also indicate that our labeling method lead to high erytrocyte labeling efficiency over time, with an average value of yield around 73.47%.

The purity of labeling was ≥95% in all the samples, confirming the efficiency of the labelling method.

Assessment of training

Training and learning programmes were scored by key objective areas with a mean value of 5 (4.8, 5.8, 4.8 for each area respectively) also in the context of high operator turn-ver.

The education programme was well received by the operators and made it possible to achieve rapidly the main goals of the labelling technique.

In all the performed exams two indipendent nuclear physicians assessed images as high-quality ones also allowing hybrid imaging (SPECT/CT) to increase accuracy in localizing labeled RBCs accumulation, expecially to detect splenosis foci (16) or extramedullary hematopoiesis.

Discussion

As in the case of other nuclear medicine examinations, the final result of a a scan obtained with ^{99m}Tc-RBCs is dependent on the labeling efficiency (Hunter and Pezim, 1990; Kuehne, 1999; Wieseler et al., 1994) in order that the impurities do not interfere with the quality of the image or result in an unacceptably high radiation dose to the patient. Therefore, it is important that high quality images be acquired starting from an excellent radiopharmaceutical preparation (Marson et al., 1998; Poter. 1983). Our "totally *in vitr*" labeling procedure showed high LE with a mean value of 73.67% as well as a labeling purity >95.22% in all the measured samples, ensuring the absence of macro-aggregates until the final product.

The results emerged from our study also indicate that a specific standardized training modality associated to our labeling method lead to highly efficient and reproducible procedure over time, easy to implement in clinical routine.

Personnel involved in preparation and release of radiopharmaceuticals has to be appropriately trained in quality systems, current good radiopharmacy practice (cGRPP) and the specific regulatory requirements (17).

Increasing staff turn-over related to the progressive reduction of financial resources in the National Healthcare System, makes it mandatory to implement rigorous competency based Radiopharmacy Training assuring to operate safely and effectively in the "hot" laboratory, to reduce the risk to patients as many of the radiopharmaceuticals come in the form of injections and/or formulations containing radioisotopes. In our Department growing attention is focused on a standardized delivery of learning and training, customized for the specific competences of the multidisciplinary team. Training modality, learning outcome and staff validation goals are defined in the Validation Master Planof the Department (8). Running 3 consecutive batches for each procedure used in the Radiopharmacy is performed for each new operator prior to initiation of clinical activityand after the standardized training. Periodic assessments of the effectiveness of training programme is taken (every 6 months) and after any significant change in the methods or reagents.

Conclusions

Our results show that the "totally in vitro" labeling of heat-damaged red blood cells with Tc-99m can be easily implemented in routine clinical practice despite a "multistep" complex procedure requiring cell manipulation if a standardized operator training is regularly performed and assessed, encouraging continuous update of core competencies in hot laboratory staff, also in the context of high operator turn-over. Our labeling method appears highly reproducible, efficient, and stable over time, due to implementation of a comprehensive QA system targeting all the critical steps of the process, including staff skills.

Finally, high labeling efficiency and purity of this procedure, allows to obtaining high-quality hybrid (SPECT/CT) images.

Authors' contributions:
SM and AS carried out the labeling procedures, participated in the data analysis and drafted the manuscript. AS carried out

the labeling procedure and collaborated in collecting data, GB, MS and LR conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Authors' information:
Authors of this paper are competent in many different disciplines from basic science to clinic. Cell. based procedures are complex and require a multidisciplinary approach and different skills related to radiochemistry (SM, AS), nuclear medicine (GB, MS, LR).

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

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Original article

Could Bet v 1 affect sensitization molecular pattern in children?

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Summary. *Background:* Allergy is characterized by allergen-specific IgE production. Molecular-based allergy diagnostic allows to define the precise sensitization profile. Bet v 1 is the major allergen of the PR-10 family. It has been reported that pan-allergens could affect the sensitization panel in adults. *Objective:* This study aimed to evaluate the impact of Bet v 1 sensitization on clinical presentation in a sample of children with Bet v 1-sensitization; oral allergy syndrome (OAS) or anaphylaxis (ANA) were considered. *Methods:* Serum IgE molecular components were assessed by ISAC method. Sera and clinical data from 132 children, 91 males (68.94%) and 41 females (31.06%), mean age 9.08 years (3.45 years), were analyzed. *Results:* Bet v 1-sensitized children were frequently, but not exclusively, sensitized to other molecules belonging to PR-10 family. However, there was no significant difference concerning IgE levels between children with or without food allergy and between children with OAS and ANA, but hazelnut only for generic food allergy. *Conclusions:* The present study demonstrates that Bet v 1 sensitization may affect the sensitization pattern in children living in Genoa, a Mediterranean city located in a birch-free area, but it is unable to discriminate patients from a clinical point of view. So, ISAC test should be integrated with more precise IgE assay. (www.actabiomedica.it)

Key words: allergen-specific IgE, Bet v 1, molecular component, oral allergy syndrome, anaphylaxis

Introduction

Sensitization, such as the production of allergen-specific IgE, has to be considered the main biomarker of allergic disorders. Sensitized subjects usually produce IgE to more allergens over time (1,2). So, polysensitization is an important phenomenon which may occur in 90% of sensitized people (3,4).

The knowledge of the allergenic molecular profile has impressively changed the work-up in allergic patients. The molecular-based allergy diagnosis is built on the assessment of allergen molecules. This methodology allows precise definition and characterization of the sensitization profile by detecting the genuine major allergens and excluding false reactivity to pan-allergens (5,6). Pan-allergen could be defined as allergen molecules shared by different allergen sources. The main pan-allergens involved in pollen allergy are: pathogenesis-related protein group 10 (PR-10), profilin, and lipid transfer protein (LTP); however, profilin is without clinical relevance in most patients (7-9). PR-10 was first identified in pollens of *Fagales* order, mainly birch, and further in cross-reacting fruits and vegetables (10). In the PR-10 family, the major allergen is Bet v 1, mainly contained in the pollens of the European white birch (*Betula verrucosa*) and cross-reacting with other tree pollens of the *Betulaceae* family, including alders, hazels, hornbeams, hazel-hornbeam, and hop-hornbeams (11).

In our geographic area, Genoa city overlooking the Mediterranean Sea, *Betulaceae* allergy (BA) is

very common (12). However, this area is paradoxically birch-free, but other PR-10-related pollen allergens are present, i.e. hazelnut and hornbeam, that may act as primary sensitizer.

Interestingly from a clinical point of view, the serum level of IgE to Bet v 1 may be able to discriminate mere sensitization from true allergy (13). In addition, it has been reported that patients with pollen allergy and oral allergy syndrome (OAS) have a peculiar molecular pattern depending on the geographical area they live (14). On the other hand, patients with pollen allergy and anaphylaxis are usually sensitized to LTP (15). So, we tested the hypothesis concerning the definition of different molecular patterns in children with BA and OAS or anaphylaxis (ANA). Therefore, the present study investigated the allergenic molecular profile in children living in Genoa and allergic to Bet v 1 with the aim of analyzing their molecular patterns also considering OAS or anaphylaxis to foods comorbidity.

Material and Methods

Patients

This retrospective study considered children suffering from respiratory allergy. They went to the Laboratory of the Istituto Giannina Gaslini of Genoa (Italy) for serologic assessment between July 2012 and April 2014. We analyzed the findings of serum allergen-specific IgE assessed by the ISAC method. We selected children with allergic rhinitis and/or asthma and Bet v 1 positivity.

OAS and ANA to foods were diagnosed as previously defined according to validated criteria (15).

The Review Board of the Istituto Giannina Gaslini approved the procedure. The patients' parents gave a written informed consent.

IgEAssay

Serum IgE were measured by ISAC test according to the manufacturer's recommendations (ThermoFisher Italy, Milan, Italy). Twenty μL of the patient's serum were incubated on the microchip containing 112 allergen spots. After 1-hour incubation, slides were washed and a monoclonal anti-IgE antiserum labeled with a fluorochrome was added and incubated for 1 hour. Then, slides were re-washed and the chips were analyzed by a Laser Scan Confocal microarray reader (LuxScan 10K/A, CapitalBio, Beijing, China). A microarray Image Analyser immediately analyzed the findings. All samples were identified using a single barcode. The results were calculated by the software. The ISAC score was expressed as ISAC Standardized Units (ISU), ranging from 0 to 100.

Data and Statistical analysis

The ISAC score was reported as ISAC Standardized Units (ISU-E), which ranges from 0 to 100 ISU. Positive finding, such as sensitization, was defined a value >0.3 ISU, according to the manufacturer's rules.

Within each group i.e. patients without OAS nor ANA (OAS/ANA- patients), patients with OAS only or ANA only (OAS/ANA + patients), patients with OAS only (OAS + patients) and patients with ANA only (ANA+ patients), the number of positive tests was evaluated. IgE levels were non-normally distributed (as evaluated by the Shapiro-Wilk test) and summarized as medians with lower and upper quartiles (LQ and UQ). IgE levels in sensitized patients (i.e. those with a positive test toward a specific allergenic molecule) were compared using the Mann U Whitney test. 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

Sera and clinical data from 132 patients, 91 males (68.94%) and 41 females (31.06%), mean age 9.08 years (3.45 years, range 0-17 years), were analyzed.

In the whole Bet v 1-positive population, rMal d 1, rCor a1.01, rPru p 1 represented the most commonly recognized PR-10, with over 80% of Bet v 1 positive patients sensitized to at least one of these allergenic molecules, with high or moderate median levels of IgE towards these molecules (Table 1). Sensitization to other PR-10 proteins were less frequent with 38% of

Table 1A. Frequency of positivity (sensitization) to different plant food allergenic molecules and IgE median serum levels (ISU-E) in allergenic-specific sensitized patients

Allergeric molecule	No.	%	Median (LQ-UQ) levels in positive pts
rMal d 1 - PR-10 protein	105	79.55	5.3 (1.6-12.85)
rCor a1.01 - PR-10 protein	87	65.91	5 (2.5-12.25)
rPru p 1 - PR-10 protein	87	65.91	4.6 (1.85-9.7)
rAra h 8 - PR-10 protein	50	37.88	1.85 (1.2-4.95)
rGly m 4 - PR-10 protein	50	37.88	2.75 (0.95-6.1)
rPru p 3 - Lipid transfer protein (LTP)	41	31.06	1.3 (0.65-3.2)
nJug r 3 - Lipid transfer protein (LTP)	37	28.03	1.6 (0.8-4.25)
rApi g 1 - PR-10 protein	32	24.24	1.8 (1-3.95)
nJug r 2 – Cupin	31	23.48	0.9 (0.5-2.1)
nAct d 1 - Cysteine protease	27	20.45	1.7 (1.25-3.25)
nJug r 1 - 2S albumin	27	20.45	2.3 (1.75-6.25)
rAra h 9 - Lipid transfer protein (LTP)	25	18.94	1.1 (0.6-4.85)
rCor a 8 - Lipid transfer protein (LTP)	23	17.42	1.1 (0.55-6.1)
nCor a 9 - Cupin	15	11.36	0.9 (0.65-1.5)
nAct d 8 - PR-10 protein	13	9.85	0.7 (0.6-1.1)
nAra h 6 - 2S albumin	12	9.09	2.6 (0.9-14.45)
rAra h 2 – 2S albumin	12	9.09	2.4 (1.15-13.55)
nSes i 1 - 2S albumin	10	7.58	3.65 (0.7-6.35)
nGly m 6 – Cupin	9	6.82	2.4 (0.65-4.35)
rAra h 1 - Cupin	8	6.06	4.3 (0.8-9.1)
nAct d 2 - Thaumatin-like protein	7	5.3	5.6 (3.65-8.55)
nGly m 5 – Cupin	7	5.3	1.4 (0.95-3.05)
rTri a14 - Lipid transfer protein (LTP)	7	5.3	0.8 (0.6-7.3)
rAna o 2 - Cupin	7	5.3	0.4 (0.4-2.25)
nAra h 3 – Cupin	4	3.03	6.95 (-)
nTri aaA - Alfa-amilase/trypsin inhibitor	4	3.03	1.2 (-)
rBer e 1 - 2S albumin	2	1.52	0.6 (-)

pts sensitized to rAra h 8 or to rGly m 4, 24% to rApi g 1, and less than 10% to nAct d 8 (Table 1A). No patient was sensitized to nAct d 5, rTri a19 nor to nFag e 2 (data not shown).

In addition, it was calculated how many patients were at least positive to one molecule belonging to the most important pan-allergen families, including PR-10, LTP, storage proteins, Cysteine protease, Thaumatin-like protein, and α-amilase/trypsin inhibitor. Table 1B shows the frequency of sensitizations: 89.39% of Bet v 1-positive children were sensitized to one PR-10 molecule; 39.39% to Storage proteins; 34.09% to LTP; 20.45% to Cysteine protease.

Table 1B. Frequency of positivity (sensitization) to different plant food allergenic molecule families in allergenic-specific sensitized patients

Allergenic molecule family	No.	%
PR-10 protein	118	89.39
Storage protein (Cupin and/or 2S albumin)	52	39.39
Lipid transfer protein (LTP)	45	34.09
Cysteine protease	27	20.45
Thaumatin-like protein	7	5.30
Alfa-amilase/trypsin inhibitor	4	3.03

Comparison between OAS/ANA- and OAS/ANA+ subjects

Table 2A shows the median levels of plant food allergenic molecules in allergenic-specific sensitized patients. We found higher levels of IgE towards rCor a1.01 in OAS/ANA+ patients as compared to OAS/ANA- patients: 6.9 (3.25-14.15) and 3 (1.35-8.05), respectively (p=0.041). Similarly, all the other PR-10 proteins i.e. rMal d 1, rPru p 1, rAra h, 8 rGly m 4, rApi g 1, and two LTPs, i.e. rAra h 9, rCor a 8, were higher in OAS/ANA+ as compared to OAS/ANA-

Table 2A. IgE serum levels (ISU-E) to different plant food allergenic molecules among the different groups of allergenic-specific sensitized patients

	OAS/ANA-	OAS/ANA+	P value	OAS+	ANA+	P value
rMal d 1 - PR-10 protein [No. 105]	3.3 (1.7-11.75)	7.1 (1.55-14.95)	0.27	7.2 (1.65-16.3)	4.8 (0.8-16.55)	0.44
rCor a1.01 - PR-10 protein [No. 87]	3 (1.35-8.05)	6.9 (3.25-14.15)	**0.041**	6.5 (3.05-12.25)	8.6 (5.2-16.9)	0.46
rPru p 1 - PR-10 protein [No. 87]	3.15 (1.1-8.2)	5.2 (2-11.15)	0.10	4.9 (2.1-11.35)	6.9 (1.65-9.8)	0.89
rAra h 8 - PR-10 protein [No. 50]	1.7 (0.85-4.45)	2.1 (1.3-5.05)	0.24	2.95 (1.2-5.35)	1.7 (-)	0.41
rGly m 4 – PR-10 protein [No. 50]	2.5 (0.68-6.38)	3.1 (1.0-5.9)	0.64	3.5 (1.1-6.25)	1.3 (0.75-3.7)	0.19
rPru p 3 - LTP [No. 41]	1.2 (0.7-2.35)	1.3 (0.6-9.45)	0.51	1.1 (0.75-9.45)	2.2 (0.5-14.55)	0.53
nJug r 3 - LTP [No. 37]	1.6 (0.7-4.25)	1.6 (0.85-6.65)	0.70	1.35 (0.65-6.3)	3.55 (-)	0.08
rApi g 1 – PR-10 protein [No. 32]	1.0 (0.8-3.4)	2.4 (1.3-4.8)	0.10	2.5 (1.2-4.8)	18 (-)	0.55
nJug r 2 – Cupin [No. 31]	0.8 (0.4-2.85)	0.9 (0.55-1.25)	0.95	0.9 (0.55-1.25)	0.85 (0.45-2.9)	0.93
nAct d 1 - Cysteine protease [No. 27]	1.7 (1.35-3.25)	1.3 (0.9-3.25)	0.30	1.3 (0.9-2.15)	5.5 (-)	0.42
nJug r 1 - 2S albumin [No. 27]	2.85 (1.9-6.25)	2.3 (1.6-7.45)	0.61	2.05 (1.2-3.25)	11.1 (-)	0.16
rAra h 9 - LTP [No. 25]	0.8 (0.45-3.05)	1.45 (0.65-7.6)	0.17	0.7 (0.6-9.15)	4.1 (-)	0.20
rCor a 8 - LTP [No. 23]	0.85 (0.45-2.3)	1.2 (0.55-8.45)	0.35	0.7 (0.45-11.75)	3.65 (-)	0.33
nCor a 9 - Cupin [No. 15]	0.7 (-)	0.9 (0.65-2.75)	0.37	0.85 (-)	1 (0.55-4.85)	0.53

Table 2B. Frequency of positive test to different plant food allergenic molecule families among the different groups of patients.

	OAS/ANA-	OAS/ANA+	P value	OAS+	ANA+	P value
PR-10 proteins [No. 118]	47 (81.0%)	71 (96.0%)	**0.0058**	57 (98.3%)	14 (87.5%)	0.12#
Storage proteins [No. 52]	22 (37.9%)	30 (40.5%)	0.76	19 (32.8 %)	11 (14.9 %)	**0.015**
LTP [No. 45]	21 (36.2 %)	24 (32.4 %)	0.65	17 (29.3 %)	7 (43.8 %)	0.27
Cysteine protease [No. 27]	11 (19.0 %)	16 (21.6 %)	0.71	13 (22.4 %)	3 (25 %)	0.75
Thaumatin-like protein [No. 7]	2 (3.5 %)	5 (6.8 %)	0.46#	5 (8.6 %)	0	0.58#
Alfa-amilase/trypsin inhibitor [No. 4]	1 (1.7 %)	3 (4.1%)	0.63#	1 (5.2%)	2 (12.5 %)	0.12#

but without reaching the statistically significance. No other statistically significant difference was found between the two group of patients.

Comparison between OAS+ and ANA+ subjects

Table 2A also reported median levels of plant food allergenic molecules in sensitized patients who had OAS or ANA, analyzed separately. No statistically significant difference was observed between the two groups of patients however, OAS+ patients tended to have higher IgE levels towards some PR-10 proteins such as rMal d 1, rAra h 8, rGly m 4 whereas ANA+ patients tended to have higher IgE levels towards PR-10 proteins such as rCor a1.01, rPru p 1, rApi g 1, towards LTPs i.e. rPru p 3, nJug r 3, rAra h 9, rCor a 8 or towards other families of allergens (i.e. nAct d 1 and nJug r 1).

Considering the pan-allergen families, sensitization to PR-10 molecules was more frequent in children with OAS and/or anaphylaxis than in OAS/ANA- group (p=0.0058), as reported in Table 2B. In addition, there was a difference between OAS+ and ANA+ children about sensitization to Storage protein family (p=0.015).

Discussion

The assessment of IgE to pan-allergens may be useful in the allergy work-up. In this context, a clinical question is: can pan-allergens affect the sensitization pattern? A previous study, conducted in adults, showed that sensitization to a pan-allergen (i.e. Bet v 1, Pru p 3, and Bet v 2) entails higher odds to have other sensitizations (12). In addition, the co-sensitization pattern

depended on the basis of the sensitizing pan-allergen family primer. As *Betulaceae* allergy is very common in Genoa, curiously a birch-free geographical area (14), we focused our attention on Bet v 1 to test the hypothesis that sensitization to the major allergen of PR-10 family, such as Bet v 1, could affect the sensitization pattern in children and the clinical outcomes.

The current study shows that children with Bet v 1 sensitization very frequently present associated sensitization to other PR-10 plant food allergens. However, sensitization also to other allergenic molecular families was detectable in these Bet v 1-positive children, mainly concerning LTP. On the other hand, the serum levels measurement showed a single statistical difference between children with or without food allergy, concerning Cor a 1 (hazelnut): in fact, children with food allergy had higher level than children without food allergy. However, there was no difference between children with OAS and children with ANA.

So, the current pediatric study provided findings consistent with a previous one, conducted on adult patients living in central and southern Italy (birch-free area), demonstrating that there are specific relationships between sensitization patterns and clinical characteristics in subjects with Bet v 1 sensitization (15).

Anyway, the current study had some limitations: it was retrospectively conducted on a selected patient population sample, such as living in a particular geographic area, and there was no follow-up. In addition, this study did not consider possible confounding factors, such as passive smoking status, parasite infestation, environmental exposures, and seasonal variations. Finally, it has to be considered that ISAC is an immunoassay and that the result can be conditioned not only by the entity of the immune-response, but also by the homology of the sequence, by the amount of allergen in the assay, by the folding of the recombinant protein and the availability of epitopes, and the correlation between component homology and percentage of positive results is not very highly significant. Therefore, the most important message of this study is that ISAC method is not a precise diagnostic tool in clinical practice. In other words, ISAC test may be useful for a preliminary evaluation of molecular pattern in allergic subjects, but the work-up should be ever integrated by more precise IgE assessment, for example by ImmunoCap assay.

In conclusion, the present study demonstrates that Bet v 1 sensitization may affect the sensitization pattern in children living in Genoa, a Mediterranean city located in a birch-free area, but it is unable to discriminate patients from a clinical point of view. So, ISAC test should be necessarily integrated with more precise IgE assay, e.g. ImmunoCap method.

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

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Original article

Clinical monitoring of safety and functionality of a non-medicated patch for pain alleviation associated to dysmenorrhea

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Summary. FIT® Lady patch is an easy-to-use class I medical device, developed to relieve pain associate to menstrual period, without pharmacological substances. The patch is based on far infrared (FIR) electromagnetic waves reflection properties that normally are emitted from the body, as a consequence of body heat dispersion between the difference of cutaneous body and ambient temperature. Consequently, infrared (IR) waves are reflected and resorbed at cutaneous level and thermal energy again locally introduced leads to a better microcirculation. Although biological IR waves properties and mechanisms of action are extensively studied, there are still few references on patches based on FIR properties. The aim of this study was the evaluation of FIR technology applied to FIT® Lady patch thought to be used to alleviate pain associated to menstrual period (dysmenorrhea). The FIT® Lady patch medical device (active patch) was evaluated in comparison with a placebo patch, in order to assess its action in reducing pain related to menstrual period in 40 women patients enrolled according to specific inclusion/exclusion criteria. This study confirmed a good tolerability of the product, by demonstrating the ability to significantly reduce inconvenience and feeling of pain. The mineral that was responsible of the reflection activity (titanium dioxide), conveniently entrapped in a patch, was able to work without any active substances in contact with and absorbed from the skin. (www.actabiomedica.it)

Key words: FIR action, FIT patch, menstrual pain, topic treatment

Introduction

There are several symptoms related to gynecologic disorders, one of which affects half of women during their reproductive period: dysmenorrhea. Dysmenorrhea is pain associated to menstruation and is characterized by spasmodic cramping pain in the lower abdomen, that can disseminate into the lower back in some cases. These symptoms can negatively impact the normal activities of women (1).

In literature there are several works studying the application of IR in between biological waves to support different pathologies and/or conditions, such as dysmenorrhea (1-2), fibromyalgia (3), chronic psychosomatic associated pain (4), or to achieve a general wellness conditions (5).

In a recent single blind randomized study conducted on patients affected by knee osteoarthritis (6), the efficacy in reducing pain and joint effusion of a plaster cast containing substances that emit at far infrared (FIR) wavelength was evaluated. From ultrasound inspections it emerged a 40% reduction of patients with joint effusions after being treated with FIR technique, which was not observed in the group treated with placebo, highlighting that FIR technique can efficaciously be used as a no pharmacological option to treat different pathologies, as osteoarthritis of knee.

In another double blind randomize study with placebo (7), the efficacy of FIR technique was evaluated by considering myofascial neck chronic pain.

After a week of treatment, intensity of pain considerably decreased in all patients treated with or without FIR technique, without showing any significant difference between groups. However, a significant difference was observed in terms of muscle rigidity, which was more evident in treated group compared to the control, allowing to hypothesize a long term treatment as an effective solution to muscle rigidity problems.

A study by Wong et al. (8) evaluated the reduction of pain intensity of FIR radiation effect on post-operative patient after total knee arthroplasty. 40 patients were randomly selected to be treated with control or with tested treatment. Pads with FIR technology were placed in different acupoints including the experimental group, from the third to the fifth day after operation. The analgesic effect was evaluated with a pain intensity scale (Numeric Rating Scale, NRS). At the end of treatments, the group treated with FIR showed a reduction in pain intensity.

Although biological IR waves properties and mechanisms of action are extensively studied, there are still few references on patches based on FIR properties.

The present clinical study conducted on FIT® Lady patches was intended to demonstrate the efficacy in reducing pain associated to dysmenorrhea in women without pharmacological active substances with an easy-to-use patch. For this purpose, a titanium dioxide powder was entrapped in patches (active patches), in order to be able to reflect electromagnetic IR wavelengths normally emitted from body. These wavelengths are intended to be reabsorbed, reintroducing the energy in order to improve the wellness conditions of patients, by decreasing pain perception.

Materials and Methods

This study was carried out from April to June of 2017 as a monocentric comparative double blind study and it was conducted at Dermo-Cosmetic and Medical R&D Center of Bio Basic Europe Srl on 40 female patients affected by painful menstrual period. One group (20 patients) was treated with a placebo patch (FIT A), while the other group was treated with the active patch (FIT C) containing mineral powder. Patients were unrolled following specific inclusion and exclusion criteria: the criteria for inclusion were women in fertility period of life, with particular painful menstrual period, while patients showing a sensitivity to one component of patch formulation were excluded.

FIT® Lady patches are 100% polypropylene nonwoven fabric patches mixed with an acrylic adhesive mass containing biomaterials, in particular metal dioxide (titanium and aluminum), that is able to refract IR wavelength interval between 4 and 21 mm (in particular at 11 mm). This powder composition is called AT5.05.

Every patient had to place 3 patches respectively: one on the right ovary, one on the left ovary and the last one at L3 vertebra lever, and keep them in position for 5 days.

Patches had to be used on intact skin in order to avoid any associated risks and adverse effects.

The improvement in quality of life of patient was evaluated as a reduction of discomfort. During the clinical study, the skin tolerability of patches was evaluated by measuring potential changes overtime throught the clinical scores reported in Table 1.

Patients enrolled for the study were examined both at the beginning and at the end of the treatment protocol by the same operator and evaluation tool.

First evaluation: starting the treatment;

Last evaluation: at the end of the treatment (after 5 days).

The experimental data model was defined through quantitative parameter variation after the application of both types of treatment and by analyzing the same patient of a group or belonging to a different group, before and after treatments.

The aim of this clinical study was to evaluate differences between data results, in terms of significantly different, before and after the treatment (after 5 days).

The results were collected relatively to the evaluation scale, and by single patient by taking into account the decrease of pain caused by menstrual period.

The statistical analysis of clinical parameters was carried out through non-parametric Friedman test wich uses a sensitivity threshold of 5% with a numeric evaluation originated by a VNS scale (values from 0 to 10).

Then personal evaluations were collected (self-assessment) in accordance with VNS scale (from 0 to 10 values). The statistical analysis of infra group self-assessment was carried out according to T-student test with a threshold of 10%.

Results

In Figure 1 it emerges that both placebo (FIT A) and active (FIT C) patches were able to maintain the integrity of skin after treatment, demonstrating a good tolerability.

In the present study, the decrease of painful perception (as a sensorial parameter) associated to menstrual period was evaluated according to a Visual Numeric Scale, VNS (from 0 to 10) at the beginning (time 0) and at the end (after 5 days) of the treatment (Table 2). The clinical parameters were gathered by the experimenter. The graphic tendency is represented in Figure 2.

The results obtained by VNS showed that there were no significant differences between the two patient groups (FIT A and FIT C) at time 0. On the contrary, a significant statistical difference was observed between group FIT A and group FIT C after 5 days of FIT Lady patch application.

After 5 days of the medical device application, it was observed a reduction of painful perception of:

- 28% in FIT A group;
- 85% in FIT C group.

Table 1. Clinical scores of skin tolerability evaluation

Skin alterations (erythema and oedema)			
Erythema		Edema	
No erythema	0	No edema	0
Slight erythema (hardly visible)	1	Very slight edema (hardly visible)	1
Clearly visible erythema	2	Slight edema	2
Moderate erythema	3	Moderate edema (about 1mm raised skin)	3
Serious erythema (dark red with possible formation of light eschars)	4	Strong edema (extended swelling even beyond the application area)	4

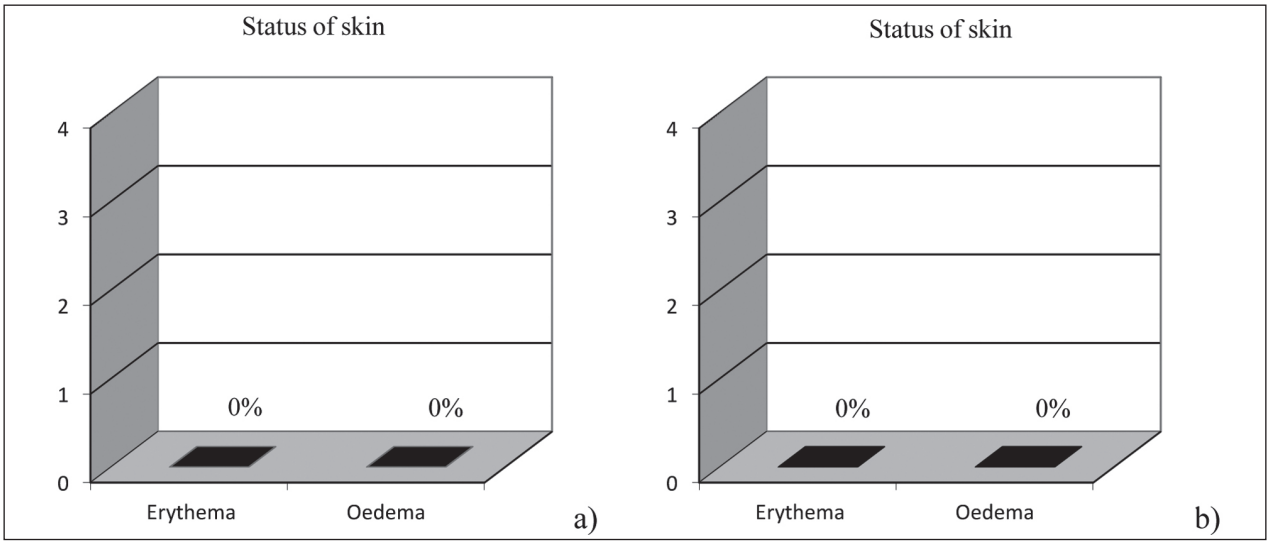

Figure 1. Evaluation of skin tolerability in accordance with the clinical scores reported in Table 1 after treatment with patches for 5 days with placebo FIT A (a) and test product FIT C (b)

Table 2. Evaluation of pain intensity related to menstrual period of patient enrolled for the study by means of VNS scale (from 0 to 10) at the beginning (T0) and after 5 days of treatment (T5)

	Attenuation of painful sensation intensity during menstrual period			
Panellists' code	T0 – FIT A	T0 – FIT C	T5 – FIT A	T5 – FIT C
1	5	5	5	4
2	4	4	4	0
3	5	6	3	2
4	6	5	5	0
5	4	6	2	1
6	5	6	2	0
7	6	7	3	0
8	4	5	3	2
9	6	3	5	0
10	3	8	2	1
11	4	8	3	0
12	7	8	6	3
13	5	7	5	2
14	6	6	4	0
15	4	9	2	1
16	5	7	2	0
17	6	8	3	2
18	8	6	6	0
19	7	7	5	0
20	6	8	6	1
Average	**5,3**	**6,5**	**3,8**	**1,0**

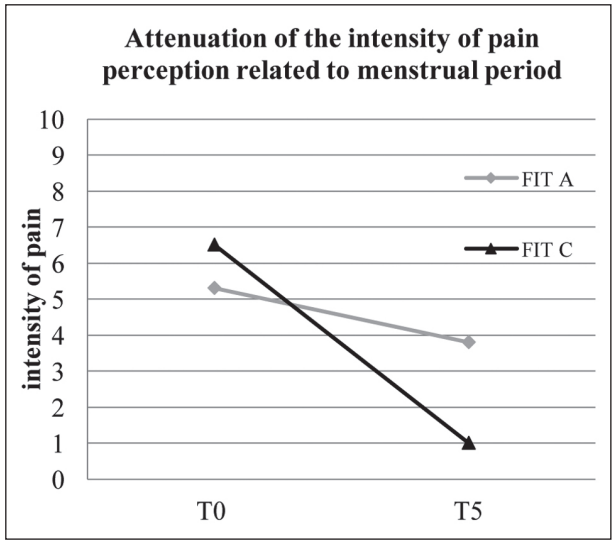

Figure 2. Evaluation of painful intensity detected by patient in accordance with a VNS sensorial scale (from 0 to 10) at the beginning (T0) and at the end (T5) of the treatment

Self-evaluation questions before (Table 3) and after (Table 4) treatment are reported. Results are presented as the average of answers given following the VNS scale.

In Figure 3 results obtained from the self-evaluation questionnaire after treatment with FIT A and FIT C patches are reported and compared to each other after 5 days.

Statistical significant differences were noted between the FIT A group and FIT C group for all questions results, only Q2 and Q5 gave the same results.

Discussion

FIT® Lady patches were developed to alleviate pain discomfort associated to dysmenorrhea. It has to be considered that dysmenorrhea is characterized by pelvic pain disseminated into lower back with spasmodic cramping, generally accompanied with nausea, fatigue and insomnia. Those clinical situations can result in loss of working hours and productivity at work, or in general can affect women's lifestyle [9], and unrelieved pain can cause damaging physiological effects.

As evidenced in literature, FIR, used at human biological IR wavelength, is a extensively studied technology used to support therapies for different pathologies or to facilitate the improvement of patient's wellbeing, also as an alternative method to useless pharmacological treatments.

FIR technology has generally been recommended as an effective, safe, and non-pharmacologic alternative to promote human health [10–11], by improving body metabolism through the resonance absorption process of the human body.

This technology was proved to be effective in different muscular-skeletal discomfort situations thanks to the application of FIT plasters based on FIR technology, as it relieved painful symptoms, by producing a myo-relaxing action on the site of application [6]. Plasters were applied to the human body taking advantage of reflecting IR normally emitted and increasing the skin's surface microcirculation.

Ricci et al. conduced a pilot study on plasters base on FIT technology containing AT5.05 powder that demonstrated to have an increased radiance when temperature augmented obtaining a maximum dif-

Table 3. Self-evaluation questionnaire according to the VNS scale (visual numeric scale) by the 20 patients treated with the tested patch FIT A (placebo)

	Questions	Average (VNS)
Q1	Evaluate the relief sensation respect to menstrual pain during product use.	**5.50**
Q2	Evaluate the practicality of the product application	**6.95**
Q3	Evaluate the comfort of using the product	**6.80**
Q4	Evaluate the adhesiveness of the product (even after contact with water)	**7.05**
Q5	Evaluate the presence of glue at the end of the treatment	**0.50**
Q6	Overall opinion	**6.25**
Q7	Give the evidence of a possible purchase	**6.15**

Table 4. Self-evaluation questionnaire according to the VNS scale (visual numeric scale) by the 20 patients treated with the tested patch FIT C (FIT Lady)

	Questions	Average (VNS)
Q1	Evaluate the relief sensation respect to menstrual pain during product use.	**8.00**
Q2	Evaluate the practicality of the product application	**7.75**
Q3	Evaluate the comfort of using the product	**8.00**
Q4	Evaluate the adhesiveness of the product (even after contact with water)	**7.90**
Q5	Evaluate the presence of glue at the end of the treatment	**0.35**
Q6	Overall opinion	**8.10**
Q7	Give the evidence of a possible purchase	**8.55**

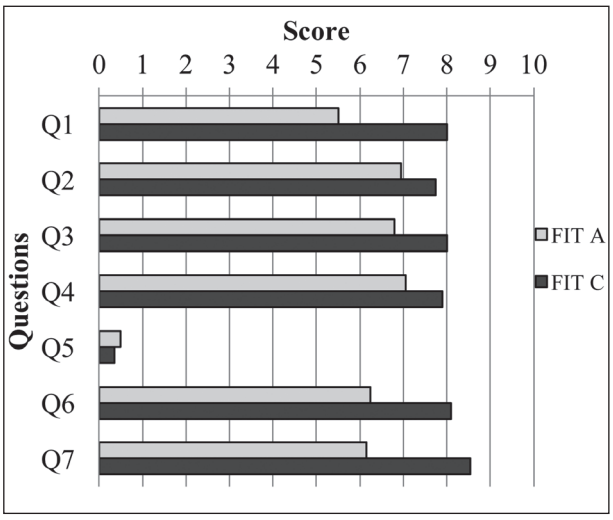

Figure 3. Average results of values recorded in accordance with VNS scale obtained from patient treated for 5 days both with placebo patch (FIT A) and "active" patch (FIT C)

ference in emission within the wavelength 9-12 nm, where 11 nm is considered the emission wavelength of the body's infrared radiation. In accordance with their preliminary study, they demonstrated that FIT plasters increased functionality and reduced pain when applied to patient with degenerative tendinopathy [12].

Given these premises, FIT Lady patches underwent a clinical study on 40 women with particular painful menstrual period. For this evaluation patches were applied for 5 continuous days on volunteers whom were asked to give a feed-back on alleviation of intensity of pain associated to menstrual symptoms in accordance with a VNS scale.

FIT® Lady Patches were manufactured with AT5.05 powder, made up of biomaterials, metal dioxides (titanium) in prevalence, that are able to reflect within the infrared range (between 4 and 21 nm). FIT® Lady patches (FIT C), based on FIR technology, had demonstrated a good tolerability and ability to relieve menstrual pain if compared to the placebo patches (FIT A). Moreover, the product was able to improve the quality of life of women during their menstrual period.

Those evaluations were based on patients' testimonies on pain feeling. The self-assessment of patient enrolled for this study is to be considered the only possible measurement of pain, since pain is a personal and com-

plex perception based on a physiological transmission of stimuli to brain and subjective experience of it. Several studies had evidenced that there is an underestimated perception of pain when data are collected from external person, such as doctors and nurses. The WHO defined pain as "an unpleasant sensation and emotive experience, that is associated to a potential or real tissue damage, or in any case, described in relation to this damage".

In this case, the application of an evaluation tool was fundamental in order to improve the relationship and the communication between patient and doctor and to get a better diagnosis. Among different scales, the one-dimensional (VAS, NRS, VRS) is a valid tool of evaluation.

In this study, VNS (Visual Numeric Scale) scale was used to get the self-evaluation of patient. This scale is a combination of a VAS scale (Visual Analogic Scale) and a NRS scale (Numeric Scale). The results gathered from this study on Class I Medical Device demonstrated a good cutaneous tolerability, without any erythema or oedema episodes after 5 days of application. Moreover, the results obtained from the self-evaluation questionnaire based on VNS evidenced that FIT® Lady patches were able to give a general wellness perception to patients.

Conclusions

In conclusion, FIT® Lady patches, based on FIR technology, had proved to be well tolerated and to be an useful support to treat painful symptoms associated to menstrual period, as demonstration by the improvement in the patients quality of life.

Based on the safety and good tolerability of the product and the promising results obtained, we can stated that FIT® Lady patches may be a valid alternative to "non-medicated pain relief" treatment during dysmenorrhea.

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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

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Nursing Summary: designing a nursing section in the Electronic Health Record

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Summary. The introduction of new information technologies in healthcare led to major changes in the field of tools for managing and evaluating the assistance. In Italy, an example of applying new technologies to the healthcare context is the realization of Fascicolo Sanitario Elettronico (FSE). The FSE is a tool that collects online data and health and socio-health information that make up the patient's clinical history. The aim of this review is to analyze which components are needed to organize and structure the information and data within the "Nursing Summary". Literature searches were conducted using the following available online Databases: CINAHL, PubMed and Cochrane Library. The searches were conducted by analyzing publications from the last five years (2012-2016). The process of selection of articles led to the choice of 14 research studies. Additionally, national guidelines were analyzed, concerning official documents and technical specifications for the development of projects of FSE. The analysis of the scientific literature showed that nursing data in the EHR can be used to develop some Clinical Decision Support Systems. Relevant were also used to clarify how the nursing data could be structured in the "Nursing Summary". The research findings have identified which could be the main components of a possible nursing section to integrate the FSE. This project is proposed as a preliminary study that needs further development. (www.actabiomedica.it)

Key words: Electronic Health Record, nursing information system, electronic nursing documentation

Introduction

In the process of modernization of health, there are many initiatives aimed at improving the efficiency of health services and at the simplification of the exercise of the right to health of person. An example is the introduction of information and communication technologies (ICT) in healthcare. The use of ICT in healthcare has led to important changes in the field of tools used for the management and evaluation of assistance. One of the major changes concerns the transition from paper-based electronic-based information systems such as the Electronic Health Record (EHR). An EHR is an electronic information system that contains retrospective, simultaneous and future information about the person's clinical history; these data and information are stored in digital format and are accessible and shared, both by the different professionals who take charge of the person and by the person himself (1, 2). One of the main objectives is to have a complete and exhaustive collection of information that will ensure a better continuity of care and the development of a citizen-centered healthcare system.

In Italy, an example of electronic documentation, with similar structure and purpose of the EHR is the Fascicolo Sanitario Elettronico (FSE), one of the lead-

ing eHealth projects developed over the past years. The FSE is a tool that collects electronic data, concerning health and socio-health information that makes up the medical history and health of the person (3, 4). The patient's clinical history is available both for the user himself and for all healthcare professionals involved in taking care of the patient. The FSE is, therefore, a tool that contains information from the different healthcare professional. For this reason, the ministerial guidelines (3) defined sanitary profiles enabled to enter the information and how can they access the information (reading and/or writing) depending on the type of data entered. The data that can be entered have been divided into five categories: personal, administrative, prescriptive, clinic and consensus. Specifically for the nursing figure, the ministerial guidelines specify only those that should be the skills for reading personal, administrative, prescriptive and consensus data and for writing clinical and consensus data. The role of nursing documentation, and the use of data and information deriving from the assistance provided is not yet specified.

This suggested the idea of proposing the integration of the FSE with a dedicated nursing section. The nursing documentation is a key tool to ensure continuity of care and communication with other healthcare professionals. Specifically, the nursing documentation is the instrument that represents the ability of nurses to communicate health status, regarding the needs and responses of patients to the received care.

In addition, some studies suggest how nursing electronic documentation and, data sharing could contribute to the improvement of care and continuity of care (2, 5, 6). As stated in the study of Westra et al. (7) nursing information sharing is an important contribution to the welfare of the person, as they evaluate aspects of human response to health problems, emphasizing not only the clinical data but also the emotional, social and psychological aspects of the individual with respect to its state of health. The value of nursing data emerges, along with how their sharing could contribute, to the improvement of the assistance granted, in a multidisciplinary approach to the patient.

Considering these elements, seems appropriate to propose the integration of the FSE with a dedicated nursing section, titled "Nursing Summary". This section should consist of a single electronic document in which are gathered the salient information of the health condition of the person, in relation to the received nursing care. Shared in the FSE, nursing data and information could implement the quality of care provided, allowing both the nurses and other health professionals to base clinical decision-making on a broader collection of information. The aim of this paper is to analyze which components are needed to organize and structure the information and data within the "Nursing Summary".

Methods

To carry out the work, a literature review (8) approach was conducted.

The following research question has been elaborated: "What is present in scientific literature about the use and integration of electronic health data pertaining to nursing?". Several keywords have been chosen and then combined through Boolean operators "OR" and "AND". The used keywords are: electronic health records, electronic patient records, computerized patient record, electronic health information, electronic medical records, EHR and nursing.

Literature searches were conducted using the following available online Databases: CINAHL, PubMed and Cochrane Library. The searches were conducted by analyzing publication from the last five years (2012-2016). The choice to analyze only studies published over the past five years is due to the fact that, in the Italian context, the use of electronic information system represents a novelty, while in the international context is a more consolidated practice. For this reason, it was decided to analyze the most recent international literature regarding studies on electronic information system. Titles and abstracts returned by the search were read and assessed.

The results obtained from the research were imported into Endnote® database, eliminating duplicates and selecting only results in English language. Were considered only articles in English, because of not understanding of other languages such as Portuguese or Spanish.

The selected studies concern the development of instruments or the employment of methodologies

for a better management and use of nursing data in electronic documentation. The following studies were not considered of interest: nursing data usage in connection with specific diseases; nursing education; the satisfaction or the nurses› perception about the use of electronic health tools. Moreover, many of the studies were not included in the final selection because they were focused on implementing standardized nursing terminologies within the electronic health documentation. Exclusion criteria were added concerning the type of study (dissertations, editorials and posters). Irrelevant articles were excluded with respect to the title and the abstract, as the suitable results were analyzed by reading the full text. By reading the full text, studies that did not focus on the implementation or use of nursing data in the EHR have been excluded (Figure 1).

Finally, national guidelines concerning official documents and technical specifications for the development of projects of FSE were analyzed (3, 4, 9, 10).

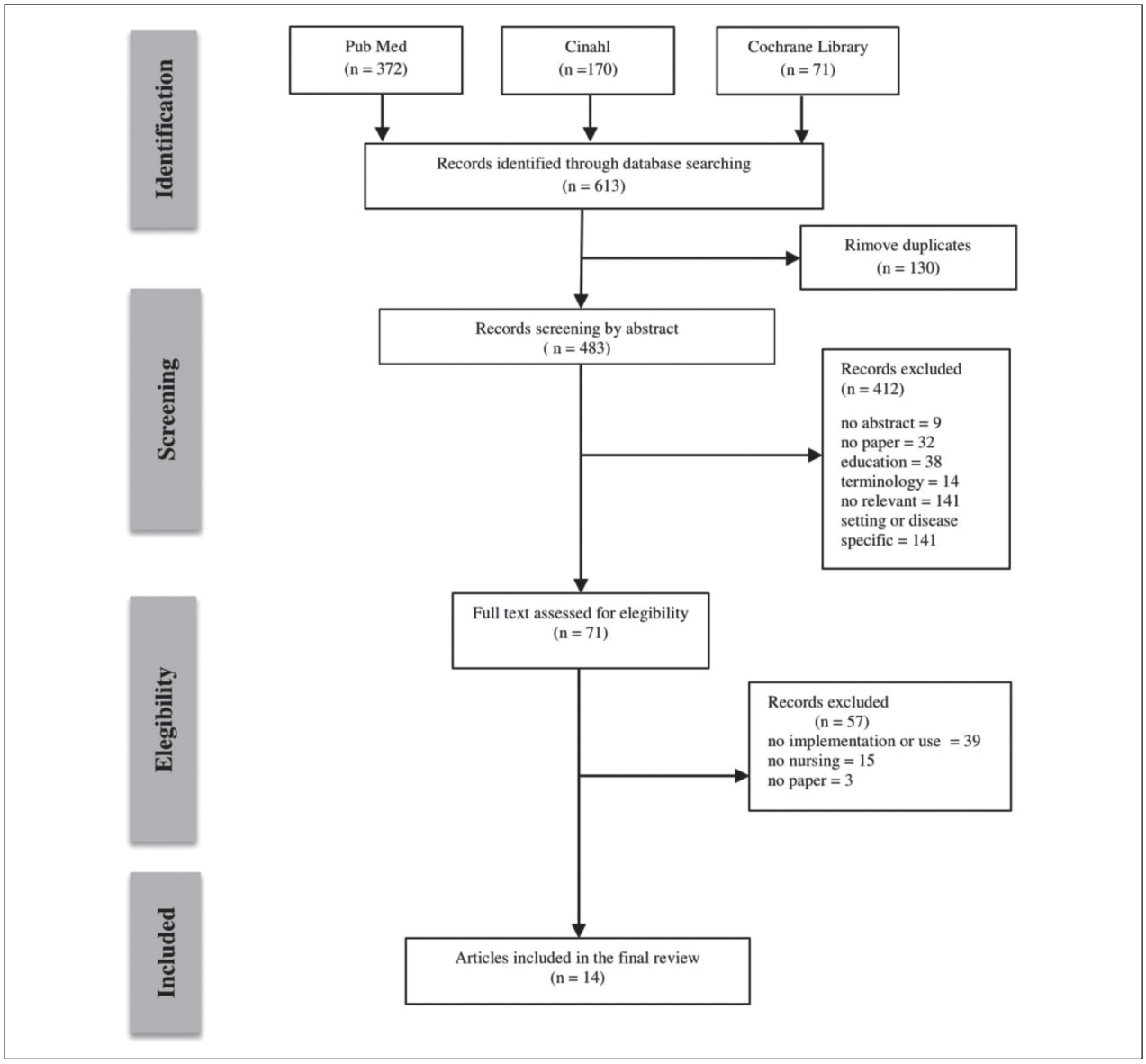

Figure 1. Flow chart

Results

The process of selection of studies was created according to the four phases of the PRISMA method (11). From the initial 483 identified references, and after the elimination duplicates, 14 relevant studies were considered (Figure 1).

The results obtained from the research of the scientific literature, have highlighted the lack of sufficient studies concerning the use of nursing data for the realization and implementation of tools or nursing sections within the electronic health records.

We considered studies that dealt with the implementation of tools or methodologies that make the information and data related to nursing, standardized and encoded so that they can be used for secondary assessments (e.g. administrative, statistical or economic) or to complete clinical decision support system. For this reason four studies were included in the final selection (12-15) that focus on a specific disease or setting of care (exclusion policy during screening). These studies were included as they dealt with the development of tools or methodologies that use nursing data present in the EHR, such as support to nursing care.

Most of the relevant studies (12, 13, 16-19) focus on the importance of encoding the nursing data contained in the databases of the EHR.

Three of the analyzed studies (14, 20, 21) focus on the development, of predictive models to be inserted in the electronic health records in order to improve and support the clinical practice, by analyzing the nursing data contained in the EHR. Two further studies (15, 22) develop instead specific software applications as tools to support and improve clinical practice. Specifically, the study of Topaz et al. (15) concerns the development of a Natural Language Processing (NLP) through an automated analysis of free text phrases that processes coded information. The study of Wilbanks & Langford (22) instead analyzes the possibility to develop a dashboard for hospital medical documentation to guide the nursing practice.

Two further studies included in this article (23, 24) are related to the development and validation of electronic nursing documentation tools that provide standardized data, which can be shared and used within the EHR.

Finally, the study of Looman (25) uses nursing data contained in the electronic documentation to get more information about the patients followed through a system of telenursing.

The results obtained from the literature review were used to understand which are the main components that the "Nursing Summary" should present in order for the information and data contained therein to be represented and shared within the FSE.

The main information regarding relevant articles were arranged in a table of data extraction (available upon request).

Discussion

The objective of the review is to analyze which components are needed to organize and structure the information and data within the "Nursing Summary". The results obtained are used to understand which are the main components that the "Nursing Summary" should have and how to organize them, to propose their integration in the FSE.

The "Nursing Summary" section is an electronic document that summarizes key information regarding the health condition of a person in relation to nursing care. To propose its integration into the FSE is necessary to establish a standard, organization of the document and to compare ways to share information, as set out in the ministerial guidelines (3, 4).

Defining standards of information means to define the contents, in order to ensure the ability to represent and use the information in the nursing section. One of the main requirements for this to happen is the use of standardized nursing terminology (16, 20, 26, 27) that encodes the data and makes it reusable. The use of a standardized terminology also allows representing the concepts and terms with which nurses document their assistance. For these reasons it is standardized nursing terminology define priority through which the information will be collected and stored. The study of Westra and colleagues (28) suggests choosing a standardized nursing terminology recognized by ANA, taking into consideration the context of the provision of care. For this reason, it is essential to perform a thorough examination of the clinical and organizational

setting in which will feed the nursing section and then choosing the most suitable language.

Even within ministerial guidelines (3, 4), is recommended the encoding of all data and information within the FSE, to ensure semantic interoperability between different regional systems. Semantic interoperability allows preserving the meaning and structure of information so that even the interpretation occurs correctly (29). In the choice of a standardized nursing terminology, it is therefore essential to consider a language that is mapped to a "reference terminology". The "reference terminologies" in fact make sure that the terms proper to discipline are connected with similar terms of meaning so that it is possible to share information between different professionals and between different information systems (28).

In addition, the possibility of having data on nursing in a standardized format and coded allows the use of high-quality information to produce new knowledge. For example, the analysis of data could be used to create predictive models or dashboard, to be included in the documentation, to improve and support the clinical practice (20-22).

After the definition of the contents, the source from which the information is collected must be defined. The information in the "Nursing Summary" is drawn from the nursing care plans (12, 16, 24), found in the clinical documentation used in the various health care setting of the National Healthcare System. The care plans content can be organized in different ways: either according to a specific setting of care, or based on the choices made at organizational level by healthcare facilities. What characterizes nursing care planning -and represents its methodological basis- is the process of nursing (23, 24). This provides a logical and systematic model for the planning and delivery of assistance that focuses on the person's needs. The data recorded by the nurse are then not only linked to the clinical patient dimension, but also to other dimensions that are extremely important to understand the state of the health of the person (30): the psychological and social dimensions (7). From this perspective, one can easily understand why it is proposed to insert the latter types of information in the nursing section. Rather the issue could be that the amount of available information in the care plans can vary, depending on the clinical case and its complexity. For this reason, it is proposed that the nurses extrapolate the salient information in the "Nursing Summary" through an automated computer process. The nurses will choose what information might be useful to achieve the well-being, in a perspective of multi-disciplinary assistance, according to their professional knowledge and skills.

The aim is to give a concise overview of the state of health of the person, in order to guide nurses and other healthcare professionals in the management of care. To facilitate the consultation of the information, these are organized into categories (16). The main "Nursing Summary" categories considered appropriate are:

- Personal data: here should be incorporated the biographic information that enables the identification of the patient.
- Social-relational unity: this category should contain information on the social life of the client, such as a household, caregiver or support figures, taking into account their relationship with patients.
- Lifestyle: this section should contain with information regarding the main daily habits and lifestyle, such as the physical activity or smoking attitude.
- Nursing diagnosis: it concerns the most relevant nursing diagnosis defined according to the general health of the person.
- Nursing interventions: it covers the main actions taken by the nurses for charitable objectives to reach the wellness of the person.
- Outcome: it refers to major ratings at discharge and to more information about the activation of educational pathways.

The nursing information grants a wider vision of the general health of the patient. This would allow to expand the vision on the assisted, and to implement effectively a multidisciplinary and holistic approach to the care of the person. To clarify how the "Nursing Summary" could improve the provision of care, we can take as an example the context of home care, where different professionals provide a set of health services. In this context, where continuity of care is very important, the ability to share accurate information about the person is of paramount importance.

Another aspect to consider is the proper functioning of the exchange of information between the nursing documentation, the "Nursing Summary" and the FSE.

In view of the necessity of data sharing they should be stored in a database. This will ensure interoperability of different regional systems, as required by the ministerial guidelines (4). Regarding the "Nursing Summary", it makes sense, then, to determine the methods of communication between different databases (which will contain the nursing information) and the registry or regional repository to ensure information sharing.

Finally, in case of sensitive information it, is necessary to respect the regulations in force about data privacy and the legal value of the produced documents. According to the guidelines of the Ministry of Health (3, 4), the use of information contained in the FSE and the handling of sensitive data are regulated in the «Personal Data Protection Code» (9), which refers to the procedures for information recording and to a set of needed electronic documentation. Ministerial guidelines stipulate further that what is present in the «Guidelines on the subject of Electronic Health Record and Health File» of the Italian Data Protection Authority must be respected (10) for what concerns the sharing of electronic documentation.

Conclusion

The aim of proposing a nursing section within the FSE is linked to the possibility that nursing data can help to improve care and continuity of care for the health of the person. The literature analysis suggested that inputting nursing data, into electronic documents, reduces the dispersion of information, encouraging their sharing with other healthcare professionals. This is one of the elements supporting this proposal. The use of data contained in the "Nursing Summary" could contribute to a multidisciplinary care management, by allowing a more effective communication between different health professionals. Better communication can also contribute to a better formulation of multidisciplinary therapeutic and educational plans.

Another essential element in favor of the use of nursing data in electronic information systems is the possibility of having a broader and comprehensive view of the state of health of the person, contributing to a holistic view of the person and care.

Furthermore, the information collected and stored in a standardized format can be used to support the management, planning and evaluation of nursing care regarding quality, workload assessment (31) or complexity of care, as well as to support studies and scientific research.

In the current context, in which the development of eHealth, marks the transition from paper to electronic, the need for coded data and information becomes huge. The "Nursing Summary" could therefore represent an opportunity to improve the visibility of nursing interventions and their value within the care process. It could also represent an opportunity to increase the knowledge and professional skills in the management of electronic information tools.

Since this work is intended as a preliminary study to conceive and analyze the potential characteristics of the "Nursing Summary", further investigation and research is 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

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VBAC: antenatal predictors of success

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Summary. To determine antenatal factors that may predict successful vaginal birth after Caesarean section (VBAC), to develop a relevant antenatal scoring system and a nomogram for prediction of vaginal birth after caesarean delivery. A non recurring indication for previous Caesarean section (CS), such as breech presentation or foetal distress, is associated with a much higher successful VBAC rate than recurrent indications, such as cephalopelvic disproportion (CPD). Prior vaginal deliveries are excellent prognostic indicators of successful VBAC, especially if the vaginal delivery follows the prior CS. A low vertical uterine incision does not seem to adversely affect VBAC success rates as compared to a low transverse incision. Maternal obesity and diabetes mellitus adversely affect VBAC outcomes. Foetal macrosomia does not appear to be a contraindication to VBAC, as success rates exceeding 50% are achieved and uterine rupture rates are not increased. An interpregnancy interval of <24 months is not associated with a decreased success of VBAC. Success rates decrease when interval increases. Twin gestation does not preclude VBAC. Post-dates pregnancies may deliver successfully by VBAC in greater than two-thirds of cases. There are few absolute contraindications to attempted VBAC. Attempted VBAC will be successful in the majority of attempted cases. (www.actabiomedica.it)

Key words: vaginal birth after Caesarean, predictors of success, antenatal, TOL, success rates

1. Introduction

Before 1970s, the phrase "once a Caesarean, always a Caesarean" dictated obstetrics practice, in fact Caesarean section (CS) rates steadily increased throughout the twentieth century. Repeating a CS came to account in almost 40% of all CS. This has implications not only at an economic level, but also in terms of maternal and neonatal morbidity. Studies have demonstrated that neonates of mothers who undergo elective repeat CS can be at greater risk of respiratory morbidity. Maternal complications associated with elective repeat CS include placenta accrete, visceral injury, intensive care unit admission, hysterectomy, blood transfusion, and a longer duration of hospital stay. In 1981, vaginal birth after CS, was recognized as a safe and acceptable option after a previous low transverse Caesarean delivery (1), though vaginal birth after caesarean (VBAC) is not without its own risks, which include uterine rupture, endometritis, blood transfusion. Since that time, encouraging women to attempt VBAC has been one of the strategies used in an attempt to reduce Caesarean delivery rates. Increasing experience with VBAC has caused a gradual easing of selection criteria for trial of labour (TOL), reflected in the clinical practice guidelines. In 1988, ACOG published 'Guidelines for vaginal delivery after a previous caesarean birth' endorsing vaginal birth after Caesarean delivery (VBAC)-TOL as it became clear that this procedure was safe and did

not appeared to be associated with appreciable excess perinatal morbidity, compared with elective CS. Subsequently, first in 2007 and then in 2015, Royal College of Obstetricians and Gynaecologists (RCOG) published new Guidelines for VBAC that planned VBAC is a clinically safe choice for most women with a single previous lower segment caesarean delivery (2). This guideline provides evidence-based recommendations on best practice for the antenatal and intrapartum management of women undergoing planned VBAC. There are predictive factors that can prenatally determine a patient's probability of successful vaginal delivery and these are summarized in the following sections. Trial of labour after CS is defined as an attempt at vaginal delivery in women with a previous caesarean section (3). A successful trial of labour after caesarean section (TOLAC) is defined as spontaneous or instrumental (assisted by vacuum or low forceps) delivery to a woman undergoing TOLAC. An unsuccessful TOLAC is defined as failure to achieve a vaginal birth after caesarean section in women undergoing a TOLAC and the delivery ending by emergency CS.

2. Antenatal factor associated VBAC

2.1 Factors associated with previous obstetrical history

2.1.1 Indication for previous CS

Several studies (4, 5), were conducted to evaluate the influence of previous CS on VBAC success. The indications of previous CS were divided into recurrent and non-recurrent group. Recurrent indication for a caesarean delivery is defined as poor labour progress, secondary arrest, prolonged second stage, failed induction of labour and macrosomic baby. Whilst non-recurrent indications include foetal distress, malpresentation such as transverse or oblique lie, breech presentation, severe preeclampsia, placenta previa, and abruption placenta. Successful rates for women whose first caesarean delivery is performed for a nonrecurring indication (breech, non-reassuring foetal well-being) are similar to vaginal delivery rates among nulliparous women (6). Prior operative delivery for cephalopelvic disproportion (CPD)/failure to progress is associated with success rates ranging from 50% to 67% compared to prior CS for breech presentation, which is associated high success rate of 89%. In a large multicentre study, VBAC were significantly lower following CS for CPD than for breech or foetal distress (7). Nevertheless, about two-thirds of women with a history of CS for CPD will achieve a successful vaginal delivery. Women with non-recurrent indications were statistically more likely to have successful trial of labour compared to those with recurrent indications. This could be due to the element of cephalon-pelvic disproportion, which reduces the likelihood of vaginal birth. Hence, they concluded that indication of previous CS is an important predictor of success of trial of labour (8).

2.1.2 Birth weight after CS

Women who underwent CS for CPD may often be counselled to consider elective repeat CS if the estimated foetal weight in the current pregnancy is larger than that of the pregnancy that required CS. A birthweight of 4kg or more is associated with an increased risk of uterine rupture, unsuccessful VBAC, shoulder dystocia, and third- and fourth degree perineal laceration. For women with no prior vaginal delivery undergoing VBAC labour when neonatal birthweight is 4kg or higher, the VBAC success rate is reported as less than 50%. However, third trimester ultrasound is a poor predictor of macrosomia in decision making regarding VBAC (2). Nonetheless, 60% to 70% of women who attempt VBAC with macrosomic foetus are successful (9). Birthweight difference between first pregnancy (delivered by caesarean) and second pregnancy with attempted VBAC clearly influences successful rates. Concluding, pregnancy estimated foetal weight (EFW) of less than 3.5 kg at 36 weeks has a higher rate of VBAC (10).

2.1.3 Cervical dilatation achieved before prior CS

Maximal cervical dilatations achieved prior to CS for CPD or for arrest disorder may be prognostic of future vaginal delivery rates. The arrest in the 'first stage' corresponding to 9 cm or less, and the arrest in the 'second stage' corresponding to full dilatation. A history of the arrest in the second stage is associated with a higher chance of VBAC, rather than the arrest

in the first stage, that is associated with 65% of success of VBAC. Women should not be discouraged from attempting VBAC solely based on the cervical dilatation achieved prior to CS in the previous pregnancy.

2.1.4 History of failed trial of operative vaginal delivery before prior CS

Those women that had had an emergency cesarean delivery in their first pregnancy have a lower VBAC success rate. Even though successful VBAC appears more likely among women with previous caesarean for dystocia at 8 cm or more compared with women with previous caesarean for dystocia at less than 8 cm. Several studies have shown that failed operative vaginal delivery resulting in CS is not an absolute contraindication to VBAC, in fact in a prospective cohort study (11) VBAC was successful in about 80%, in those women who had CS in the second stage of labour. The success rate for VBAC was high in women who had a prior CS due to an unsuccessful instrumental delivery.

2.1.5 Number of previous CS

Women with a history of two previous low transverse CS remain candidates for TOL. There is conflicting evidence regarding any increased risk of uterine rupture with TOL after more than one prior CS, but this factor does not seem to impact negatively on vaginal birth rates. Several studies have shown similar rates of VBAC success with two previous caesarean birth (VBAC success rate of 62-75%) and single prior CS, but it must be underlined that more than half of the women two previous caesarean deliveries had also had a previous vaginal birth and 40% had had a previous VBAC (2). Women who have undergone laparoscopic or abdominal myomectomy, particularly where the uterine cavity has been breached, are at increased risk of uterine rupture, while uterine rupture after hysteroscopic resection of uterine septum is considered a rare complication. These women should be considered to have delivery risks at least equivalent to those of VBAC and managed similarly in labour (2).

2.1.6 Effect of prior vaginal deliveries

Prior vaginal delivery, including prior successful VBAC, is the strongest predictor of a successful TOL and is protective against uterine rupture following TOL (12). The success increases when women had a prior VBAC (93%) rather than a vaginal delivery prior to the caesarean birth (85%). A history of vaginal delivery in addition to a CS would appear to be a positive indicator of success in subsequent TOL. The chance of success increases with the increasing number of prior vaginal deliveries. Mercer and colleagues found that the rate of uterine rupture decreased after the first successful VBAC and did not increase with subsequent vaginal deliveries (0.87% risk after VBAC, 0.52% after 5 deliveries) (13). The possible explanation for this is multiparous women will develop efficient uterine contractions in labour and will have less problem with cephalopelvic disproportion (CPD) (14). This suggests that a previous successful VBAC (15, 16) is the single best predictor of successful VBAC than a vaginal birth before the original CS (2, 17, 18).

2.1.7 Type of previous CS scar

The type of scar depends on the type of cut in the uterus: Low transverse: a side-to-side cut made across the lower, thinner part of the uterus. This is the most common type of incision and carries the least chance of future rupture. Low vertical: an up-and-down cut made in the lower, thinner part of the uterus. This type of incision carries a higher risk of rupture than a low transverse incision. High vertical: an up-and-down cut made in the upper part of the uterus. This is sometimes done for very preterm caesarean deliveries. It has the highest risk of rupture. There is insufficient evidence to support the safety of VBAC in women with previous inverted T or J incision, low vertical incision or significant inadvertent uterine extension at the time of primary caesarean. The risk of uterine rupture with classical incision or inverted T incisions is high as 12%. RCOG recommend that VBAC is contraindicated in women with previous classical caesarean delivery (2). The type of previous incision may not always be known, especially if the operation was performed in a different country. Although in several

previous studies, it has been shown that there are no statistically significant differences between the group of women with unknown scar types and the group with known low segment incisions, caution should be exercised in these women and decisions should be mad case by case.

2.1.8 Interpregnancy interval

Interpregnancy interval was defined as time in months between caesarean in first pregnancy and the start of amenorrhea in next ongoing pregnancy. In women with one prior CS and no history of vaginal delivery, an interpregnancy interval of <2 years is non-associated with a reduced success rate of trial of labour after CS. The success rate is lower in intervals of >2 years. No association between adverse outcomes and interpregnancy interval was found (19). Short interpregnancy intervals are a risk factor for uterine rupture during TOLs. The incidence of rupture increases when inter-delivery interval of less than 18 months.

2.1.9 Uterine closure technique

Since the early 1990s, single-layer closure of the uterus has been frequently used by many obstetricians, but patients had two-layer closure of the uterine wall during their primary CS. A single-layer uterine closure technique is commonly used because it is associated with a shorter operating time and a short-term complication. It is possible that a single, continuous suture technique does not precisely approximate the tissues together because decidua can be included in the scar. Pathophysiology involved in the association of the single-layer closure technique and subsequent uterine rupture is not clear enough. Probably uterine rupture is thought to result from a biomechanical process, in which there is an imbalance between the tensile strength of the scar that maintains its integrity and the forces causing disruption (20). Several authors concluded that there were no statistically significant differences in maternal or foetal mortality in either group, single-layer or two-layer uterine closure (15).

2.1.10 Presence of Müllerian anomalies

Women with Müllerian duct anomalies according to the classification ESHRE/ESGE (21) such as, class I: dysmorphic uterus class III: dysfused uterus, class IV: unilaterally formed uterus, class V: aplastic/dysplastic uterus have significantly higher rates of CS mainly caused by foetal malpresentation. The outcome of VBAC in women with uterine malformations has been poorly studied. Foetal malpresentation is the major indication for primary and repeated CS in women with MA. Instead patients with Müllerian anomalies and foetal cephalic presentation who presents with spontaneous labour have an excellent prognosis for a successful VBAC; these patients have a significantly lower rate of failure to progress in the first stage of labour (22). The VBAC success rate is 37.6% for women with Müllerian anomalies and 50.7% for those with a normal uterus (P<.0009). Therefore, the presence of an isolated maternal Müllerian anomaly has not been thought to constitute a contraindication to VBAC (23). Women with a uterine anomaly should be counselled that, based on the small amount of data available on their relatively rare condition, their risk of uterine rupture is low, but they may have an increased risk of failed TOLAC.

2.2 Factors associated with previous medical history

Maternal demographics factors such as race, age, BMI, and insurance status have been demonstrated to impact the success of TOL. Younger women had highest success rate. Maternal age of 40 years or more is an independent risk factor for stillbirth and unsuccessful VBAC. In a multicentre study of 14.529 term pregnancies undergoing TOL, Caucasian women had an overall 78% success rate compared with 70% in non-Caucasian women such as African, American, Hispanic and other women. Married women, smokers, and those with private insurance all had a greater likelihood of successful TOL.

2.2.1 Maternal obesity

Maternal BMI is classified as underweight (<19.8 kg/m^2), normal (19.8-24.9 kg/m$^{2)}$, overweight (25-29.9 kg/m^2), or obese (≥30 kg/m^2).

Durnwald et al. was seen that VBAC success had decreased in obese (54.6%) but not overweight (65.5%) women compared women of normal BMI (70.5%). Women with a normal BMI had higher rates of VBAC success than overweight women. To determine whether change in weight between pregnancies impacts whose BMI classifications changed, they had evaluated women whose BMI classification changed before the second pregnancy. When overweight women lost weight achieving a normal BMI before the second pregnancy, there was no significant improvement in rates of successful vaginal delivery during a subsequent trial of labour. However, women of normal BMI before the first pregnancy who became overweight (BMI >25 kg/m^2) before the second pregnancy had a significant reduction in VBAC success compared with those women whose BMI remained normal between pregnancies. It is possible that adiposity accrued when a woman was overweight may not decrease enough to increase her likelihood of VBAC success comparable to those women who have always had a normal BMI. This adiposity may be disproportionately distributed in the pelvis and may alter a woman's pelvimetry, thus increasing the likelihood of dystocia. Increasing pregravidic BMI and weight gain between pregnancies reduce VBAC success. In the other study, Gupta S. et al., in a prospective observational study included 100 women with previous CS in the study group and 100 primigravidas in the control group. Various predictors of success were analysed including pre-pregnancy BMI. These support that there is a highly significant relation between BMI and success of trial of labour after previous caesarean delivery (19). Hence, maternal obesity may be a negative predictor of successful vaginal delivery (2, 15, 16, 24, 25). Women with increased BMI clearly experience decreased VBAC success rates.

2.2.2 Diabetes mellitus

Diabetic pregnancies are at increased risk for Caesarean delivery secondary to failed induction of labour, arrest of labour, foetal intolerance of labour, and foetal macrosomia (estimated foetal weight >4000 g). Diabetes complicates 2% to 3% of pregnancies. Several studies have been conducted and these data show that diabetes mellitus is associated with a reduced chance of successful vaginal delivery, both in comparison to non-diabetic women attempting VBAC, and diabetic women without uterine scars undergoing a TOL. However, reported success rates suggest that attempting VBAC in a select population of women with diabetes mellitus remains a reasonable option (6, 26, 27, 28).

2.3 Factors associated with current pregnancy

2.3.1 Macrosomia

Foetal macrosomia is a difficult diagnosis to make. There was a 'U'-shaped relationship between birthweight and the error of ultrasound estimation of foetal weight, in which the error in birthweight estimation increases for both low and high birthweight foetuses. In fact, for foetus greater than 4500g, the sensitivity and specificity of ultrasound decreases. Hence, third trimester ultrasound is a poor predictor of macrosomia on decision making regarding VBAC. In relation to VBAC labour, birthweight of 4 kg or more is associated with an increased risk of uterine rupture and unsuccessful VBAC. In women with no prior vaginal delivery undergoing VBAC when there is a suspicion of macrosomia, the VBAC success rate was less than 50% (2). Society of Obstetricians and Gynaecologists of Canada (SOGC) states that labour and vaginal delivery are not contraindicated with estimated foetal weights of up to 5000g, in absence of maternal diabetes. Suspected foetal macrosomia is not a contraindication to a TOL after CS.

2.3.2 Twin pregnancy

Women with twin gestation and one previous low transverse scar caesarean section are candidates for TOLAC. Moreover, two analyses of large populations found that women with twin gestations had a similar likelihood of achieving VBAC as women with singleton gestations as well as outcomes are similar to singletons (29).

2.3.3 Breech presentation

Women with a prior caesarean who are carrying a breech in the current pregnancy have similar rates of

successful breech versions as mothers without a uterine scar. A mother with a prior caesarean birth can elect to have a version for breech this time to avoid a caesarean. External cephalic version for breech presentation is not contraindicated in women with a prior low-transverse uterine incision who are candidates for external cephalic version and TOLAC. Moreover, the likelihood of successful external cephalic version has been reported to be similar in women with and without a prior caesarean delivery.

2.3.4 Post-dates pregnancy

Studies evaluating the association of gestational age with VBAC outcomes have consistently demonstrated decreased VBAC rates in women who undertake TOLAC beyond 40 weeks of gestation. Although the likelihood of success may be lower in more advanced gestations, gestational age greater than 40 weeks alone should not preclude TOLAC (24). A logistic regression analysis indicated that gestational age >40 weeks was an independent predictor of caesarean section (30). Trial of Labour for VBAC is a reasonable course of action if labour begins spontaneously after the due date, because the risk of uterine rupture is significantly higher with induction of labour. Hence, plans to pursue VBAC need not to be changed simply because the due date has passed. The NICE induction of labour guidelines recommend induction of labour from 41 weeks, as this reduces perinatal mortality without an increase in caesarean delivery rates, but a few data recommend whether this approach is equally valid in women with previous caesarean delivery. Thus, likelihood of successful VBAC on clinical and cervical assessment at the time to admission.

2.3.5 Preterm VBAC

Whereas postdates have been associated with a no statistically significant decrease in VBAC success, prematurity has been associated with an increase in VBAC success rate. Some studies, including NICHD study, show that VBAC success rates for preterm and term pregnancies were similar. Perinatal outcomes were similar with preterm VBAC and preterm elective repeat CS (ERCS) as well as the rates of uterine rupture and dehiscence were significantly lower in preterm compared with the term VBAC (31).

2.4 Labour status and cervical examination

Labour status and cervical examination on admission influence VBAC success. Those women who were admitted after rupture of membrane at active first stage of labour and having occipito-anterior position had a higher chance of vaginal delivery than those with occipito-posterior and occipito-transverse position, or unknown position. Presence of meconium stained liquor and labour stay lasting more than four hours after admission were associated with high failure rate of VBAC. The stronger factor determining success was cervical dilatation at admission. Those who were admitted with cervical diameter greater than 3 cm (active first stage of labour) had a strong likelihood of vaginal delivery than those admitted at cervical diameter of less than or equal to 3 cm (latent first stage of labour). Hence a favourable initial pelvic examination, consisting of cervical dilation >1 cm, cervical effacement >50% or station -1 or lower. Women who laboured spontaneously, had higher percentage of successful VBAC than those who underwent induction of labour (2, 9, 14, 32). Oxytocin augmentation had lower rate of successful VBAC rather than any intervention and may be associated with uterine rupture. Favourable Bishop's score on admission was the strongest and most significant predictor for successful vaginal birth after caesarean section and the chances of vaginal delivery after previous caesarean section improve as the Bishop's score at the time of admission increase (8). Therefore, the chance of VBAC for an individual varies based on demographic and obstetric characteristics.

2.5 The role of vaginal birth after caesarean delivery prediction models

Several pre-admission and admission-based multivariate models have been published to predict the individualised likelihood of VBAC success. Several studies have been conducted about the association between second trimester cervical length and VBAC. Furthermore, studies have been conducted to understand the role of ultrasonographic assessment of myometrial scar

thickness to predict VBAC success and uterine scar rupture. Moreover, predictive models have been created to predict the likelihood of a successful trial of labour after caesarean delivery. The probability that a woman attempting TOLAC will achieve VBAC depends on her individual combination of factors. Several investigators have attempted to create scoring systems to assist in the prediction of VBAC, but most have had methodologic limitations and have not been used widely. However, one model was specifically developed for women undergoing TOLAC at term with one prior low-transverse caesarean delivery incision, singleton pregnancy, and cephalic foetal presentation. This model uses information that is available at the first prenatal visit to generate the predicted probability that a VBAC will be achieved if TOLAC is undertaken. Predicted probability for VBAC is based on a multivariable logistic regression model that includes maternal age, BMI, race, prior vaginal delivery, history of a VBAC, Bishop's score and indication for prior caesarean delivery (15). Prediction of TOLAC success at the time of admission was highly dependent on the initial cervical examination (33, 34). This model, as well as one that provides the probability of VBAC after TOLAC using information that is not available until the admission for delivery, may have utility for patient education and counselling for those considering TOLAC at term. Although such a calculator may provide more specific information about the chance of VBAC, which can be used by health care providers and their patients to further the process of shared decision making, no prediction model for VBAC has been shown to result in improved patient outcomes. By using a proposed mean score of 4 out of 7, the scoring system had a sensitivity of 81%, specificity of 52.3% and a positive predictive value of 84.6%. Grobman and colleagues (35) as early as 2007 created a simple nomogram using factors available at the first prenatal visit. These predictive nomogram incorporates six variables including history of successful VBAC, value not included in the other models because according to some authors it is highly probable that these women would reattempt VBAC regardless of counselling. The investigators concluded that the nomogram was accurate and discriminating, and was a potentially useful tool for patient-specific rates of success and that the MFMU VBAC prediction model validated in women with one prior caesarean delivery also accurately predicts the likelihood of successful TOLAC in women with two prior caesarean deliveries (36).

2.6 The role of vaginal birth after caesarean delivery second trimester cervical length

Several studies have provided evidence that cervical length and its changes begin in the midtrimester. It can be established sonographically and it has relevance for pregnancy outcomes, but does not significantly improve the clinical value of a previously developed validated VBAC prediction model. Women have been undergoing transvaginal ultrasound between 18 and 24 weeks. A cut off of Cervical Length (CL) of 45 mm has been established and more women with CL < 45 mm had successful VBAC compared to women with long CL. Prior vaginal delivery and CL < 45mm were both significant predictor of VBAC. Hence, shorter midtrimester CL is associated with a greater chance of vaginal birth after TOLAC (36, 37).

2.7 Sonographic assessment of lower uterine segment thickness

Three layers of the lower uterine segment (LUS) can be identified on ultrasound: the chorion amniotic membrane with decidualized endometrium, the middle muscular layer, and the uterovescical peritoneal reflection juxtaposed with muscolaris and mucosa of the bladder. Full thickness is defined as the distance between the bladder wall and the amniotic cavity, while myometrial thickness is defined as the minimum thickness overlying the amniotic cavity at the level of the uterine scar. The measurement of LUS thickness antenatally in women with a previous caesarean delivery, could be used to predict the occurrence of a uterine defect, scar dehiscence or scar rupture, in women undergoing VBAC. Uterine scar dehiscence is defined as a loss of continuity of the myometrial layer without the complete rupture of the LUS, also called uterine 'window'. Uterine rupture is defined as a complete separation of the uterine scar resulting in a communication between the uterine and peritoneal cavities (38). Several studies were conducted to evaluate the

reliability of bidimensional and tridimensional ultrasonographic measurement of the thickness of LUS in pregnant women by transabdominal and transvaginal approaches LUS measurement by transabdominal approach must be performed with the women with full bladder, placing one cursor at the urine-bladder interface and the other at the decidua-amniotic fluid interface. Transvaginal approach measuring the LUS muscular layer, one cursor must be positioned at the bladder-muscular interface and the other at the muscular-decidua interface. Secondly, a volumetric acquisition must be performed for a tridimensional reconstruction. Finally, the authors conclude that Transvaginal approach is more reliable the entire LUS thickness measured compared to transabdominal approach (39). A myometrial thickness cut-off of 2.1-4.0 mm provided a strong negative value for the occurrence of a uterine defect during VBAC, whereas a myometrial thickness cut-off between 0.5 and 2.0 mm provided a strong positive predictive value for the occurrence of a uterine defect (2, 38, 39). However, there is not an ideal LUS thickness cut-off value to be used in clinical practice, but these researches support the use of antenatal LUS measurement in the prediction of a uterine defect in women undergoing VBAC. Therefore, based on the evidence, the thickness of LUS measured by transabdominal and transvaginal sonography can successfully predict the risk of scar rupture and remain the gold standard. But several studies confirm that this is not only viable predictor of rupture because composition of the scar tissue may also play a significant role in the occurrence of scar dehiscence. Magnetic resonance (MR) is another imaging modality that has a well-established role in studying the female pelvis, especially at high field strengths. MR with diffusion tensor imaging (MR-DTI) and fibre tracking reconstruction is a novel non-invasive imaging technique that could characterise tissue morphology by measuring the amount of random diffusion of water molecules throughout the tissue and is known to have the best soft tissue contrast resolution. Fiocchi et al. shows that the majority of the uteri with a Caesarean scar have altered orientation of fibers in the anterior isthmus compared to non-scarred myometrium. A significant difference between the two methods, transvaginal-ultrasound and 3t-MR, was found in the measurement of the myometrial thickness at the scar level. We suggest that 3T-MR could provide a more accurate and reliable measurement than TVUS, as the spatial resolution of the image is much higher. Upstream of the scar, TVUS measurements reported a significantly thinner myometrial layer compared to 3T-MR. Hence, MRI can be used to predict scar dehiscence and rupture (40, 41). However, the quantitative data of MRI added to morphological evaluation could help the gynaecologist predict later complications of CS, and the identification of those women who could attempt VBAC (41).

Conclusion

It has been demonstrated that women who undergo successful VBAC have lower short-term and long-term morbidity. Conversely, women who are unsuccessful following TOLAC have the highest morbidity. For this reason, Trial of labour after CS should be considered in women who have no contraindications that are relatively few, classical or T-shaped uterine incision after appropriate discussion. Identifying the best candidates using factors available to the obstetrician can increase VBAC success rate and minimize maternal morbidity. Multiple previous CS, Müllerian anomalies, maternal obesity, maternal diabetes and a short interdelivery interval are negative predictors of successful VBAC, while a non-recurrent indication for previous caesarean section, one prior vaginal birth and a spontaneous labour are positive predictor of successful VBAC. So, concluding is important individualize the risk estimation for each patient in order to make the VBAC a safe choice.

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

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New shapes and original medical creations: the dependent nature of the individual in a Nahua community in Mexico

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Summary. *Background and aim of the work:* Within of Nahua of Naupan, the impact of acculturation processes by the historical interconnection between different models of medicine has given rise to important revisions and reinterpretations of local medical culture. The main purpose of this article is the observation of dynamics and aspects related to processes of understanding, perception and management of diagnostic categories, as well as the local understanding of the person (the individual) in the rural district of Naupan, located in the North East part of Sierra de Puebla. *Methods:* The analysis presented in this work is the result of an ethnographic study carried out at the Nahua community (1,614 people) residing in the rural town of Naupan (Huauchinango, Puebla, Mexico). *Results:* The attention will be given to the synthetic analysis of the local conceptions of certain pathologies and how the individual is seen as an unstable and constantly changing aggregate, situated in a context where health-related issues are clearly linked to different levels of perceived reality. *Conclusions:* In settings where there are no systems of institutionalized medical knowledge, nosological concepts are seen in a subjective and indeterminate manner, due to the fact that in some cases they also vary considerably depending on the person. Faced with the choice of therapeutic options, the Naupeña population moves between integrating and rejecting medical concepts from different cultural horizons, through a continuous creation of knowhow that they see as more or less organized and transmissible knowledge about disease, treatments and methods of prevention and interpretation. (www.actabiomedica.it)

Key words: traditional medicine, diagnostic categories, Nahua

Introduction

Based on data collected during a five-month period of ethnographic research carried out at the Naupan (Figure 1) rural community in the Sierra de Puebla (Huauchinango, Puebla, Mexico), this article explores the local conceptions of specific pathologies that refer to the individual as an unstable aggregate of balances connected to the environmental, social, individual and supernatural ecosystem, within which health-related problems are tied to the fluidity of individual boundaries – this is the concept of a body that is perpetually open and subject to the continuous

Figure 1.

and constant interaction between the person's inner and outer forces.

The research focused first and foremost on processes regarding the exchange, merging, inclusion and conflict between medical knowledge from different cultures. During our work, the resulting pluralism of concepts, diagnostic categories, choices, and therapeutic strategies proved to be truly dynamic, based on the continuous adaptive facets that members of society developed in order to deal with a condition that was considered as pathological in the community. In fact, within the same medical system, there is a multitude of knowledge, traditions and practices that can not, aside from rare cases, be easily classified or traced back of solid theories related to disease, cure for diseases, and health. Based on this viewpoint, the author saw the Naupan's medical system as a dynamic complex, within which members of a particular society move (1, 2).

Methods

The analysis presented in this work is the result of an ethnographic study carried out at the Nahua community (1,614 people) residing in the rural town of Naupan (Huauchinango, Puebla, Mexico). The study, promoted and supported by the Italian Ethnographic Mission in Mexico, was aimed at understanding the conceptualisations and beliefs of the population in relation to the cognitive-values of local medicine, which greatly differs from the medicine that has been imported there in the last thirty years, part to the biomedical model that was adopted by the primary health centres within the territory. The survey in this project included participant observation, 50 structured and semi-structured individual interviews and 10 group meetings. Participant observation was carried out in biomedical healthcare facilities as well as those where local medicine practiced, including private homes and places of worship such as religious sites, markets, etc. Discussions and interviews were in Spanish (80% of the population speaks Spanish in addition to local Nahuatl), and in specific cases we used the help bilinguals. All interviews were recorded and transcribed, and in some cases we required the support of bilingual members of the community.

In order to understand the dynamics that determine the complexity of health and disease in the culture of local medicine, we needed to identify the following focal points for our analysis: (a) local population's representations on the concept of the body/health/illness/healing; (b) the complexity of the disease as an event that can be broken down into disease, illness and sickness; (c) the identification of therapeutic prototypes that would be useful in creating a systematic analysis on the use and methods of local healthcare resources.

Results

The complexity of the person, which at certain moments of their existence is spread out within the surrounding ecosystem, makes them subject to the influence of external forces that can bring them to experience conditions of discomfort and danger. If different studies have been shown essentially that there are permeable boundaries of the body that allow the "soul-components" of the person to move around and act out beyond that of the individual self, here the authors have often chose to dwell on just how much the external forces, ethereal and intangible, can interact with the alteration of the person's health, from malicious actions against internal components to external projections of the individual and the community (3).

The addictive nature in people is the consequence of a concept of the responsibility that invests human relationships with social norms and values, whereby the individual evil is understood not only as an attack on the social and moral order, but also as a break from the harmony of the relationship between the body, the person and the ecosystem.

The spiritual energy that can abandon or attack the body of a person when this person is not careful or is in a difficult moment constitutes the interpretative aspect of illness that defines the "extra-human' etiology of varying symptoms, the neglecting of which would likely trigger a dangerous aggravation that could lead to death (4, 5).

The person – the individual – is placed in a context where extra-human forces have a strong influence on social and individual destiny. The idea of a fragmented world, criss-crossed by a vast complexity of benevolent or malevolent entities, finds the principle of unity through the Catholic idea of one God. From this idea

of unity - which, as we will explain below, is more quantitative qualitative - everything in the ecosystem is divided into countless beings with many different characteristics, usually anthropomorphic, whose raw material has a "thin", light density, unlike the "heavier" living beings and earthly creations. The uniqueness of God determines the design of the main figure of the supernatural world, embodied in turn in the rich pantheon of Catholic saints.

The polar opposite of God is the figure of the Devil, in the classic Catholic ideology strictly inferior to God and the saints. To simplify the complex structuring of supernatural forces, which in the Nahuatl cosmology rule the world and influence the fate of all living creatures, we would like to propose a list of some of these entities, all hierarchically submissive to God:

1) the pantheon of Catholic saints (*santos*);
2) the patrons of places (*dueños de lo cerros, de las cuevas, de los ríos, de los pozos,* etc.);
3) the winds (*aires*).

The *aires* are not actually completely distinct entities compared to the two prior ones. Often, in fact, they represent the means by which they arise and act in the environment, interacting with the individual. In many other cases, however, they are associated with malignant powers, and consequently represented as emanations of forces or entities belonging to the world of the dead, the middle world, and therefore also that of Evil. *Santos* and *dueños*, on the hand, can be defined as secondary deities (compared to the singularity of God), because, according to the divine plan, they determine the fate of the individual and the community. Precisely for this reason, *santos* and *dueños* are addressed, through divination, by special rituals and are essential for producing diagnosis and identifying a particular cure, and therefore they also determine the healing of any disease. However, both are not mere benevolent entities and indeed they often inflict a certain evil upon those who dare to show disrespect towards them (6).

There are many ways in which the individual may cause irascibility. For example, the *dueño del río* can scare a child, near water, behaves in such a way that shows lack of respect for authority. At this point the *dueño* will make sure to scare the child to cause the release of the *tonal* and capture him, holding him hostage until he has received due compensation for the offense. To accomplish this clearly punitive taks, the *dueño* may make use of *aires* to capture and hold the *tonal*, which will remain imprisoned in the place where the crime happened.

During this research on the spiritual world inhabited by these figures, which are generally anthropomorphic, we focused particular attention on what the *aires* represent to the Naupan, and more specifically, on the pathological aspects in which they are directly involved.

The *aires*, in nahuatl called *yeyekame*, can be divided into four different categories:

a) climatic phenomena of air currents;
b) harmful emanations that are absorbed by the body;
c) intentional emanations of extra-human or human entities;
d) personified evil entities with their own will.

Each of these categories of *aires* turns out to be potentially harmful to the individual and each of them correspond to definite pathological classes.

a) The *aires*, when associated with climate or weather, are forces that could be categorized as those above, called secondary deities, which are seen as a basic element of the agricultural cycle and associated with seemingly natural aspects of air currents. Consequently, they are separated into *yeyekame itstik* (literally. "cold winds") and *yeyekame totonke* ("hot winds"). As seen in literature (4), hot and cold are the two opposing elements that in Nahuatl thinking constitute the cardinal idea of the polarization of elements, bodies and substances, according to one of the fundamental interpretative frameworks of illnesses afflicting the person. Accordingly, the *aires*, which are also connotations of thermal qualities, can be determinate in how pathogens, penetrating inside the body, cool the person and lead to general symptoms such as headache, cough, fever and colds, attributable to *yeyekame totonke* as well as *yeyekame itstik*. The latter, however, are considered to be more harmful than the others and can generate chronic illnesses if not treated promptly. Their accumulation, in any point of the body, is extremely harmful, even if, based ethnographic data we collected, we

found evidence that those interviewed mainly identified *yeyekame itstik* as a cause of bone pains, of just about any type:

> *Onechuitek yeyekatl itstik* (Literally, "The cold air hit me") [...] It usually reaches them in the bones, anywhere in the body, it goes through the skin, and then: "What else can it touch?" Then the bone, and sometimes it does not stop moving. The bones can become very cold, for example a tomato when you do not put it in the refrigerator it is soft, but when you put it the refrigerator it is very hard – much like bones when enters the air enters. [...] Knees start to hurt mainly [...] then legs, knees sometimes, sometimes shoulders and elbows being to ache [13/01/2010 B.C.].

b) Whether the bones or other parts of the body are affected by this category of *aires*, the cure includes empirical treatments aimed at halting the pathogenic nature of the entity through the thermal principle of opposition, which in a symbolic-mechanical conception focuses on expelling, through the use of contrast, the element of *aire*.

c) When defining this category of *aires* as harmful emanations that are absorbed by the body we are referring to the subtle and ethereal substance that is associated with the emanations of mothers or newborn babies. Children, in their first days of life, represent the liminal state of the human beings, and therefore they are still tied to the world from which they came – the world of the dead that carries the harmful forces. In this regard, the ceremony of the *lavado de manos* ("Hand washing") is quite meaningful. This lustral ceremony follows the classical Catholic rite of baptism, through which it aims at eliminating the contagious impurities that the child brings with it when coming from another world. Even the woman who kept the child in her womb is considered potentially contaminant for those around her. All garments that have been in contact with the new mother as well as the child are dangerous because they are impregnated with these harmful substances, in the form of *aires*, which can infect anyone who is nearby, with the exception, however, of the family and the midwife: the therapeutic specialist is the only person authorized to wash, and touch, the clothes of the mother and child. However, this type of *aire*, in addition to being connected with the harmful forces from the way of the dead, also refers explicitly to another type of impurity: that of sexual relations. The newborn is also the result of the sexual act by which his parents conceived him or her, and it carries the contaminant "dirtiness". The aspect of the contamination regarding the sexual act (the power of "force"), along with the contamination from a world that is different from that of the living, are closely interconnected. In order to clarify the complex features of these *aires*, we should consider the role of dogs, which next to that of the mother and newborn, is constantly cited by those interviewed as a cause of contamination. Dogs are also connected to the world of the dead, and are described as nocturnal animals that communicate with the souls of the dead, yet they are also considered highly impure and dirty because they mate frequently in public places, in front of everyone.

These different aspects of *aires* are described and characterized through a "bad smell" from liquids that the mother loses in the days after birth, as well as the dirtiness of stray dogs that populate the streets of Naupan. Therefore, insofar as *aires* recall the idea of dirt, impurity and consequently the contaminant, they would appear to belong to the so-called "*aires de basura*" (7).

Contamination that occurs by means of this category of *aires* is commonly represented as the entrance, within the body, of a strong and disgusting smell that causes the disease called *nitlatlak* (lit. "I'm burnt"):

> It is a smell is found in the throat, which then goes into the stomach and remains in the body causing the creation of different saliva [...] it happens because we feel disgusted and want to vomit. [...]: Nitlatlak means "I'm burnt", but not burnt by fire, it's as if the body was burning from the inside [18/2 / 2010, L. B.]

That notwithstanding, these quotes appear to highlight that the "bad smell," understood in a generic sense, is the cause of *nitlatlak*, while the pathogen is always traced back to emanations, more or less odorous, of mothers, babies, and dogs. For this reason, the agent of evil is not the smell, but more likely the nature of some living things (dogs, mothers, and infants), which determines this connection with *aires*. We would also like to point out that the disease is not caused only by inhaling these impure *aires*, but that contamination can occur, otherwise, through direct contact with

the bodies and objects that are infested. Thereofre, the *aire* is introduced into the body through inhalation or through casual contact, attaching itself in the trachea or in the stomach, and leading to weakening in the individual through the symptoms of lack of appetite, body swelling, and yellowing of the skin.

d) As mentioned in the introduction of this part of study, the *aries* are often the medium used by extra-human entities (*santos* and *dueños*) in order to punish the individual or the entire community for some outrage or offense committed. Another case is that in which the entity associated with the natural phenomenon (rainbow) infests any source of water with harmful *aires*, causing a noteworthy urinary tract inflammation called *mal de orín*. In this category of *aires*, we can also include the entity that the ritual specialist emanates from himself in order to pass or cure a disease. In the previous paragraph, we referred to the soul-components present in the practices of ritual specialists. The emanations that he produces can be regarded as *aires* – breaking away, in a voluntary manner, from the body casing, they roam the surrounding ecosystem with the aim of damaging a possible enemy. Although so far we have only highlighted what concerns the harmfulness of these forces, the fact remains that they can be malicious as well as benevolent. *Dueños*, *santos* and ritual specialists can address and pass their emanations with the aim of freeing the individual from a specific disease or, more generally, from a misfortune.

e) Malignant forces may also have their own will. In this case, we are referring to *aires* understood as souls of the deceased, the damned, or demonic entities. All the souls of the deceased, including those of who died of natural death, remain for some time near the dead body and their home before embarking on the journey to the afterlife. Those who have died in a sudden or violent death, before their time, are destined to wander the world of the living until the time comes for their ultimate departure. They are tormented souls who, taken from their own bodies, are persistently trying to get inside of the living, damaging the vital force and leading them to death.

Each of these harmful entities has their own will. They often turn aggressive towards those who are still alive, and especially towards more vulnerable people, such as children and the sick. Generally, they are encountered at night outside of the village, where anyone can run into them or suffer the attack. Otherwise, the places where they are concentrated in greater density are cemeteries, crossroads, and caves. The types of diseases that can arise are numerous, and often with horrible symptoms. The diseases are generally caused by the spirit's intrusion into the body, which may cause a widespread malaise (tremor, insomnia, nausea, cyanosis, convulsions, etc.) as well as pain or dysfunction of various parts of the organism, or even the onset of behavioural or psychological alterations. This type of *aires* usually intentionally harms its victim, generally driven by envy and resentment against the living. However, there is also the possibility that a morbid state is manifested in those who encounter these entities in a chance meeting. Not infrequently, in fact, people may come across the spirit of a deceased who eventually scares the person, damaging their soul forces. In most cases, however, the *aires* are invoked and congealed by ritual specialists who, on behalf of third parties and through an action of witchcraft, instruct the evil entities to damage the chosen enemy.

By analyzing these different categories of *aires*, we can see that the first two include pathogens that are harmful in a completely random way, in a manner devoid of any kind of intentionality and personification. With regard to the last type, it is evident that there is the will to act against someone, either on their own initiative or on commission. In fact, these are *aires* given the name *a'mo kuale yeyekame* (literally meaning "bad winds"), indicating their close relationship with demons, precisely *a'mo Kuali* ("not good"). Evil, this "not good", is associated with two days in particular, Tuesdays and Fridays, when divine forces, inherently beneficial, are absent or become less vigilant. As a matter of fact, these days are reserved for witchcraft, and all possibly beneficial or curative activities are suspended. The expression *a'mo kuale yeyekame* is used by the Nahua of Naupan to distinguish demonic spirits, damned souls, dead souls and soul-projections of ritual specialists from atmospheric or climatic phenomena and harmful emanations that do not have the intent to harm people. Therefore, certain pathologies are accepted as events that lack voluntary causes, as in the

case of *yeyecame itstik*, which enter the body simply because in a given situation the individual is found to be vulnerable to this particular disease-causing agent. As for the *a'mo kuale yeyekame*, however, the personification and intentionality of the pathogen are nearly always obvious. They can often manifest themselves in a visible manner, taking on human characteristics, so much so that whoever comes across them is not able to immediately recognize their ethereal nature. Only ritual specialists, through divination, can identify the true essence of the entity that the sick person has encountered. Divination is in fact the only means by which we can determine the nature of the evil at hand. If the cause is recognized as a result of the action of the *a'mo kuale yeyekame*, the cure often consists in the ritual of lustral *limpia*, a process of "cleaning" that is carried out in order to rid the body of the foreign entity:

> For example, one day a shadow appeared, and it quickly hid behind the bathroom and I went over to see it, but I found nothing. I got scared because I saw a lady who then disappeared behind the room and then nothing was left of her, so I went into the house and the baby started crying non-stop, and I couldn't calm him down because he had been seized by "*mal aire*". [...] The baby kept crying all day and all night. So even though we did not see anything that was hurting him or making him sick, we went to see *curandera* because they can see ... Donna Angelita [ritual specialist] immediately realized that the child was not well and said: "He has got the *mal aire*, I have to urgently cleanse him, otherwise who knows what could happen." She carried out the *limpia* [symbolic ritual of cleansing] with turkey egg, wax, herbs and many other things. She watched the child using the *copal* [divination tool commonly used by ritual specialists] and confirmed that the child had *a'mo kuale yeyekatl*, or *mal aire*. After this operation the child gradually calmed down. He was saved and is now doing great. [29/12/2009, R. G.]

The *a´mo kuale yeyekame* are extracted [removed] from the body of the sick through the use of elements (e.g. eggs and wax) that absorb them, hold them or attract the harmful ethereal substance, only to be then abandoned in remote areas, or returned to the place where evil comes from, including crossroads, cemeteries, and caves.

Discussion

Based on this approach, it is quite clear that in the description of *aires* as pathogenic agents, the author has given greater importance to a viewpoint that focuses, with regard to the representation assigned to them, on the dichotomous distinction through which two dominant directions can be identified in the causal explanation of the onset of the disease: "naturalistic" and "personalistic" (8, 9) In the first case, we are dealing with aetiologies that explain the illness in an impersonal manner – using systemic terms – such as loss of strength and balance, or as the breakdown of normal living conditions. In the latter, the aetiologies refer to the active and intentional actions of a real or non-human agent acting on the patient. This binary opposition can be addressed through various analytic tools that arise from the debate on aetiology, which began at the dawn of research in medical anthropology. Moreover, in capturing the instrumental character of this dichotomy, the author has attempted to highlight the different attributes given to the *aires*, making a net distinction – as far as general pathological agents are concerned – between a random dimension, in which certain nosological phenomena are accepted as "natural" events, with no specific motives, and a causal principle of to which other nosological phenomena are attributed to the responsibility of malicious, human and/or non-human powers. The benefit of this perspective is to allow us to understand the multiple factors of pathogenic forces, even at a purely descriptive level. On the other hand, if we apply it as an analytical tool to reconstruct causes that are attributed to specific phenomena of illness, we would be faced with a dichotomy where naturalistic viewpoints would concentrate on empirical causality of "how", while the personalistic view would call into question solely the psychosocial and moral dimension of "why", regarding the responsibility and fault that led to the harmful entity's aggression. The reality – with regard to the causality of the disease – is too complex to be linked to one of these two etiological spheres. Therefore, we can see that the event of an illness does not reach completion solely in this "casualty", as it may seem as far as diseases connected to "natural" pathogens; and much less when, as in the case of the disease called nitlatlak, harmful emanations are

in a clear relationship with the world of dead, and specific "causes" directly or indirectly affect the individual in each alteration to the normal state of health.

Therefore, if there is still some margin for this case, it should in any case be placed within the complex intertwining of the causes related to the axiological principle of the responsible causality. Gilles Bibeau (10) proposed the "theory of responsibility" as complementary term to the "theory of persecution", more closely linked to imprudence and mistakes committed by the individual, or to powerful attacks directed by enemies against him or her. The first theory emphasizes the implication of the subject in the onset of his or her problem, while the second refers to the cultural language used to translate this implication. The responsibility of the sick person in the explanation of evil does not play a fundamental role, only in cases where behavioural and moral irregularities can be easily traced back to the individual, and therefore the illicit action committed to a person in the community or to a non-human entity. The latter, cited several times before, refers to the irritability of *dueños* or *santos* as the result of an offense committed against them. On the other hand, through the theory of responsibility, it is possible to emphasize the importance of the "root [initial] cause" in every pathological state. Attention to the underlying cause of illness, which is often overlooked in anthropological studies, is an integral part of this distinction – once again proposed by Bibeau with regard to the Classification of Diseases (11) – between a historical "root cause", Linked to social and spiritual aspects, and a functional "agent cause", which may simultaneously involve empirical and spiritual elements. The majority of etiological systems described in anthropological literature refer to "agent causes" and therefore mirrors an excessive importance of causation that is seen on a social level, at the expense of aetiological perspectives activated individually and expressed by "root causes". The individual or community that is responsible for the "root cause" is always expressed in the biography of the patient, who even if they believes they are ill - because they were accidentally exposed to cold air currents (yeyekatl itstik) – they can find the reasons for the disease based on personal responsibilities that can even be traced back to their distant past.

Therefore, Sindzingre and Zempléni (12) are quite right when they remind us that in defining a certain affliction, we must consider at the same time three causative elements, in this way proposing an effective perspective through which we can bring together the various approaches to which we have made reference in the analysis of the etiological categories. The two authors propose a distinction among: an occasional "cause" that may correspond to the pathogen (the "how"); an "agent", which is an non-human entity, a witch doctor, or a person in general (the "who"); and finally an "origin", whether it is an offense committed or an injustice they have suffered (the "why"). In the same year, in 1981, Janzen emphasized the importance of considering "multiple causality" in the analysis of etiological concepts, recovering in a critical manner what Evans Pritchard had already said in 1937 regarding the analysis of Azande medicine: e.g. that witchcraft would act together with other natural or supernatural secondary causes. Returning to Marc Augé's discourse, the disease must be analyzed as a "a disorder that is biological and at the same time social", highlighting the interconnection of that which is natural, non-human and social, all coexisting within the same etiological dimension (13).

The multiplicity of etiological criteria is also evident in the therapeutic itself, where there may be no correspondence between the type of aetiology and the type of care, so that a type of "natural" aetiology may also correspond to a cure that is based on mystics and rituals, or vice-versa.

In light of these dense complications, in addition to the pathogenic agents, the two large etiologic categories through which the Naupan population carries out an initial classification of pathological states, distinguishing them in the *Maldad* (literally "Disease of Evil") and *kokolistle Dios* (literally "Disease of God"). In this type of classification, the native population explains the disease based on its origin, using an aspect of "interdigitation" (14) which brings together the two conceptual universes in a perpetual dialectical overlapping. The illness is experienced in a completely subjective manner, as a regret and rethinking of the person's existential condition, through which he or she gives meaning to their life's story – stories where there are illicit actions committed or suffered, in relation to the visible ecosystem (relationships between oneself and

the community) and that which is invisible (the relationship between the person and the outside world) through which both categories are interpreted. In nosological categories of the *Naupeños,* the illness is interpreted on a case-by-case basis, depending on facts, feelings and actions in which the patient is involved and that as a whole have contributed to creating the causative chain – and the onset of disease is the its ultimate expression. The terms used to explain the disease are related to the person who is ill, not the pathological condition. Furthermore, it goes without saying that the very definition of a disease is based on the culturally and socially determined subjective manner of dealing with and conceiving the alteration of the normal state of health (15).

Therefore, with regard to the classification structure, as shown by the same denominations in the case of *kokolistle Maldad*, the reference to that which is malicious or evil is made to correspond to particularly serious pathological conditions produced by either non-human or human agents that are voluntary and involuntary. On the other hand, "diseases of God", also referred to as the natural *kokolistle* (literally "natural diseases"), include, in particular, those kinds of pathologies whose understanding has been transmitted from biomedical nosology, and more generally correspond to all those illnesses that are considered initially mild, although they may still reach conditions that are severe or chronic, even becoming fatal.

1. Kokolistle Maldad:

a) Non-human pathogenic agent: it includes all the diseases in which the *a'mo kuale yeyekame* are involved, either through a voluntary attack or by a random encounter, involving the loss of vital components or the intrusion of harmful entities;
b) Human pathogenic agent: all diseases caused by voluntary human action, such as witchcraft, or involuntary actions, caused by uncontrollable harmful emanations.

2. *Kokolistle Dios:*

a) Pathologies caused by the breakdown of the body's physiological balance;
b) Pathologies that can attributed to the body's mechanical and physical dysfunction;
c) Pathologies caused by contagion through physical contact or by inhalation of harmful ethereal substances, or by the bite of some insects;
d) Illnesses caused by different types of emotional states.

There are other factors, apart from the causes, that determine the affiliation of each disease to one of the two macro-categories we have presented in this paper. The first factor that we would like to examine – due to its relevance to the fundamental position in our research – concerns the inclusion, within the category of the *koklistle Dios*, of all the pathologies whose understanding is derived from biomedical nosology. In fact, biomedicine can only treat "Diseases of God" successfully, while local specialists claim their ability to intervene for both classes of diseases. The family group (in the field of self-care) and the local therapist are the people, when making the diagnosis, who will initially decide whether the patient is facing a disease of divine or evil origin. Therefore, classification will not depend on the type of disease, but above all on factors such as the severity and violence of the pathological attack, its duration and its resistance to biomedical drugs. The parameter of seriousness and gravity, in relation to the first manifestation of illness or malaise, is indicative of the nature of the disease. The sudden onset of serious illnesses generally represents causes related to human or non-human malefic implications. Just as the persistence and resistance of the disease also leads to the search for the meaning behind the various levels of causation in which, in addition to the responsibility of the individual, may also lead to attributing the cause of illness to persecutory attacks (16).

Conclusions

In order to avoid unwanted stretching or embellishment of medical interpretation and classifying requirements, Robert Pool (1993) offers a solution. He underlines the belief that traditional and biomedical nosological systems are more indeterminate than scholars are willing to admit. In fact, there is not always a correlation between the type of aetiology and the type of care and therefore, for example, we must

recognize fact that a "natural" aetiology can safely follow a cure with strong symbolic foundations. In settings where there are no systems of institutionalized medical knowledge, nosological concepts are seen in a subjective and indeterminate manner, due to the fact that in some cases they also vary considerably depending on the person with whom we are speaking (17). Moreover, we must not forget that the same institutionalized knowledge and understanding of biomedicine, based on the experimental method, can not in the slightest way be regarded as immutable and unchanging, since it is itself subject to continuous variations. Therefore, only by accepting the principle of indeterminacy can we observe the semantic wealth of local aetiologies and nosological categories (18). In fact, the most important aspect regarding the complexity of how an individual is represented in Naupan is the "fluidity" of individual boundaries, with regard to the constant interaction between the external and internal forces connected to the person (19).

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A child of two mothers: what about the father? Italian overview

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Summary. Surrogacy techniques allow for the birth of children who are then raised by parents who may have no genetic or biological connection with them at all. Italian legislation on medically assisted procreation bans such practices, under national criminal codes, and yet the intended parents' ability to legally register children born abroad via surrogacy has not been affected by such legislation. Italian jurisprudence has acknowledged the parental status of same-sex couples, following the same path outlined by the European Court of Human Rights. The paper's author elaborates on court decision n. 145/2018, from the Naples Court of Appeals, which has stated that surrogacy children may be connected to their intended parents merely by virtue of "mental" elements, based on affection, harmony and listening enjoyed by the child within the family setting. The author is critical of that view, in light of conflicting research findings on children growing up in same-sex families. In that regard, the author argues that even though homosexual couples may well turn out to be good parents, families made up of fathers and mothers still constitute the best scenario for the children, from a social perspective. It is however necessary for lawmakers to step in and better regulate an utterly sensitive area of law, one that might engender adverse repercussions on the children's well-being, in terms of growth and psychological development, following their becoming part of homosexual families. (www.actabiomedica.it)

Key words: artificial reproduction, natural motherhood, parental relationships, surrogacy contract, surrogate motherhood, adoption

Introduction: A few preliminary remarks

The issue of surrogacy is arguably one of the most controversial to have come to the fore over the past years (1); such a procedure enables those with sterility and infertility issues, which have always been rife among heterosexual couples, to achieve pregnancy even when they cannot resort to homologous/heterologous fertilization and experience practical or procedural difficulties when trying to adopt a child, mostly because law no. 183/1984 sets an age limit for prospective parents as well as the number of children declared abandoned and therefore available for adoption.

Surrogacy has been resorted to even by same-sex couples wishing to have children. Surrogate motherhood is an ancient practice, even mentioned in the Holy Bible (Genesi, 30,3) (2). The new aspect, however, is that it has been medicalized, given how it is achieved through medically assisted procreation procedures. The widespread use of such techniques has over time brought about a commercial trend relative to the practice itself, with the creation of a new "market", ranging from the creation of more and more dedicated clinics to the provision of legal counselling when drawing up contracts to be submitted to those couples interested in availing themselves of the procedures. Estimates set the value of the global "reproductive market" in excess of 6 billion dollars a year (3).

European landscape

There is a wide ranging variety of different national regulations on surrogacy across the world. In Europe, there seems to be a climate of hostility to the practice, especially when it entails a contract that is basically commercial in nature. The European Parliament, via resolution no. 2009, on 13th December 2016, decried surrogacy (4, 5). On 2nd February 2016, human rights organizations in Paris, along with politicians and scientists, have signed the charter of Paris, calling on European nations to respect the international conventions for the protection of human rights that they have ratified and to oppose firmly any form of legalization of surrogate motherhood at a national or international level.

Each and every European Union member state has passed various laws of its own, often conflicting from one another. Austria and Germany have banned it altogether. Norway, on the other hand, has passed no law specifically targeting surrogacy, yet the gestational surrogate mother may not receive donor oocytes. Switzerland specifically forbids surrogate motherhood. France has made it legal to donate oocytes, at the same time banning gestational surrogacy, criminally prosecuting both surrogate mothers and intended parents. Lastly, Spain has outlawed surrogacy and all contracts related to it are deemed null and void, whether financial compensation may be thereby included or not (6). Britain, via the 16th July 1985 "Report of the Committee of Inquiry into Human Fertilization and Embryology" acknowledges the value of surrogacy as a means to solve sterility and infertility issues. The Surrogacy Arrangements Act allows for the elaboration of surrogacy agreements, provided that they be of the altruistic type and that parties in the deal are the intended parents and the voluntary surrogate mother; it also entails criminal liability arising from any form of commercial or brokering activities. In Italy, law no. 40/2004 makes surrogacy illegal, under article 12, viewing the practice as a breach of public order, carrying criminal sanctions (7). On account of that ban, several couples, in an effort to get around the ban, resort to surrogacy abroad, in nations where it is indeed legal, bringing the newborn child back to Italy and trying to legally register him or her as their own. Law n.40/2004 does not deal with the legal soundness of surrogacy procedures that take place abroad, at the request of Italian citizens, and says nothing as to the feasibility of legally registering the children thus born. Still, the courts have stepped in to fill that vacuum, producing rulings that come across as confused and contradictory. In some instances, the mother who had declared the child born via surrogacy as her own has been sentenced for false statement, and with the cancellation of her name from the birth certificate (Brescia Courthouse, 26th November 2013). In other cases, the judges decided to record in the civil status registry came with the replacement of the intended mother's name with the name of the woman who had born the child (Bari court of appeals, 13th February 2009). Most recent legal trends and court decisions make it possible for birth certificates of children born abroad via surrogacy to be legally registered, owing to the lack of specific legislation on the subject (Civil Supreme Court, 20th September 2016, no. 19599).

Biological truth as opposed to social-affective truth

The issue of whether the family status of children born via surrogacy ought to be acknowledged is closely related to the legality of adoption by same-sex couples. Debate is ongoing on that utterly sensitive issue, one that is rife with complexities from the doctrinaire and legal perspectives. In order for the rights of surrogacy children to be properly enforced, European judges have referenced the principle enshrined in the Hague Convention on the International Recovery of Child Support and Other Forms of Family Maintenance and in article 24 of the Charter of Fundamental Rights of the European Union, which states «In all actions relating to children, whether taken by public authorities or private institutions, the child's best interests must be a primary consideration». According to the judges, the child's best interests are to be intended in an evolutionary fashion, i.e. not necessarily in traditional family setting with clearly defined parental roles, but rather as the interest in maintaining the personal interrelationships established abroad through the adoption by the intended parents. According to the Court, the notion of family life may comprise that develop-

ing in a homosexual relationship (8), hence sharing the biological origins of the children is no longer to be considered to be a necessary requirement (9). Modern assisted reproduction techniques have made it possible to acquire parenthood at will, and for children to be regarded as someone's offspring by virtue of an affective, mental, harmony-based connection with their homosexual intended parents, experienced by the children in the family setting, no more based on having been conceived and born from a heterosexual, traditional couple. The bond between children and their parents thus goes beyond the natural, biological tie, thus creating the "social parent" figures, in addition to biological ones. Indeed, a child's birth, his or her physical production do not represent grounds to turn the biological parents into real parents. Birth and being born are physical events, which are expected to turn a parental relationship into a social fact (10, 11).

Establishment of parental relationships of children born through surrogacy within the Italian legal framework

European courts have granted a somewhat wide margin of appreciation to member states, while urging them to uphold the parental relationships of surrogacy children, especially in cases where one of the intended parents coincides with the biological one. Any failure to acknowledge such prerogatives would run afoul of article 8 of the European Convention on Human Rights, from the standpoint of one's individual rights, enjoyed by the children, to personal identity and to respect for their private life (10, 11). In order to comply with the ECHD's recommendations, the Italian judiciary has widely interpreted the principle of "adoption in particular circumstances" (article 44, letter d, law no. 184/1983), dictating that even singles or same-sex couples be granted the right to adopt a minor, provided that pre-adoption be impossible due to, for instance, the lack of a requirement such as abandonment of the minor (Rome juvenile court, 30th July 2014). The Italian magistrates have argued that the peculiar status of such adoptions warrants the legality of the child being adopted by the parent's partner as well. Such a prospect would be in the child's best interest, who has grown up and taken care of by both partners forming the couple. The judges argue that failing to legally acknowledge such a relationship would conflict with the child's best interest. Hence, at the same time, the courts provide protection for those parental relationships that are not based on biological ties, but rather grounded in consent, thus prioritizing the *favor affectionis* (favor affection) over *favor veritatis* (favor the truth).

The Italian Constitutional Court has borne out the view according to which surrogacy «is a blot on the dignity of women and deeply undermines human relationships» (Constitutional Court, 18th December 2017, no. 272). Nonetheless, it has also stated that one's origins should not be limited to and determined by genetic connections, but rather it should take on legal and social meaning. Hence, beyond the biological relations between parents and children, in cases of those born via assisted reproduction techniques (whether by homologous or heterologous fertilization), the parental bond may outweigh the biological one. It is incumbent upon the courts to strike the right "balance" between genetic and parental connections, and such a balance must dovetail with the children's best interests.

As a matter of fact, the guiding principle needs to be the minor's best interests, rather than the parents' (whether biological or intended ones).

A set of criteria may serve as a beacon light in order to guide those consequential decisions: 1) the length of the parental relationship that has been established, 2) the methodology of conception and gestation, 3) the availability of legal means in order to give rise to a legal connection between children and intended parents.

Ruling no. 145/2018, from the Naples Court of Appeals, appears to be particularly significant in that regard (12). The facts: a same-sex couple made up of two women, both with a solid professional background (an entrepreneur and an attorney), affluent (they had just bought the house where they resided), got married in Spain and entered into a civil union in Italy, pursuant to law no. 76/2016. They then decided to enlarge their family by means of heterologous fertilization, undergone by one of the two women. A child was born, who was raised by his two "moms". The partner who shared no biological tie with the child attempted to have her family connection with the child legally sanc-

tioned, and applied to the juvenile court of Naples, in order to officially adopt him (through stepchild adoption), in pursuance of art. 44, lett. d), law no. 184/1983. The Naples court, however, turned down her application, because even though the child's biological mother had consented to her partner adopting him, she had not waived her exclusive parental responsibilities towards the child. The records in fact reflected both women' s intention to exercise full parental prerogatives. The court of appeals overturned the ruling, since the "intended" parent, the one who has consented to the medically assisted procreation procedure, thus determining the child's birth, is to be viewed as a parent, even in absence of genetic ties. Intended parents, in fact, may not withdraw their consent and shirk the responsibilities that they have acquired. The courts reasoning goes that the biological mother's partner is not some sort of "third parent", but rather a second parent: she has taken up that role by granting her consent to the heterologous MAP procedure that her partner had undergone.

The court concluded that the child's best interest was to live with and be brought up by his two mothers, and argued that the following criteria had been met:

1. A steady affective relationship had been formed between the two women who had then gone on to plan their family, sharing the parenthood-centered project. The women' s respective families had accepted the child.
2. The women were economically dependable, owned the house where they lived and undersigned a life insurance policy with their child as beneficiary.
3. The child had settled well in a school already attended by the twin daughters of two mothers.
4. Law enforcement agencies had checked and vouched for their good conduct.
5. Social services officials, in their report on the case, remarked that the two women "in a thoughtful and timely fashion, had explained to their child that he was conceived through the seed of a kind and generous gentleman, which had joined the egg in his mom's belly. Such explanations had obviously been well understood and elaborated by the child, who, as of today, does not look troubled or unsettled in saying: "I have two moms, I have no dad, but plenty of friends, uncles and aunts that I can rely on and play with".

Going over that narrative, one may well conclude that the child's life is uneventful and trouble-free. The judges have therefore ruled that the child's best interest is well served by living with his two mothers. I disagree with that conclusion for the following reasons. The judges write: the child (who is now in elementary school) has struck social workers as being "well groomed and neat, sociable ... he addresses his parents as "mammina" and "mammona" ("mommy" and "big mama"), he hugs them both, smiling, on occasions, he has been observed to use different tones of voice according to which mother he was addressing, and at any rate, he seems well aware of the different roles held by each one of his mothers within the family setting (13).

Therefore, the child is aware of the different situation that he is in compared to his peers, he is conscious of the different roles played by his two mothers: he draws that distinction by addressing them as "mammina" and "mammona", as observed before, and his tone of voice varies according to which one of his mothers he is talking to. In light of those considerations, one should ask: is it really in the child's best interest to be raised by two women?

Discussion

Current scientific literature centered on homosexual parenting is split in two strands: the psychoanalytic doctrine revolving around the Freudian oedipal triangle (father, mother and child), according to which it is essential for proper child development to be able to identify fatherly and motherly figures within the family (14). As Eugenio Borgna contends, child identity develops through an identification process that involves both their psyche and their parents' sexualized bodies. Children recognize themselves and envision their future reflecting in and relating to male and female traits belonging to a father and a mother, whether they be biological or foster parents. Should such sexual diversification no longer be there, the child's very well-being would be in jeopardy. Children have a remarkable ability to adapt; however, they lead better lives

when they have a chance to live through their childhood with their biological fathers and mothers, as it is reflected in available scientific research studies on the subject. Children undoubtedly need a mother and a father, two clearly defined and distinct polarities, sexually defined as well, in accordance with nature (15). At the other end of the spectrum lies the theory that good parenting is unrelated to the parents' sexual orientation, rather on the climate and attention they devote to their children, which sets good families apart from bad, dysfunctional ones. The implication is that the right "mental pairing" outweighs sexuality, and paternal and maternal functions are somehow interchangeable, and can be exercised irrespective of any reference to the sexualized body (16). Such a theory holds that parenthood is not bound to biological factors, but rather to the mindset, and could therefore be termed "mentalization" of parenthood (17). The issue raised by such a theoretical framework is no longer whether same-sex couples are indeed capable of effectively bring up children, but rather how they can rear them. In that sense, homosexuality is a condition that does not foreclose the ability to discharge parental functions and duties (18). The numerous studies that have been conducted on the topic produced conflicting findings. The reason for such discrepancies might be that different research studies do not take into account the same factors: the couple's socioeconomic background and level of education, for instance. Other studies were flawed in that they were too small-scale to be statistically significant.

Some studies seem to point to the alleged tendency of children raised in same-sex-parented families toward depression, ill-health, unemployment, infidelity, drug and alcohol abuse, sexual self-victimhood and unhappy childhood memories (19). Other reviews have concluded that the same-sex parented children analyzed in those studies grew up and did as well emotionally, socially and educationally as their peers raised by heterosexual couples. The very same researchers, however, concede that in drawing up the research, factors such as socioeconomic extraction and the educational levels of couples and children were not accounted for (20).

The above mentioned theory seems to fuel the conviction that having children is to be considered a right. However, even the desire to have a child, as commendable and deserving as it may be, cannot necessarily be viewed as a right. Children do not and cannot constitute a "right", no one can stake a claim to parenthood because children are not objects to flaunt: they are gifts bestowed upon their parents by life. The word "parent" translates into "he/she who has generated" in many languages (in Italian, *genitore*). Every human being is generated from male and female gametes, with no exception possible. Men and women are biological fathers and mothers: they convey their genetic backgrounds into their children's bodies, and that includes physical characteristics and temperamental inclinations that will accompany them for all their lives. No child, therefore, can be born from a couple of women or men. For that reason, although I am aware that gay couples can raise and take care of children just as well, or even better than heterosexual couples, I still believe that "acting as parents" is different from "being parents". A family with small children is different from one with grown-up children. Being reared in a family devoid of fatherly or motherly figures could ultimately be harmful to minors, because the natural bond is inextinguishable (21).

A few closing remarks

Surrogacy entails the commodification of women's bodies, who are bound by a contract to hand over the babies that they kept in their wombs for nine months and born. Many nowadays discuss surrogacy and the pain and anguish experienced by those who cannot have children. Few however seem to wonder what a surrogate mother must feel when she is required to relinquish her newborn child and hand him or her over to a couple of strangers, a baby that is her biological child, and what consequences the children may experience when they find out about the biological mothers that nourished them for nine months and that should have been the ones who would never betray them, and yet forsook them at birth.

The debate centered on the legal standing and validity of surrogacy agreements is particularly passionate worldwide. It is undeniable the most heart-felt issue is providing protection to children born through surrogacy, often circumventing bans and restrictions codified in national laws.

I do agree with the studies that have concluded that the condition that best serves the children in their personal development is when biological and social truths coincide: that is a family where fathers and mothers are integrated with each other in a harmonious climate, for their children's sake. In my view, children raised in same-sex-parented families serve the couple's interest in "completing" their union through the child. In actuality, such a child is the "choice" of two adults who have him born and already orphaned of one parent. When topics of such great social relevance are discussed, which affect the right of children to grow up in a safe and protected environment, the rights of adult couples or partners are trumped. First and foremost, there are the children's best interests. For the time being, we cannot rely on a large enough number of research studies that could enable us to conclude that growing up in in same-sex-parented families may cause psychological damage in children. Nevertheless, that is not tantamount to concluding that growing up in such families is as positive an experience for children as growing up in heterosexual-parented families (22).

In a stance characterized by caution, along the lines of the precautionary rule (23), the risk of trauma for children cannot be ruled out. A social and political reflection needs to be made, in order to prevent *de facto* situations from escalating, leading to a normalization of surrogacy, despite its exploitation of women' s bodies and the its leading to births of children in a condition of diversity, compared to others. In order to make opposition to this practice effective, international agreements ought to be made, aimed at dissuading and deterring citizens of nations that ban surrogacy from traveling to countries where it is legal and punishing brokering activities.

As for children already born through surrogacy, a procedure ought to be outlined for the purpose of recognizing such children, which has to be compliant with the rules of children's rights enforcement, particularly article 7 of the United Nations Convention on the Rights of the Child, which entitles children to get to know the women who bore them after nine months in their wombs (24).

Lastly, innovative legal solutions are urgently needed that will take into account the blatant evolution undergone by families over time, and acknowledge that at this juncture, lawmakers should start a discussion on how to consider such changes in the realm of adoptions as well, making adoption-related procedures easier.

Conflict of interest: The author declares that he 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

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Case report

Dysplasia spondyloepiphysaria and patella dislocation: a case followed over 10 years

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Summary. Dysplasia spondyloepiphysaria means altered form and seizure of the vertebral bones and the epiphyseal bone regions. Pathologies related to this disease are: scoliosis, short stature, eye problems, articular deformities. We report a case of tarda form. The main problem was habitual and later fixed dislocation of both patellae together with valgus deformities of the knees. We describe the surgical procedures to gain reduced patellae and correction of the mechanical axis. (www.actabiomedica.it)

Key words: spondylopeiphyseal dyplasia, patella dislocation, femur osteotomy, hemiepiphyseodesis

Introduction

Spondyloepiphyseal dysplasia is a rare disorder with particular deformities of the vertebral bones and epiphyseal segments. There are two variants: congenita and tarda form. They differ in the inheritance pattern and the age of onset (4).The patient described in this report suffered from the tarda form. The clinical aspects of the tarda form are: manifestation from 6-12 years, short stature (height <160 cm), short neck, enlarged chest, short arms, scoliosis, kyphosis, lumbar hyperlordosis, reduced hip function, early osteoarthritis of hip and knee. Severe myopia is common (about 50%). About one quarter of people with this condition have hearing loss (1-4).

Case description

Boy born in 2000; at 7 years he started treatment with Cheneau brace for scoliotic deformity and gained good clinical results during the following years. He has short stature and severe myopia. From age 7-10 he presented lateralisation of the patellas with concomitant valgus deformity of both knees. The x-ray at 10 years showed a markedly lateralised right patella (figure 1). During the following 3 years the patellae dislocated completely (figure 2).

At age 13 we applied eight-plates for temporary hemiepiphyseodesis on the medial femoral and tibial side of the knees to correct the valgus deformity. The main deformity was on the femoral side, but we applied the plates on the tibial side too, because we wanted to reach faster correction of the main axis accepting an initially oblique joint line. The purpose was to remove the tibial plates former to achieve a correct joint line regarding the mechanical axis. Contemporary we did a lateral release, a duplication of the medial joint capsule and distalization of the vastus medialis (Madigan procedure). At the end of the procedure the patellae were correctly reduced (figure 3,4). During the following year the mechanical axis improved, was very good on the left side but remained still in valgus on the right side. The patellae were reduced, but the right has tendency to lateralize. After 1,5 years from the first surgical procedure we removed the tibial eight-plates and re-positioned the right femoral plate (figure 4). During the following 6 months the boy had perfect function on the left knee, but subsequently the right patella dislocated and he complained permanent pain.

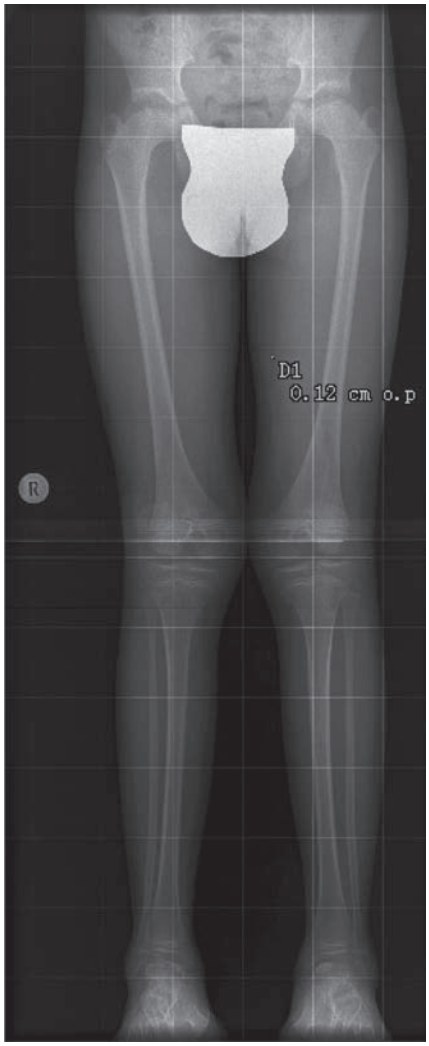

Figure 1. Age 10 years: valgus of the knee and subluxation of the right patella

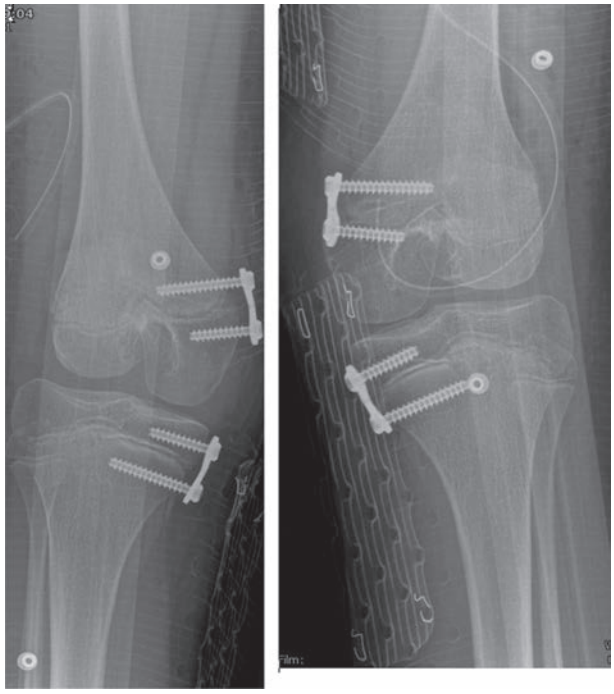

Figure 3. Plates on both knees for temporary hemiepiphyseodesis, lateral release and medial capsule duplication con distalization of the vastus medialis (Madigan) and reduction of the patellae. Age 13 years

It was mandatory for us to get a perfectly reduced patella also on the right knee. So at age 15 we re-operated the boy. The procedure was: lateral release of the patella, correction of knee axis with femoral supracondylar varus osteotomy. The patella still remained in a slightly lateral position. So we medialized the tuber-

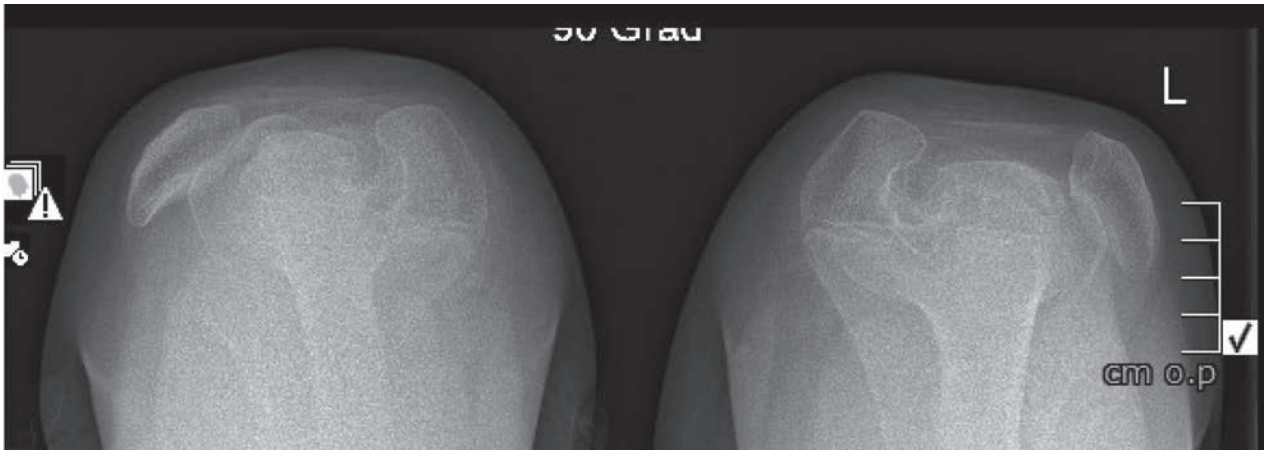

Figure 2. Both patellae are dislocated laterally (age 13 years)

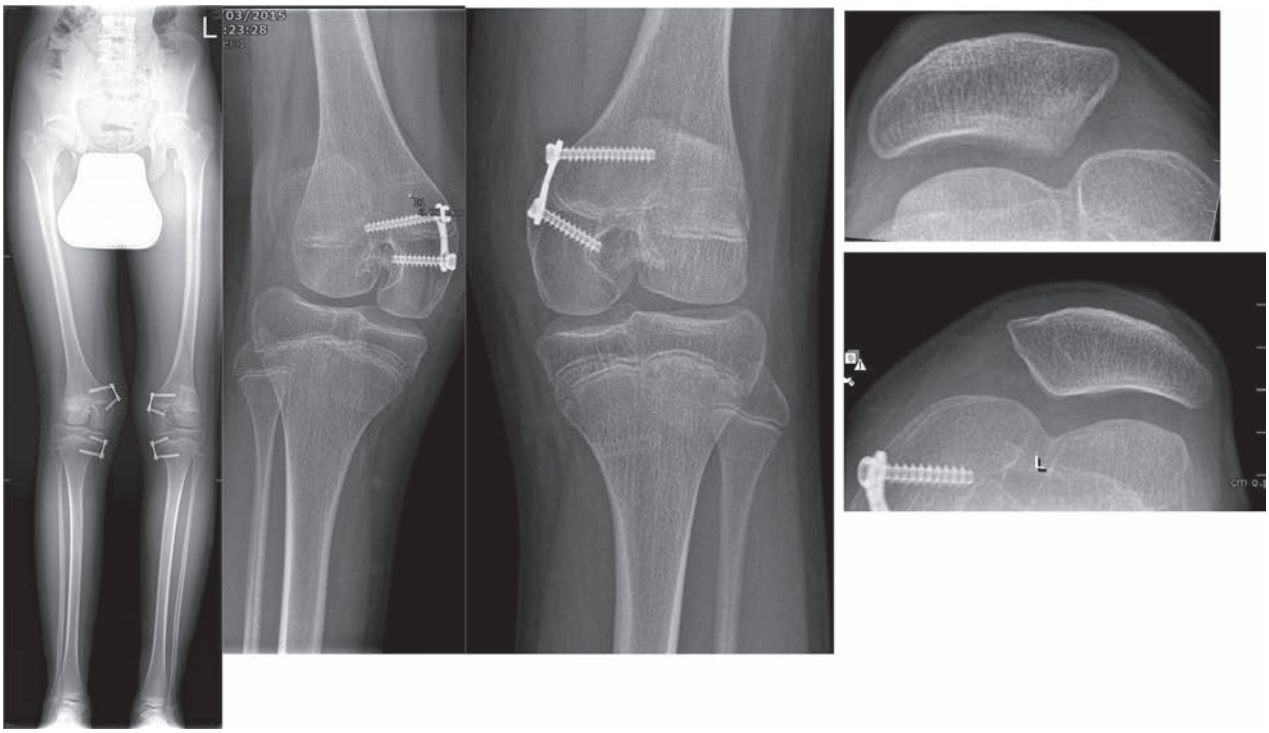

Figure 4. After 1,5 years (age 14,5 years) with hemiepiphyseodesis the mechanical axis improved, we removed the tibial eight plates and re-positioned the right femoral eight-plate. Good clinical function, but right patella has still tendency to lateralize

ositas tibiae until the patella movement in extension-flexion was perfectly aligned (figure 5). Clinically the boy had a perfectly centered patella also on the right knee, persistence of light extension deficit and absence of pain. During the last year of growth the boy developed varisation of the rigth knee (figure 6) and at age 16,5 (closed growth plates) we corrected definitely the deformity with femoral valgus and extension osteotomy (3.5 plate) see figure 7.

At 17 years the boy presented a complete knee function, stable knees (mediolateral) and absence of pain. The patellae were perfectly aligned without any tendency to lateralize or medialize during knee function (figure 8).

Discussion

We presented a boy with spondyloepiphyseal dysplasia. The scoliosis was completely controlled by a Cheneau brace. The most difficulties we encountered were a progressive valgisation of the knees with patella dislocation. On the left knee a soft tissue procedure (modified Madigan) with temporary hemiepiphyseodesis produced a correctly aligned knee with reduced patella.

On the right knee the soft tissue correction and growing correction through the hemiepiphyseodesis wasn't sufficient to maintain the patella reduced and to correct the valgus. When we tried to re-position the plate on the medial right femur the screw hasn't enough stability in the small condyle, so we corrected the axis by an varus osteotomy of the distal femur. The pathologic traction of the patella tendon pulled the patella still lateral with the risk of dislocation. The boy was 15 years old and with open growing plates: there is contraindication for osteotomy of the tuberositas tibiae. Partial tendon detachment wasn't expected to gain satisfactory medial traction, full detachment would had quite the same damage to the tuberositas tibiae and knee like the osteotomy. Therefore we executed medial sliding osteotomy of the tuberositas tibiae.

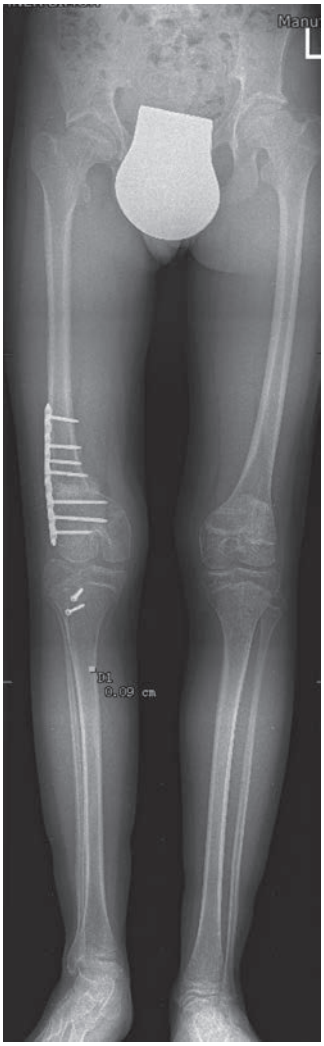

Figure 5. Femoral varus osteotomy with 3.5 plate. Medialisation of the tuberositas tibiae (age 15 years)

This produced a stable and correctly aligned patella. Afterwards the boy hasn't anymore patella dislocations and was painfree. However in the remaining year of growth the knee developed a varus deformity and with closed growing plates we brought the knee axis to a more physiologic value by supracondylar valgisation and extension osteotomy. We associated extension correction because the boy had still some degrees of extension deficit on the right knee, not fully corrected after the patella alignment. The clinical results were excellent: full range of movement of the right and left knee, absence of pain, walking without limping. Fortunately the tuberositas osteotomy didn't produce any negative aspect.

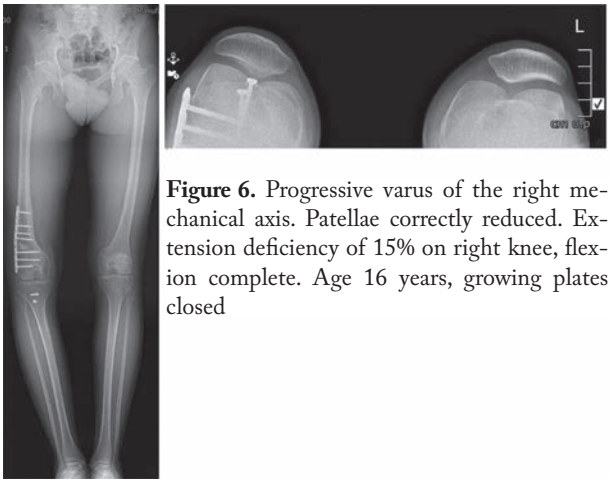

Figure 6. Progressive varus of the right mechanical axis. Patellae correctly reduced. Extension deficiency of 15% on right knee, flexion complete. Age 16 years, growing plates closed

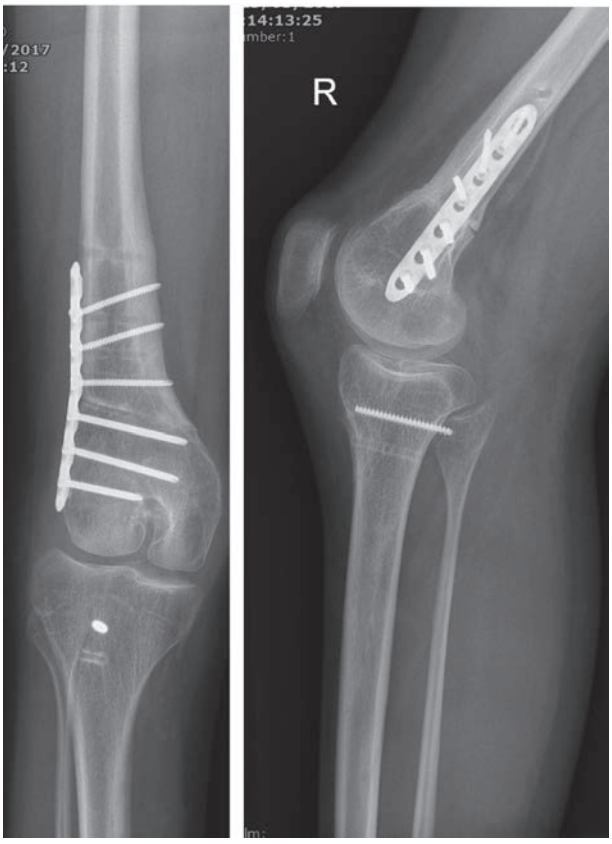

Figure 7. Femoral valgus and extension osteotomy. Age 16,5 years

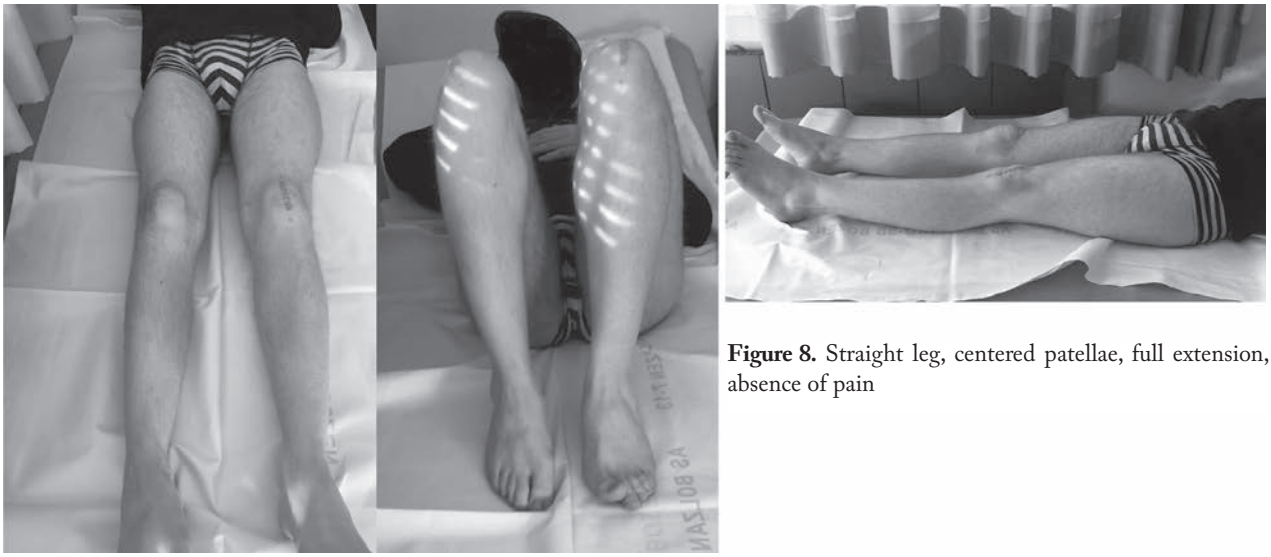

Figure 8. Straight leg, centered patellae, full extension, absence of pain

Conclusion

The patella dislocation and valgus deformity in this case of spondyloepiphyseal dysplasia required on the left knee a soft tissue recosntruction and simple hemiepiphyseodesis. The right knee was completely different: soft tissue procedures and hemiepiphyseodesis weren't sufficient and bony procedures were necessary. The osteotomy of the tuberositas tibiae produced a stable and correctly aligned patella.

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

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Case report

Hypersensitivity pneumonia and HIV infection in occupational settings: a case report from northern Italy

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Summary. We describe a case of relapsing hypersensitivity pneumonitis (HP) manifesting as a reconstitution inflammatory syndrome (IRIS) in a HIV infected patient receiving antiretroviral therapy (HAART). The patient, who works as a farmer since the early 20s, was diagnosed with HP at age 23: after an initial steroid therapy, a long lasting clinical regression followed. At age 32, HIV positivity was diagnosed, with HAART starting only at age 38 (initially, lamivudine 300 mg/daily + zidovudine 300 mg b.i.d.). In the following 15 years, CD4+ count remained <500 cells/μL until therapy was shifted to ritonavir 100 mg b.i.d + fosamprenavir 700 mg b.i.d. A six-months long increase in the CD4+ count (>600 cells/μL) with undetectable viral load then followed. Eventually, the patient developed cough and slowly worsening dyspnoea. Laboratory exams (serum T cell lymphocyte count 83%, CD8+ 45-51%; serum IgG for *M faeni*=78 mg/L and *P notatum* >200 mg/L) and high-resolution computer tomography (HRCT) were compatible with relapsing HP. The working tasks were modified avoiding any contact with allergens, then achieving a 6 months long clinical regression. Detectable HIV load (62 copies/mL) was identified at follow-up, and emtricitabine 200 mg/tenofovir disoproxil fumarate 245 mg s.i.d. was added to HAART. Respiratory involvement newly relapsed. HAART was shifted to emtricitabine 200 mg/tenofovir disoproxil fumarate 245 mg s.i.d. and raltegravir 400 mg b.i.d. Within several weeks, signs and symptoms resolved almost completely (peripheral oxygen saturation >95%: CD4+ count remained >600 cells/μL with CD8+ count steadily <50% and CD4+/CD8+ ratio >55%). (www.actabiomedica.it)

Key words: immune reconstitution inflammatory syndrome, HIV infections, Farmer's lung, alveolitis, extrinsic allergic, occupational medicine

Introduction

Hypersensitivity pneumonitis (HP), also known as extrinsic allergic alveolitis (EAA), is a complex syndrome resulting from respiratory exposure to various antigens, such as animal, insect, bacterial, protozoal and fungal proteins, and low-molecular-chemical compounds (i.e. isocyanates, zinc, dyes), triggering an exaggerated immune response with subsequent involvement of small airways and lung parenchyma (1-4). Farmer's lung is a well-known occupational disease, being the classical and most studied example of HP/EAA (4). Even though available data are significantly heterogeneous, it is estimated that 0.5 to 3% of all farmers will eventually develop HP/EAA during their lifetime (5), with an incidence of 0.9 cases/100,000 person-year (6).

The pathogenesis of HP/EAA is poorly understood, resulting from a combination of immune-complex-mediated (type III) and T-cell mediated (type IV) hypersensitivity reactions in susceptible people (7). In particular, T-cell-mediated immune response is suspected to be the cornerstone of the HP/EAA pathogenesis (7).

T-cells are among the specific targets of the Human Immunodeficiency Virus (HIV). As HIV can directly infect and kill cells directed against specific antigens, leaving decreased numbers of cells available to participate in host defence, it eventually impairs the metabolic or secretory functions of effector cells. As a consequence in patients with advanced HIV, HP/EEA should be very infrequent (8-9). In the last decades, many HIV patients have received highly active anti-retroviral therapy (HAART) and experienced long-lasting improvements in their immune status that ultimately allow a better quality of life. Therefore, HIV patients receiving HAART should be more prone to professional exposition to HP/EAA associated antigens, and the restoration of the immune system should in turn raise the possibility that such exposure may elicit associated hypersensitivity reactions (9).

However, only one case of HP/EAA in HIV patients has been reported (9). Moreover, no case at all has been reported from the WHO European region, where estimated prevalence of adult HIV infection ranges between 0.2% (central Europe) and 0.7% (Eastern Europe) (10), and the relatively easy access to up-to-date anti-retroviral therapies should bring at least some serendipitous associations, making patients with HP/EEA+HIV a far from infrequent report for occupational physicians.

Case history

The patient is a 54 year-old male farmer. He started working 14 year-old at the parent's farm in Northern Italy, a still active medium-size mixed enterprise, with large cereal and vegetable cultures producing the fodder to fed around 400 milk and beef cattle. During his early 20s, the patients performed directly all tasks associated with animal feeding, in particular managing the stores of hay, grain and fodder.

At the age of 23, he suddenly developed a flu-like syndrome, with fever, chills, myalgia and dry cough. The symptoms were elicited by the managing of the stable, worsened during the first hour of the working day and substantially improved at the end of the working shift. Eventually the patient developed progressive weight loss and dyspnoea, with a late stage of severe breathlessness and cyanosis. A chest X-ray demonstrated a patchy airspace disease, with numerous ill-defined small opacities, sparing apices and bases. Laboratory exams showed high level of IgG, detecting specific serum antibodies for *Micropolispora faeni* and *Penicillum notatum.* A diagnosis of HP/EAA was then proposed. After a prolonged therapy with i.v. steroids, he experimented a significant clinical regression. Since this episode, the patients returned to his daily tasks, all stable tasks performed wearing a filtering facemask. Initially, he achieved a substantial control of clinical symptoms without immunosuppressive therapy.

At age 32, he developed a lymphadenopathy of inguinal region lasting more than 5 months: in the same time period, he lost approximately 10 kg in weight. Laboratory exams identified HIV positivity, with a retrospective staging CDC A2. For undisclosed reasons, the patient delayed starting the HAART until age 38, after the diagnosis of Kaposi sarcoma and subsequent restaging of the HIV infection to AIDS CDC stage C2. As the HAART was started (initially, lamivudine 300 mg/daily + zidovudine 300 mg b.i.d.) a steady state was reached, but the CD4+ count remained largely lower than 500 cells/μL. In April 2012 (CD4+ cell count = 366 cells/μL), therapy was shifted to ritonavir 100 mg b.i.d + fosamprenavir 700 mg b.i.d, achieving a slight but significant improvement in CD4+ cell count, steadily higher than 600 cells/μL, with persistent state of non-detectable HIV load.

Starting November 2012, during the annual health survey performed by the occupational physician, the patients complained that work shift associated cough and dyspnoea had recently reappeared, progressively worsening despite any significant modification of working habits or tasks. At physical examination, the patients showed tachypnea, mild tachycardia, and whole lung decreased fremitus, without crackles or any other pathologic respiratory sound. Oxygen saturation was stable to 92% at rest whereas pulmonary functions tests (Table 1) showed a pattern of obstructive disorder, non-reversible, with slight reduction of FVC (4.04 L) and more severe involvement of FEV1 (2.43 L, 62.3% of reference value) with a FEV1/FVC of 60%. Also FEF_{25-75} was involved, with a reduction to 1.05 $L*s^{-1}$ (26.1% of the predicted value). Suspecting a relapse of the HP/EAA, further evaluation was per-

Table 1. Time trend of pulmonary function tests values

		Reference	2011	2012	2013	2014
FVC	*L*	4.90	3.77	4.04	4.51	3.83
FEV1	*L*	3.91	2.70	2.43	2.80	2.27
PEF	$L^{*}s^{-1}$	9.21	7.65	7.32	8.00	8.11
FEV1/FVC	*%*	79.7	71.6	60.0	62.0	59.0
FEF25-75	$L^{*}s^{-1}$	4.03	1.97	1.05	1.29	0.99
DL_{CO} (adj)	$mL/mmHg^{*}s^{-1}$	30.8	-	-	23.4	18.2

formed. At the blood count, a persistent leucocytosis was found (WBC >10,000 cells/μL; 63-65% of them neutrophil granulocytes and 25-27% lymphocytes). T cell lymphocytes were at upper normal limit (83%) with a persistent increased representation of CD8+ T cells (45-51%). Specific serum IgG antibodies determinations showed abnormal high values for *M faeni* (78 mg/L, normal values <30 mg/L) and most notably *P notatum* (>200 mg/L; normal limits <30 mg/L). High-resolution computer tomography (HRCT) chest scans showed patchy ground-glass opacities and patchy areas of mosaic perfusion, more profuse at the apices, without evidence of nodular lesions (Figure 1). Eventually, the relapse of HP/EAA was confirmed.

Stating the underlying immunodeficiency status and the previously unsatisfying response to HAART, the patient did not receive any steroid and the doses of the antiviral medications were not changed. Following a consultation with the occupational physician, his tasks were modified in order to avoid any contact with hay or cattle fodder, rather carrying out open-air field activities.

Following this initial intervention, during November 2013 the symptoms apparently regressed: chest physical examination was deprived of any clinical sign; oxygen saturation was stable at 95% at rest and also at pulmonary function tests showed a slight improvement (Table 1). However, DL_{CO} stated a moderate reduction of the alveolar transfer rate (23.4 ml/$mmHg^{*}s^{-1}$). Contextually, CD4 count showed a slight drop, decreasing to 566 cells/μL, associated with the evidence of a detectable HIV load (62 copies/mL in January 2014).

Stating the increased viral count, in February 2014 the antiviral therapy was improved adding a third medication (i.e. emtricitabine 200 mg/tenofovir disoproxil fumarate 245 mg s.i.d.). In the following months, viral load dropped to a non-detectable status and CD4+ count reached 661 cells/μL (March) and eventually 787 cells/μL (April). Unfortunately, HP/EAA symptoms newly relapsed: in May 2014, the patient contacted his occupational physician, who reported diffuse crackles at the upper lung fields, associ-

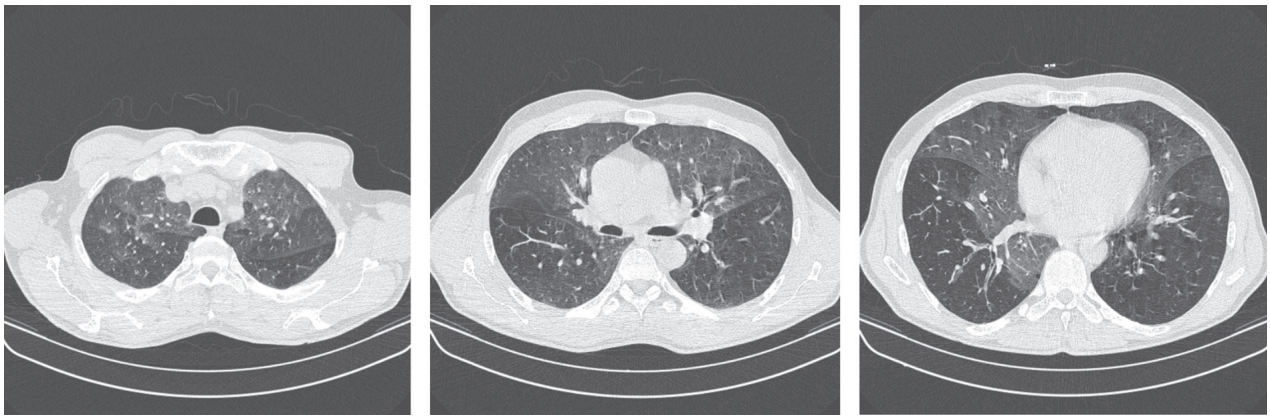

Figure 1. HRCT performed at first HP/EAA relapse, patchy ground-glass opacities and patchy areas of mosaic perfusion, more profuse at the apices, without evidence of nodular lesions. The image, without contrast media, evidenced some reactive lymph node (diameter <15 mm) at the lung ila

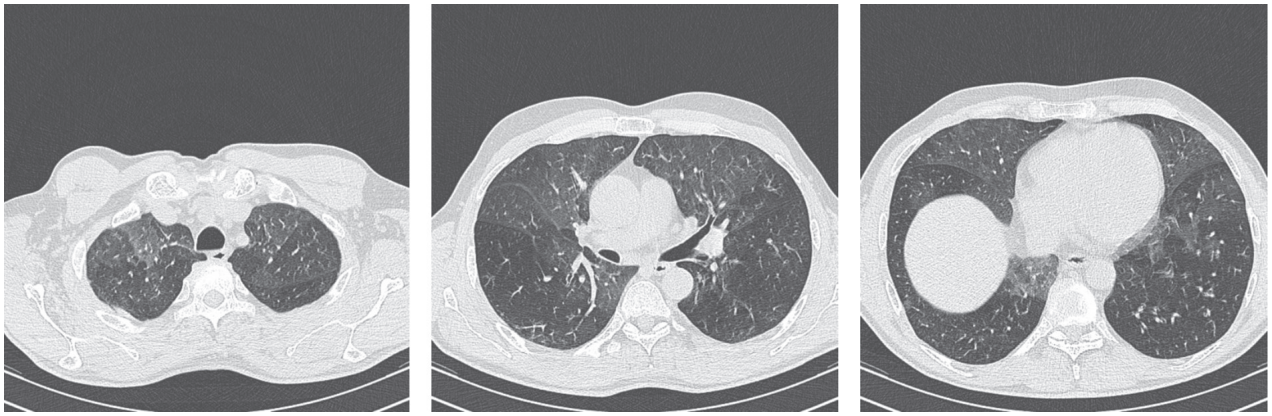

Figure 1. HRCT performed immediately after the second HP/EAA relapse, showing more diffuse patchy ground-glass opacities and patchy areas of mosaic perfusion, with some nodular lesions and air trapping.

ated with decreased peripheral oxygen perfusion (91% at rest) and severe involvement of pulmonary function tests (Table 1), with worsening obstructive pattern (FEV1 2.27 L, FVC 3.83 L, FEV1/FVC 59%) and a more severe deficit of DL_{CO} (18.2 ml/mmHg*s^{-1}, 59.1% of the predicted value). New chest scans were performed (Figure 2). The exam showed a more profuse pattern of ground-glass opacities, now associated with diffuse air trapping and some centrilobular nodules.

Because the apparently erratic relapses regularly followed improvements of CD4+ T cell count and the disappearing of the viral load, an association between the HAART and the HP/EAA was then suspected, similar to immune reconstitution inflammatory syndrome (IRIS) (11). The HAART was eventually modified with emtricitabine 200 mg/tenofovir disoproxil fumarate 245 mg s.i.d. and raltegravir 400 mg b.i.d. Within several weeks, symptoms resolved almost completely and peripheral oxygen saturation was stable >95%, despite the CD4+ count remained >600 cells/μL. Interestingly, with the new therapy, CD8+ count remained steadily <50%, with a CD4+/CD8+ ratio always >0.55. However, the patient was unable to restart stable activities, because direct, massive contact with hay and fodder almost immediately elicits symptoms such as a flu-like syndrome: after a consultation with his occupational physician, the work restrictions were then confirmed as a precautionary measure, with a definitive attribution to open-air field activities.

Discussion

In the case report we presented, both diagnoses of HP/EAA and HIV were previously stated, with a prolonged follow-up (up to 30 years). The clinical features of the two documented and more recent relapses of HP/EAA were consistent with the underlying diagnosis and showed a clear association with working environments housing the causative antigens (i.e. *M faeni e P notatum*) and task requiring high level professional exposures. The main clinical topic of this case report actually states on the relation between HIV/AIDS stage and HP/EAA activity, or more specifically on the consequences of HAART on an occupational disease with an immunological aetiology such as HP/EAA.

Acute or subacute HP/EAA in immune-depressed patients is unlikely: not only the total number of immune cells may be extensively affected, but also HIV-infected cells may shift their activity from immunostimulation to immunosuppression, such as a shift from Th1 to Th2 cytokine production. Moreover, HIV infection may interfere with the ability of circulating immune cells to migrate into the lungs and to interact with antigens in the alveolar spaces (8). On the other hand, insurgence of HP/EAA during HAART was previously described (9), and is consistent with an improved status of the immune system.

We think that point should be particularly stressed. The patient had an early diagnosis of HP/

EAA, with prolonged survival despite no significant drug therapy was established after the first hospital admission: prompt identification of inciting agents in HP/EAA may increase the overall survival up to a median 18.2 years (12), but the very long "honey-moon" experienced by our patients is in fact remarkable. We suggest that HIV infection, hypothetically following HP/EAA insurgence of no more than 5 years, had undermined the immune system, actually "freezing" its natural history. When HAART re-established a satisfactory T cell count, not only CD4+ cells were involved: CD8+ lymphocyte count has also been shown to increase when patients respond to HAART and CD8+ are well known effector agents of tissutal events associated with HP/EAA. A still low-level occupational exposure became therefore sufficient to reactivate the natural history of HP/EAA, with subsequent relapses every time CD4+ cell count became enough to trigger CD8+ activity. Even though the T-cell count did not show a very dramatic increase after the application of HAART, particularly in the second episode of HP/EAA reactivation, this presentation is somehow similar to cases of profound, pathological inflammatory reaction frequently identified in patients receiving HAART and previously housing either subclinical or previously recognized microbes, or immune reconstitution inflammatory syndrome (IRIS) (11). IRIS is usually described in the settings of Mycobacterial of cryptococcal infections, but also some autoimmune disease such as Graves diseases were described (11).

In conclusion, availability of effective treatment for HIV infection has changed many aspects of the disease, and also the perspective of the occupational physician: not only patients are surviving longer, but they also retain a prolonged active, working life. If the priority of the occupational physician in the '80s and early '90s was to restrain every possibility of HIV infection in the working settings, now he must be aware that HIV/AIDS patients may interact in new and in some way unpredictable term with their working environment. In the present day context, where atopic professional diseases are acquiring the status of a world-wide priority in occupational health settings, HIV/AIDS patients in HAART should be strictly monitored, because every slight improvement in the immune status may elicit severe clinical consequences, potentially undermining the fitness to work or, more critically, the labile health status of HIV+ subjects.

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

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Case report

Everything in the "right" place: multifocal transient ST segment elevation in patient with single coronary artery arising from the right Valsalva sinus

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Summary. Single coronary artery (SCA) is a rare coronary anomaly that occurs with an incidence of 0.024%. We report the case of an 83-year-old woman with a Lipton's type 3 SCA, which is the rarest anomaly within this group, occurring only in the 0.004% of general population. The clinical presentation of this patient was chest pain at rest with multifocal transient ST segment elevation as a marker of multifocal ischemia secondary to severe three vessels coronary artery disease (CAD). This patient was proposed for coronary artery bypass grafting (CABG) with an excellent mid-term outcome. (www.actabiomedica.it)

Key words: coronary anomaly, coronary artery disease, congenital heart disease, ischemic heart disease, cardiac surgery, chest pain

Case presentation

We report the case of an 83 years old woman with hypertension, dyslipidemia and diabetes that had complained chest pain at rest for several months. Her physical examination, electrocardiogram (ECG) and transthoracic echocardiography (TTE) at rest were unremarkable. Stress-echocardiography was scheduled to assess for inducible ischaemia.

On the day of stress echocardiography, a few days after suspension of beta blocker, the patient was symptomatic for typical angina at rest. During chest pain ECG showed ST segment elevation in the anterior leads (figure 1a) with TTE evidence of apical and anterior hypokinesia. Complete regression of symptoms, ECG ST-elevation and segmental kinetic abnormalities were observed with sublingual nitrate and intravenous beta-blocker administration. Troponin dosage was within the limits. Dual antiplatelet therapy was started in addition to Fondaparinux, statin and intravenous beta blocker that was subsequently switched to oral administration and adequately titrated.

The patient underwent coronary angiography (figure 2), that demonstrated a SCA arising from a single ostium in the right coronary sinus, branching into left anterior descending artery (LAD), left circumflex artery (LCX) and right coronary artery (RCA). The SCA presented significant ostial stenosis (80%) with evidence of some thrombus inside (Ellis type B2); significant stenosis were also present in the proximal segments of LAD, LCX and RCA.

Due to the severity and multifocality of the atherosclerotic lesions of the patient's SCA, involving ostium and proximal segments of the three coronary arteries, CABG was scheduled, suspending Ticagrelor and starting continuous infusion of Tirofiban. During the following days of hospitalisation the patient remained asymptomatic for angina and breathlessness. On the day

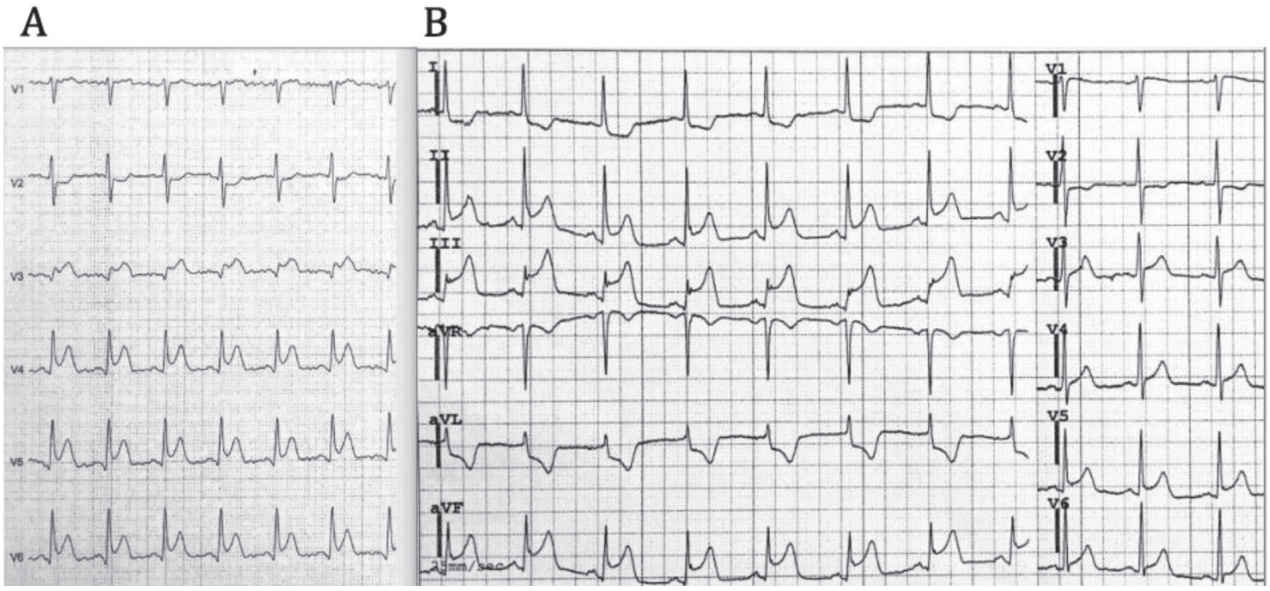

Figure 1. Electrocardiogram of the patient at rest on the first day of clinical observation demonstrating a transient ST segment elevation in the anterior leads (1a) and on the day of surgical intervention with a transient ST segment elevation in the inferior leads (1b)

of the intervention, Tirofiban infusion had been suspended six hours before. Subsequently, she complained the onset of nausea and chest pain with ECG evidence of ST segment elevation in inferior leads (Figure 1b). Therefore, timing of the intervention was speeded up and the patient underwent emergent CABG.

Triple venous coronary artery bypass graft was carried out in absence of peri-procedural complications and with an excellent short-term in-hospital outcome. At 6 months follow-up the patient is fine, without symptoms and in hemodynamic stability.

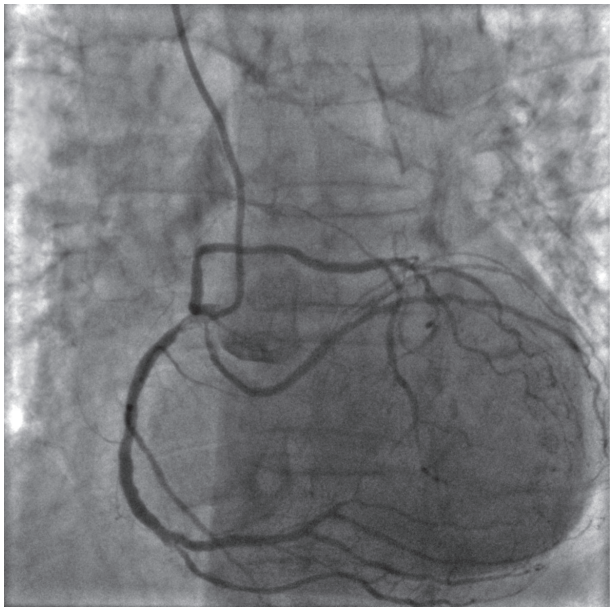

Figure 2. Coronary arteries angiography demonstrating a SCA arising from the right coronary sinus branching into LAD, LCX and RCA with a severe three vessels disease with a prevalent proximal atherosclerosis

Discussion

In 1979 Lipton et al. sub-grouped SCA into three categories (1). Type 1: a single vessel that follows the course of a normal left coronary artery (LCA) or RCA; type 2: LCA arises from the proximal portion of RCA or vice-versa; type 3: one single trunk originating from the right sinus of Valsalva from which LAD, LCX and RCA arise separately.

Our patient had a type 3 SCA which is the rarest anomaly in this group occurring only in the 0.004% of the general population and the clinical presentation was very unusual.

Indeed clinical presentation of coronary arteries anomalies is usually quite different and a wide spec-

trum of severity can be covered: it is often a casual finding in totally asymptomatic patients, or it can show up with stable angina, chest pain at rest (2), with syncope or sudden cardiac death (SCD); the latter is the most feared clinical expression of SCA and it is usually associated with the anomalous origin of the coronary artery from the opposite side of the aorta (ACAOS), involving left anomalous coronary artery arising from the opposite sinus (L-ACAOS) in 57% of cases and right anomalous coronary artery ACAOS (R-ACAOS) in 25% of cases (3-5).

This types of anomaly have been evaluated with IVUS imaging, demonstrating an intramural proximal intussusception of the ectopic artery at the aortic-root wall (3).

Clinical management of SCA is not well established but much depends on the clinical presentation of the patient. There is no standard treatment for isolated SCA without atherosclerotic coronary artery disease, even if much attention must be given to young athletes with this anomaly above all in ACAOS cases that are more often related to SCD. In stable conditions screening tests should be performed in the suspicion of a coronary artery anomaly, in particular in young people; several imaging techniques could be used, above all cardiac magnetic resonance (CMR); in comparison transesophageal echocardiography (TOE) is more invasive and less accurate, whereas cardiac tomography (CT) results in more precise imaging but the need of ionizing radiation is unacceptable in children and young people.

In our patient SCA was associated with severe coronary atherosclerosis of the ostium and proximal segments with simultaneous multifocal plaque destabilisation , thus leading to significant ischemic symptoms with evidence of multi-leads transient ST segment elevation.

Due to the complexity and severity of the pathology and the multifocal atherosclerotic lesions of the patient's SCA, surgical treatment was chosen with a very good outcome for the patient.

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

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Letter to the editor

"Medicine does not have to cure the poor". The thought of Giovanni Bianchi (1693-1775)

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The Report "Universal health coverage" of the Director-General of the WHO indicates that "at least half the world's population still lacks access to essential health services; some 800 million people spend more than 10% of their household budget on health care; almost 100 million people are pushed into extreme poverty each year because of out-of-pocket health expenses" (1).

World Health Statistics of 2018 indicate that each year, billions of dollars are spent on research and development into new or improved health products. However, low-income countries received only 0.3% of all direct grants (2).

Children from disadvantaged environments and exposed to a range of early childhood adversities are at increased risk for chronic health problem (3, 4).

Does medicine have to cure everyone in need? Today, the debate on this issue is characterized by different positions that are confronted with the limits of the laws of the economy and bioethical principles in order to trace the boundaries between what can be offered and to whom, compared to what we must renounce to offer. Socio-economic inequalities are linked with unequal exposure to social, economic and environmental risk factors, which in turn play an integral role in influencing on health inequalities (2).

The doctor Giovanni Bianchi (Rimini, 1693-1775), one of the great Italian doctors of his time, had no doubts. He was convinced that it was futile and unjustified to treat poor people and to admit them to hospital. No one should have to invest medical knowledge, labour, and money in the poor. He was satisfied in asserting his thoughts especially in his writing; the doctor should let the poor die and to only take care of people of rank, nobility or wealth. He had written this several times. In particular, in a letter dated November 13th 1759 to Leopoldo Caldani (1725-1813), he considered the inoculation of smallpox for the poor who, he believed, represented an unjustified burden on the State, and that it would have been better to let them die. "Here we have too many insignificant unemployed people, that serve as a burden on the State, so I think we can call on providence that many of them perish, as it would be dangerous to nurse their health in a hospital" (5). Despite breathing the atmosphere of enlightenment, the Italian doctor, also aware of the teachings of Bernardino Ramazzini on occupational diseases, did not seem to observe the dictation of Hippocrates and for this reason he disgusts us as this, in our eyes, is deplorable insensitivity. But, he was not the only doctor to appear cruel. Probably, the same insensitivity was shared by other doctors and it was also found to be an abundant opinion in other powerful people of that time.

From this, we know that a physician, through ignorance or indifference, was willing to let labourers die and not grieve. But if a person of rank succumbs to death in his hands, he is inconsolable and spends days justifying and consoling himself. In the message for the Second World Day of the Poor on the 18th November 2018, Pope Francis remembers "the poor hear voices scolding them, telling them to be quiet and to put up with their lot. These voices are harsh, often due to fear of the poor, who are considered not only destitute but also a source of insecurity and unrest, an unwelcome distraction from life as usual and need to be rejected and kept afar" (6).

Is the logic of exclusion just a problem of the past? Do medical practioners of today have the courage and the strength not to appear as equally insensitive?

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Review

Autoimmune diseases in Turner syndrome: an overview

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Summary. Turner syndrome (TS) results from a sex-chromosomal anomaly characterized by presence of one normal X chromosome and the loss of the second X-chromosome in phenotypic females. Autoimmunity has been recognized as one of the more prominent characteristics of TS. The risk of autoimmune diseases in patients with TS is approximately twice as high as in the general female population. The spectrum includes, Hashimoto's thyroiditis, coeliac disease (CD), type 1 diabetes (T1DM), alopecia areata, inflammatory bowel disease, juvenile rheumatoid arthritis and some cutaneous disorders as vitiligo and Halo nevus. This review will address the autoimmune disorders associated with TS, their pathophysiologic mechanisms and clinical characteristics. (www.actabiomedica.it)

Key words: Turner syndrome, autoimmune disorders, pathophysiologic mechanisms, clinical characteristics

Introduction

Turner syndrome (TS) results from a sex-chromosomal anomaly characterized by presence of one normal X chromosome and the loss of the second X-chromosome in phenotypic females (1). The initial description by Henry Turner in 1938 included short stature, sexual infantilism, cubitus valgus and pterygium colli (2). The phenotype includes short stature, primary ovarian failure, some physical features resulting from consequences of fetal lymphedema and skeletal abnormalities. Congenital cardiovascular defects, osteoporosis, endocrine and metabolic disorders and hearing loss are recognized contributors for increased morbidity and mortality and decreased life expectancy in this syndrome (3).

Increased prevalence of autoimmunity in women

In general, autoimmune diseases are more common in women than men and the explanations remain uncertain. Estrogens seem to impact the course of human autoimmune disease. Pregnancy has also been suspected of contributing to excess autoimmunity in women explained with retention of allogenic fetal cells (4). Another factor implicated in excess autoimmunity in women involves the process of X chromosome inactivation, wherein one of the two X chromosomes undergoes inactivation or transcriptional silencing during early embryonic development. This typically results in tissue mosaicism in which approximately 50% of cells express the maternally-derived (XMat) and 50% express the paternally-derived (XPat) X chromosome. It has been proposed that X chromosome inactivation may be skewed during thymic development resulting in predominant expression of only one set of X chromosome encoded self-antigens. This may lead to inadequate thymic deletion of autoreactive T-lymphocytes, which in turn leads to impaired "self" antigen recognition and tolerance. The risk of initiation of an autoimmune reaction would be enhanced if such autoreactive T cells encounter XPat or XMat specific antigens in peripheral tissues (5, 6).

Mechanism of autoimmunity in women with Turner syndrome

Autoimmunity has been recognized as one of the more prominent characteristics of TS (7, 8). The risk of autoimmune diseases in patients with TS is approximately twice as high as in the general female population (9). The increased risk of autoimmunity in patients with TS has also been attributed to X-chromosome haplo-insufficiency, maternal origin of the X-chromosome, excessive production of pro-inflammatory cytokines (IL-6), decrease in anti-inflammatory cytokines (IL-10, TGF-β), or hypogonadism. The impact of three copies of genetic material on the long arm of the X-chromosome and an increased incidence of AD in girls with the iXq karyotype have also been suggested (10,11). The excess of autoimmune antibodies is likely to result from the X chromosome defects. It has been demonstrated that genes located in the X chromosome, including a major histocompatibility complex (MHC) locus in the long arm, are involved in regulation of the immune response and altered immune tolerance (12).

Moreover, discrete disturbances in both humoral and cellular immune responses have been reported and a genetic basis has been proposed, although not established uniformly (13). More recent data suggest that in Brazilian patients with TS, the PTPN22 C1858T polymorphism may be an important genetic factor predisposing to autoimmune disease risk (14). Another study from USA showed that autoimmune susceptibility in Turner Syndrome is due to an alteration in the expression of the X-linked FOXP3 gene. FOXP3 is important in the development of regulatory T cells, and complete loss of FOXP3 expression has been shown to result in severe autoimmunity (15).

Autoimmune diseases in Turner syndrome

Morbidity secondary to autoimmunity ranks among the more prominent syndrome-associated characteristics, where an estimated 50% of the middle-aged patients suffer from Hashimoto's thyroiditis, and the prevalence increases with age. Other diseases of possible autoimmune aetiology also prevail with an increased risk of coeliac disease (CD), type 1 diabetes (T1DM), alopecia areata, inflammatory bowel disease, juvenile rheumatoid arthritis, idiopathic thrombocytopenic purpura, psoriasis and vitiligo. Furthermore, an increased frequency of cobalamin deficiency was reported, although this was not shown as secondary to pernicious anaemia with autoantibody production (16).

1. Autoimmune thyroid diseases and thyroid autoimmunity

Thyroid autoimmune diseases are characterized by abnormal lymphocytic activation, directed against self-antigens, i.e. thyroglobulin (Tg) and thyroperoxidase (TPO). They encompasses Hashimoto's thyroiditis (HT), a predominantly T cell mediated disease and Graves' disease, characterized by a primarily humoral response and the presence of anti-thyroid stimulating hormone (TSH) receptor antibodies (17). The relationship between thyroid disease and TS was first suggested by Atria *et al.* (18) in 1948 when they reported the postmortem findings of a small thyroid gland with lymphocytic infiltration in a young TS woman. Most HT forms evolve into hypothyroidism, although at presentation patients can be without clinical hypothyroidism and present with subclinical hypothyroidism which is a biochemical condition characterized by serum TSH above the upper limit of the reference range and serum FT4 levels within the reference range (19, 20). Interestingly, regarding the putative influence of karyotype on clinical features, some studies reported an association between autoimmune thyroiditis and the X isochromosome karyotype (21).

In the general population, the diagnosis of thyroiditis is based on clinical evidence of thyroid dysfunction, whereas in patients with TS, functional evaluation is done periodically, regardless the clinical picture, which allows the detection of subclinical changes (13).

2. Celiac disease

Since the 1970s, several reports have indicated an association between TS and celiac disease. The incidence of CD increases 11-fold in TS (22). The prevalence of antiendomysial antibodies positivity detected by screening in TS is 4.2% (23). Reviewing the data

in the TS population, serological screening appears to be an effective method of identifying subclinical CD. Most of the patients diagnosed with TS who also have growth retardation do not respond to growth hormone therapy if they have coexisting CD. On the other hand, some of the patients with CD who have persistent growth retardation and pubertal immaturity despite a gluten-free diet are diagnosed with TS afterwards (24). The available data and publications indicate that screening for CD should be performed in patients with TS, and intestinal biopsy should be carried out in patients with positive results (25).

3. Type 1 diabetes mellitus

In a Danish study, Type 1 diabetes used to appear the most common AID associated with TS (26). The reason for the increased incidence of diabetes in TS women is probably due to deranged insulin secretion by mechanisms that are not entirely clear. It has been suggested that the abnormalities of the X chromosome may influence immune tolerance, leaving TS patients more susceptible to autoimmune disease (27).

4. Skin manifestations

TS has been associated with several cutaneous abnormalities including an increased frequency of pigmented nevi, but few reports consider nevi in detail. Halo nevus (HN) is clinically defined as a melanocytic nevus surrounded by a halo of depigmentation. Vitiligo, a dermatologic disorder characterized by the presence of depigmented patches on the skin, has been described in the list of cutaneous findings associated with TS. In contrary to the common belief, Halo nevus, rather than vitiligo, is the typical dermatologic finding of Turner's syndrome (28).

5. Inflammatory bowel diseases

Inflammatory bowel diseases (IBD) affects millions of people around the world and the peak of incidence occurs between 15 and 30 years old. Two chronic disorders represent this group of diseases: CD (Crohn's disease) and UC (ulcerative colitis). It is unclear why autoimmune diseases have an increased incidence in TS patients, but hormone therapy often used to treat these patients, seems to be a susceptibility factor to IBD occurrence (29, 30).

6. Rheumatic diseases and other immune-related conditions

Juvenile idiopathic arthritis (JIA) is an autoimmune condition that might be associated with Turner syndrome. The prevalence seems to be at least six times greater than would be expected if the two conditions were only randomly associated (31). Other investigators believe that it is important to consider the diagnosis of Turner's syndrome in girls with JIA, recognizing that characteristic radiographic findings such as metacarpal shortening are usually present. Conversely, suspicion of an underlying inflammatory arthritis is warranted in search for radiological findings consistent with JIA in girls with TS and joint symptoms (32). There are scarcity of literature addressing this association and the reports are relatively old.

Conclusions

In conclusion, autoimmune diseases are prevalent in patients with TS. Despite the importance of early detection and treatment of AD, literature reports are ambiguous, and studies related to girls with TS are very few. Further study of autoimmune disorders in people with Turner syndrome may contribute to the better understanding of mechanisms in the pathogenesis of autoimmune conditions more generally. Moreover, there are no current clear guidelines for management of the several autoimmune disorders in Turner's Syndrome.

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

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Review

Long-term effects and significant adverse drug reactions (ADRs) associated with the use of gonadotropin-releasing hormone analogs (GnRHa) for central precocious puberty: a brief review of literature

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Summary. Central precocious puberty (CPP) is defined as an early pubertal development that occurs before the age of 9 years in boys and 8 years in girls. It results from premature activation of the hypothalamic-pituitary-gonadal axis. Gonadotropin-releasing hormone agonists (GnRHa) have been the gold standard therapy for CPP for more than 30 years. These compounds have a high affinity for the pituitary LHRH receptor and are resistant to enzymatic degradation. Through continuous stimulation, GnRHa inhibit the pulsatile secretion of gonadotropin, resulting in hormonal suppression, cessation of pubertal development, and normalization of growth and skeletal maturation rates. The goal of therapy is to halt pubertal progression and delay epiphyseal maturation that leads to improvement of final adult height. There are no widely accepted guidelines for how long to continue treatment with a GnRHa for CPP, and individual practice varies widely. Furthermore, conflicting results have been published on the long-term effects of GnRHa therapy in patients with CPP. Therefore, we reviewed the current literature focusing our attention on the long-term effects and the significant adverse drug reactions (ADRs) observed during treatment with GnRHa in patients with CPP. Our review may provide the necessary data to enable clinicians to administer GnRHa in the safest and most appropriate way. Further studies are necessary to identify the mechanisms of development of potential adverse drug reactions related to GnRHa therapy in CPP. (www.actabiomedica.it)

Key words: precocious puberty, gonadotropin-releasing hormone analogs, long-term effects, significant adverse drug reactions (ADRs), Hartwig and Siegel severity scale

Introduction

Precocious puberty (PP) is one of the most common reasons for referral to pediatric endocrinologists. PP is defined as the development of secondary sexual characteristics before the age of 8 years in females and 9 years in males (1-3). The overall incidence of sexual precocity is estimated to be 1:5,000 to 1:10,000, with the female-to-male ratio being approximately 10:1 (1).

Central precocious puberty (CPP) results from premature activation of the hypothalamic-pituitary-gonadal (HPG) axis) unlike peripheral precocious puberty, where the HPG axis is not involved.

Although the precise mechanisms triggering the onset of puberty are unclear, the earliest known biochemical change during puberty is increased production of kisspeptin produced by arcuate nucleus and anteroventral periventricular area of the hypothalamus.

This step is critical to puberty initiation. Neurokinin B and dynorphin from the same neurons stimulate and inhibit the release of kisspeptin respectively, and hence these kisspeptin, neurokinin and dynorphin neurons have now been recognized to be central to puberty initiation (1-4).

In females, CPP more frequently is idiopathic while in boys is more likely to be due to a pathological source (1-3). Risk factors for CPP include a history of international adoption, as well as congenital or acquired CNS insults. Several genetic syndromes are associated with CPP (4).

Apart from recognized genetic syndromes, from 5.2% to 27.5 % of cases have been reported to be familial and segregation analysis has suggested an autosomal dominant transmission with incomplete sex-dependent penetrance (4,5). Currently, mutations in the kisspeptin system, MKRN3, and DLK1 have been identified in sporadic and familial cases of CPP. In familial CPP, *MKRN3* defects were found in about 30% of families while in patients with apparently sporadic CPP, *MKRN3* defects were detected in about 8% of cases. In theses cases, genetic counselling should be considered in affected patients and their families (6).

The earliest clinical manifestation of central puberty in girls is usually breast development (thelarche), followed by pubic hair (pubarche). The pubertal growth spurt typically occurs during Tanner stage II-III, with the first menstrual period usually occurring at Tanner stage IV. In boys, the initial clinical sign of central puberty is testicular enlargement and the pubertal growth spurt happens later than in girls (7).

Gonadotropin-releasing hormone analogs (GnRHas) are the treatment of choice for children with CPP. Treatment aims to halt physical maturation, to prevent an early menarche, to retard skeletal maturation, to improve final adult height, to avoid psychosocial/behavioural sequelae, and to relieve the parents of the associated anxiety (8-11).

Good predictors of height outcomes include younger chronological age (CA), younger bone age (BA), greater height standard deviation score for CA at initiation of therapy (11-14) and a higher predicted adult height using Bayley-Pinneau tables (15). A suppression of luteinizing hormone (LH) to < 3 mIU/mL in patients on GnRHa therapy may be a reasonable target in patients on GnRHa therapy (16).

Although GnRHa therapy appears to be both well tolerated and effective in pediatric patients; there are no widely accepted guidelines for how long to continue treatment with a GnRHa for CPP. Individual practice varies widely among endocrinologists. Furthermore, conflicting results have been published on the long-term effects of GnRHa therapy in patients with CPP. These included a higher incidence of polycystic ovary syndrome (PCOs), changes in body composition, metabolic profiles and bone mineral density (16-21). Moreover, short term side effects such as headaches, hot flushes, mood swings and injection site reactions (rashes, bruising and sterile abscess formation) have been reported in the literature.

Therefore, we reviewed the current literature focusing on the long-term effects and the significant adverse drug reactions (ADRs) observed during treatment with GnRHa in patients with CPP. As long-term studies of male CPP patients are scarce, this review mainly addresses female CPP patients.

Gonadotropin releasing-hormone analogs (GnRHa)

First synthesized in 1980, GnRHa desensitize and down-regulate GnRH-receptors, suppress gonadotropin secretion, and eventually reduce gonadal hormones to pre-pubertal levels (9,11,22,23).

Basically, the native GnRH molecule is modified at least at the glycine 6 position, where it is substituted by another amino acid resulting in a super-agonistic effect. Prolonged exposure of the pituitary to a GnRHa paradoxically results in inhibition of gonadotropin secretion.

In 1986, the first long-term study of daily GnRHa treatment in 27 children (21 female and 6 male), treated for 2-4 years, showed a reduction of growth velocity to pre-pubertal levels, improved the advancement of skeletal maturation, and increased the predicted adult height (PAH) (24,25).

GnRHa are available as rapid-acting or long-term depot preparations. The long-acting preparations available include: leuprolide, triptorelin and goserelin,

given every 3-4weeks or as a long-acting depot at 10 to 12-weekly intervals. The monthly (leuprolide 3.75 mg or triptorelin 3.75 mg) or 3-month depot leuprolide 11.25 mg are the most common formulation used to treat CPP as they cause a steady release of the drug without relevant side effects (1-4,8).

All these preparations are synthetic analogues of naturally occurring gonadotropin releasing hormone (GnRH) which possess greater potency than the natural hormone.

All depot preparations are available as lyophilized powder along with separate reconstituting fluid in a composite syringe. It is important to inject the preparation immediately after re-constitution, to avoid solidification and injection failure. Injection should always be administered deep intramuscularly, preferably in the gluteal region.

In the United States a histrelin implant that causes pubertal suppression for more than a year has been approved and successfully used. While short-acting intranasal preparations such as nafarelin are available for daily administration, these are less efficient and there are significant difficulties with compliance, which limit their use substantially as a first-line treatment.

In Europe, triptorelin depot is widely used at 28-day intervals, even though some authors have reported shorter frequency intervals of administration (21- 26 days). It is usually administered at a dose of 3.75 mg (approximately 60-75 μg/kg) for children weighting more than 20 kg; and a half dose has been employed in patients weighting less than 20 kg. Some authors have used higher doses (100-120 μg/kg/21-25 days) (26).

Leuprolide depot is used at different doses in Europe (3.75 mg/28 days) and in the USA (7.5- 15 mg/28 days) (21,27). The dose of leuprolide required for gonadal suppression is unclear, with higher doses employed in the USA (7.5 mg monthly) compared to European countries (3.75 mg monthly). This was addressed in a trial comparing the effect of 7.5 mg leuprolide monthly against 11.25 mg and 22.5 mg, 3-monthly, in girls with gonadotropin dependent precocious puberty (28). The study demonstrated that at 6 months, greatest suppression was observed in the 22.5 mg group, but the effects were similar at 1 year. Thus, the initial use of higher-dose leuprolide may be worthwhile, particularly in girls weighing more than 30 kg.

Results on goselerin depot (10,8 mg, 3 monthly) are mainly from the United Kingdom and limited to girls (56 females and 6 males) (29).

Long-term Effects

a. Linear growth during the treatment

A recent consensus document of 30 experts from Europe, the USA and Canada concluded that the efficacy of GnRHa in increasing adult height is undisputed only in girls <6 years old with early-onset CPP (9) but does not improve final height in girls beyond 8 years of age, and there is only modest improvement in final adult height (FAH) in girls aged 6-8 years (30,31). Carel et al. (32) also pointed out that continuing GnRHa treatment beyond 11 yr of age in girls did not improve FAH and could may potentially decrease it.

During treatment with GnRHa, it is frequently observed that height velocity decreases, even below pre-pubertal levels. The effects of GnRHa treatment on the growth hormone (GH)-IGF axis remain controversial. To compensate for the reduced spontaneous or stimulated secretion of GH and IGF-1 during GnRHa therapy, it would be logical to add recombinant human GH (rhGH) in combination with GnRHa.

Several groups have studied the effect of the addition of rhGH to GnRHa in children with CPP. The overall analysis of the data failed to indicate any benefit of combined therapy, while " individual reports suggested that in specific instances combined therapy may be beneficial in preserving or reclaiming growth potential and improving adult height " (33).

Nevertheless, a recent meta-analysis searched randomized controlled trials (RCTs) and clinical controlled trials (CCTs) adopting GnRHa therapy and GnRHa plus rhGH combination therapy to treat CPP girls. A total of six RCTs (162 patients) and six CCTs (247 patients) were included. Compared to the GnRHa therapy group, "the combination therapy group achieved taller final height, greater progression of final height compared with target height and larger height gains. No severe adverse effects to treatment were reported" (34).

b. Weight changes during treatment

Several reports have demonstrated that treatment with GnRHa in patients with CPP was associated with an increase risk of obesity but others have not confirmed these observations.

In summary, to date the reported results on changes in the BMI values of CPP patients before, during and after treatment are inconsistent. Table 1 summarizes the data reported in the literature from 1991 to 2019. Therefore, long-term prospective controlled research is required to evaluate the weight changes in these subjects.

c. Metabolic changes

Currently there is a relatively little research concerning changes in body composition and metabolic profiles in CPP patients following GnRHa treatment. It seems that in the normal-weight group there are no

Table 1. Review of body mass index (BMI) changes before, during and after GnRHa treatment

Authors and references	Results
Kamp GA et al. J Clin Endocrinol Metab. 1991;72:301-7.	The increased BMI SDS during treatment seems to be a transient phenomenon.
Boot AM et al. J Clin Endocrinol Metabol. 1998;83:370–3.	The Authors performed dual-energy x-ray absorptiometry (DEXA) before and during treatment with GnRHa in girls with CPP and early puberty. Their findings showed that BMI SDS, fat mass, and percent of body fat for chronological age increased during GnRHa therapy.
Heger S et al. J Clin Endocrinol Metab. 1999;84:4583-90.	Many CPP patients were obese prior to GnRHa treatment but experienced no changes in BMI SDS following treatment. The BMI SDS before treatment correlated strongly with the BMI SDS after treatment discontinuation.
Palmert MR et al. J Clin Endocrinol Metab. 1999;84:4480-8.	Obesity occured at a high rate among children with CPP, but did not appear to be related to long term pituitary-gonadal suppression induced by GnRHa administration.
Feuillan PP et al. J Clin Invest. 2001; 24:734-6.	The increased BMI, at initial presentation and during therapy, persisted after discontinuation of therapy and progressed to frank obesity.
van der Sluis IM et al. J Clin Endocrinol Metab. 2002;87:506-12.	After an initial increase of percentage body fat during treatment, percentage body fat decreased and normalized within 1 yr after cessation of treatment.
Arrigo T et al. Eur J Endocrinol. 2004;150:533-7.	23.8% of CPP patients were obese prior to GnRHa treatment but experienced BMI decreases after at least 2 years of treatment.
Paterson WF et al. Clin Endocrinol (Oxf). 2004;61:626-34.	The mean BMI SD scores of CPP patients increased from 0.93 to 1.2. The frequency of overweight increased from 41% to 59%, and the frequency of obese patients increased from 28% to 39%.
Traggiai C et al. Eur J Endocrinol. 2005;153:463-4.	The Authors compared 29 ICPP girls with 45 healthy girls with normal onset puberty. Regarding BMI SDS few changes were observed during the first year of therapy, while an increasing trend was observed at the end of therapy and a complete recovery after 2.5 years of the end of therapy.
Pasquino AM et al. J Clin Endocrinol Metab. 2008;93:190-5.	CPP patients maintained their previous BMI SDS during treatment regardless of the overall increase in BMI after GnRHa treatment.

(continued)

Table 1 *(continued).* Review of body mass index (BMI) changes before, during and after GnRHa treatment

Authors and references	Results
Glab E et al. Pediatr Endocrinol Diabetes Metab. 2009;15:7-11.	No significant correlation between overweight and obesity at the end of treatment and the duration of the therapy, and with the duration of CPP before introduction of GnRHa therapy was observed.
Magiakou MA et al. J Clin Endocrinol Metab. 2010;95:109-17.	No difference in the BMI SDS between GnRHa-treated group and a nontreated group was observed. Therefore, it appears likely that GnRHa treatment is not associated with an increase in fat mass
Ko JH et al. Horm Res Paediatr. 2011;75: 174–9.	The Authors assessed the percentage of body fat with DEXA method, at baseline and after one year of GnRHa therapy in 121 Korean girls and concluded that GnRHa therapy does not increase the prevalence of obesity in girls with CPP.
Yoon JY et al. J Korean Soc Pediatr Endocrinol.2011;16:165- 71.	BMI z-score increased from 0.26 ± 1.03 to 0.4 ± 0.89 during a year of GnRHa treatment.
Wolters B et al. Horm Res Paediatr. 2012;78:304-11.	Patients who were normal-weight at the start of the GnRHa treatment, exhibited an increase in BMI z-score (0.08±1.02 at baseline vs. 0.40±0.85 at the end of treatment vs. 0.41±0.89 at 6-month follow-up). In the overweight group, there was an insignificant change in BMI z-score (2.01±0.69 at baseline vs. 2.03 ± 0.54 at the end of treatment vs. 1.9 ± 0.51 at 6 months after the end of treatment).
Lee SJ et al. Chonnam Med J. 2012; 48:27-31.	BMI z-score of a Korean girl with CPP significantly increased from 0.58± 1.18 to 0.96 ± 0.83, after 18 months of GnRHa treatment.
Sorensen K et al. Eur J Endocrinol. 2012;166:903-10.	A year of GnRHa treatment increased BMI from 18.1 to 18.6 kg/m2.
Karamizadeh Z et al. Acta Med Iran. 2013;51:41–6.	GnRHa therapy cause central obesity and hyperlipidemia. The maximum weight gain of was observed at sixth months of therapy.
Gillis D et al. J Pediatr. 2013; 163: 532–6.	34 girls with CPP treated with a GnRHa were evaluated before, and the end of treatment until menarche. Changes of BMI-SDS was not significant in neither group.
Anik A et al. Indian J Endocrinol Metab. 2015;19:267-71.	GnRHa treatment did not induce significant changes in BMI z-score for chronological age, but it increased BMI z-score for bone age. The percentage of overweight/ obese CPP patients increased from 59.4% to 65.7%, after a year of treatment.
Arani KS and Heidari F. Int J Endocrinol Metab. 2015 July; 13(3): e23085. DOI: 10.5812/ijem.23085v2	The prevalence of obesity was significantly different between study groups at baseline and at sixth and 12th months of therapy (P = 0.11, P = 0.068, and P = 0.052, respectively).
Chemaitilly W et al. Clin Endocrinol (Oxf). 2016;84:361-71.	Obesity was more prevalent at the last follow-up than at the completion of GnRHa or the puberty onset (37,7%, 22,6% and 20,8%, respectively, P = 0.03).
Park J et al. Ann Pediatr Endocrinol Metab.2017; 22:27-35.	GnRHa treatment increased BMI z-score within a year of treatment, regardless of the subject's obesity status.
Arcari AJ et al. J Pediatr Endocrinol Metab. 2019;32:181-6.	An increase of BMI in girls with normal weight was observed.

changes in insulin resistance, whereas a tendency to develop an insulin resistance was detected in patients who at the start of the treatment were overweight or obese (Table 2). Different diagnostic criteria, race/ethnicity, age at follow-up, and potential for bias make comparison of studies difficult, but concern continues for long-term endocrine and metabolic outcomes (35,36). Therefore, long-term prospective controlled research is required to evaluate the changes in obesity and insulin resistance in subjects with CPP.

d. Bone mineral density (BMD) and bone markers

Although suppression of ovarian activity has been associated with BMD reduction during GnRHa treatment (37), recent studies have shown no changes in bone mineralization among CPP patients who had received 3 years of GnRHa treatment (38). Antoniazzi et al. (39) reported that although the BMD decreased during GnRHa treatment, this was reversible and preventable with calcium supplementation. Furthermore, restoration of BMD after cessation of treatment has been also documented (26). As in normal girls and adolescents, exercise and adequate nutritional intake would be helpful for bone mass formation in CPP patients.

Regarding bone turnover markers in CPP patients, the expression of carboxy terminal telopeptide of type 1 collagen (ICTP), a bone resorption marker, and procollagen type 1 C-terminal propeptide (PICP), a bone formation marker, increased prior to GnRHa treatment but decreased during a 6-month treatment period and stabilized after treatment. Bone age-adjusted bone turnover markers were also normalized 2 years after treatment cessation. On the other hand, a report indicated that no changes in age- and bone age-adjusted BMD-SDS was observed during GnRHa treatment (40).

In brief, the long-term BMD studies in CPP patients proposed that although BMD levels decreased during GnRHa treatment, the bone mass was sufficiently preserved after treatment.

e. Menarche, menstrual cycles and polycystic ovary syndrome (PCOS)

Regarding reproductive function, studies indicate that menstruation occurs on average 16 months after the treatment of CPP is withdrawn (with a variation of 2 to 61 months). Regular ovarian cycles occur in 60% to 96% of the patients, and infertility has not been reported (9,26). However, there are concerns that PCOs may occur more often in those with CPP than in those with normal puberty (41). The reported frequencies vary and conflicting data on the long-term risk of developing PCOS in conjunction with CPP remain (Table 3).

PCOS is observed in 5%-10% of women of reproductive age and is characterized by anovulation, hyperandrogenism, and polycystic ovaries (42,43). Severe

Table 2. Review of the metabolic changes reported in patients with precocious puberty treated with GnRHa

Authors and references	Results
Taşcilar ME et al. Turk J Pediatr. 2011;53:27-33.	An exaggerated elevation in trunk fat mass and insulin resistance (IR) in GnRHa-treated ICPP children was observed.
Sorensen K et al. Eur J Endocrinol. 2012;166:903-10.	Fasting insulin, first phase insulin release and mean plasma insulin during oral glucose tolerance test in CPP patients increased after a 52-week period of GnRHa treatment, whereas whole body insulin sensitivity index decreased, indicating an insulin resistance.
Park J et al. Ann Pediatr Endocrinol Metab.2017;22:27-35.	No changes were observed in QUICKI and HOMA-IR within a year of treatment in the normal-weight girls with CPP.
Arcari AJ et al. J Pediatr Endocrinol Metab. 2019;32:181-6.	GnRHa did not affect BMI, insulin index and lipid profile. However, an increase of BMI in girls with normal weight was observed.

Table 3. Review of PCOS prevalence in girls with precocious puberty before, during or after treatment with GnRHa

Authors and references	Results
Boepple PA. In: Savage MO, Bourguignon J-P, Grossman AB, eds. Frontiers in paediatric neuroendocrinology. Oxford, London,Edinburgh, Cambridge, Carlton: Blackwell. 1994: pp. 23–9.	PCOS was reported in approximately half of the patients treated with GnRHa.
Bridges NA et al. Clin Endocrinol (Oxf) 1995; 42: 135-40.	The prevalence of PCOS among CPP patients was 24%, compared with 2% in an age-matched control group.
Lazar L et al. Eur J Endocrinol. 1995; 133: 403-6.	A significant number of girls with CPP develop PCO-like syndrome at a relatively young age.
Baek-Jensen AM et al. J Pediatr.1998; 132:105–8	The Authors did not observe PCOS during or after treatment with GnRHa.
Heger S et al. J Clin Endocrinol Metab.1999; 84:4583–90.	No increased incidence of PCOS in GnRHa-treated patients with CPP compared with the normal population was reported.
Chiavaroli V et al. Eur J Endocrinol. 2010;163:55-62.	The prevalence of PCOS and hyperandrogenemia was significantly higher in GNRHa-treated adolescents than in untreated adolescents (36 and 14.5% respectively, P=0.04; 56 and 23.6% respectively, P=0.01).
Magiakou MA et al. J Clin Endocrinol Metab. 2010;95:109-17.	21% of subjects evaluated between ages 16 and 32 had PCOS, using the National Institutes of Health criteria.

insulin-resistant obesity, premature adrenarche, and sexual precocity in childhood are some of the known risk factors of PCOS (43,44).

In summary, the prevalence of PCOS among CPP patients varies depending on the characteristics of the patients, durations of treatment and follow-up period, and differences in the PCOS diagnosis standards. It is unclear whether this association is due to the hyperinsulinemia or premature adrenarche already present at CPP onset or a result of an abnormal hormonal response to GnRHa treatment (45). A comparison with a control group of CPP patients through a long-term evaluation from diagnosis to post-treatment adulthood is needed to determine the causative factors of PCOS in patients treated for PPC (45).

f. Psychosocial changes

One of the most common concerns about PP in girls is the potential for adverse psychological consequences. Numerous studies have reported an association between early normal puberty and adverse psychological, behavioural, and social outcomes in girls (46-51).

Although many studies have examined early maturity or puberty, little is known about psychosocial changes in girls with CPP receiving treatment with GnRHa (Table 4). The available results are reassuring regarding concerns of adverse psychological consequences of early puberty in girls. However, long-term prospective studies are needed in order to further elucidate the psychological impact of PP on girls and their mothers.

Adverse Drug Reactions (ADRs) associated with the use of GnRHa

Bone pain, micturition problems, hypersensitivity (itching, skin rash, fever), gynecomastia, flushing, depression, easy and quick to anger, headache, nausea, muscle pain, joint pain, excessive sweating, fatigue, sleep disturbances, pain at the injection site, predisposition to hypertension, and thrombosis are the adverse

Table 4. Review of psychosocial changes in girls with precocious puberty before and during treatment with GnRHa

Authors and references	Results
Xhrouet-Heinrichs D et al. Acta Paediatr. 1997; 86:808–15.	Some behavioral and affective characteristics were observed in girls with PP. During treatment with long acting triptorelin, problematic behavior and functioning decrease slightly, particularly in the few girls showing breast regression.
Officioso A et al. J Pediatr Endocrinol Metab. 2000 Jul;13 Suppl 1:835-9.	Ten adolescent girls aged 14 years treated for ICPP were evaluated. All the adolescents had a negative body image compared with age-matched controls and expressed a strong inhibition of their femininity. Their poor body image was reflected by their low self-esteem.A psychological support was recommended.
Mul D et al. Acta Paediatr. 2001;90:965–71.	The psychological evaluation did not reveal any consistent abnormalities in adopted children with early puberty. Treatment with GnRHa with or without rhGH did not increase emotional and behavioural problems in adopted children, nor was their self-perception decreased.
Zheng F et al. Zhejiang Da Xue Xue Bao Yi Xue Ban. 2008; 37: 289- 94.	The authors compared the psychological behavior of girls with ICPP before and after treatment by GnRHa. They found that the self-esteem scale, and body-esteem scale score in ICPP were significantly lower compared to controls (P <0.05).
Kim YJ et al. Ann Pediatr Endocrinol Metab 2013;18:173-8.	The psychological assessment did not exhibit a significant difference except with scores for sociability and behavior problems.
Choi MS et al. Ann Pediatr Endocrinol Metab. 2016; 21:155-60.	Patients with PP had distorted perception about their body image and breast development that seems to contribute to depression score.
Schoelwer MJ et al. Horm Res Paediatr. 2017; 88:347-53.	Girls with CPP completed psychological assessments at baseline and after 1 year along with their mothers. All girls were treated with GnRH analogs. Psychological measures were normal in all girls.

drug reactions (ADRs) observed in adults (52-57). In children, the available evidences show that GnRHa in general are safe and effective long- term (58,59). However, some significant ADRs in children treated with GnRHa for CPP have been reported (60).

ADRs are basically defined according to the World Health Organisation as: "any response to a drug which is noxious, and unintended, and which occurs at doses normally used in man for prophylaxis, diagnosis or therapy of disease, or for the modification of physiological function " (61).

Relevant studies indexed in Pubmed and Google Scholar were selected using the search terms: "precocious puberty/early puberty, GnRH analogue, GnRHa safety and adverse events". For the classification of ADRs severity we choose the Hartwig Siegel assessment scale (62).

a. Vaginal spotting/bleeding (Hartwig and Siegel severity scale: Level 1)

Continuous stimulation of the pituitary gland results in a short period of pubertal stimulation, followed by down regulation of GnRH receptors, pituitary desensitisation and reduced gonadotropin synthesis. Therefore, the first injection of GnRHa is associated with a transient surge in LH and FSH resulting in a transient increase in estradiol levels, which then rapidly drops following down regulation of GnRH receptor, usually within a fortnight (63).

This transient surge in estradiol may result in vaginal spotting/ bleeding, in a small number of female patients, following the first injection due to discontinuation of the estrogen support of the proliferative and stable endometrium.

Eight of the 28 (28.5%) girls, aged 6.5-11 years, with idiopathic CPP treated by Yeshaya et al. (64) every 28 days with an intramuscular depot GnRHa developed vaginal bleeding after GnRHa administration. Of these, prolonged vaginal bleeding of 11-13 days occurred in four girls, three recurrent episodes occurred in one during the second injection, and in one other girl the 4th episode occurred after 6 months of treatment. The episodes resolved spontaneously and necessitated no further treatment.

However, other researchers have suggested the use of an anti-androgen [cyproterone acetate, given (usually) for the first six weeks of therapy at a dose of 70 mg/m^2/day], or a prostanoid receptor antagonist or a co-injection of depot medroxy-progesterone acetate (MPA) with the first dose of GnRHa (65,66).

b. Local side effects (Hartwig and Siegel severity scale: Levels 1 and 3)

Local side effects, including pain at the injection site and flares usually are mild, although some may persist for several months and can leave significant scarring. Rare cases of subcutaneous nodules and sterile abscess (SAs) formation related to GnRHa, affecting the compliance with the treatment, have been observed (60, 67-69).

Lee et al (70) reported a prevalence of SAs formation in 4 out of 621 patients (0.6%) with CPP and early onset puberty, who were receiving monthly long-acting GnRHa (leuprolide acetate, triptorelin acetate) (70). In one patient, SAs occurred following leuprolide acetate depot therapy and also developed after a switching the treatment to triptorelin acetate depot. The fact that one patient had SAs formation following treatment with 2 different long-acting GnRHa can suggest that the cause could be attributed to the antibody formation against the same type of biodegradable polymers (lactic acid glycolic acid copolymer) present in the depot formulations (69,70).

c. Slipped capital femoral epiphyses (SCFE) (Hartwig and Siegel severity scale: Level 5)

Slipped capital femoral epiphyses (SCFE) occur mainly in boys in late childhood or adolescence. The incidence is 0.33/100,000 to 24.58/100,000 children 8 to 15 years of age. The single most significant risk factor for SCFE is obesity. Other risk factors include male sex, periods of rapid growth, and prior radiation therapy. The average age of onset is 11.2 years in females and 12.0 years in males. Approximately 25% (range: 8-50%) of cases are bilateral. Delay in the diagnosis of SCFE is associated with higher rates of complications, including femoral head osteonecrosis (71,72).

Five events of SCFE associated with GnRHa occurred in children during or shortly after the drug discontinuation (70,73,76). Inman et al. (76) suggested that a lack of adequate sex hormone exposure at a " critical period " of bone formation may result in a weakened epiphysis that becomes susceptible to slipping. In addition, the increase in growth velocity after

Table 5. Hartwig and Siegel severity scale

Level description
Level 1: An ADR occurred but required no change in treatment with the suspected drug.
Level 2: The ADR required that treatment with the suspected drug be held, discontinued, or otherwise changed. No antidote or other treatment requirement was required. No increase in length of stay
Level 3: The ADR required that treatment with the suspected drug be held, discontinued, or otherwise changed, and/or an antidote or other treatment was required. No increase in length of stay.
Level 4: Any level 3 ADR which increases length of stay by at least 1 day.
Level 5: Any level 4 ADR which requires intensive medical care.
Level 6: The adverse reaction caused permanent harm to the patient.
Level 7: The adverse reaction either directly or indirectly led to the death of the patient.

stopping GnRHa , subsequently results in a reduction of the shearing force needed for the displacement of the epiphysis.

d. Pseudotumor Cerebri (PTC) (Hartwig and Siegel severity scale: Levels 5)

Pseudotumor cerebri (PTC), also known as idiopathic intracranial hypertension, is a disorder with increased intracranial pressure and associated headaches, papilledema, vision changes, or pulsatile tinnitus in the setting of normal imaging and cerebrospinal fluid (CSF) studies. Children of both genders are affected equally before puberty (77). Males (aged 12 to 15 years) have an annual incidence of 0.8 per 100,000; females aged 12 to 16 years have an annual incidence of 2.2 per 100,000 (78). PTC can be classified as either primary (when there is no clear causal factor) or secondary to cerebral venous thrombosis or changes in the composition of the CSF. Proposed mechanisms involve the vascular, hormonal, and cellular systems. The first-line treatment is acetazolamide. The most concerning complication of PTC is permanent vision loss because of compression of the optic nerve secondary to elevated intracranial pressure (77).

Pseudotumor cerebri (PTC) secondary to use of leuprolide acetate is an extremely rare event with only two cases reported in the literature (79,80).

Summary of case 1 presentation reported in the literature (Reference 79)

A 9-year-old girl with PP was treated with leuprolide acetate (3.75 mg). After the 4th dose, she presented headache and hypertension (130-155/85-110 mmHg). There were no causes underlying the hypertension such as cardiac, renal, or endocrine. Neurological examination was normal except for bilateral papilledema. Cranial magnetic resonance imaging was normal and the orbital section of MRI revealed bilateral optic nerve enlargement. Cerebrospinal fluid (CSF) opening pressure was elevated. Triptorelin therapy was stopped and acetazolamide was started. The patient improved and the CSF pressure and fundoscopic examinations returned to normal (79).

Summary of case 2 presentation reported in the literature (Reference 80)

A 9-year-old girl with PP was treated with leuprolide acetate (3.75 mg). After 4 month, she complained of holocranial headache, transient visual obscuration followed by progressive visual loss. After 6 months, she persisted with holocranial headache and progressive visual loss associated with ocular deviation. Neuro-opthalmological examination revealed severe visual loss and bilateral papilledema. Cerebrospinal fluid (CSF) analysis showed opening pressure of 45 cm H2O. The most likely diagnosis was PTC associated with leuprolide acetate. Treatment was started immediately with oral acetazolamide and leuprolide was discontinued. Unfortunately, acetazolamide induced a metabolic acidosis. A ventriculoperitoneal shunt was performed to control intracranial pressure as an alternative to acetazolamide treatment. The follow-up of 18 months showed CSF pressure of 14 cm H2O, stabilization of visual acuity and resolution of papilledema (80).

e. Hypertension (HTN) (Hartwig and Siegel severity scale: Levels 5)

According to the instructions for GnRHa use, issued by the manufacturer, arterial hypertension is considered an infrequent complication (81).

Hypertension has been reported in girls with CPP (82-85) and in girls with gender dysphoria (86), likely due to loss of the vaso-protective properties of estrogens (87). The authors concluded that although estrogen depletion may play a role in the pathogenesis of triptorelin-induced HTN, this aspect should be further investigated. Furthermore, clinicians should be aware of the possibility, although rare, of HTN developing during triptorelin administration in childhood, specifically in patients at increased risk of HTN, such as those with Williams-Beuren syndrome (Table 6) (83).

f. Anaphylactic reactions (Hartwig and Siegel severity scale: Levels 5)

Anaphylaxis is defined by the European Academy of Allergy and Clinical Immunology (EAACI)

Table 6. Summary of patients with central precocious puberty developing arterial hypertension during GnRHa treatment

Authors and references	Results
Calcaterra V et al. Indian J Pediatr. 2013;80:884-885.	A 7-year-old girl with triptorelin-treated CPP, who developed reversible HTN with secondary concentric left ventricular hypertrophy, requiring transient antihypertensive therapy.
Siomou E et al. Pediatr Nephrol. 2014;29:1633–1636	A 10-year-old girl with a Williams-Beuren syndrome and CPP who developed HTN with triptorelin treatment. In that case, blood pressure totally normalized, without any anti-hypertensive medication once GnRHa was discontinued.
Palma L et al. J Pediatr Endocrinol Metab. 2018 Aug 8. pii: /j/jpem. ahead-of-print/jpem-2018-0210/ jpem-2018-0210.xml. doi: 10.1515 /jpem-2018-0210	A girl with CPP who developed HTN from treatment with GnRH-a (triptorelin). HTN subsided once triptorelin was interrupted. Consequently, the Authors hypothesized that the hypertension was related to triptorelin treatment.
Sifaki L et al. Front Pediatr. 2019 Mar 19;7:74. doi: 10.3389/ fped. 2019.00074.	A 10-year-old girl with CPP during treatment with triptorelin, developed an asymptomatic stage II HTN. Initial workup showed no renal, thyroid, or electrolytes abnormalities. A complete normalization of her blood pressure was obtained without any medication.

as a severe, life-threatening generalised or systemic hypersensitivity, characterised by its rapid onset with life-threatening airway, breathing and/ or circulatory problems (88).

The incidence of anaphylaxis in children worldwide varied widely, ranging from 1 to 761 per 100 000 person-years for total anaphylaxis and 1 to 77 per 100 000 person-years for food-induced anaphylaxis. Gender and ethnicity are demographic risk factors associated with anaphylaxis in children (89).

Whilst drug-induced anaphylaxis is more commonly reported in adulthood, less is known about the role of drugs in pediatric anaphylaxis. Antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs) are the main elicitors in drug-induced anaphylaxis in children. Anaphylactic reactions to GnRHa agonists are exceedingly rare (90-92).

Summary of case 1 presentation reported in the literature (Reference 93)

An 8-year-old girl who was diagnosed with CPP was receiving triptorelin acetate treatment uneventfully for 6 months. To evaluate the efficacy of the treatment, an LH-RH stimulation test with gonadorelin acetate was planned. Within 3 min after intravenous administration of gonadorelin acetate, she lost consciousness and tonic seizures began in her hands and feet. She was immediately treated with epinephrine (0.01 mg/kg; 1:1000), high flow supplemental oxygen (6-8 L/min), IV diphenhydramine (1 mg/kg), and IV methylprednisolone (1 mg/kg). Her vital signs recovered within 30 min. When her medical history was more deeply investigated, her parents recalled that there were several skin reactions along with pruritus after a previous gonadorelin acetate injection. A skin test with gonadorelin acetate was planned during the follow-up, however, it could not be performed due to the unwillingness of her parents (93).

Summary of case 2 presentation reported in the literature (Reference 70)

An 8.4-year-old girl with CPP was treated with triptorelin acetate depot SC injected at four-week intervals. Immediately after the sixth injection, she developed dizziness, headache, whole body redness, and chest tightness. Subsequently, she lost consciousness. Blood pressure (BP) could not be measured at that time. All the above symptoms were relieved within several minutes without any particular treatment. Anaphylaxis was considered to have occurred (70).

In conclusion, although the occurrence of anaphylactic reactions to GnRHa are very rare, it can have serious practical implications. Therefore, clinicians should be aware of the potential association of GnRH analogs with systemic reactions, should recognize that recurrent anaphylaxis may occur due to the long half-life of these therapeutic agents in tissue and recommend GnRHa administration under proper conditions, even if there is no history of previous systemic hypersensitivity reactions.

Conclusions

Since 1981, GnRHa administration has been the standard treatment for CPP. GnRHa suppress LH and FSH and thereby induce a marked inhibition of gonadal activity. This treatment is generally considered to be safe and well tolerated in children and adolescents. The most commonly reported drug reactions were pain, swelling, and urticaria at the injection site. Most events were mild, and there was no interruption in study procedures from these ADRs. Nevertheless, whatever is the frequency of these side-effects, clinicians using these treatments should be aware of the possibility of significant local and general ADRs that can lead to treatment withdrawal in the most severe cases.

We hope that our review may provide the necessary data in order to enable clinicians to administer GnRHa in the safest and most appropriate way. Further studies are necessary to identify the mechanisms of development of potential adverse drug reactions related to GnRHa therapy in CPP and the potential risk of causing prolonged QT, as reported in adults (94).

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

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Original article

Long-term prednisone versus hydrocortisone treatment in children with classic congenital adrenal hyperplasia (CAH) and a brief review of the literature

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Summary. *Background:* Debate still exist about the safety of long-term use of prednisone (PD) versus hydrocortisone (HC) for treating children with congenital adrenal hyperplasia -21OH D (CAH). Despite recent developments in congenital adrenal hyperplasia -21OH D (CAH), several issues related to patient growth and final height remain unsolved. Debate still exist about the safety of long-term use of PD versus HC for treating children with CAH. The mechanism by which glucocorticoid therapy interferes with growth is complex and multifactorial. Relatively slight supra-physiologic levels may be enough to blunt growth velocity. An increased risk of developing obesity is another possible consequence of hyper-cortisolism in children with CAH. *Objectives of the study*: To evaluate the anthropometric and biochemical effects of long-term PD versus HC treatment in children with CAH-21OHD. A brief review of the literature is also reported. *Patients and Methods:* This retrospective study evaluated linear growth and biochemical data of thirty children with classic CAH (19 females and 11 males), who were on PD (n=22) or HC (n=8) treatment, since their first diagnosis. Clinical data included age, gender, duration of therapy, dose of HC and or equivalent dose of HC in the PD group, blood pressure, height (Ht) and weight. Ht-SDS and BMI were also calculated. Biochemical data included measurement of 17- OH progesterone, cholesterol, triglycerides (TG), HDL, LDL, fasting glucose, and insulin concentrations. HOMA-IR was calculated. Carotid intima-media thickness (CIMT) was measured using high-resolution B-mode ultrasonography. Thirty normal age matched children were used as controls for the anthropometric and CIMT data. *Results:* The age of children and duration of treatment did not differ among the two treatment groups. After a mean of 6 years of treatment, the Ht-SDS and BMI did not differ between the three groups of children. The equivalent hydrocortisone dose of children on prednisone was significantly higher than the dose for the hydrocortisone group. Both systolic and diastolic blood pressures (BP) of children on PD was slightly higher compared to those on hydrocortisone group. However, the BP of the 2 treatment groups was not different compared to control children. Fasting blood glucose, homeostatic model assessment insulin resistance (HOMA-IR), plasma TG, HDL, and cholesterol did not differ among the two treatment groups. LDL levels were significantly higher in the PD group versus the HC group. The mean CIMT did not differ among the two treatment groups but was significantly higher in the treated groups versus controls. There was a significant linear correlation between BMI-SDS and CIMT (r=0.37, p=0.047). *Conclusions:* Children with CAH-21OHD who were kept on PD therapy for 6.4±2.7 years, since the begin-

ning of diagnosis, have maintained normal linear growth. No difference in BMI, HOMA-IR, or CIMT was detected among the two treated groups. The efficiency, safety and convenience of a single daily dose of PD could be a good and relatively safe alternative to HC for the continuing medical treatment of patients with CAH-21OHD. However, more prospective studies across childhood and adolescence are necessary to draw definitive conclusions. (www.actabiomedica.it)

Key words: congenital adrenal hyperplasia (CAH), long-term corticosteroids treatment, growth, blood pressure, glucose, insulin, carotid intimal thickness, lipid profile

Introduction

Congenital adrenal hyperplasia (CAH) is caused by the loss or severe decrease in activity in one of the five steroidogenic enzymes involved in cortisol biosynthesis. In 90-95% of all cases a 21-hydroxylase deficiency (21-OH) is found (1). It is caused by mutations in the 21-hydroxylase gene (CYP21A2), which encodes the steroid 21-hydroxylase (p450c21). This enzyme converts 17-hydroxyprogesterone (17-OHP) into 11-deoxycortisol, and progesterone into 11-deoxycorticosterone, which are then converted into cortisol and aldosterone, respectively. Disturbance of cortisol production causes accumulation of precursors of cortisol by stimulation of ACTH, and these precursors lead to a pathway for adrenal androgen. Overproduction of androgens causes virilization, accelerated growth, advanced skeletal maturation, and early epiphyseal fusion (1,2).

Patients with classic CAH, especially those with the salt-wasting form, also need mineralocorticoids, and infants usually need sodium chloride supplementation. Non- salt-wasting 21-OHD may be diagnosed on genital ambiguity in affected females, and/or later the occurrence of androgen excess in both sexes.

Traditional treatment consists of substitution of cortisol to reduce excessive androgen production and its consequences. Physiological cortisol production is estimated to be 5-6 mg/m^2/d. The biological criteria to optimize treatment are controversial. Some authors use mainly clinical development (growth velocity and bone age). Others measure hormone levels: serum 17-OHP and/or serum Δ 4 androstenedione and/or testosterone which are believed to be the most sensitive index of biochemical control. Renin, aldosterone and potassium are useful to monitor mineralocorticoid treatment (1). Undertreatment with steroids leads to androgen excess with advancement of bone age and reduced final height (FH). Overtreatment may impair growth through the growth-inhibiting effects of steroid and may predispose to obesity, hypertension and osteoporosis (1-5).

Hydrocortisone (HC) is the preferred glucocorticoid (GC) in children with CAH due to potential concerns of linear growth suppression associated with longer-acting and more potent GC formulations (1, 6, 7). However, individual differences in treatment needs make this difficult to evaluate the effect on linear growth.

A randomized controlled trial (RCT) showed that 25 mg/m^2 depressed growth in children with CAH compared to 15 mg/m^2 of HC and suggested that full suppression, or even normalization, of plasma concentrations of 17-hydroxyprogesterone and androgens should not be considered a treatment goal, but instead an indication of corticosteroid treatment excess (7). Reliable results using relatively smaller HC doses and mineralocorticoid replacement with fludrocortisone have been reported, when combined with antiandrogens (flutamide) and aromatase inhibitors (testolactone). However, this complex and expensive multi-drug scheme is demanding for routine use, especially in third-world countries (8).

Up till now, debate still exists about the difference in the effect on growth and metabolism when different GC formulations are used for treatment of CAH (9-15).

Tendency for overtreatment increases when potent longer-acting GC formulations, such as prednisone or dexamethasone are used. The bioequivalence dose ratio is based upon anti-inflammatory potency, because other clinical equivalence tables are not yet clearly available and can lead wrong estimation of the proper doses. Even "physiologic doses" may impair growth velocity and restrict final height.

Several mechanisms may be involved in this consequence: GC formulations interfere with the normal interactions in the growth hormone (GH)/IGF-1 signaling cascade at the level of the hypothalamus, pituitary, and target organ.

In human studies, excess GC formulations (pharmacological dose) causes a decrease in GH response to growth hormone releasing hormone (GHRH) and a paradoxical increase in IGF-1 levels, inducing a state of GH resistance. In addition, GC formulations may prompt apoptosis of chondrocytes through activation of caspases and inhibit the phosphatidylinositol 3 -kinase (PI3K) signaling pathway (9-14).

On the other hand, the use of long acting synthetic GC analogues to treat CAH-21OHD, may suppress ACTH more efficiently. Prednisolone (PD) has a molecular structure that resembles that of cortisol, with the C1-C2 double bond determining a longer half-life and possibly permitting single daily dose administration. It is convenient commercially available formulation (homogeneous oral solution) that permits fine therapeutic adjustments (15, 16).

This study aimed to investigate retrospectively the long-term effects of two different glucocorticoid regimens (PD versus HC) in children with CAH-21OHD with special reference to growth, lipids, insulin resistance, blood pressure and carotid intimal-media thickness. A brief review of the literature is also reported.

Patients and methods

Thirty children with classic CAH-21OHD (19 females and 11 males), attending the Endocrinology Clinic of Alexandria University Children's Hospital (Egypt) were enrolled in our study. Thirty healthy age and sex matched children were used as controls. Anthropometric measurements were assessed, including: weight, height (Ht), body mass index (BMI), and Ht-SDS. Patients with other diseases, or those receiving drugs that could affect linear growth were excluded.

All data were extracted retrospectively from the patients' charts. The diagnosis of CAH-21OHD was based on both clinical symptoms and signs, and later on hormonal analysis and comprehensive genotyping.

Twenty-two children with CAH-21OHD were on continue treatment with a single morning dose of oral PD and eight were on daily oral dose of HC, divided in two daily doses. The doses were adjusted every 4 months to keep average plasma 17-hydroxyprogesterone concentration around 21 nmol/L (695 ng/dL). In addition, oral fludrocortisol (100-150 μg) was given to all patients as replacement mineralocorticoid therapy.

Laboratory investigations done during the last visit included: assessment of electrolytes, 17OHP, fasting glucose and insulin concentrations, lipid profile and homeostatic model assessment insulin resistance (HOMA-IR). High-resolution B-mode ultrasonography was performed to measure the carotid intimal-media thickness (CIMT) and to evaluate the color Doppler flow characteristics of the carotid arteries. A CIMT value more than 0.9 mm or over the 75th percentile were considered abnormal (17).

The Ethical Committee of Alexandria College of Medicine approved the study. Informed consent was obtained from all patients and controls before the study.

Statistical analyses were performed with the parametric t test when the data were normally distributed and nonparametric Mann-Whitney U test when the data were not normally distributed. Spearman's correlation coefficient was calculated to evaluate correlation of different variables with the Ht-SDS. Statistical analyses were done with the SPSS 10.0 software (SPSS Inc., Chicago, IL). A P value <0.05 was considered statistically significant.

Results

Comparison between the two groups of children with CAH-21OHD (PD versus HC) and controls is shown in table 1.The age of children and duration of treatment did not differ among the two treatment

Table 1. Comparison of different variables between CAH patients and controls

Variables	Type of steroid treatment		P1*	P2*	
	Hydrocortisone (n=8)	Prednisone (n=22)	Controls n =30		
Age (years)	7.4±3.3	6.4±2.7	6.28±2.75	0.37	0.511
Systolic BP mmHg	96.5±13.4	98.5±6.9	97.47±7.65	0.01	0.828
Diastolic BP mmHg	61.3±8.3	63.9±4.2	63±4.34	0.01	0.677
Height (± SD)	-0.3±1.3	-0.7±1.6	-0.25± 0.80	0.25	0.249
BMI (kg/m^2)	19.0±3.6	18.7±5.1	19.8±5.42	0.57	0.399
Total daily dose (mg/m^2/day)	15.2±3.7	5.5±1.1	ND	<0.001#	ND
17- OH progesterone nmol/L	26.8±70.0	3.0±3.3	ND	0.00	ND
Cholesterol mg/dL	144.5±20.4	162.0±30.9	ND	0.06	ND
TG mg/dL	69.1±27.6	74.1±25.5	ND	0.65	ND
HDL mg/dL	53.5±5.3	55.1±11.0	ND	0.69	ND
LDL mg/dL	72.9±20.5	91.3±27.0	ND	0.04	ND
Fasting glucose mg/dL	73.8±7.2	73.9±11.4	ND	0.97	ND
HOMA-IR	1.1±0.5	1.2±0.8	ND	0.73	ND
CIMT Mean (mm)	0.54±0.07	0.51±0.06	0.044±0.004	0.21	<0.001#

Legend=CAH: congenital adrenal hyperplasia; * P1: hydrocortisone vs prednisone; ** P2: Patients versus controls; CIMT: carotid intimal thickness; TG: triglycerides; HOMA- IR: homeostatic model assessment insulin resistance; # p ≤ 0.05

groups. Ht-SDS and BMI did not differ between the three groups of children. The equivalent HC dose of children on PD was significantly higher compared to the dose given to the patients on HC treatment. The systolic (SBP) and diastolic (DBP) blood pressure of children on PD was slightly higher compared to HC group (p: 0.01). However the blood pressure of both groups of patients was not statistically different compared to controls.

Fasting blood glucose, HOMA-IR, plasma TG, HDL and cholesterol did not differ between the two groups of patients. LDL levels were significantly higher in the

PD group versus the HC group. The mean CIMT did not differ among the two treatment groups but was significantly higher in the treatment groups versus controls (<0.001).

Correlation studies showed a significant linear correlation between BMI-SDS and CIMT (r: 0.366, p: 0.047). However, CIMT did not correlate with other variables including: age, total daily dose (mg/m^2/day), 17- OHP, LDL, fasting insulin, and TG concentrations or HOMA-IR (Table 2). Ht-SDS was not significantly correlated with the total daily dose (mg/m^2/day) or 17-OH progesterone level (r=0.22 and -0.08, respectively; p>0.05)

Table 2. Correlation between mean carotid intimal thickness (CIMT, in mm) and different parameters in our patients with CAH

Cases group	Mean CIMT	
	r	p
Age (years)	0.292	0.117
Total daily dose (mg/m^2/day)	0.349	0.059
BMI- SDS	0.366*	0.047*
17- OH progesterone	-0.214	0.257
HDL (mg/dL)	-0.021	0.910
LDL (mg/dL)	-0.214	0.257
Fasting Insulin (mIU/L)	0.053	0.780
HOMA-IR	-0.007	0.971
TG (mg/dL)	0.164	0.385

Legend=CIMT: carotid intimal thickness, in mm;
HOMA- IR: homeostatic model assessment insulin resistance;
*p ≤ 0.05

Discussion

HC acetate, is most similar to endogenous cortisol and therefore is considered as a potentially good therapeutic option for treating children with CAH-21OHD, in whom there is a risk of suppression of growth with the use of other longer acting synthetic GC preparations (6). Adherence to treatment is a cru-

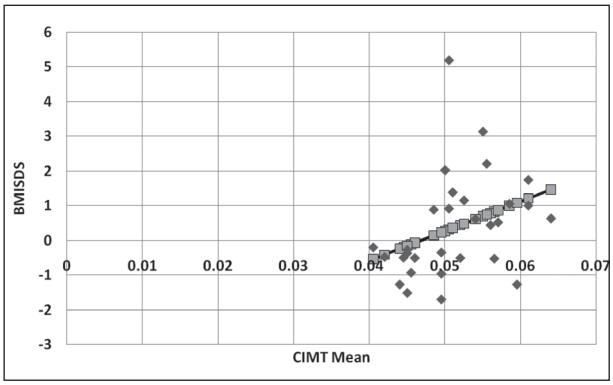

Figure 1. Correlation between CIMT and BMISDS in children with CAH (r=0.336, p=0047)
Legend=CIMT: carotid intimal thickness, in mm; BMI: body mass index, in SDS

cial issue to attain satisfactory results in children with CAH. Hence, it is of fundamental importance to seek a therapeutic scheme that adapts to the daily life of the patient and their parents. However, HC has a short biological half-life, requiring a maximum of 8-hour doses intervals. This hampers adherence to treatment and may lead to intermittent non-suppression of ACTH with the consequence of frequent androgen excess production (18). Additionally, the oral preparation of HC is not commercially available in many areas in Egypt. The liquid form is unstable (suspension) and precipitates in non-alcoholic diluent. These characteristics make it challenging to administer and leads to dosing mistakes in young children. The possibility of using single dose daily medication with GC with intermediate half-life has been proposed.

PD, an intermediate half-life GC, has been suggested as a therapeutic option in children and adolescents with 21OHase deficiency. It is a synthetic GC structurally like cortisol, whose only molecular difference is the existence of a double bond between carbons 1 and 2. This feature prolongs its plasma half-life and allows its use in a single daily dose. The pharmacokinetic profile of PD showed similarity to the published profile of dual-release HC. Therefore, once-daily PD has been suggested as a replacement to patients on treatment with HC. Some data suggest that PD profile is superior to the current standard of thrice-daily HC because mimicking the physiological cortisol profile and promoting a better compliance (19, 20).

In fact, Johannsson et al. (21) found an obvious patient preference for once-daily regimens vs thrice-daily regimens. Furthermore, the recommended initial dose of 3 mg /m², represents one fourth to one fifth of the HC dose. Its oral presentation is commercially available as a solution, which facilitates the acquisition and administration in children and the cost is lower when compared to formulated hydrocortisone (22-25).

Traditionally, PD bioequivalence to HC has been quoted at 1:4, although there are some evidences that the true value is closer to somewhere between 1:6 and 1:8, at physiological doses (25). The physiological dose of PD is uncertain. For anti-inflammatory effects PD is four-fold more potent than HC. Whether this relative potency is also true for the GC and mineralocorticoid effects is less clear. Using luciferase transactivation assay in CV-1 cells transfected with the human GC receptor, PD was 1.7-fold more potent than HC. When transfected with the human mineralocorticoid receptor, HC was 4.2-fold more potent than PD.

Bioequivalence of PD and HC derived from growth data in patients with CAH suggest that PD is from 6 to 8-fold more potent than HC (26, 27). However, the drawback of using PD is the potentially higher risk of negative impact on growth and metabolism (28-31).

Our study compared different anthropometric and lab. variables in CAH-21OHD children on long-term treatment with PD (5.5±1.1 mg /m² daily, equivalent to a HC daily dose of 22 mg/m²) versus HC treatment (15.2±3.7 mg/m²), from the diagnosis of CAH. The Ht-SDS and BMI did not differ between children on PD, HC and controls (Ht-SDS=-0.7, -0.3, -0.25, respectively; BMI=19.1, 18.7, and 19.8 Kg/m², respectively). Serum 17 OHP was significantly lower in children on PD versus HC treatment.

In support to our data, Leite et al. (15) evaluated the growth parameters in 15 children with CAH. In the first year of treatment, HC (17.5 mg /m ²/ day, divided in three doses) was used, followed in the second year by a morning daily dose of PD (3 mg / m² / day). The comparison between the two treatments during this relatively short period showed no significant difference in relation to Δ Ht- SDS, Δ bone age SDS and Δ BMI- SDS. No significant difference was observed in the serum levels of Δ 4 androstenedione (32).

Caldato et al. (22) evaluated 44 patients previously diagnosed as having the salt-losing or simple-virilizing forms of CAH-21OHD were randomly assigned to two groups, stratified according to sex, age, and pubertal status, in order to assess the clinical benefits of a one year treatment period with a single morning dose of PD, as compared to a three times a day (TID) HC replacement therapy. Growth velocity (expressed in SDS) was preserved in the PD group (from 1.2 to 1.2 in all; 0.79 to 1.13 in pre-pubertal children, whereas a slight increase occurred in the pre-pubertal HC-treated patients (from 1.1 to 1.9); Ht-SDS for bone age increased significantly in the PD group. Thus, patients with CAH-21OHD treated for one year with a single morning dose of PD appeared to achieve a better clinical control than those on TID HC. However, long-term follow-up is still necessary to demonstrate individual clinical benefits upon final stature and fertility.

In another prospective cross-over study done in 13 prepubertal children with classical CAH-21OHD, HC did not offer significant advantage in achieving biochemical control of disease. Replacing HC with PD, in the evening, resulted in similar hormonal control of the disease (33).

Khadilkar et al. (34) compared growth parameters of children with CAH treated with PD or HC. HC had a less negative growth effect than PD and patients treated with HC from the beginning showed near normal growth.

Linder et al. (35) hypothesized that alternate day prednisone therapy might be more efficacious in the treatment of CAH. To evaluate this hypothesis, they studied an 11-yr-old girl with salt-losing 21-OHD with alternate day PD therapy (20 mg every other day) for over 3 yr. This treatment regimen caused sustained adrenal androgen suppression and allowed normal growth and pubertal development, despite persistently elevated plasma ACTH and 17-OHP levels.

To evaluate the impact of HC dosage, age at diagnosis, compliance, genotype and phenotype on growth and height outcome in CAH patients, Grigorescu-Sido et al. (36) studied 37 patients (17 had completed growth and 20 were still growing). Doses >20 mg/m^2/day during the first year and >15 mg/m^2/day during age 1-5 and at puberty resulted in significantly lower final height- SDS, predicted final height-SDS and in a greater height losses. The Authors concluded that HC substitution in CAH patients should be kept at the lowest efficient level, if possible <20 mg/m^2/day during the first year and <15 mg/m^2/day until age 5 and during puberty. Similar recommendations were reported by Bonfig et al. (14).

In summary, most of the studies in the pharmacotherapy of CAH-21OHD are done in heterogeneous group of CAH, with different degree of enzyme deficiency and different age group. This makes interpretation of these clinical data difficult.The management of patients with CAH involves replacement of glucocorticoids and suppression of ACTH secretion and thereby controlling the excess androgen secretion from adrenals. A variety of glucocorticoids (HC, PD, and dexamethasone) and dosage schedules are used for this purpose in children and adults (37). In children the typical dosing of hydrocortisone is 10-15 mg/m^2/day administered orally in three divided doses. Infants may temporarily require doses up to 20 mg/m^2/day to reduce the markedly elevated sex steroids.

Similarly higher doses (up to 15 mg/m^2) are required during puberty. Doses must be individualized by monitoring growth, bone age, and hormonal levels.

Excess GCs intake can lead to increased weight gain, hypertension, osteoporosis / osteopenia and early onset diabetes. Furthermore, cardiovascular function and the elastic properties of major arteries are disturbed in children and adolescents with 21-hydroxylase-deficient CAH (38).

Our patients on PD therapy had slightly but significantly higher SBP and DBP, and higher plasma LDL compared to those on HC. These may impose an increased risk on developing further lipid abnormalities and atherosclerosis on the long-term treatment (39,40). In support of our blood pressure findings, other studies reported higher systolic blood pressure and abnormal blood pressure profile in children with CAH. (40,41).

A recent systematic review and meta-analysis suggested that, compared with controls without CAH-21OHD, individuals with CAH had increased SBP, DBP, insulin resistance, and carotid intima thickness (42). No statistically significant difference was noted in fasting blood glucose or lipids. The Authors

were unable to draw conclusions regarding the effects of several important variables such as sex, glucocorticoid type and dose, fludrocortisone dose, and genotype (42). Nevertheless, there are no evidence of actual morbidity or mortality due to cardiac events. Long-term prospective studies are warranted to assess strategies for reducing cardiovascular risk in individuals with CAH-21OHD.

However, in CAH-21OHD patients, intima media thickness of abdominal aorta (AIMT) and common carotid arteries (CIMT) are influenced by androgens and obesity. CIMT is an independent predictor of future cardiovascular risk and is positively correlated with body mass index (BMI) and systolic blood pressure (43-45). In our children with CAH-21OHD there was a positive significant correlation between CIMT with BMI. Furthermore, in our study, an increased of CIMT has been observed in our patients with CAH-21OHD, both on HC and PD, compared to control children. On the other hand, our study did not detect difference in CIMT between patients on HC compared to patients on PD.

It was observed that cardiovascular risk factors detected in childhood such as elevated LDL-c, SBP, and BMI were associated with increased CIMT and that progression to atherosclerosis may be predicted in childhood independent of risk factors identified later in adulthood (46). Our children on PD therapy had slightly higher LDL and blood pressure compared to those on HC therapy.

In a small group of prepubertal children with classic CAH, serum insulin concentrations were reported to be significantly higher than those of healthy counterparts (47). However, in our patients on PD and HC treatment, fasting glucose level and HOMA-IR were normal.

Varying data have been reported on triglyceride levels, with one study reporting higher levels in a group of prepubertal patients on GC treatment compared with age-matched controls and another showing a lipid profile in CAH children similar to that of controls (48, 49). Our patients on PD and HC had normal triglyceride levels with no difference among the two groups.

In brief, our children with CAH-21OH who were on single daily dose of PD for a 6.4±2.7 year, since the beginning of their diagnosis, maintained a normal linear growth with no or minimal effect on statural growth compared to TID therapy with HC. No difference in BMI, fasting glucose level, HOMA-IR, or CIMT was detected among the two groups of patients. Mild increase in BP, LDL levels was detected in the PD group versus the HC group. 17-OHP levels were significantly lower in the PD group versus HC group suggesting a better biochemical control. Consequently, when the convenience of a single daily dose, compliance and cost-effectiveness are considered, PD appears to be a good and relatively safe alternative choise to HC.

We did not include in the present study the impact of PD or HC on bone mineral density (BMD) of our patients. GC therapy using pharmacological doses has a detrimental effect on bone, which is known to be strongly dose dependent.

Longitudinal studies in patients with CAH are scarce: a study including 15 patients with CAH reported an increase in L1-L4 BMD but a decrease of femoral neck BMD after 8-10 years (50); another study also showed a mixed response (51). Although, Jääskeläinen and Voutilainen et al. (52) reported that adult patients substituted with HC were less often over-treated and had better BMD Z-score means than patients substituted with PD or dexamethasone, no longitudinal study has examined the effect of different GCs in detail. Because the potential risk for osteoporosis remains, we suggest to monitor and eventually to treat bone disease, even in young patients.

In conclusion, the aim of CAH treatment is the replacement of mineralocorticoids and glucocorticoids and the normalisation of elevated androgen concentrations. Long-term treatment with glucocorticoids will improve the androgen symptoms but may result in long-term complications, such as obesity, insulin resistance, hypertension, osteoporosis and fractures. However, optimising replacement therapy in patients with CAH-21OHD remains challenging. Furthermore, management of CAH requires parents to administer oral steroids, typically HC, up to three times daily within school and activity schedules, supplementing maintenance doses with oral "stress dosing" during times of illness and an emergency intramuscular (IM) injection of HC when a child is unable to tolerate oral

medications and/or if signs of adrenal crisis are present. Prednisone is a potentially good alternative when HC is not available or once daily dose is required for improving compliance. "These patients should therefore be carefully followed-up, from childhood through to adulthood, to avoid these complications and to ensure treatment compliance and tight control of the adrenal androgens, by multidisciplinary teams who have knowledge of CAH" (53).

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

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Original article

Evaluation of the antimicrobial activity of novel composite plastics containing two silver (I) additives, acyl pyrazolonate and acyl pyrazolone

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Summary. *Background:* Public health systems today face the dual challenges of controlling infections and curbing the increase in antimicrobial resistance manifested in drug-resistant microorganisms in hospitals and elsewhere. In the last ten years, research has been conducted to develop new materials with antimicrobial properties to be used in medical devices, increasingly found to harbour critical nosocomial infections. *Methods:* Two next-generation composites using the antimicrobial qualities of silver were tested against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* with the purpose of evaluating their antimicrobial and antifungal activity. These tests applied the standardized method according to ISO-2216: Plastics-Measurement of Antibacterial Activity on Plastics Surfaces. Testing was carried out using polyethylene (PE) enriched with $AgNO_3$ as a positive control and PE as a negative control. *Results:* The antimicrobial activity of the composites proved to be between medium (bacteriostatic) and very good (bactericidal). In particular, PE2 showed the highest scores against all microorganisms, with values ranging from good to very good. Instead, PE1 had lower scores, with a value of medium for *Escherichia coli* and slight for *Candida albicans*. Statistical analysis carried out with the t-test for unpaired data showed a statistically significant difference between the positive control and the other polymers ($p < .0001$). *Conclusions:* Based on our findings, we conclude that the test, conducted to ISO-2216 standards, could be extended to include fungal strains and that the new composites could be used to produce antimicrobial surfaces for medical devices, for example, intubation tubes, urinary catheters, vascular prostheses, and mechanical heart valves. This would reduce the risk of microbial contamination and biofilm formation, ensuring better health outcomes for patients treated with these devices. Further testing should be done to evaluate potential future applications of these composites and the possibility of adding fungal strains to the IS0-2216 standard. (www.actabiomedica.it)

Key words: antimicrobial resistance, silver composites, nosocomial infections, antimicrobial activity

Introduction

Even today, infectious diseases, especially in hospitals, continue to present a major public health problem with socio-economic and cultural consequences (1-5). Even though the standards of health care and medical technology have risen significantly, the World Health Organization (WHO) declared that infections occur in 15% of patients under medical care in hospital, particularly in Intensive Care Units (ICUs) (2,6).

In 2015, the European Center for Disease Control reported that 8.3% of hospitalized patients in

intensive care for more than two days acquired at least one healthcare associated infection (HAI). Specifically, 6% of patients were affected by pneumonia, 4% by bloodstream infections (BSI), and finally 2% by urinary tract infections (UTIs) (7). Intubation is the cause of infection in 97% of patients, catheters have been associated with 43% of BSIs, and urinary catheters with 97% of UTIs (7). Regarding the most common bacteria, *Pseudomonas aeruginosa* was identified in pneumonia episodes, *Staphylococcus spp* in bloodstream infections, and *Escherichia coli* in urinary catheter infections. In fact, the surfaces of medical devices that remain in place for days provide a substrate for biofilm production and the growth of these microorganisms (4-7). In the last decade, *Candidemia* has also been defined as a severe and often life-threatening infection. Candida strains can cause invasive candidiasis (IC) in tertiary care hospitalized patients and also in catheter-associated urinary tract infections (CAUTI) (11,12).

Hospital-acquired infections can be caused by contamination due to incorrect conduct on the part of healthcare workers, for example, failure to wash their hands properly, to store food at the correct temperature, or prepare it in properly hygienic conditions (11-14). This superficial attitude can be attributed to the high levels of stress to which these professional categories are subjected and the speed required for the performance of their duties (15-24).

Another factor in the spread of infections is the public's limited awareness and understanding of the proper use of medicines. Better education about this would improve the effectiveness of treatment and reduce costs for patients and society, thus avoiding risks for health and the waste of resources (25, 26). One of the first steps to take in encouraging the correct use of medicines is to improve the public's health literacy, defined by the WHO as "the cognitive and social skills which determine the motivation and ability of individuals to gain access to, understand and use information in ways which promote and maintain good health" (27,28). One area in particular to be improved is the understanding of the medical terminology commonly used in manuals or package inserts.

Furthermore, in the last decade the over-prescribing of antibiotics or their misuse by patients has increased the risk of hospital infections caused by drug-resistant microorganisms (29). The WHO has recorded an increase in antimicrobial resistance (AMR) among clinical bacteria and has defined this phenomenon one of the most critical threats for human health, in particular in vulnerable patients (29).

At the same time, the European Center for Disease Control highlighted the very rapid increase of infections caused by bacteria like carbapenemase-producing Enterobacteriaceae (CPE) in patients hospitalized in Italian and in European institutions from 2014 to 2017 (30-32). Therefore, public health services are facing a double challenge: controlling infections and limiting AMR diffusion (33, 34).

As part of the efforts to tackle these problems, researchers have been developing polymers with antimicrobial activity, and in the last decade, a new group of materials including antimicrobial peptides (AMPs), cationic synthetic polymers, and nanoparticles has been created (7, 35). In particular, antimicrobial-biocompatible polymers have been developed for medical devices in order to prevent the formation of biofilms and the insurgence of nosocomial infections, and as such provide an innovative method to combat these problems that is both gentle and safe. Two types of antimicrobial polymers can be used for the production of medical devices: polymers that already exhibit antimicrobial activity and polymers that are modified to confer antimicrobial properties (10).

In particular, among the wide range of existing antimicrobial plastics, metal-polymer-nano composites and in particular silver-polymers are the subject of increasing interest (36,37). Silver compounds are known to show strong antibacterial activity towards a broad spectrum of bacteria, and the interest in silver derivatives and their potential application as antimicrobial agents has led researchers to investigate different classes of ligands in order to obtain novel silver(I) complexes. The aim of this study was to evaluate possible antibacterial and antifungal activity of new silver-containing polymers (I) synthesized by the Chemistry Department of the University of Camerino (UNICAM), specifically as a potential antibacterial and antifungal agent to embed in plastics. The results obtained against *Escherichia coli* and *Staphylococcus aureus* were compared with the results obtained against *Candida albicans*.

Methods

1. Novel composites containing silver (I) acyl pyrazolonate additives

The polyethylene composite materials named PE1 and PE2 were prepared, by the Chemistry Department of UNICAM. PE1 was obtained by mixing, at 150 °C, the complex $[Ag(Q^{fb})]_n$ with polyethylene in a 1:1000 weight ratio, the final product was shaped into 50x50 mm square (10 mm thick). PE2 was similarly obtained by using the complex $[Ag(Q^{cy})]_n$ which differs only for the R substituent (Figure 1). The detailed preparation procedure and the complete characterization have been previously reported (39, 40).

The antimicrobial action of the composite materials PE1 and PE2 has been tested against two microbial strains (*E. coli, S. aureus*) and one fungal strain (*C. albicans)*.

Molecular structures of silver(I) acylpyrazolonato additives 1 and 2, respectively used in the composite polyethylene materials PE1 and PE2.

2. ISO-2216: Plastics-Measurement of Antibacterial Activity on Plastics Surfaces

The antimicrobial activity by contact exerted by each of the PE composites was measured according to ISO standard-2216 (*ISO-22196: Plastics – Measurement of Antibacterial Activity on Plastics Surfaces*) (38). Square pieces of the composites measuring 50x50 mm (10 mm in thickness) were tested against two bacteria strains and one fungal strain in triplicate: (Gram-positive) *Staphylococcus aureus* ATCC 25923, (Gram-negative) *Escherichia coli* ATCC 25922 and the fungal strain *Candida albicans* ATCC 24433. Unloaded PE samples were used as negative control; PE loaded with $AgNO_3$ was used as positive control. The appropriate culture medium was inoculated with the test microbes and cultivated for 24 h at 35±1°C under aerobic conditions to achieve the concentration of 10^7 CFU/mL. Bacterial suspensions (0.4 mL) were inoculated onto the test surface (in triplicate) and the inoculums were covered with a piece of polyethylene film (40x40mm), gently pressed down to spread the inoculum to the edges. The Petri dishes containing the inoculated test specimens were incubated at (35±1)°C with a relative humidity of no less than 90% for 24±1h. After the incubation time, the inoculum was processed by adding 10 mL SCDLP broth (Soybean casein digest broth with lecithin and polyoxyethylene sorbitan monooleate). From the SCDLP broth, tenfold serial dilutions were made in phosphate-buffered physiological saline (PBS-saline) and aliquots of 1 mL for each dilution were placed in Petri dishes, and 15 mL of plate count agar (PCA) was added to disperse the bacteria. The inverted Petri dishes were incubated at (35±1)°C for 48 h. After incubation, the number of colonies in the Petri dishes was counted. The number of bacteria surviving on the specimens tested was compared to the number of colonies present on the negative controls. Antimicrobial performance (R) was determined according to the Japanese industrial standards method (*JIS L-1902: 2002, Testing Method for Antibacterial Activity of Tex-*

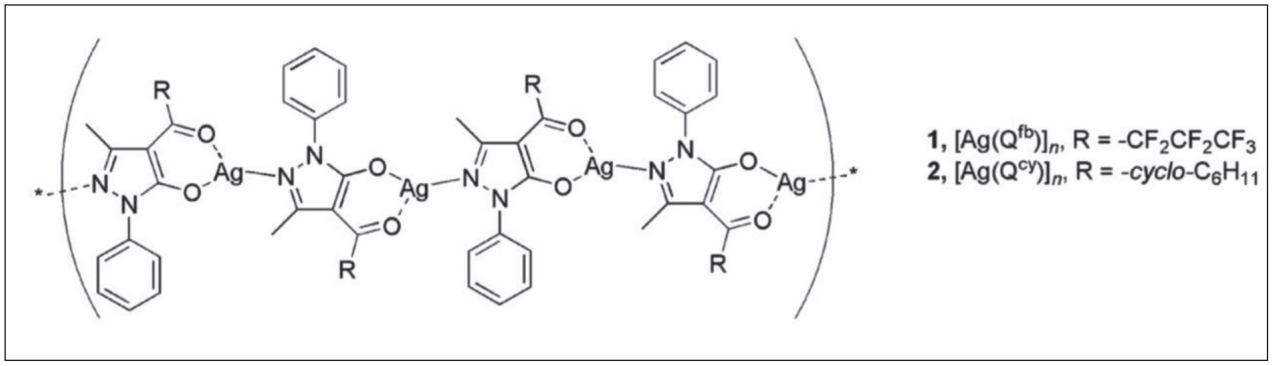

Figure 1.

tiles) (41) and based on the following classification: no antimicrobial activity = ≤0.5 log microbial growth reduction (<68.4% reduction); slight antimicrobial activity = 0.5-1log microbial growth reduction (<68.4% to<90% reduction); medium antimicrobial activity = >1 to ≤ 2 log microbial growth reduction (90% to <99% reduction); good antimicrobial activity = 2 to <3 log microbial growth reduction (99% to <99.9% reduction); very good antimicrobial activity = >3log microbial growth reduction (>99.9% reduction). In addition, the % of reduction was interpreted for bacteria strains in terms of the bactericidal activity (>99.9% of inoculum reduction) or bacteriostatic activity (90 to 99.9% of inoculum reduction) of each composite.

3. Statistical analysis

To verify whether there may be a difference between the resilience and coping values before and after rescue, the unpaired t-test was used and data was processed with the XLstat software (*XLSTAT. Statistical software and data analysis add-on for Excel. Addinsoft (2017)*) (42).

Results

To verify the antimicrobial activity of the loaded composites by contact, the results of the experiments were elaborated according to the JIS L-1902:2002: *Testing Method for Antibacterial Activity of Textiles* (41), applying the standardized method (ISO-2216) (38). After an incubation of 24h, the data obtained relating to microbial charge found on different types of composites was transformed into logarithmic units to calculate antimicrobial performance as Log reduction (R value). The evaluation of bactericidal, bacteriostatic and antifungal activity provided us with much more detailed information about the behavior of the substances tested against two microbial and a fungal species.

In general, an inhibition of microbial growth on the contact surface between the PE composite squares and the inoculums of the microorganisms was revealed.

Our data show that within 24h of exposure, both PE1 and PE2 exhibited antimicrobial activity against both bacteria, ranging between a medium and very good level, in line with the behavior shown by the positive control ($PEAgNO_3$). Tested against *C. albicans*, PE1, unlike PE2, showed only slight activity, measuring between 68% and 90%. The positive control ($PEAgNO_3$) achieved a medium-good antibacterial performance against all bacteria strains.

In detail, tested against *E. coli*, composites PE1 and PE2 show bacteriostatic activity (<99.9%), with PE2 performing better than PE1 (Figure 2). In fact, PE1 achieved an R value from 90% to <99%, corresponding to good antimicrobial performance. The R value of PE2, instead, was within 99% to 99.9%. In addition, compared with the positive control ($PEAgNO_3$), PE1 achieved only 1.59 log microbial growth reduction, whereas PE1 showed a value of 2.81 against the 2.21 reached by $PEAgNO_3$ (Figure 2).

Tested against *S. aureus,* composites PE1 and PE2 achieved very good antimicrobial performance, corresponding to bactericidal activity largely exceeding a log value of 3. Instead, $PEAgNO_3$ showed only a medium antimicrobial performance, with a range of antimicrobial performance from 90% to <99% (Figure 3).

Tested against *Candida albicans,* only PE2 achieved good antimicrobial performance. PE1 showed only slight antimicrobial activity. The observation that PE2 and $PEAgNO_3$ had similar activity suggests that the ISO standard test can be used to test new polymers against fungi (Figure 4).

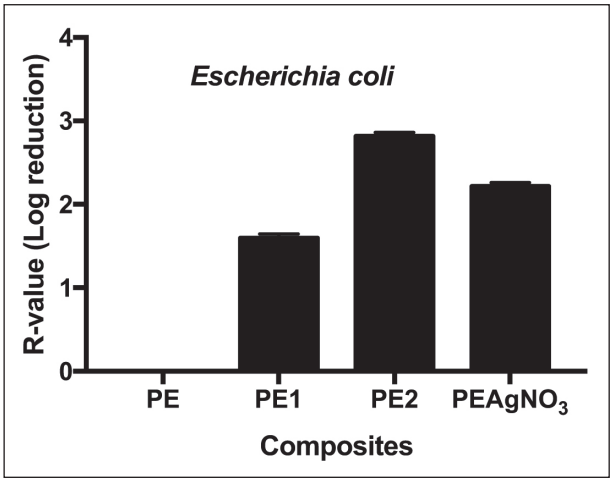

Figure 2. R-values obtained for *Escherichia coli;* unpaired t test of PE1 vs PAgNO3 was t=131.5 with a ***p value< .0001, and of PE2 t=127.3 with a ***p value <.0001

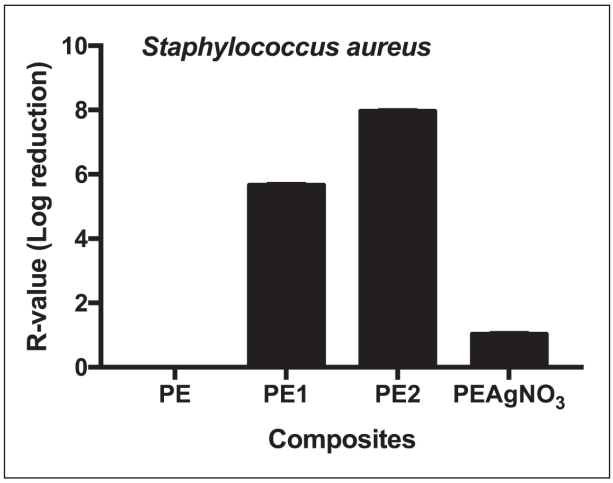

Figure 3. R-values obtained for *Staphylococcus aureus*; Unpaired t test of PE1 vs PAgNO3 was t=983.6 with a ***p value< .0001, and of PE2 t=1041 with a ***p value <.0001

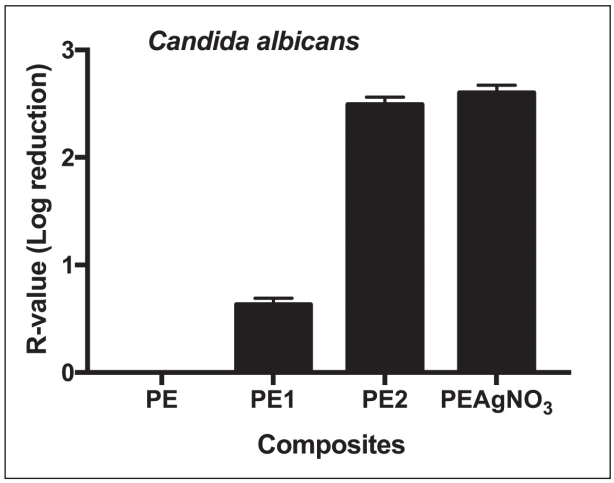

Figure 4. R-values obtained testing PE, PE1, PE2 and PAgNO3 against *Candida albicans;* unpaired t test of PE1 vs PAgNO_3 was t=575.29 with a ***p value<.0001, and of PE2 t=26.99 with a ***p value <.0001

Discussion

All experiments performed on the composites against two bacterial and one fungal strain in accordance with the ISO 22196:2007 (38) confirmed the antimicrobial and antifungal activity of all polymers tested. In general, the results showed that the composites embedded in PE maintain the broad-spectrum activity of silver, targeting both gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria and the *Candida albicans* fungus.

The positive results of the contact test, [standardized by the ISO 22916:2007 (38)], against two bacteria strains and also against a fungal strain (*Candida albicans*) suggest that these composites can be used against fungal strains as well. In depth, analysis of the results indicates some differences between the antimicrobial activities displayed by the various PE composites. The most important result was recorded for PE1, which showed very good antimicrobial performance measured as bactericidal activity against *Escherichia coli* and *Staphylococcus aureus*, and the highest performance (good) against *Candida albicans*. Even though PE1 showed only medium and slight performance against *Candida albicans* and *Escherichia coli* respectively, it performed very well against *Staphylococcus aureus*.

The higher performance of PE1 may indicate that the mechanism of interaction between this composite and the bacteria cell is stronger than that of the PE2 composite. The silver in the structure of polymer 1 probably exhibits stronger antimicrobial activity. In fact, the hypothesis that silver may act against microorganisms (43) is based on the observation that the oxidized form of silver (Ag^+) can bind strongly to thiols, phosphates, and other electron donating functional groups, in an interaction that disrupts the microbial cell membrane, causing leakage of cellular contents and finally cell death. A consequence of this interaction is the disruption of the cell membrane, followed by the leakage of cellular contents and finally microbial cell death (44).

Our results confirmed the possible application of the novel polymers to control nosocomial infections and AMR. In fact, new polymers characterized by antimicrobial activity are being investigated for use as materials for medical devices (45). In particular, silver has been widely used as an alternative antimicrobial agent in medical devices such as vascular prostheses, urinary catheters, and mechanical heart valves, and the results obtained in the present research confirm this application (46,47).

In addition, this feature is useful in that the various PEs can be used in place of plastic composites of AgNPs, in order to avoid the negative side effects of the latter, such as silver release and harmful environmental impact. “Contact action by polymer/polymer composites,” achieved by embedding insoluble silver

(I) coordination polymers in a polymeric matrix offers a new concept in the field of plastics endowed with permanent antimicrobial activity. Thus our composite plastics may prove useful in a number of different situations in which the accumulation of unwanted material is often overlooked.

Conclusions

In the last decade, there has been increased research to develop new antimicrobial polymeric materials free of negative effects on human health. Study of the mechanisms of interaction between such polymers and microorganisms should move forward with the use of standardized protocols. For this reason, we tested the antimicrobial and antifungal activity of two silver (I) coordination polymers, applying the standard methodology reported in ISO-22196: Plastics – Measurement of Antibacterial Activity on Plastics Surfaces. For future clinical applications, it will be important to test the composites against clinically isolated microbes, especially multidrug-resistant strains, and evaluate the *in vitro* and *in vivo* biocompatibility of composites.

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Author's contributions: SS designed the study and interpreted the results. PF contributed to the manuscript writing. GI contributed to the manuscript revising. IL conducted the microbiological tests. MF coordinated the synthesis of polymers and helped to critically review the manuscript. DNC designed the study, interpreted the results, and contributed to critical revision of 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

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Original article

First surveillance of malaria among seafarers: evaluation of incidence and identification of risk areas

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Summary. Malaria is a potentially deadly parasitosis. Due to its geographical distribution, seafarers are particularly exposed to it. This study is an epidemiological analysis on the morbidity of malaria onboard commercial ships without a physician, with the purpose of proposing solutions aimed at limiting further infections in this delicate and difficult working environment. *Methods:* We examined 17,212 health records of patients embarked on ships assisted by the CIRM from 2011 to 2015. All the diagnosed diseases have been categorized based on the ICD-10 classification system by the WHO. This study analyzed the cases of suspected malaria and the cases of malaria confirmed by clinical tests. The geographical distribution of infections and the drugs chosen by physicians are also assessed. *Results:* Although a reduction in malaria cases was appreciated during the 5-year period taken into consideration, this disease still covers an important percentage of all infectious diseases that occurred onboard. This proves that it is a major health problem for seafarers. *Conclusions:* In order to protect seafarers' population, strengthen malaria prevention and control programs, as well to improve the availability of antimalarial drugs onboard ships is necessary. This may ensure rapid and efficient health interventions in case of need. (www.actabiomedica.it)

Key words: malaria, prophylaxis, merchant ships, seamen, travel

1. Introduction

Malaria is the deadliest parasitosis in the world; every year it kills about 400,000 people, gaining the second place as a cause of infectious death, after tuberculosis (1).

It is transmitted by the female mosquitoes of the genus *Anopheles*, and caused by a parasite of the genus *Plasmodium*, Phylum *Apicomplexa*. Human malaria is primarily caused by four species of Plasmodium: *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium vivax*, but also, occasionally, by *Plasmodium knowlesi* (2-4). Among these, the most lethal is *Plasmodium falciparum*, with the highest mortality rate among infested subjects, and mainly widespread in Sub-Saharan Africa (5). Malaria manifests itself as an acute febrile illness, with an incubation period of 7 days or more, and with a symptomatology and clinical evolution whose severity depends on the species that has transmitted the infection (5, 6). Malarial starting symptomatology, which can be rather bland, includes fever, chills, headache, muscular aching and weakness, vomiting, cough, diarrhea, and abdominal pain. Subsequently, very serious complications may arise, such as symptoms related to organ failure, such as acute renal failure, pulmonary edema, generalized convulsions, circulatory collapse, coma and death (6).

About 40% of the world population lives in areas where malaria is endemic, such as in tropical and subtropical areas, and at an altitude of less than 1,800 meters (5, 7-9).

The remarkable geographical distribution of malaria causes travelers (10) and sailors to be particularly exposed to this disease.

In fact, a category of people particularly at risk is that of seafarers, especially those embarked on commercial ships that take long journeys around the world, for relatively long periods of time, and without a physician on board (11-15). These workers sail all over the world, and for work reasons they are often forced to visit malaria risk areas. Moreover, the work on ships is associated with an increased risk of illness and injury (16-21). This would seem to be due to the specific conditions of the sea, to the scarce possibilities of escaping from danger, exposure to extreme climatic conditions, psycho-physical stress and so on. Then, malaria among seafarers can have a double risk: the disease itself, and therefore the malaise and consequent disability of the subject, and the difficulty of diagnosis and therapy onboard ships (22).

Although many studies have shown a significant reduction in morbidity from infectious diseases in recent years, malaria still remains one of the biggest health problems related to sailors (11). Seafarers, by the nature of their job, cannot avoid malarial regions. That's why it emerges that it is essential to act in terms of prevention, avoiding the bite of the mosquito, and especially through chemo-prophylaxis, to be performed and maintained throughout the period in which the subject is potentially exposed to the disease or for the entire period of stay in the malarial area, and at least until 4 weeks after return or departure (2).

According to the World Health Organization's most updated guidelines, in malarial areas with multidrug-resistances, the most effective medicine has turned out to be the Malarone, which comes from the association of 250 mg of Atovaquone and 100 mg of Proguanil in a tablet (23).

Epidemiological investigations in the maritime sector are slightly uncommon in literature; even more rare are the investigations focusing on malaria; as a matter of fact, in this field epidemiology has often focused on small samples of the working population. Similar studies published in the last 10 years are also lacking (11, 13, 24-28).

The aim of this study is to carry out an epidemiological investigation on the morbidity of malaria on board commercial vessels, on the basis of the electronic health records provided by the International Radio Medical Centre (CIRM), paying particular attention to the geographical distribution of infections, and proposing strategies aimed at preventing further and future infections in this delicate and difficult working environment.

2. Methods

The period considered in this paper is from 1st January 2011 to 31st December 2015; in this time frame, the CIRM has assisted 17.212 patients on board commercial ships; for each case assisted by the Centre, a digitalized medical file, called "electronic record", has been established and updated following every contact with the ship. These files represent the basis of the investigations conducted in this study. Subject of this study are the crew members of commercial ships sailing in international waters. In these vessels there is no medical or health personnel on board, and medical assistance is provided through tele-assistance techniques.

All the diagnosed diseases have been categorized based on the International Statistical Classification of Diseases and Related Health Problems (10th Revision) by the World Health Organization (WHO) (29). Among the different ICD-10 categories, we took into consideration the diagnoses included in the ICD-10 Class "I", called "Certain Infectious and Parasitic Diseases", a category to which malaria belongs, as parasitosis. In this way, we were able to perform a quantitative comparison between cases of malaria and cases of other infectious and parasitic diseases.

In this paper, malaria diagnoses are distinguished in "suspected unconfirmed cases" and "cases confirmed by clinical tests". After a first telemedical contact with a competent CIRM doctor, a pre-diagnosis is proposed depending on the patient's symptoms and on physiological parameters directly measured on board. In the event that the symptomatology is ascribable to malaria, immediate confirmation is requested through clinical analyses, to be carried out at the nearest port. The performance of these laboratory tests has made it possible to distinguish confirmed cases of malaria from those who, despite having a symptomatology attribut-

able to this disease, have not had anything to do with it, and which we have called "suspected unconfirmed cases". In a second phase, through the identification of every single case of malaria, we were able to analyze the coordinates relating to the call position at the time the assistance request was formulated. This made it possible to analyze the geographic distribution of Plasmodium infections registered by us, and then to compile a cartography highlighting the areas with the highest endemic risk of malaria for seafarers. All data was analyzed with standard statistic methods; Microsoft Excel was the software used for information processing and result analysis. Data are expressed in the text as means ± SD.

According to informed consent, data were anonymized before being used for research purpose. The survey is a part of the project called Health Protection and Safety on Board Ships (acronym: HEALTHY SHIP). It is a project of disease prevention and health protection on board sailing ships through information campaigns on the major health risks for seafarers and on their prevention (30), approved by Comitato Etico Fondazione Centro Internazionale Radio Medico (C.I.R.M. Foundation ethic, scientific and medic Committee).

3. Results

The results of the epidemiological analysis conducted in this study are summarized in Table 1.

In Table 1 malaria diagnoses are compared with the diagnosis of other infectious and parasitic diseases included in the ICD-10 category I (Certain infectious and parasitic diseases), and also reported in percentages (%). This table therefore allows us to evaluate the morbidity of malaria and compare it with other infectious and parasitic diseases that occur on board. Fortunately, there were no deaths due to malaria among the cases of assistance taken into consideration in this study.

Whenever a request for assistance comes from a patient with malarial symptoms, the assistant doctor also orders to perform an appropriate laboratory test to diagnose malaria. The "confirmed by clinical tests" cases are those whose clinical examination has returned a positive result; on the other hand, we are talking about cases of "suspected unconfirmed" malaria, and then of pathological conditions of different etiology but which showed a symptomatology attributable to malaria.

In most cases, prescribing doctors, pending the results of laboratory tests, prescribed antipyretic drugs for the control of symptoms, especially those of a febrile kind and malaise. In particular, the most administered drug (75% of all cases) was paracetamol, with a dosage of 0.5-1g three times a day, followed by 200 mg ibuprofen tablets (for the remaining 25% of all cases).

Following the confirmation of malaria, made possible by clinical laboratory tests, the pharmacological choices of CIRM physicians, strongly influenced by the availability on antimalarial drugs on board, showed chloroquine as the drug of first choice (107 prescrip-

Table 1. Number of ICD-10 class I diagnoses, and number of malaria cases on board commercial ships assisted by the CIRM in the period 2011-2015

Year	Number of patients on board ships assisted by the CIRM	Number of ICD-10, category I cases	Number of "Suspected unconfirmed" Malaria Cases N(%)	Number of Malaria Cases "Confirmed by clinical tests" N(%)
2011	2561	80	2 (2,50%)	27 (33,75%)
2012	3120	84	3 (3,57%)	35 (41,67%)
2013	3428	152	5 (3,29%)	33 (21,71%)
2014	3908	200	4 (2,00%)	31 (15,50%)
2015	4195	154	2 (1,30%)	20 (12,99%)
Total	17212	670	16 (2,39%)	146 (21,79%)

tions, corresponding to 73,29% of all cases), followed by the artemether (19 prescriptions, 13,01% of all cases) then by the mefloquine (13 prescriptions, 8,90% of all cases), and finally the malarone (7 prescriptions, 4,80% of all cases).

According to the geographic coordinates concerning the calls made to the CIRM by the different naval units, we have created a world plan where the positions occupied by the ships are indicated with a special symbol, at the time of the request for assistance (Figure 1).

Considering the incubation times of malaria (7 days or more) (5, 6), and that the request for assistance is performed at the onset of symptoms, we have analyzed the routes travelled by the ships in the 7 days before the request for assistance. Then, thanks to this information, we created a map that highlights the areas at risk of malaria through different colors (Figure 2).

4. Discussion

As shown in Table 1, the malaria cases accounts for a significant proportion of the total, especially in the years 2011 and 2012 where it represents respectively 33,75% and 41,67% of the total cases of all infectious and parasitic diseases diagnosed on board.

This is cause for concern, since the ICD-10 I category also includes relatively less dangerous and rather common pathologies (such as topical fungal infections, bacterial and viral infections) (29), and the fact that malaria represents as overall 21,79% of the total cases of infectious diseases (in the 5 years analyzed) means that this parasitosis still represents a great threat to the health of seafarers.

On the other hand, the reduction of malaria cases, between 2013 and 2015 (Table 1) is encouraging, and suggests that if the correct prevention rules are followed correctly, a significant reduction of malaria cases onboard commercial vessels can definitely become an achievable goal.

According to the results of this report, by observing the geographic areas at risk of malaria in Figure 1 and Figure 2, we can see that the coasts of West Africa represent the area with the highest risk of contracting malaria.

The risk was intermediate nearby the coasts of the Middle East, while it was lower in Central America and South-East Asia. Looking in detail at Figure 2 we also notice a higher risk of contracting malaria in those

Figure 1. Geographical position of the vessels at the time of request for assistance

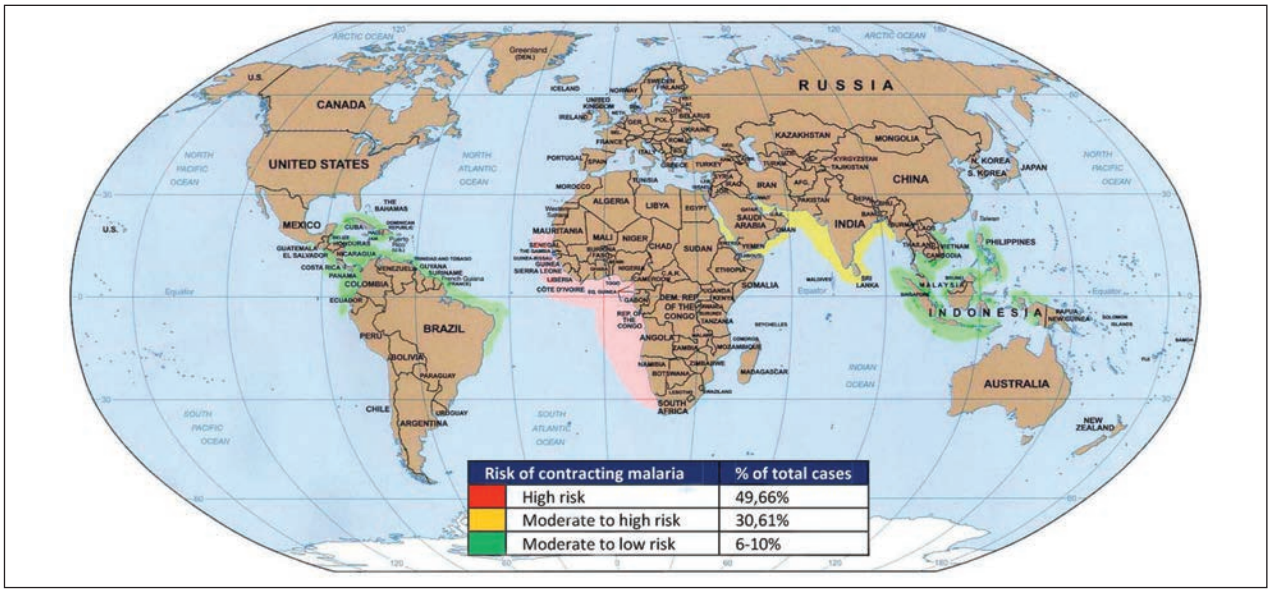

Figure 2. Geographic areas at risk of malaria infection for seafarers (based on the number of reported malaria cases)

where the risk of *P. falciparum* is just as serious (31).

From an analysis of the scientific literature and the international guidelines, it emerges that Malarone is the most effective drug against *P. falciparum*, but also the most efficient medicine against multidrug-resistant Plasmodium (23).

Despite this, it was the least administered among all antimalarial drugs (4.88%), as reported in the results. The choice of drugs considered as non-first choice for a given kind of Plasmodium is probably a consequence of the lack of a multiple choice possibility between different antimalarial drugs in many national medical chests (23, 24). The minimum amount of drugs that each ship should have on board is indicated in two publications: The International Medical Guide for Ships (32), and the Medical First Aid Guide for use in Accidents Involving Dangerous Goods (33), both by The World Health Organization (WHO). According to these two important documents, the main nations have regulated their on-board pharmacies (34).

The existing lack of homogeneity in terms of drugs in the medicine chest can seriously compromise the quality of assistance given to seafarers, and exposes patients to an absolutely non-negligible risk, as malaria is a deadly disease, especially if not properly treated.

5. Conclusions

For work reasons seafarers may not avoid malaria risk areas. That's why the right prevention and surveillance must be considered mandatory onboard ships navigating in endemic zones. These procedures can consist of the identification of samples tested in laboratories, the analysis of suspected infections, and the identification of subjects at risk. The accurate reports that follow will ensure that all cases of infection are reported and documented, with the purpose of performing notification and epidemiological analysis. The results of this analysis showed a reduction in the number of cases of malaria among seafarers over the years of study, highlighting the fact that reducing the cases of infection by this disease is an achievable goal. Therefore, strengthening prevention and creating control programs, along with a standardization in terms of quality and quantity of drugs that commercial vessels must have on board (in particular for more recent and effective medicines), is necessary to ensure a rapid and efficient health intervention in case of need, and reduce the spread of this parasitosis, especially in the delicate maritime sector.

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Availability of Data and Materials: The data that support the findings of this study are available from C.I.R.M. (Centro Internazionale Radio Medico) but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of C.I.R.M. (Centro Internazionale Radio Medico).

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

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Original article

Continuity of care for patients with hip fracture after discharge from rehabilitation facility

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Summary. *Background and aims of the work:* Hip fracture is a dramatic event especially in the elderly and the return to the pre-fracture functional and social state is often difficult to achieve. In the post-acute, the intensive rehabilitation period aims to recover as much autonomy as possible to these patients, but not always its duration is sufficient to ensure an effective and lasting result in returning home, hence the need for additional rehabilitation services. Our aim was to evaluate the use of additional rehabilitation services by patients who underwent hip fracture after an intensive rehabilitation treatment period performed at our hospital. *Methods:* This is a retrospective cohort study. We involved patients aged 45 years and older, admitted at our intensive rehabilitation, who joined a rehabilitation program for a hip fracture. *Results:* Our results showed how the use of further physiotherapy is associated with the type of surgical intervention and with higher Cumulative Illness Rating Scale CIRS scores. Similarly, the loss of autonomy is associated with the type of intervention, the increase in CIRS and the duration of the physiotherapy, and negatively associated with the duration of each session. The re-hospitalizations for each cause is positively associated with CIRS and negatively associated with the further use of physiotherapy. *Conclusions:* Our conclusion is that rehabilitation needs a personalized schedule, because the real discriminating factor in the management of frail patients should therefore be the quality, and not the quantity (i.e. longer session), of the rehabilitative intervention prescribed. (www.actabiomedica.it)

Key words: hip fracture, rehabilitation outcomes, post-acute care, hip fracture and surgery

Introduction

The fracture of femur, a dramatic event especially in the elderly, has an important impact both on the clinical level, as a factor of acquired comorbidity, and on the social level, through the reduction of the independence and autonomy of the patient (1, 2). The return to the pre-fracture functional and social state is often difficult to achieve and frailty that characterizes these patients has different expressions: the reduction of the ability to carry out daily activities both simple (Activity Daily Living, ADL) and instrumental (Instrumental Activity Daily Living, IADL), increased disorientation, possibility of further falls, new accesses to the Emergency Room. In the post-acute period, the intensive rehabilitation period aims to restore as much autonomy as possible to these patients, but not always its duration is sufficient to ensure an effective and lasting result on return home (3, 4). It is also interesting to note that, as emerges from the literature, one of the first factors involved in determining the optimal recovery of the patient is the time gap between the fracture event and the surgery (5, 6). The scientific community has in fact established its desirable duration of maxi-

mum 48 hours, since the increase in this time gap increases the patient's risk of intra- and post-operative complications, delaying and compromising his/her optimal5 overall functional recovery (7-9).

In the literature, several studies have analyzed the impact of rehabilitation on the functional outcome of the patient, and on the possibility of discharging a patient to home, without the onset of further early complications: the risk of new hospitalization or institutionalization is in fact high in the light of the type of patients who typically face a fracture of the femur, as elderly and with one or more comorbidity (10). It is interesting to note that in general only a small percentage of people return directly to their home without any need for rehabilitation or stay there even after a long time after discharge from the rehabilitation facility (11). Moreover, strictly related to the functional outcome that we aim to achieve at the time of compilation of the Individual Rehabilitation Project, is the setting in which the patient can perform rehabilitation: when it is conducted in specialized facilities rather than at home better results are more likely to be achieved for aspects such as balance, muscle strength and pace (12). This is based on the possibility of using specialized machinery and of being followed more intensely by an expert operator, thus enabling more lasting results to be achieved: the patient is thus put in a position to deal more safely with the various activities of daily life. For each patient, independence and the greatest possible functional autonomy in returning home are in fact the primary outcome of interest and their achievement must guide the choices by various specialists who make up the team when they take charge (13).

Objective of the study

The primary aim of the study was to evaluate the use of additional rehabilitation services by patients after discharge from our facility. The analyses were conducted considering the morbidity of the patients at the time of hospitalization, measured by the CIRS (Cumulative Illness Rating Scale) (14-18), the functional autonomy prior to the fracture event, the type of surgery and the time elapsed between the fracture and the surgery.

The following secondary outcomes were also considered:

- possible loss of autonomy at the time of the interview, compared to the one before the fracture;
- any new hospitalization, with reasons for doing so;
- consequences of any new falls;
- mortality of patients, in relation to the timing and type of surgery performed.

Methods

Study design

Observational, single-center, retrospective, cohort, epidemiological study with convenience sampling.

Patients

Patients undergoing surgery for a fractured femur between 1st May 2013 and 31st May 2016 for whom telephone contact was available were contacted to answer to a questionnaire drafted ad hoc to reconstruct their care needs over time. Patients were contacted by telephone between 4 months and 48 months after surgery. A maximum of three interview attempts were made for each patient. If the patient died at the time of the interview, the information provided by the caregiver was taken into account. The study finished at the end of June 2018.

Patients over 45 years of age, both male and female, who demonstrated the following inclusion and exclusion criteria, were contacted:

Inclusion criteria

- Surgically treated femur fracture.
- Intensive rehabilitation treatment performed at our hospital and provided according to current legislation, which provides for a physiotherapy treatment lasting a total of 380 minutes per patient per week (19).

Exclusion criteria

- Patients who have not joined the rehabilitation program due to non-compliance and/or worsening of clinical conditions that have led to an early transfer to acute care.

- Patients who did not give their consent to participate in the study at the time of telephone contact.

For all patients, the following informations were also considered at the time of hospitalization: the state of morbidity measured by the CIRS (Cumulative Illness Rating Scale), the functional autonomy prior to the fracture event, the type of surgery and the time elapsed between the fracture and the surgery. The CIRS scale has been arbitrarily evaluated and the scores obtained in the different items have been added and kept rough.

Statistical methods

Data have been reported in terms of absolute frequencies and percentages or median and interquartile range if appropriate. To evaluate the factors associated with the use of additional physiotherapy and loss of autonomy, univariate and multivariable logistical models were used. The results were reported in terms of odds ratio (OR) and 95% confidence interval (95% CI). For outcomes that assess the time of occurrence of an event, i.e. hospitalization for any cause and death, the Cox proportional hazard regression model was used, as well as the results reported in terms of hazard ratio (HR) and relative 95% confidence intervals. Interest times were calculated from the date of surgery to the date of the event for patients who had the event, otherwise to the date of last contact. The crude cumulative incidences (CCIs) for re-hospitalization for each cause and for re-hospitalizations only due to a new fall have been estimated using a method appropriate to the competitive risks.

Results

Of the 850 patients who underwent surgery/rehabilitation at our facility in the reporting period and considered for the purposes of this study, 445 had a telephone contact available and answered. Of these, 424 agreed to the interview. Further seven patients were excluded from the study because they could not be evaluated (n=2 with no date of surgery available, n=1 with less than 45 years and n=4 with a telephone interview less than 4 months after surgery). The remaining 417 patients are divided into males (N=58) and females (N=359), with a median age of 83; the rough median value of the CIRS scale was 17. Of the surgical procedures, 234 patients underwent surgical fixation, 149 placed an endoprosthesis and 34 a total replacement: 198 patients waited less than two days for surgery. Considering the autonomy before the femur fracture, 309 patients were able to leave the house on their own, 70 used to leave house only with someone, 38 moved mainly indoors and only 1 patient was bedridden or sitting in a wheelchair.

Of the patients included in the analysis, 333 (80%) had recourse to further physio-kinesiotherapy after discharge, mainly on the instructions of the physiatrist (73%) (Table 1). Of the 84 patients who did not use physiotherapy, 68 returned to their homes, 11 went to nursing homes, 4 to acute care hospitals and one to extensive rehabilitation.

The logistic regression analysis (Table 2) shows how the use of further physio-kinesiotherapy is asso-

Table 1. Characteristics of patients who underwent further physiotherapy. *Median (IQR)

Variable	N (%)
Duration of physiotherapy, weeks (n=333)	6 (4, 10)*
Number of sessions per week (n=332)	3 (2, 5)*
Duration of a session, minutes (n=333)	
≤30	77 (23)
31-60	242 (73)
60	14 (4)
Addressing physician (n=333)	
Physiatrist	244 (73)
General practitioner	16 (5)
Orthopedist	4 (1)
Patient's own choice	69 (21)
Return to home (n=333)	
No	214 (64)
Yes	119 (36)

Table 2. Results from univariable and multivariable logistic regression models of further physiotherapy.

	Univariable			Multivariable		
	OR	95% CI	P	OR	95% CI	P
Sex						
Female	1			1		
Male	0.68	0.36 to 1.30	0.24	0.66	0.34 to 1.27	0.21
Age (years)						
5-unit increase	0.98	0.85 to 1.12	0.75	0.93	0.81 to 1.08	0.35
Intervention			0.02			0.01
Surgical fixation	1			1		
Endoprosthesis	0.78	0.47 to 1.32	0.36	0.79	0.47 to 1.34	0.39
Total replacement	0.33	0.15 to 0.72	0.005	0.30	0.13 to 0.67	0.003
Interval between fracture and intervention						
≤2 days	1					
>2 days	1.13	0.70 to 1.83	0.61			
Autonomy before fracture			0.37			
Patient used to leave house on his own.	1					
Patient used to leave house only with someone.	0.94	0.49 to 1.81	0.86			
Patient moved mainly indoors, or was bedridden, or sitting in a wheelchair	0.58	0.27 to 1.23	0.16			
CIRS						
5-unit increase	1.19	1.02 to 1.38	0.03	1.20	1.03 to 1.39	0.02

ciated with the type of intervention, in particular the total replacement versus surgical fixation (OR=0.33, 95%CI=0.15 to 0.72, P=0.005) and with higher CIRS scores (OR=1.19, 95%CI=1.02 to 1.38, P=0.03). Similarly, the loss of autonomy is associated with the type of intervention and the increase in CIRS, as well as the increase in age (Table 3). For patients who have done further physiotherapy, in the multivariable model the loss of autonomy is also positively associated with the duration of the physiotherapy in weeks (OR=1.05, 95%CI 1.00 to 1.10 for each one week increase) and negatively associated with the duration of each session (31-60m vs <30m OR=0.50, 95%CI=0.28 to 0.89; >60m vs <30m OR=0.15, 95%CI 0.04 to 0.64), while the CIRS score has no longer a significant effect.

Of the patients interviewed 151 (CCI = 50%) have a new hospitalization: 74 (CCI = 22%) for a new fall and 77 for other reasons (25 for cardiovascular causes, 20 for infectious causes, 7 for neurological causes, 6 for tumors and 17 for other causes). The new re-hospitalization for each cause is positively associated with CIRS and negatively associated with the further use of physiotherapy (Table 4). Of the 74 patients re-hospitalized for new falls, 8 reported fractures to the previ-

Table 3. Results from univariable and multivariable logistic regression models for loss of autonomy

	Univariable			Multivariable		
	OR	95% CI	P	OR	95% CI	P
Sex						
Female	1			1		
Male	0.71	0.41 to 1.26	0.24	0.73	0.40 to 1.33	0.30
Age (years)						
5-unit increase	1.39	1.23 to 1.59	<0.0001	1.38	1.21 to 1.58	<0.0001
Intervention			0.009			0.05
Surgical fixation	1			1		
Endoprosthesis	0.87	0.58 to 1.31	0.51	0.78	0.51 to 1.20	0.27
Total replacement	0.25	0.11 to 0.61	0.002	0.33	0.13 to 0.83	0.02
Interval between fracture and intervention						
≤2 days	1					
>2 days	1.02	0.70 to 1.50	0.91			
CIRS						
5-unit increase	1.13	1.00 to 1.28	0.04	1.16	1.02 to 1.31	0.02
Further physiotherapy						
No	1					
Yes	1.56	0.95 to 2.54	0.08			

ously operated limb, 39 to the limb contralateral limb, 13 other fractures and 14 did not report any significant consequences. No significant associations emerged between the duration of post-discharge physiotherapy, in terms of number of sessions, and further falls or new fractures (data not shown).

From the Cox regression model (Table 5) the risk of mortality is positively associated with age, loss of autonomy and shorter time between fracture and surgery.

Discussion

The results of the study were in line with the literature, confirming the important clinical-functional implications that the fracture of the femur, and the consequent loss of autonomy associated with it, has on the patient especially elderly (20-22).

The finding of a low mortality in relation to an intervention timing of more than 48 hours, is, however, in clear contrast with the literature, we probably think we can attribute this result in part to a 'selection bias' induced by the difficulties in contacting patients or their caregivers and the possible inaccuracy with which data were collected regarding the days elapsed between fracture and surgery.

Patient mortality was found to be particularly related with advanced age and reduced recovery of functional autonomy. This result could be attributable to a general physical deconditioning of the subject, with a relative reduction in cardiopulmonary performance, an

Table 4. Results from univariable and multivariable proportional hazard regression models for re-hospitalization

	Univariable			Multivariable		
	HR	95% CI	P	HR	95% CI	P
Sex						
Female	1			1		
Male	1.14	0.73 to 1.76	0.57	1.13	0.72 to 1.75	0.60
Age (years)						
5-unit increase	1.04	0.94 to 1.14	0.46	1.04	0.95 to 1.15	0.36
Intervention			0.98			
Surgical fixation	1					
Endoprosthesis	0.97	0.69 to 1.36	0.85			
Total replacement	0.97	0.53 to 1.77	0.91			
Interval between fracture and intervention						
≤2 days	1					
>2 days	0.78	0.57 to 1.08	0.13			
Further physiotherapy						
No	1			1		
Yes	0.68	0.47 to 0.98	0.04	0.64	0.44 to 0.94	0.02
CIRS						
5-unit increase	1.11	1.01 to 1.23	0.04	1.13	1.02 to 1.25	0.02

increase in secondary complications related to hypomobility and bedding. The results also suggest a possible protective effect (although not significant) for the positioning of endoprosthesis with respect to the surgical fixation, while the small number does not allow conclusions to be drawn on the total prosthesis intervention.

In addition, again with regard to the type of surgery, it was found that patients undergoing arthroplasty (total replacement) have found a lower need for physiotherapy than those undergoing surgery for osteosynthesis (surgical fixation) and endoprosthesis (Table 2).

The recovery of the autonomy prior to the operation is more conditioned by a lower age, a lower CIRS score and the type of surgery performed (Table 5). In fact, it was noted that the reduction in autonomy was statistically associated with surgical fixation, which would justify in the latter patients the greater need for physiotherapy sessions (Table 3).

It is also interesting to note that, of the 151 patients who needed access to an emergency room after discharge from our facility, 77 presented a new fall as an index event. Of these, 60 patients had a new fracture, of which more than half (39 patients) had a contralateral limb fracture. The duration of the physiotherapy carried out after discharge from our facility, in terms of number of weeks, was positively related with the loss of autonomy; on the contrary, having carried out longer physiotherapy sessions (for example >30 minutes) was negatively correlated with it. These data can have a twofold key: first, the need for further physiotherapy emerges for those patients with more compromised

Table 5. Results from univariable and multivariable proportional hazard regression models for overall survival

	Univariable			Multivariable		
	HR	95% CI	P	HR	95% CI	P
Sex						
Female	1			1		
Male	1.41	0.65 to 3.05	0.38	1.90	0.87 to 4.12	0.11
Age (years)						
5-unit increase	1.41	1.15 to 1.74	0.001	1.28	1.02 to 1.61	0.04
Intervention			0.07			0.14
Surgical fixation	1			1		
Endoprosthesis	0.52	0.26 to 1.03	0.06	0.51	0.26 to 1.03	0.06
Total replacement	0.21	0.03 to 1.53	0.12	0.45	0.06 to 3.38	0.44
Interval between fracture and intervention						
≤2 days	1			1		
>2 days	0.46	0.25 to 0.86	0.02	0.46	0.24 to 0.86	0.02
Autonomy before fracture			<0.0001			
Patient used to leave house on his own.	1		<0.0001			
Patient used to leave house only with someone.	5.66	2.82 to 11.34	<0.0001			
Patient moved mainly indoors, or was bedridden, or sitting in a wheelchair	7.22	3.31 to 15.73				
Autonomy after fracture			<0.0001			
Patient used to leave house on his own.	1					
Patient used to leave house only with someone.	2.05	0.46 to 9.17	0.35			
Patient moved mainly indoors.	13.85	4.04 to 47.55	<0.0001			
Patient was bedridden, or sitting in a wheelchair.	33.85	10.05 to 114.06	<0.0001			
Loss of autonomy						
No	1			1		
Yes	7.55	3.19 to 17.89	<0.0001	6.48	2.69 to 15.57	<0.0001
CIRS						
5-unit increase	1.02	0.85 to 1.24	0.80			

starting clinical conditions (higher CIRS scores, Table 2), therefore also likely with more limited prospects of recovery; secondly, the importance of the "posology" of physical exercise is confirmed, according to which a longer rehabilitation period is not necessarily associated with greater recovery. The quality (intensity - in the sense of longer sessions) of the rehabilitation intervention is therefore more effective in improving the outcome than the quantity of the same.

However, it emerges that the rate of re-hospitalization was higher in two categories of patients: first, in those with higher values of pre-morbid CIRS, confirming that the fracture of the femur probably overlapped a pre-existing clinical picture of fragility; secondly, in those patients who, following surgery, did not undergo further sessions of physiotherapy. This fact, from our point of view, highlights how rehabilitation can positively affect not only the strictly neuromotor aspects, but also cardiovascular, respiratory, etc. aspects, reducing the risk of secondary problems to hypomobility and/or bedding (Table 4).

Study limitations

Our study has some limitations. Firstly, respondents may not have been sufficiently precise in recalling certain aspects of their rehabilitation. Above all, it has not been possible to carry out a more in-depth analysis with regard to the different discharge settings.

Moreover, the selection of the sample induced by the availability of a contact may have influenced the homogeneity of the sample with consequences on the interpretation of the results. In this sense, it would be interesting to carry out a new study considering also the long-term outcomes.

Conclusions

The results obtained from our study confirm that the femur fracture is a dramatic event for each patient in terms of loss of autonomy with greater impact on the most fragile individuals, regardless of the precociousness of intervention, and predisposes to adverse outcomes such as re-hospitalization.

Further physiotherapy after discharge from the specialist facility has generally been effective in reducing the likelihood of new hospitalizations. However, the non-evidence of association between falls or new fractures and the intensity of rehabilitation sessions can have important results in daily clinical practice: rehabilitation, in fact, as any therapy, must be prescribed with an appropriate and personalized schedule, depending on the clinical condition of the patient and the rehabilitation goals that you want to achieve (23, 24). Since high 'quantities' of rehabilitative treatment are not necessarily associated with better functional outcomes, the real discriminating factor in the management of fragile patients must therefore be the quality of the rehabilitative intervention prescribed.

Clinical messages

- rehabilitation is essential after hip fracture in the elderly
- rehabilitation is a therapy that must be carefully prescribed by the specialist doctor
- rehabilitation must be qualitative rather than quantitative

Ethics approval and informed consent: Ethics approval from Ethics Committee of Milan Area B dated 10.05.2016, reference number 268_2016. Informed consent was acquired for all patients.

Author contributions: LP and AVC originated the idea for the study and contributed to its design, DC and SE are responsible for the data collection. DC, SE and SC drafted the manuscript, MF and IA conducted the statistical analysis. All authors read, edited 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

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Briefing on

Esitazione ed obbligo vaccinale in Emilia-Romagna: il caso della vaccinazione MPR

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Vaccine hesitancy and mandatory immunizations in Emilia-Romagna Region: the case of MMR vaccine

Summary. *Background and aim:* An increase of vaccine hesitancy has spread worldwide and lead to reduction in coverage rates. The trivalent Measles-Mumps-Rubella (MMR) vaccine has been one of the most targeted leading due to possible (but never proven) adverse effects. This resulted in an increase of measles cases. The aim of the study is to analyze the 24-months coverage rates for the MMR vaccine in Emilia-Romagna Region (RER) between 2007 and 2018 and to correlate any significant changes to index events. *Methods:* Official aggregate data on vaccination coverage at 24-month provided by the RER and the Italian Ministry of health were analyzed and discussed. *Results:* From 2012 to 2015 a significant reduction in vaccination rates has been registered. In the following years an increase was recorded temporarily related to national and regional laws. *Conclusion:* The mandatory vaccination strategies seem to be in the short period effective in RER to counteract the growing population neegative attitudes towards vaccination and mitigate vaccine hesitancy. (www.actabiomedica.it)

Key words: MMR vaccination, vaccine hesitancy, mandatory vaccination, Italy

Riassunto. *Premesse e obiettivi:* Un aumento dell'esitazione vaccinale si è diffusa in tutto il mondo e ha portato alla riduzione significativa dei tassi di copertura. Il vaccino trivalente morbillo-parotite-rosolia (MMR) è stato uno dei più interessati a causa di presunti (e mai dimostrati) effetti collaterali e il calo della copertura di questa vaccinazione ha portato ad un aumento dei casi di morbillo. L'obiettivo della ricerca è quello di analizzare i tassi di copertura per il vaccino MMR a 24 mesi di età nella Regione Emilia-Romagna (RER) tra il 2007 e il 2018 e di correlare eventuali cambiamenti significativi ad eventi esterni. *Metodi:* Sono stati analizzati e discussi i dati aggregati ufficiali sulla copertura vaccinale a 24 mesi forniti dalla Regione e dal Ministero della Salute. *Risultati:* Dal 2012 al 2015 è stata registrata una riduzione significativa dei tassi di vaccinazione. Negli anni seguenti è stato registrato un aumento temporalmente correlato all'entrata in vigore delle leggi nazionali e regionali. *Conclusioni:* Le strategie di vaccinazione obbligatorie sembrano essere efficaci nel breve periodo nella RER per contrastare l'atteggiamento della popolazione nei confronti della vaccinazione e mitigare l'esitazione vaccinale.

Parole chiave: vaccino MMR, esitazione vaccinale, vaccinazioni obbligatorie, Italia

Introduzione e obiettivi

Come bene noto, nell'ultimo decennio si è verificato a livello mondiale un aumento dell'esitazione vaccinale con conseguente riduzione dei tassi di copertura (1, 2). Il vaccino trivalente Morbillo-Parotite-Rosolia (MPR) è stato maggiormente penalizzato a causa di presunti effetti collaterali (autismo), e il calo della copertura di questa vaccinazione ha determinato un aumento dei casi di malattia in Italia con conseguente richiamo ufficiale dell'OMS. La Regione Emilia-Romagna (RER), pur avendo avuto in passato una delle più elevate coperture per il vaccino MPR (3), non è stata risparmiata dal fenomeno della esitazione vaccinale e anche dell'aumento di casi di malattia. Nel 2008 sono stati registrati 180 casi di morbillo, passando da un tasso di incidenza inferiore allo 0,5 al 4,6 per 100.000 abitanti. Negli anni successivi si sono verificate altre ondate epidemiche, in particolare negli anni 2010 (129 casi), 2011 (198 casi) e 2014 (208 casi) che hanno interessato soprattutto i giovani-adulti non vaccinati o vaccinati con singola dose. Per la rosolia invece si è assistito ad un unico anno epidemico, il 2008, nel quale sono stati registrati complessivamente 499 casi di rosolia (4).

Tale contesto ha indotto le autorità sanitarie regionali ad anticipare, rispetto ai provvedimenti nazionali in itinere, l'entrata in vigore di misure coercitive. Infatti, il 25 novembre 2016 è stata emanata la Legge Regionale n.19 che ha introdotto l'obbligo vaccinale per l'accesso ai servizi educativi e ricreativi. Nel 2017 sono poi entrati in vigore il nuovo Piano Nazionale di Prevenzione vaccinale (PNPV) 2017-2019 e la Legge Nazionale n.119 che ha esteso l'obbligo a 10 vaccinazioni con divieti di accesso per i bambini non vaccinati a nidi e scuole materne e sanzioni per i non vaccinati nella scuola dell'obbligo (5).

L'obiettivo di questo lavoro è analizzare l'andamento temporale dei tassi di copertura per il vaccino MPR a 24 mesi di età nella RER e correlarli con eventuali fattori esterni che potrebbero avere influito sull'andamento.

Materiali e metodi

Sono stati considerati come indicatori i dati sulle coperture vaccinali per MPR a 24 mesi, diffusi dai report dalla RER e del Ministero della Salute in forma aggregata (6, 7). La popolazione di riferimento è relativa ai residenti al 1° gennaio di ciascun anno di rilevazione. I dati presentati relativi alle coperture vaccinali della RER negli anni successivi al 2016 provengono dall'Anagrafe Vaccinale informatizzata Regionale, quelli precedenti venivano forniti in forma aggregata dalle singole Aziende Usl. Il trend generale delle coperture vaccinali è stato correlato con i due importanti provvedimenti legislativi (LR del 2016 e Legge Lorenzin del 2016) e con la sentenza del Tribunale di Rimini del 15 marzo 2012 (8) che ha sostenuto la possibile associazione fra vaccino e autismo determinando paure nella popolazione e sviluppo dei movimenti novax nella RER (9). È stata anche identificata temporalmente un›importante epidemia di meningite nella confinante Regione della Toscana per l'attenzione che i mass-media hanno riservato a questo evento correlandolo alla esitazione vaccinale (10,11).

Risultati

Il trend delle coperture per MPR in RER (Figura 1), sostanzialmente stabile tra il 2007 e il 2012 e tra i più elevati a livello nazionale (3), subisce un decremento significativo a partire dall'anno 2013 fino a raggiungere il minimo storico nell'anno 2015, con un decremento del 5,4% in soli tre anni. In media dal 2007 al 2015, ogni anno sono stati vaccinati circa 700 bambini su 100.000 in meno. A partire dall'anno 2016 si è osservato un incremento che ha portato i tassi di copertura al 93,5% nel 2018, che risulta il miglior dato del periodo storico considerato.

Per quanto riguarda i dati delle singole province della RER (Tabella 1) non si osservano particolari variazioni rispetto al trend regionale, con coperture più basse nell'Area Vasta della Romagna (in particolare le province di Rimini, Cesena e Forlì, note per la presenza dei movimenti anti-vax) e più elevate nelle province del Nord.

Discussione e conclusioni

I tassi di copertura per il vaccino MPR a 24 mesi nella RER hanno registrato un'importante riduzione tra il 2012 e il 2015 che hanno indotto le autorità sa-

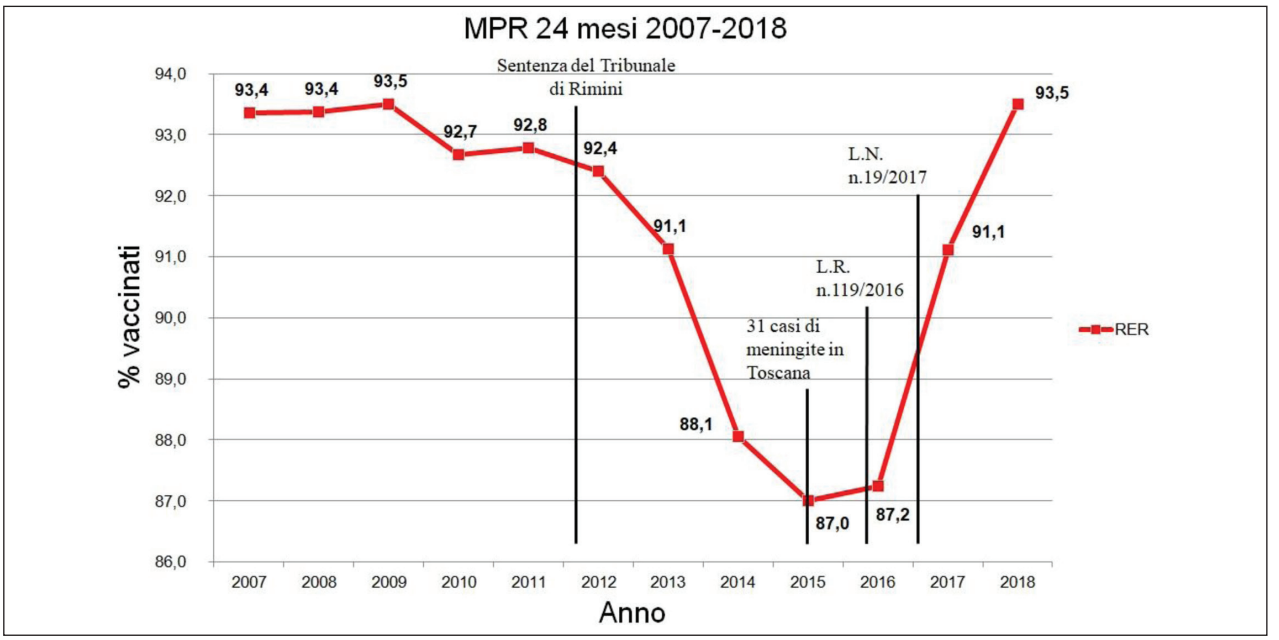

Figure 1. Tassi di copertura vaccinali nel periodo 2007/2017 in relazione ad eventi indice

Tabella 1. Copertura della vaccinazione MPR a 24 mesi nelle diverse AUSL e Aree vaste

AUSL	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Piacenza	97,4	97,0	97,6	96,7	94,1	94,3	93,0	91,8	90,9	88,8	90,9	93,0
Parma	93,3	92,8	94,3	93,8	93,9	93,2	93,1	87,5	89,0	89,1	92,8	94,1
Reggio Emilia	93,0	93,0	93,4	94,1	93,3	92,8	90,8	88,5	87,8	89,7	92,6	94,5
Modena	94,1	94,6	94,5	94,2	93,9	93,7	92,1	90,2	90,2	89,1	92,5	94,3
Totale Emilia Nord	**94,0**	**94,1**	**94,5**	**94,4**	**93,8**	**93,4**	**92,1**	**89,3**	**89,4**	**89,2**	**92,4**	**94,0**
Bologna	92,7	93,1	92,6	91,9	92,4	91,4	91,8	89,1	86,3	86,7	90,9	93,7
Imola	97,1	96,0	96,0	96,2	96,6	95,0	94,0	89,7	90,1	89,3	92,9	94,4
Ferrara	95,9	96,6	97,0	95,2	95,6	94,7	94,4	92,0	89,2	88,8	92,1	93,5
Totale Emilia Centrale	**93,9**	**94,2**	**94,0**	**93,2**	**93,5**	**92,6**	**92,6**	**89,8**	**87,4**	**87,4**	**91,3**	**93,9**
Ravenna	95,0	94,1	94,2	93,0	93,4	93,3	91,2	89,3	89,3	90,0	93,8	95,7
Forlì	92,8	92,6	92,9	90,5	90,5	91,6	87,7	86,5	81,5	83,8	90,2	90,9
Cesena	89,3	89,5	89,9	86,0	90,6	89,1	86,6	81,0	77,4	80,6	87,3	91,7
Rimini	88,2	87,8	87,2	85,2	85,6	87,3	84,2	77,5	77,7	77,6	82,3	88,3
Totale Romagna	**91,5**	**91,2**	**91,1**	**89,0**	**90,1**	**90,4**	**87,7**	**83,7**	**82,1**	**83,3**	**88,4**	**91,6**

nitarie regionali ad assumere provvedimenti specifici, al di là delle iniziative nazionali. Pur dovendo considerare i tempi di latenza tra i provvedimenti emanati e il rilievo delle coperture vaccinali a 24 mesi, si può notare come, nell'anno successivo all' entrata in vigore della Legge RER 19/2016, la copertura per MPR sia aumentata del 3,9% e, nell'anno successivo all' entrata in vigore del PNPV 2017-19 e della Legge 117/2016, di un ulteriore 2,4%. Ciò avvalora l'efficacia dei provvedimenti normativi, accompagnati peraltro da cam-

pagne informative e iniziative politiche a diversi livelli a favore delle pratiche vaccinali. Anche la cessazione di sentenze non basate su teorie scientifiche, alcune campagne mediatiche a supporto delle vaccinazioni e l'emanazione del PNPV 2017-2019 possono avere contribuito al rialzo dei tassi di copertura (12, 13).

Resta il dubbio che l'obbligo vaccinale - efficace nel breve periodo - possa limitare la fiducia nei confronti della vaccinazione e portare, nel lungo periodo, ad un'attenuazione degli effetti positivi deie provvedimenti coercitivi (13). Anche per questo motivo il miglioramento delle conoscenze sulle vaccinazioni (health literacy) rimane l'obiettivo principale da raggiungere, in coerenza con il vigente PNPV (5). E ciò al fine di migliorare la fiducia dei genitori e permettere di compiere scelte consapevoli e responsabili, per contrastare la diffusione delle malattie infettive prevenibili. Infatti la comunicazione e l'informazione sono le componenti essenziali delle strategie per indirizzare l'esitazione vaccinale e per il successo di qualsiasi programma di immunizzazione (14, 15).

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