EUROPEAN JOURNAL OF ONCOLOGY

GIORNALE EUROPEO DI ONCOLOGIA

Official Organ of the Italian Society of Tumours
SIT
Prevention, Diagnosis, Therapy

Organo Ufficiale della Società Italiana Tumori
SIT
Prevenzione, Diagnosi, Terapia

European Foundation of Oncology
and Environmental Sciences "B. Ramazzini"
Fondazione Europea di Oncologia
e Scienze Ambientali "B. Ramazzini"

PUBLISHER
MATTIOLI 1885 - SPA
CASA EDITRICE

Autorizzazione del Tribunale di Parma n. 14/97 del 11/6/1997 - ISSN 1128-6598

The European Journal of Oncology is indexed by Excerpta Medica (EMBASE) and the Elsevier BioBASE
Il Giornale Europeo di Oncologia è recensito su Excerpta Medica (EMBASE) e su Elsevier BioBASE

EUROPEAN JOURNAL OF ONCOLOGY
GIORNALE EUROPEO DI ONCOLOGIA

CONTENTS/INDICE

VOLUME 9 - N. 4 - 2004

NINTH COLLEGIUM RAMAZZINI STATEMENT

Preventing chemical accidents: lessons learned since the Bhopal disaster in 1984 /
Prevenire gli incidenti nell'industria chimica: le lezioni apprese dal disastro di Bhopal del 1984 213

TENTH COLLEGIUM RAMAZZINI STATEMENT

Call for action on the genocide in Darfur / ***Richiesta di intervento per il genocidio nel Darfur*** 215

EDITORIAL / ***EDITORIALE***

Public perceptions of science / ***La pubblica percezione della scienza***
M. Sorsa 217

CRITICAL REVIEWS / ***RIVISTE CRITICHE***

ANATOMIC SITES / *SEDI ANATOMICHE*

Colon / *Colon* (C18.9)

Enteral expandable metal stents for the treatment of neoplastic large bowel obstruction /
Le protesi metalliche autoespandibili nel trattamento dell'ostruzione neoplastica del grosso intestino
A. Piemontese 219

Breast / *Mammella* (C50.9)

Breast reduction and cancer: state of the art and future trends / ***Riduzione della mammella e cancro: stato dell'arte ed orientamenti futuri***
B. Palmieri, A. Costa 223

ARTICLES ON ORIGINAL STUDIES AND RESEARCH / ***ARTICOLI SU STUDI E RICERCHE ORIGINALI***

GENERAL TOPICS / *ARGOMENTI GENERALI*

HLA genotyping, non classical HLA-I-G soluble molecules and cytokine production in 28 established human tumour cell lines. Considerations for *in vitro* biological investigations and human anti-tumour vaccine therapy / ***Tipizzazione HLA, produzione di molecole solubili di HLA-I-G e citochine da parte di 28 linee cellulari tumorali umane. Considerazioni per studi in vitro e vaccinoterapia antitumorale umana***
C. De Vinci, R. Rizzo, A. Mazzuca, L. Melchiorri, M Ferrari, L. Giacomelli, G. Pizza, O.R. Baricordi 231

ANATOMIC SITES / *SEDI ANATOMICHE*

Colon / *Colon* (C18.9)

La magnificazione d'immagine nella diagnosi endoscopica dei tumori epiteliali del colon. Criteri classificativi e indicazioni per il trattamento endoscopico (mucosectomia) dell'*early colon cancer* / *Image magnification in endoscopic diagnosis of epithelial tumours of the colon. Classification criteria and indications for endoscopic treatment (mucosectomy) of early colon cancer*
L. Saragoni, A. Saragoni, C. Cortini, E. Ricci 237

Breast / *Mammella* (C50.9)

Il carcinoma localmente avanzato della mammella. Indicazioni alla mastectomia / *Locally advanced breast cancer. Indications for mastectomy*
G. Fabiano, A. Pezzolla, G. D'Eredità, M.A. Filograna, S.T. Massa, F. Ferrarese 245

CLINICAL CASE REPORTS / *RESOCONTI DI CASI CLINICI*

Spleen / *Milza* (C42.2)

Resoconto di un caso di angioma a cellule littorali della milza e revisione della letteratura / *Report of a case of splenic littoral cell angioma and review of the literature*
R. Ricco, M.G. Lecce, T. Cipriani, F. Sanguedolce, F. Giotta, A.M. Dalena, M. Carlucci 249

BRIEF COMMUNICATIONS / *COMUNICAZIONI BREVI*

Who uses alternative medicine: unconventional cancer treatment in Italy after the Di Bella scandal / *Chi usa la medicina alternativa: terapie antitumorali non convenzionali in Italia dopo lo scandalo Di Bella*
S. Tanneberger, A. Panetta, E. Strocchi, F. Pannuti 253

OBITUARY / *NECROLOGIO*

Professor Feng-sheng He
Y.-x. Zheng, Z.-l. Xia 257

ANNOUNCEMENT / *ANNUNCIO*

Second international NIVA course "Ethical, scientific and social aspects of predictive testing in occupational health practices", Nagu, Finland, May 2005 / S*econdo corso internazionale NIVA "Aspetti etici, scientifici e sociali dei test predittivi nel campo della medicina del lavoro", Nagu, Finlandia, maggio 2005*
259

ANNOUNCEMENT / *ANNUNCIO*

Collegium Ramazzini International Scientific Conference "Framing the Future in the Light of the Past: Living in a Chemical World", Bologna, Italy, September 2005 / *Convegno Scientifico Internazionale del Collegium Ramazzini "Progettare il Futuro alla Luce del Passato: Vivere nel Mondo della Chimica", Bologna, settembre 2005*
261

NINTH COLLEGIUM RAMAZZINI STATEMENT

PREVENTING CHEMICAL ACCIDENTS: LESSONS LEARNED SINCE THE BHOPAL DISASTER IN 1984

PREVENIRE GLI INCIDENTI NELL'INDUSTRIA CHIMICA: LE LEZIONI APPRESE DAL DISASTRO DI BHOPAL DEL 1984

On the 20th Anniversary of the Bhopal disaster in India, the Fellows of the Collegium Ramazzini express our condolences to and compassion for the more than 500,000 people who were harmed by the toxic gas tragedy in 1984. We praise the continued work of many community members and their supporters who have sought to alleviate the consequences from this disaster. Lessons learned from the Bhopal incident and others must be used to prevent similar events.

At least 2,500 children, women and men were killed suddenly by the release of toxic gases from a runaway chemical reaction at a production facility in the Indian state of Madhya Pradesh on the early morning of December 3, 1984. People exposed to methyl isocyanate and other toxic gases suffered injuries to their eyes and respiratory tract, and some also suffered neurological effects. The gas release damaged animals, plants and the ecosystem. The long-term consequences of the exposure are still unfolding: thousands of residents suffer chronic diseases with multiple symptoms and impairments that undermine the health and productivity of the community. Governmental and private organizations have provided clinical facilities for the care of the victims, though not enough to satisfy the medical and social needs. The site has not been remediated, and remains a source of toxic chemicals that continue to contaminate air, water and soil and endanger public health.

The Bhopal tragedy is the world's worst, reported chemical disaster, but it is not unique. Other major incidents have occurred, e.g., the dioxin release in Seveso, Italy in 1976, the ammonium nitrate explosion in Toulouse, France in 2001 and the hydrogen sulphide poisoning in Chongqing, China in 2003. Major disasters have prompted considerable advances in science, technology, administration and regulation at national, regional and international levels. Notably, these include the ILO Convention concerning the Prevention of Major Industrial Accidents (No.174), and a multi-stakeholder framework for an integrated approach in worldwide management of chemical risk. Further global progress in this direction is now more urgently needed in the light of increasing market pressures and the prospect of terrorism.

The manufacture, transportation, usage and disposal of hazardous chemicals have increased rapidly over the last few decades in both developing and developed countries. Of 11 million known chemicals, about 100,000 are currently produced on an industrial scale with more than 1,000 new chemicals entering the market each year. For more than 85% of the 2,500 chemicals generated in quantities greater than 1,000 tons per producer per year, little or nothing is known concerning human and environmental health effects.

Major chemical runaway reactions, explosions, fires, leaks and spills have followed increasing industrialization worldwide, with particularly severe incidents occurring in newly industrialized countries. The public and private infrastructure for oversight, control, planning, mitigation and response are generally insufficient. Documented consequences include fatalities, injuries, emergency evacuation, environmental contamination, and also long-term health *sequelae* among children, including those of exposed parents.

Early warning signs and lessons from major disasters are too often ignored. Consequently, incidents continue to occur. Because effective surveillance and independent investigations are largely absent, incident trends, patterns of occurrence and underlying causes are neither identified nor corrected. Inadequate economic incentives, weak public and private policies, and insufficient resources for effective governmental intervention impede the development and deployment of appropriate prevention strategies.

The Collegium Ramazzini reviewed these issues at an international conference held in Carpi, Italy, on October 28-29, 2004. Physicians, engineers, and public health officials representing academia, national governments, industries, non-governmental organizations, the European Environment Agency, the International Labour Office, and the World Health Organization participated in the discussions.

On the basis of these deliberations, the Collegium Ramazzini calls for:

for the Bhopal community:

- better clinical management of the long-term consequences; fair settlement of remaining legal claims regarding causes, consequences and remediation; expanded scientific studies to assess harm and implement recovery from the 1984 disaster;

for the Global Community (governments, chemical enterprises, workers, scientific and medical professionals):

- improved effectiveness of public and private policies, compliance auditing and enforcement, and allocation of resources sufficient to prevent unintended chemical releases and their consequences;
- expanded programmes of mandatory toxicity testing that examine long-term effects of commercial chemicals on human health and the environment and that systematically examine all toxicological impacts, including the neglected areas of reproduction and development;
- expanded national and international incident surveillance programmes, and increased independent, multidisciplinary investigations of incident root causes and consequences;
- primary prevention approaches based upon inherently safer chemical production, use, distribution, handling and disposal to reduce risks from catastrophic incidents whether attributable to mismanagement or intent;
- strengthened management systems based on the ILO guidelines on occupational safety and health management systems (ILO-OSH 2001);

– enhanced worker and community rights to know about and to participate in decisions regarding chemical hazards, risks, and measures to prevent, respond to and recover from incidents at facilities engaged in production, use, distribution, handling and disposal of hazardous chemical products;
– land use planning to ensure separation from residential and public areas of commercial facilities that produce, use, distribute, handle and dispose hazardous chemicals, and facility planning to ensure on-site separation of incompatible chemical hazards and other precautionary measures to reduce risks to workers and the community;
– capacity building among all stakeholders for emergency prevention, preparedness and response to ensure global harmonization of safer production, use, distribution, handling and disposal of hazardous chemicals;
– education of health care providers to ensure that occupational/environmental health and toxicology are incorporated into basic and continuing medical education.

TENTH COLLEGIUM RAMAZZINI STATEMENT

CALL FOR ACTION ON THE GENOCIDE IN DARFUR

RICHIESTA DI INTERVENTO PER IL GENOCIDIO NEL DARFUR

The Collegium Ramazzini has followed with alarm the progressively worsening catastrophe in Darfur in western Sudan, where, according to the US Secretary of State Colin Powell[1], genocide has been perpetrated. A reported 70,000 Darfurians have died in refugee camps, and one million refugees have been displaced in Darfur since early 2003[2,3]. Many more may have been killed before reaching the camps[4].

Reports from Physicians for Human Rights, Human Rights Watch and the US Holocaust Memorial Museum all indicate that mass executions, rapes, expulsions, and destruction of entire villages have taken place in Darfur. Entire populations appear to have been targeted principally on the basis of their racial or ethnic identity. For some time there has been abundant evidence to indicate that these actions have been condoned, tolerated and even organized by the Government of Sudan.

Genocide, as defined by the UN 1948 Convention on the Prevention and Punishment of the Crime of Genocide, is the killing or destroying of populations on the basis of their racial or ethnic identity. Genocide is the most extreme assault on human rights, and governments carrying out, permitting or condoning genocide forfeit their sovereign rights. When genocide is declared by reputable authorities to be occurring, international action to halt it is mandated.

In all genocides, there are formidable barriers to early detection and stopping of mass killing, because the evidence is buried and concealed in mass graves[5]. Suppression of evidence and repression of those who could give evidence is inherent to genocidal scenarios. Therefore, where genocide is suspected, the case for intervention is imperative, and the burden of proof is on those held responsible to show otherwise[6]. To date, there is no convincing evidence to indicate that the mass killing, expulsions and rape in Darfur have stopped and that its population is no longer the target of organized mass violence.

As an international professional society committed to the protection of the right to human health of all human beings, the Collegium Ramazzini calls for immediate action to stop the mass killing, expulsions, rape and destruction in Darfur and protect the health and safety of refugees from this region. The Collegium Ramazzini declares that there is an international responsibility to act immediately, forcefully and effectively to stop genocide. Furthermore, the Collegium condemns the use of the term "ethnic cleansing" to describe the situation in Darfur or elsewhere where genocide is taking place. This term has become a pretext for avoiding the decisions mandated by the term genocide and sanitizes evil.

There are now reportedly some 3,000 members of an international peace keeping force assigned to protecting the people of Darfur. There is urgent need for additional peacekeepers to prevent more killing, rapes and mass expulsions and also for more international aid – water, food, shelter and health services – for refugees. Relief, even if effective, will by itself not stop the extermination of populations targeted by their ethnic status. There can be no effective work on the long-term ecological, environmental, social and political macrodeterminants of the Darfur catastrophe until there is effective protection of the population of Darfur.

The Collegium Ramazzini calls upon all the governments in the world, the European Union, the Organization of African States, the United Nations and the international community not only to provide emergency relief to the victims of the Darfur crisis, but to take whatever actions are necessary to stop what all credible authorities have characterized as genocide.

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Eur. J. Oncol., vol. 9, n. 4, pp. 217-218, 2004

Editorial/Editoriale

Public perceptions of science

La pubblica percezione della scienza

Marja Sorsa
Former Professor, Finnish Institute of Occupational Health, and former Director, Ministry of Education and Science, Helsinki, Finland

The rapid developments in molecular genetic technologies during the last decade have made science a topic of interest, debate and suspicion among the general public, since the various applications of science increasingly touch everybody's life and future prospects. This is a new incentive for the scientific community to intensify communication with the general public, administrators and decision makers, who all want to be involved in discussions on the directions of future science and its ethical validity.

Economic values and profit making are today easily overriding issues like human integrity, privacy and justice in many societal sectors; e.g. in public health care, in protection of the environment or in occupational safety and health. The decisions are often difficult and far reaching; thus thorough debate and ethical analysis are necessary among all stakeholders. Scientists are the natural stakeholders in ethical debates on the advances of science and technology. This means a step down from the "ivory tower" where the scientist knows best about his field and its future and where ethical debates may be considered a waste of time and a hindrance to freedom of research.

Science barometers are becoming an increasingly important tool to measure awareness, attitudes and general interest of the public in science matters and progress of research. A worrying development in many countries seems to be the increasing public mistrust and unconcern in science. The EU Commission has for years been following the views of Europeans on science, especially modern biotechnology, through interview studies performed in the member states. A general level of science knowledge has been screened with some quiz questions. Surprisingly, 35% of the 15,000 European citizens interviewed answered "true" to the statement: "Ordinary tomatoes do not contain genes, while genetically modified tomatoes do" (Eurobarometer 52:1). In a later survey (Eurobarometer 55:2) a clear mistrust attitude was observed, not only against animal experimentation, gene technology and genetically modified foods, but also towards science in general. Over half of the interviewed Europeans, especially the younger generation, did not care to follow science news in the media and were not interested in scientific issues.

Some smaller countries, like Finland, where science barometer surveys were carried out in the years 2001 and 2004, show better results of trust in science. Even if on the statement concerning public funding of research [which is about 1% of the Gross Domestic Product (GDP)] 49% of the respondents hold the view that " a lot of useless research is conducted with taxpayers' money in Finland", the views on science and technology were generally very positive. Finnish people trust in science, researchers and universities. Only minimal changes in opinions had occurred during the 3 years in between the two surveys. Genetically modified foods were still considered "unsafe" by about half of the Finnish respondents, but over 40% approved the application of gene technology for human benefit and over 60% agreed that controlled animal experiments are sometimes necessary and should not be banned.

Communicating science information to the general public is a challenge to the educational institutions and libraries, to the media and journalists but also to the researcher community. There are many difficulties to be resolved in educating the public, but the task has to be taken, because this is also a prerequisite for balanced ethical debates between all stakeholders. The national and regional ethics committees have an important guiding rôle for structuring the debates with their recommendations and opinions on timely research innovations; presently these include issues like human cloning, stem cells, gene patenting, biobanking and predictive genetic testing.

In all countries the media are getting an increasingly important rôle as an opinion leader also in science issues and the potential applications of research results. Researchers should take a more active part in informing journalists about their findings and educate the journalists to understand the value of scientific news. The doorstep to the press and the other media is too often too high for good prospects in science, while "bad" news, somebody's mistakes, personal deficits or misunderstandings about horrifying hazards get easily to the headlines.

Received/Pervenuto 22.9.2004 - Accepted/Accettato 6.10.2004
Address/Indirizzo: Dr. Marja Sorsa, CA ConsultArt Ltd, Menninkaisentie, 8B, Espoo 02100, Finland - E-mail: marja.sorsa@transmix.fi

So science has achieved a new social contract with society. This governance might have its effects on the directions and priorities in research, at the same time maintaining the essential element of freedom in research, but within the ethical framework. This is a new challenge to the scientific community, to enlarge its competence to communication with the public and to ethical thinking and debating. Bioethics has developed from the public awareness that scientific and technological progress have important societal implications which need to be analysed and discussed among the various stakeholders.

In our high hopes for the flourishing prospects of new scientific advances in the service of humankind, it might be wise to be reminded of a certain humbleness and not be blinded by the speed of advance. Not everything that can be done in science needs to be done.

"Give me the strength and the will to broaden my knowledge. Steer me from the idea that all is within my reach" are the wise words from 800 years ago of an Egyptian rabbi and doctor Maimonides (1135-1204), still relevant even for today's scholars and researchers.

Eur. J. Oncol., vol. 9, n. 4, pp. 219-222, 2004

Enteral expandable metal stents for the treatment of neoplastic large bowel obstruction

Le protesi metalliche autoespandibili nel trattamento dell'ostruzione neoplastica del grosso intestino

Antonio Piemontese
Unit of Gastroenterology, Bellaria Hospital, Bologna, Italy

Summary

The onset symptom of up to 30% of patients with a primary colorectal tumour consists in a large bowel obstruction. These patients are often severely ill, with a very high surgical risk; the traditional approach to pathology includes, first, resection of the tumour and creation of a colostomy and, thereafter, re-anastomosis. Correct staging of the disease could limit the treatment to palliative surgery. Self-expanding metal stents have been developed for the treatment of colorectal neoplastic stenosis. Definitive palliation may include patients with extensive local and/or metastatic disease or high risk candidates for surgery, while the stent will permit clinical stabilization with decompression and bowel cleansing with the aim of performing a one-step operation without the need for a temporary colostomy. Eur. J. Oncol., 9 (4), 219-222, 2004

***Key words*: enteral metal stent, colorectal obstruction, "bridge to surgery"**

Riassunto

L'esordio di un tumore primitivo del colon-retto consiste, in quasi il 30% dei pazienti, in una occlusione acuta del grosso intestino. Questi pazienti sono spesso in gravi condizioni, con un rischio chirurgico molto elevato; il tradizionale approccio a questa patologia comporta in prima istanza la resezione del tumore con confezionamento di una colostomia, ed in seguito la re-anastomosi. Una corretta stadiazione della malattia potrebbe comportare la necessità solo di un trattamento palliativo. Le protesi metalliche auto-espandibili sono state sviluppate per il trattamento delle stenosi colorettali di origine neoplastica. La palliazione definitiva potrà interessare i pazienti con malattia localmente estesa e/o metastatica o i candidati ad alto rischio per la chirurgia, mentre la protesi permetterà la stabilizzazione clinica con decompressione e la pulizia del colon, allo scopo di effettuare un intervento in un solo tempo senza la necessità di una colostomia temporanea. Eur. J. Oncol., 9 (4), 219-222, 2004

***Parole chiave*: protesi metallica intestinale, ostruzione colorettale, "ponte per la chirurgia"**

Introduction

Colon cancer has become one of the main causes of death in western countries[1].

In spite of the information campaigns to sensitize people and social screening programmes for the prevention and early diagnosis among high risk groups and the general population, 30 out of 100 patients with colon cancer are admitted to hospital with a subocclusion or a complete occlusion as onset symptoms[2,3].

In these conditions surgical treatment brings about major risks, complications, and a high mortality[4-6]. Many of these patients, if correctly staged, should be submitted to endoscopic palliative treatment alone instead of surgical treatment[3,7].

Since their introduction by Dohomoto in 1991[8], endoscopic expandable metal stents have become a useful tool for their ability to provide direct palliative recanalization in inoperable patients.

In 1994 Tejero first used this kind of stent in order to transform emergency surgical treatment for acute obstructive colorectal cancer into an elective operation[9].

Some procedures have recently been developed under endoscopic and fluoroscopic guidance in order to avoid surgical treatment in patients inoperable due to general conditions or the stage

Received/Pervenuto 30.8.2004 - Accepted/Accettato 15.9.2004
Address/Indirizzo: Dr. Antonio Piemontese, Via del Colle 62, 40068 San Lazzaro di Savena (BO), Italia

of disease, as well as to decrease the percentage of high risk operations.

Since 1991 several different expandable metal stents have been used (the first ones were generally oesophageal stents). More recently specific stents have been adopted with a peculiar metallic structure allowing the stent, once placed and completely dilated, to maintain the correct shape, even when undergoing compression or torsion phenomena due to bends in the colon; these new stents are known as "enteral stents".

Review

A systematic review of the main articles on this topic until June 2002, selecting 32 series from Medline and other databases is presented in Table 1.

The total number of patients undergoing stenting treatment is 727, but technical success (right placement across the lesion, no early migration) was achieved in 670 patients (92.2%). Technical failure occurred in 57 (7.8%) cases; it consisted in failure to gain access or to pass right through the stenosis, wrong or incomplete stent placement or expansion, or major complications during stenting, such as perforation, requiring interruption of the procedure.

Table 1 - Case series with successful stent positioning

Author	Year	Number of successfully stented patients		
		Total	Palliation	Bridge to surgery
Rey *et al*[10]	1995	11	11	0
Saida *et al*[11]	1996	12	0	12
Canon *et al*[12]	1997	13	9	4
Tejero *et al*[13]	1997	38	13	25
Arnell *et al*[14]	1998	7	7	0
Baron *et al*[15]	1998	23	14	9
Choo *et al*[16]	1998	18	8	10
De Gregorio *et al*[17]	1998	24	24	0
Knopfle *et al*[18]	1998	8	0	8
Tack *et al*[19]	1998	9	9	0
Wallis *et al*[20]	1998	7	5	2
Wholey *et al*[21]	1998	10	4	6
Desroches *et al*[22]	1999	8	2	6
Diaz *et al*[23]	1999	16	16	0
Fava *et al*[24]	1999	9	7	2
Lobato *et al*[25]	1999	41	41	0
Mainar *et al*[26]	1999	66	0	66
Adamsen *et al*[27]	2000	8	8	0
Camunez *et al*[28]	2000	70	37	33
Cole *et al*[29]	2000	25	17	8
Law *et al*[30]	2000	24	18	6
Miyayama *et al*[31]	2000	8	8	0
Repici *et al*[32]	2000	15	15	0
Rocca *et al*[33]	2000	14	4	10
Tamim *et al*[34]	2000	10	6	4
Zollikofer *et al*[35]	2000	26	8	18
Ben Soussan *et al*[36]	2001	16	16	
Knopfle E *et al*[37]	2001	21		21
Montes-Lopez *et al*[38]	2001	23	14	9
Spinelli *et al*[39]	2001	36	33	3
Aviv *et al*[40]	2002	13		13
Martinez-Santos *et al*[41]	2002	41	17	24
Total		670	371	299

Right placement not followed by clinical improvement, such as bowel movements or decompression, 96 hours after the procedure occurred in 32 patients (4.4%).

In conclusion, the procedure was effective and clinical success (decompression) was obtained in 638 patients (87.7% of cases).

Perforation occurred in 25 patients, but only 2/3 required surgical treatment, while 1/3 was treated successfully by medical therapy.

Bleeding and pain requiring medical therapy (haemotransfusion and analgesic drugs) arose in few cases (4 and 5 respectively), all successfully managed. Mortality was less than 0.4%. Total major complications (perforation, severe bleeding, death) occurred in 34 out of the 727 patients treated.

Discussion

The classic strategy in patients with occlusive or subocclusive neoplastic stenosis, particularly in the left colon, consists in immediate decompressive colostomy followed, when possible, by a second surgical step including colon resection and definitive anastomosis.

In a minority of cases surgeons carry out direct anastomosis when the bowel conditions are apparently favourable.

The general condition of these patients is usually very poor and the surgical risk is very high. Operating in such cases means a double surgical risk, the first decompressive intervention and second step surgical treatment being very hazardous.

Immediate anastomosis, in addition to involving higher surgical risk, brings about more frequent post-operative complications such as leakage.

Finally the long-term prognosis of preoperative stent insertion (bridge to surgery) compared with emergency operation proves not so different[11, 42].

Self-expandable stent placement allows decompression and cleansing of the colon.

After clinical stabilization, if indicated, patients can undergo "one step" surgical treatment without the need for previous colostomy, in better general conditions; otherwise the stent might be positioned as a definitive palliative treatment.

Thus the two main indications to stenting the colon are palliative treatment and "bridge to surgery".

The first should be considered in comparison with decompressive surgery, mortality through which is between 6 and 30%, with 10-36% of morbidity[43].

A two-step strategy is required in about 75% of cases in the best series.

Elective surgery allows one to decrease mortality by a percentage between 1 and 7% and morbidity by 4-14%[44].

The cost of the stenting procedure is quite limited, both during and after definitive colostomy, considering the cheaper cost of stenting in comparison with surgical treatment and the need for managing and changing the bag, as well as the complication rate of the stoma[12]. Moreover, in elderly patients, management may be very difficult and often needs help from nurses, thus increasing the cost of the entire process.

Finally the psychological impact of colostomy on the quality of life is considerable, as regards relationships, sexual activity, etc[45].

Only a few articles published up to now have addressed the

economic aspects, comparing direct surgery with surgery after stenting and with definitive palliative stenting.

Two works show a cost lower by about 28%, mainly due to the the patients on endoscopic treatment being discharged earlier than surgical patients[46,47].

Stent placement not only defers surgical treatment and ameliorates the patient's general condition, but assists correct staging of the pathology.

The staging results will orient treatment, thus avoiding surgery in non-resectable and metastatic tumours, and in the presence of heavy co-pathology. The stent will then be left in, with a palliative function instead of being a "bridge to surgery", as expected at the beginning of the process.

Non only is recanalization of the patients obtained but fistulas (recto-vaginal or recto-vesical) can also be treated by covered stent placement, as sometimes occurs in pelvic tumours. Covered stent placement is similar to uncovered placement, but migration is easier than in the latter[48]. Thus stenting with this kind of prosthesis should be avoided unless there is a clear indication in the presence of fistulas.

In the series reviewed, it should be stressed that these are mainly consecutive prospective series, but that there have been no control studies conducted comparing the two kinds of procedure (surgery *vs* stenting) as regards complications and costs. Thus our considerations are based on general data from the literature as to the complications and cost of two-step *vs* one-step treatment. Only two studies compared the long-term prognoses, confirming the advantages of the stenting technique[11,42].

In conclusion, the placement of enteral stents should be considered in the case of colorectal obstructions in order to allow for nonsurgical therapy (palliation) and for carrying out preoperative decompression.

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Eur. J. Oncol., vol. 9, n. 4, pp. 223-230, 2004

Breast reduction and cancer: state of the art and future trends

Riduzione della mammella e cancro: stato dell'arte ed orientamenti futuri

Beniamino Palmieri*, Alberto Costa**
* Department of Surgery, University of Modena Medical School, Modena, Italy
** Breast Surgery Unit, Department of Surgery, Maugeri Foundation, Pavia, Italy

Summary

The literature on extra-aesthetic aims of breast reduction surgery recently focussed on the functional effect and the correction of both physical and psychological symptoms. The problem of cancer in the long-term post-surgical follow-up is an intriguing issue. In the last 20 years some studies both on animals and humans have addressed the relationship between the amount of tissue removed and cancerogenesis of the remnant gland. We present a clinical review of published papers outlining the future trends of breast reduction procedures from an oncological perspective. Assessment of the literature data reveals that breast reduction does decrease the risk of breast and other types of cancers. The actual risk lowering for patients older than 40 is related to the amount of tissue removed. This study illustrates that breast reduction surgery should be encouraged, besides the known aims, as a preventive operation in those patients presenting degrees of breast hypertrophy and a family history of breast cancer. Eur. J. Oncol., 9 (4), 223-230, 2004

***Key words*: breast reduction, breast cancer, incidental breast cancer finding**

Riassunto

La letteratura sugli scopi non estetici della chirurgia riduttiva della mammella ha recentemente considerato gli effetti funzionali e la risoluzione dei sintomi fisici e psicologici. Il problema del cancro nel follow-up post-chirurgico a lungo termine è un argomento stimolante. Negli ultimi 20 anni alcuni studi sull'animale e sull'uomo hanno valutato il rapporto fra quantità di tessuto rimosso e cancerogenesi nella ghiandola residua. Presentiamo una rassegna clinica di lavori pubblicati che delineano gli orientamenti futuri da un punto di vista oncologico delle procedure di riduzione mammaria. L'esame dei dati della letteratura rivela che la riduzione mammaria riduce il rischio di tumori della mammella e di altre sedi. La reale riduzione del rischio nelle pazienti di età superiore ai 40 anni è in relazione alla quantità di tessuto rimosso. Questo studio dimostra che la chirurgia riduttiva della mammella dovrebbe essere incoraggiata, oltre che ai fini noti, come intervento preventivo in quelle pazienti che presentino aspetti di ipertrofia mammaria ed una storia familiare di carcinoma mammario. Eur. J. Oncol., 9 (4), 223-230, 2004

***Parole chiave*: riduzione mammaria, carcinoma mammario, reperto occasionale di carcinoma mammario**

Introduction

Breast reduction encompasses different surgical techniques, aimed at reducing the breast volume when the excessive breast size affects the quality of life.

In a previous paper[1] we reviewed several Authors' reports of subjective and objective pre- and post- operative symptoms in women with various degrees of breast hypertrophy: this extensive analysis, based on a careful evaluation of different protocols, provided evidence that, regardless of the aetiology of macromastia, reduction mammoplasty is effective in improving physical symptoms and psychological discomfort (primary hyperplasia, post-lactation hypertrophy in multiparous women, obesity, etc.).

The problem of cancer in long-term post-surgical follow-up is intriguing. In the last 20 years experimental and clinical investigations have addressed the relationship between the amount of tissue removed and cancerogenesis of the remnant breast.

Received/Pervenuto 28.8.2004 - Accepted/Accettato 20.9.2004
Address/Indirizzo: Prof. Beniamino Palmieri, Università degli Studi di Modena e Reggio Emilia, Dipartimento Misto di Chirurgia Generale e Specialità Chirurgiche, Clinica Chirurgica, Via del Pozzo 71, 41100 Modena, Italia - Tel: 0039/059/4222483 - Fax 0039/059/4224370
E-mail: palmieri@unimo.it

Animal studies

Cancer incidence in amputated breast glands has been experimentally evaluated by Klamer *et al*[2]. He administered 7,12-dimethylbenza(alfa)anthracene and a 20% fat diet to 120 Sprague-Dawley rats; two days later in 60 animals one mammary ridge was excised; in the others only a sham procedure as surgical incision was perfomed. Seventy-nine animals survived the procedures and were followed up to 77 weeks. After 15 weeks there were neoplasms in 7 of 40 breast reduced animals and 17 of 39 controls. After 77 weeks 35 of 40 treated and 32 of 39 controls were tumour affected. The carcinogenic effect of the compound was prevalent in the long run in both groups.

Jackson *et al*[3] published an investigation on 3 groups each containing 20-, 50-day-old rats treated with 5 mg dimethylbenzanthracene (DMBA). There was no further treatment in group A. A second group (B), 2 weeks after DMBA administration, underwent excision of two of the four quadrants. The third group (C) had 75% of the gland removed (one mammary ridge plus half of the contralateral ridge).

In control group A survivors (14/20), an average of 1.3 tumours per quadrant or 5.4 tumours per animal were detected. In group B survivors (50% breast amputations) there was an average of 5.4 tumours per animal (0.72 tumours per surgical quadrant, 2.0 for non surgical quadrants). In group C survivors (18/20 respectively, 9/10 on each upper or lower quadrant) there was a tumour incidence of 0.89 for the upper surgical quadrants versus a tumour incidence of 2.33 in the non surgical quadrants; and a tumour incidence of 0.48 for the lower surgical quadrants versus 2.88 per non surgical quadrants.

This investigation failed to show either an overall cancer risk reduction by surgery or a relationship between the amount of excised gland and tumour development. The total number of tumours in any animal remained fairly constant. Probably the molecules of DMBA were linked to receptors in the non-operated breast quadrants.

Nelson *et al*[4] evaluated the effectiveness of prophylactic mastectomy on breast tumour induction in spontaneous breast tumour forming C3H mice. They also assayed prolactin levels to evaluate its rôle in mammary tumour induction: 256 one month old C3H mice were divided into four groups: group 1: control; group 2: sham surgery; group 3: mammectomy 50%; group 4: mammectomy 100%. The levels of prolactin were significantly increased in all the operated groups 24 hours after surgery. Six months later the hormone levels were significantly higher in mice with tumours in groups 3 and 4 compared to tumour-free animals. There were no differences in tumour incidence between the four groups. One year postoperatively, prolactin levels were similar in the four groups. The major number of tumours was observed in the sham operated animals (89%, $p<0.01$). In summary, the breast volume reduction in this experimental model did not prevent tumour incidence. Prolactin levels rose after surgery and remained elevated in tumour bearing-mice that underwent mammectomy.

Clinical studies

Here we review some published papers (Table 1) and attempt to outline from an oncological point of view the future trends of breast reduction procedure.

The first report from Lund *et al*[5] describes 1245 women between 20 and 70 years of age who underwent different types of breast reduction in Denmark in the interval 1943-1971. They observed 18 cases of breast cancer although they had expected 30.28. This resulted in a relative risk (RR), defined as the ratio between observed and expected cancers, of 0.39.

In the first 10 years of follow-up, 5 cases were detected (7.11 expected); after 10 years 13 cases were detected (23.17 expected). The greatest advantage in cancer risk reduction was experienced in women with a removal of 600 g or more tissue (RR = 0.31, 95% confidence interval 0.06-0.91).

Nine years later Baasch *et al*[6], following up to 1990 the original group of Lund, fixed the relative risk at 0.61, and supported Lund's observation that a 600 g weight or more was the critical specimen resection to achieve a significant cancer risk reduction.

This long follow-up (19 years) on 1240 patients yielded 32 observed cancers (versus 52.55 expected); the women operated on before the age of 21 had a substantial but not statistically significant reduction in the risk of malignancy compared to the general population or other age groups. In the Copenhagen series, a 30% risk reduction was seen among women aged between 21 and 40 in long-term follow-up (20.7% of the patients 40-year-old or older were nulliparous, compared with 20% in the general population, with the number of children equal to 1.7 and mean age 24.0 at the first birth, both similar to the general population). The long-term follow-up did not show any specific trend toward cancer formation. The effect of mammography screening in women over 50 years old should explain the substantial tumour incidence reduction in the 5-year follow-up (2 cancers observed *versus* 12 expected). Under 50 this effect has not been seen (12 cancers observed versus 12.2 expected). Significant variations were observed with the type of technique, date of operation, place of surgery and quadrant excision.

In relating cancer incidence and age, it has to be stressed that younger females with large breasts are usually slimmer; older women are frequently overweight or obese. This might increase the risk of tumorigenesis**,** and the protection offered by breast reduction surgery. Not only is the glandular mass that potentially is expected to become malignant reduced, but also a large number of adipocytes (responsible for enhancing hormone co-carcinogenic potential, transforming androstenedione into oestrogens through aromatase) are removed.

A small population-based case-control report on breast reduction and cancer by Brinton *et al*[7] was part of an investigation on surgical breast enlargement which included 2174 patients and 2009 controls with breast implants but only 10 reduction mammoplasties with 13 controls. In the prosthesis group there were 36 cancers (1.1%) versus 44 (2%) of the controls showing a RR of 0.2 and a RR of 0.8 for both localized and distant tumours. The RR in reduction mammoplasties was 0.7 (95% CI 0.4-1.0).

Boice *et al*[8] examined the Danish hospital discharge registry of 7720 women that underwent breast reduction between 1977 and 1992, focussing on risk relative to age and time since surgery. The median age at time of surgery was 46 years and the mean follow-up 7.5 years. One hundred and eighty-two cancers of various types were observed in the follow-up, compared with 209 expected (standardized incidence ratio - SIR = 0.9; 95% CI 0.7-1.0). Specifically, breast cancers were reduced by roughly 50% (29 observed *vs* 53.9 expected with RR = 0.54). The patient age at surgery was meaningful, the risk being reduced at 40, and subsequently at 50 for an overall reduction of 70%.

Table 1 - Previous studies concerning clinical data[a]

First author (year)	T_N (Age)	C_F	C_E [b]	Relative risk (CI)[c]	Follow-up (year) and RR	Risk factors (RR)
Lund *et al*[5] (1987)	1245 (20–70)	18	30.28	0.59 (0.35-0.94)	- 10 years: RR = 0.70 - 10 or more years later: RR = 0.56	NR
Baasch *et al*[6] (1996)	1240 Lund's patients	32	52.55	0.61 (0.42-0.86)	As the previous study, up to 1990 (19 further years)	NR
Brinton *et al*[7] (1996)	2174 cases	NR	NR	0.5% of N had RM got RR= 0.7 (0.3-1.6)	- <5 years: RR = 0 - 5-9 years: RR= 1.44 - 10+years: RR= 0.69	- Race: RR=1.2
Boice *et al*[8] (1997)	7720 (13-79)	182	209	0.9 (0.7-1.0)	0-17 (average 7.5)	Significantly reduced risk only among 40+ y.o. women (RR = 0.5) especially >50 (RR = 0.3)
Brown *et al*[9] (1999)	27500 (15-60+)	101	165.8[d]	Breast cancer 0.61 (0.50-0.74)	Average 6.5 years At 10 years: 86 b.c. observed *vs* 147.0 expected (RR = 0.59)	Decrease in risk not highly correlated with age
Boice *et al*[10] (2000)	31910 (11-87)	161	223.9	0.72 (0.61-0.84)	0-30 (average 7.5)	Risk reduced especially for 50+ y.o. (RR = 0.57) and for those followed for 5+ ys (RR = 0.68)
Brinton *et al*[11] (2001)	31910 (11-87) 161 developed subsequently breast cancer	137	223.9	0.72 (0.61-0.84)	Up to 28 years (1965-1993)[e]	Height weight, body mass index, parity, no. of children, oral contraceptive use, hormone replacement therapy

[a] T_N: total number of analysed cases; C_F: number of breast cancers found among T_N; C_E: number of breast cancers expected among T_N; NR: not explicitly reported.

[b] The expected number of breast cancers was estimated, if anything is explicitly said, by multiplying age and calendar-time specific breast cancer incidence rates from a specific Registry by the appropriate person-years follow-up.

[c] Ratio of observed and expected numbers of breast cancers (useful as a measure of correlation between mammoplasty and risk of BC); CI = confidence interval, calculated at 95%.

[d] Calculated by using the Person Years programme (Coleman M. et al, *Int. J. Epidemiol. 15*:134, 1986).

[e] This study was accomplished by calculating the person-years of observation, begun at 3 months after the date of breast reduction and ended either at the date of death, or migration on December 31, 1993.

Jansen *et al*[12], in a retrospective study of 2576 breast reductions (Table 2), reported 4 cases (0.16%) of intra-operative cancer detection, that had escaped routine mammography, self exam and physical examination by the surgeons. This rate of incidental tumour finding at the time of reduction surgery might be explained on the basis of accuracy in pre-operative breast examination. Six hundred and sixty patients with pre-existing breast cancer detected before surgery were subsequently found not at risk for the contralateral breast (based on post-operative evaluation of the specimens) in spite of the anticipated high risk of cancer.

Brown *et al*[9], using the Canadian Institute for Health databank, identified 30,137 women submitted to bilateral (94.7%) or unilateral (5.3%) breast reduction between 1979 and 1992. The final eligible cases were 26,567 bilateral reductions and 933 unilateral reductions. Of this latter group 412 patients had prior breast cancer, 314 had prior or synchronous cancer, and 87 of these (27.7%) had specific breast cancer, with 18 cases detected during the surgery. Among the 26,567 operated women, 101 cancers were observed compared with the expected 165.8, yielding a RR = 0.61 (95% CI 0.50-0.74).

As to the other primary cancers, there were 285 cases observed and 372.5 expected with RR = 0.77 (95% CI 0.68 –0.86). Lung cancer, cervical carcinoma and non colorectal gastrointestinal tract tumours were the most frequent, but they, too, presented a well defined reduced risk to 0.59, 0.51 and 0.58 respectively. Probably, this benefit was due to post-operative lifestyle changes (stopping smoking, increasing physical activity and weight reduction).

Evaluating the breast cancer incidence in the first 10 post-operative years, 86 cases were observed vs 147 expected. The remaining 15 cases occurred 10 to 14 years after the initial surgery. Therefore, the RR in the first 10 years was 0.59 (95% CI 0.47-0.72); for the remaining 4 years it increased to 0.80 (95% CI 0.45-1.32). No difference in risk between groups evaluated by age was observed.

Tang *et al*[14] report a cohort study of breast cancer risk in breast reduction patients (27500 cases and 101 cancers found) following

Table 2 - Comparison between the studies performed by Jansen *et al* (1998) and Snyderman and Lizardo (1960)

	Jansen *et al*[12]	Snyderman and Lizardo[13]
Total number of cases	2576	5008
Breast carcinoma diagnosed during the preoperative work-up	0	5
Breast carcinoma diagnosed from surgical specimen	4	14
Total breast carcinomas detected	5	19
Incidence of malignancy in operative findings	0.16%	0.38%

a contemporary Brown report that had demonstrated a 40% reduction in the risk of developing breast cancer after breast reduction. In his retrospective survey, the author describes the diagnosis of cancer between 3 months and 13 years after mammoplasty with a median of 5 years. The median age of the patients when submitted to reduction was 50 years, and their average age at cancer detection was 55, a significantly younger age if compared with the average cancer detection age in the general population of 61. This difference may be explained based on increased attention and awareness in breast reduced women with an easier physical and instrumental exploration of the reduced parenchyma. Histology and distribution were similar to control cases, as were the surgical and oncological protocols. The survival rate at 5 years was 70% in the reduced breast and 77% in the control group, a not significant difference because the lower and upper confidence levels are overlapped.

Boice *et al*[10] investigated the oncologic impact of breast reduction on 31,910 women, excluding cancers occurring before and within 3 months of surgery. The mean age at surgery was 33 and the mean length of follow-up 7.5 years. Six hundred and sixty-two cancers were observed and 729 were expected (SIR = 0.91, 95% CI 0.84-0.98) with a reduction in breast cancer of 28% (161 detected vs 223 expected, SIR = 0.72, 95% CI 0.61-0.84). Oddly enough, lung cancer (SIR = 0.73) and melanoma (SIR = 0.72) were also reduced. Corpus uteri cancer (SIR = 1.37), thyroid (SIR = 1.39), other endocrine tumours (SIR = 1.55), parathyroid adenomas (SIR = 1.51) were increased post-operatively. The inverse association between age at surgery with cancer risk is confirmed in this study; in fact, it was respectively reduced by 24% in the group 40-49, and by 43% in the group over 50.

Brinton *et al*[11] focussed their investigation on the amount of tissue removed during reduction mammoplasty toward breast cancer risk. They examined 137 breast reduction specimens and 422 control patients. Subjects with more than 800 g removed had a 76% decreased risk relative to those that had more than 400 g of tissue removed from either breast, irrespectively of the age at surgery. Probably the "weak protection" from cancer in patients operated before 40 years is related to the lesser volume of tissue removed due to the lesser weight of young women compared with older ones. It is also possible that there is a genetic impact of cancer early in the life; thus, a longer follow-up would allow a correct evaluation of the effect on life span.

Breast reduction and cancer: incidental findings and intentional treatment

The incidental finding of breast cancer during cosmetic or reconstructive breast reduction (Table 3), has been reported by some authors. Rees *et al*[15] have previously outlined the use of careful macroscopic intra-operative fat and gland examination, with further microscopic inspection to detect unrecognised tumours.

Petit *et al*[16] operated 440 contralateral breasts for symmetric correction and observed 22 (4.4%) occult, undetected cancers in the surgical specimens. These were located in the lower quadrant-central area in 70% of the cases. Their size ranged between 3 and 16 mm, 12 of the tumours were *in situ* and 10 were ductal infiltrating neoplasms. The author stresses the option of performing this reconstructive procedure, with this oncologic view, achieving symmetry of the breasts and reducing further tumour incidence on the other side.

Jansen *et al*[12] starting from occult breast carcinoma occurring in their surgical practice collected a questionnaire from the 43% of the plastic surgeons in the New Orleans area. In 2,576 breast reduction patients no carcinomas were detected preoperatively (self breast exam, physician exam and pre-operative mammography). There were four malignancies in the surgical specimens (a rate of 0.16% for breast carcinoma). This incidental finding is significantly lower than the 0.38% of Syndeman and Lizardo[13] (Table 2). This difference might be due to better mammographic screening (85% sensitivity in detecting carcinomas across age groups).

The Keleher *et al*[17] report from the M.D. Anderson Cancer Center, found incidental breast cancer in 4 patients who underwent breast reduction, three of whom had not had a preoperative mammogram. All of the patients underwent modified radical mastectomy.

In order to improve cancer detection at the time of breast reduction surgery, the authors suggest the following age-related protocols, in accordance with the American Cancer Society[18]:

- for patients between 20 and 39, a physician breast examination with ultrasounds every 1-3 years is recommended with regularly repeated self breast exam;
- at 40 years or above, early physician breast examination, screening mammography and breast self examination should be performed regularly.

The women who have high risk of developing cancer are further subdivided into four groups:

a) group with previous thoracic irradiation: annual mammography and physician breast exam every six months, beginning ten years after radiotherapy and not before 35 years of age;
b) women with a prospective five year risk of invasive breast carcinoma of ≥ 1.7%, accordingly to the Gail model: physician examination every 1-3 years and regular breast self-exam;
c) women of ≥ 35 years of age with risk of >1.7%: mammography and physical exam plus breast self-exam are prescribed;

Table 3. Breast cancer found at the time of reduction mammoplasty

First author (year)	T_N	C_F and cancer location	Follow-up
Petit *et al* [16] (1997)	440	In RM 20 occult (4.6%) In lower and central quadrants (70%)	NR
Jansen *et al* [12] (1998)	2576	2 cases not found preoperatively by mammography and self exam	2 years free of cancer
Tang *et al* [19] (1999)	27500 17 (incidence 0.06%) had breast cancer at the time of RM (49 year old average)	Breast cancer found at 49 years old average for women undergoing RM (average age at diagnosis: 61 year old)	8 years
Brown *et al* [9] (1999)	27500 18 cases of incidental findings out of 314 bilateral RM	Breast cancer	Average 6.5 years
Keleher *et al* [17] (2003)	Incidental findings	4 cases not found preoperatively by mammography and self exam at M.D. Anderson Cancer	1-6 months

d) women at genetic risk for breast cancer (at least 2 breast cancers and 1 or more ovarian cancers in mothers and relatives; incidence of breast cancer in sisters before 50 years old, and various penetrance of ovarian mixed with breast cancer in the family). In these cases annual mammography and medical breast examination every 6 months have to be started 5-10 years before the age of the youngest relative affected. BRCA-1 and BRCA-2 mutations can involve multiple genes and thus show different expressions of tumours and their follow-up has to be individualized.

In case of an incidental finding of cancer, extensive dissection has to be performed in order to leave at least 1 cm of tumour-free breast tissue. The suspected specimen margins must be marked with ink for correct pathology orientations. Different containers for the specimen from each breast quadrant have to be sent to the pathologist, and if the suspected diagnosis is confirmed, a modified radical mastectomy with node dissection and, potentially, reconstruction should be considered. In this case Keleher suggests approaching mastectomy through the keyhole incision of the breast reduction technique removing en bloc the nipple-areola complex with a final vertical and horizontal scar. If the tumour is multicentric and cannot be safely and completely removed with the previously planned incision, the operation can be converted to a standard mastectomy with a different final scar.

Tang *et al* [19] identified 17 women with breast cancer at the time of breast reduction out of 27,500 patients (incidence 0.06%) enrolled with over 13.5 years of follow-up. The average age of this intra-operative cancer group was 49 and all the patients underwent bilateral operations. The follow-up of this specific cohort was 8 years. Thirty-five percent of the cancers were suspected at the operating table and 65% were identified by pathology. Of these, 65% were ductal, 35% were lobular carcinomas, and 29% were lymph node positive. The control group (105 women) selected from the Ontario Canada Cancer Registry Database between 1979 and 1993 had a mean age of 61 years. Eighty-six percent had ductal carcinoma, 10% had lobular carcinoma and 4% had ductal and lobular mixed together; 42% had lymph node metastases. Of the breast reduction group 33% had partial mastectomy at the time of breast reduction without further surgery and 67% had modified radical mastectomy. There were 48 partial mastectomies and 32 radical modified mastectomies in the control groups. Radiotherapy was performed in 50% of the breast reduction group and 71% of the controls. Chemotherapy and hormonal therapy compared with 60% of the control group. The 5-year survival rate was 88% in the breast reduction group and 67% in the control group. The explanation for the high incidence of lobular carcinoma (35% compared with 11% in the control), could be related to the difficulty of detection either clinically or mammographically of this multicentric tumour. Because typical breast reductions rotate the quadrants, modify the anatomy of the area to be treated with radiation, and increase the risk of tumour seeding, Tang's opinion is that the "gold standard" treatment of intra-operatively detected breast cancer should be mastectomy. In this report the cancers found during reductive surgery affect younger women with earlier diagnosis. Therefore, a minor number had involved lymph nodes and they had a better 5-year survival rate.

Breast reduction as a first choice oncologic procedure

Other authors have emphasized the rôle of reduction mammoplasty (RM) in cancer operated breast remodelling, either contralaterally, or on the same side of a previous usually cosmetically quite unpleasant quadrantectomy (Table 4).

Shestak *et al* [20] were the first to suggest the RM in four patients with macromastia and/or mammary hypertrophy and simultaneous carcinoma of the breast. The operation consisted of a keyhole marking and wide mammary gland resection with bilateral inferior pedicle reconstruction, and bilateral resection and transplantation of the nipple. The average specimen weight was 825 g. The disease-free follow-up was between 7 and 43 months. In the author's opinion, this strategy is well accepted for cosmetic reasons, as well as effective in terms of radiation targeting and of easier post-operative self-examination.

Cothier-Savey *et al* [21] suggested RM as a safe procedure for breast cancer instead of quadrantectomy, followed by chemother-

Table 4 - Combination of reduction mastoplasty with mastectomy and other oncological procedures

First author (year)	T_N (age)	Follow-up	Breast tissue removed (g)	Complication rates	Cosmetic result	Oncologic result
Shestak *et al*[20] (1993)	4	7-43 months	825 on average	NR	NR	All patients are alive and without disease
Clough *et al*[22] (1995)	20 (41-70)	1-7.5 years (mean 4.5)	248 on average	1 case: local recurrence 4 cases: metastases (same oncologic results as lumpectomy and irradiation)	At 1 year: - very good: 75% - moderate: 20% - poor: 5% (nipple areola necrosis)	3 years after surgery: only one distant recurrence
Cothier-Savey *et al*[21] (1996)	70	9-43 months (mean 21)	120-440 (mean 350)	NR	NR	NR
Smith *et al*[23] (1998)	10 (59 year old, on average)	8-37 months (no recurrence)	945 on average	No complications arising from the surgery or radiation therapy	From good to excellent	NR
Spear *et al*[24] 1998	Average age: 46.3 year old	3 to 18 months (reported only for 1 case)	711.7 on average	- Fibrous mastopathy - Fibrocystic changes and apocrine metaplasia - Oedematous breast for several months; stromal fibrosis	NR	NR

apy and radiotherapy. They evaluated 70 breast reduction and contralateral mastopexy patients operated between 1983 and 1991. The cases were matched as to age, history, stage and pathology of the tumours, etc. The 5 year overall survival was 85% with 5% local relapses (detected in an interval of 9-43 months, 21 months on average) and patient satisfaction was 81%. The morbidity-mortality rate was no greater than quadrantectomy, and postoperative treatment was the same. Of the 36 patients with a tumour diameter of more than 3 cm, local relapses were 3% and actuarial survival 79%. Among all of the patients a 5 year actuarial rate for local relapse of 8.5% was globally found, next to an average delay of illness appearance of 21 months. *In situ* cancers were found in 24.2% of the cases, while the remaining were infiltrating cancers. This last group demonstrated local relapse in 8% of the cases with an actuarial survival of 84% at 5 years. The cosmetic outcome was judged very good in 39% of the cases, fair in 36%, acceptable in 24% and poor in 1%. The nipple areola complex was always evaluated with frozen sections, in order to exclude any cancer infiltration; and the reduction specimens weighed between 120 and 440 g with an average of 350 g.

Clough *et al*[22] described 20 patients with lower quadrant cancers between 1983 and 1993, treated with remodelling mammaplasty with a nipple bearing superior pedicle, preceded (9 cases) or followed (11 cases) by radiation. The contralateral breast was rendered symmetrical at the same time. The mean resection weight was 248 g (range 40-540 g).

Pathologic examination always showed free margins and there was contralateral fibrocystic disease in 13 patients and a single case of epithelial proliferation. The only serious postoperative complication was a nipple areola necrosis that required debridement and full thickness skin graft.

With a mean follow-up of 4.5 years there was 1 local recurrence and 4 distant metastases but no difference from the oncologic point of view in comparison with similar stage-size cancers.

Smith *et al*[23] operated 10 bilateral breast reductions for breast malignancy followed by radiation therapy, between 1996 and 1998. The average amount of tissue removed was 945 g per breast. Radiotherapy started 4 weeks after surgery with 50 Gy in 25 sessions. The follow-up has been 37 months, without recurrence. The cosmetic outcome was excellent. The authors believe that in suitable women with large ptotic breasts and cancer the primary consideration should be reduction.

Spear *et al*[24] describe the RM procedure in three cases conservatively operated and radiated for breast cancer. The reductive procedure was performed 3-16 months after surgery. The technical note in this very selected group of patients was to prepare wide, short flaps, with very little undermining of the skin avoiding dehiscence and ulceration due to post-actinic fibrosis.

The overall number of cancer patients treated is not large enough to establish that this treatment could have a specific rôle in breast surgery oncological protocols. These anecdotal reports cautiously emphasize the cosmetic rôle of reducing technique in supporting the psychological distress of mastectomy by introducing a single bilateral procedure.

Discussion

The problem of breast reduction is really not only cosmetic, but also oncologic in perspective. We have evidence, based on the reports in the literature, that a critical reduction in the amount of gland and fat tissue significantly lowers the breast cancer incidence in the operated group of patients in the long run.

Based on the risk reduction philosophy, we should try to redesign the techniques of breast reduction, performing a radical, nipple sparing subcutaneous mastectomy, and remodelling the fat accordingly with reduced breast volume, introducing, if necessary, a submuscular breast prosthesis to improve the shape of the reduced breast.

In fact, the radical nipple-sparing subcutaneous mastectomy featuring two delays is a surgical procedure which has been conceived by us for prophylactic surgery in high risk BRCA1-2 positive women. It aims at complete removal of the breast gland within its capsule, sparing the nipple areola complex with a careful final (laparoscopic camera magnified) overview, establishing that only subcutaneous fat tissue is left in place.

In a preliminary outpatient procedure under local anaesthesia the vascular supply of the nipple areola complex is autonomized from the breast gland stalk, using the laparoscope and electrified forceps. Great care is utilized to create a dermal-subcutaneous capillary network that will allow upward transposition of the nipple areola complex to correct the ptosis after reduction. Four weeks after this first step, the reduction mastoplasty with radical gland ablation is performed. The subcutaneous and dermal network is preserved with a wide de-epithelization and very fine stitching.

The main cosmetic goal of RM is to correct the ptosis and to uplift the nipple-areola complex. Sometime, when the breasts are extremely large and ptotic, a free nipple-areola complex (NAC) transplant is the preferred choice. Of course, sensitivity of the nipple area is lost. Even if this is acceptable in the correction of severe megalomastia, this technique cannot be the "gold standard" for minor breast hypertrophy.

Radical nipple sparing offers a conservative alternative. The NAC can be mobilized as a subdermal–subcutaneous flap. This autonomization spares the sensitive nerve fibres, giving a satisfying functional result.

In clinically symptomatic cases of gigantomastia, breast reduction should be emphasized, not only as a restorative procedure for the patient's fitness and wellness, as we have demonstrated in a previous extensive literature review, but as an effective removal of the ageing gland tissue reducing potential cancer risk.

Women should be made aware of a total subcutaneous mastectomy, targeted to specific physical improvement and cancer prevention, with minimal change in nipple areola sensitivity and a marked substantial cosmetic improvement. Many women probably might accept the choice of this procedure, if oncologic prevention can be achieved. On the other hand cancer risk reduction has been observed in the RM operated women for other types of cancer (lung or gastrointestinal).

We should encourage breast reduction in order to improve the well-being of the patients as well as from an oncological preventive perspective. Our suggestion is removal of as much of the glandular tissue as possible, converting the classic pedicle, and partial gland resection technique, in a formal nipple sparing radical, subcutaneous mastectomy. The preliminary NAC vascular autonomization is obtained with a mini-invasive out-patients procedure that is done 2-4 weeks before the main operation.

This strategy results in a complete prevention of the breast cancer, and should be well accepted, for cosmetic reasons, by the patients with various degrees of breast hypertrophy, and a family history of breast cancer.

Acknowlegments

The Authors are grateful to Dr Richard Toranto, for English and Editorial assistance.

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Eur. J. Oncol., vol. 9, n. 4, pp. 231-236, 2004

HLA genotyping, non classical HLA-I-G soluble molecules and cytokine production in 28 established human tumour cell lines. Considerations for *in vitro* biological investigations and human anti-tumour vaccine therapy[a]

Tipizzazione HLA, produzione di molecole solubili di HLA-I-G e citochine da parte di 28 linee cellulari tumorali umane. Considerazioni per studi in vitro e vaccinoterapia antitumorale umana

Caterina De Vinci*, Roberta Rizzo**, Augusto Mazzuca*, Loredana Melchiorri**, Maura Ferrari***, Laura Giacomelli****, Giancarlo Pizza*, Olavio Roberto Baricordi**

* Immunotherapy Module, Operative Unit of Urology, Department of Urology and Nephrology, S. Orsola-Malpighi Hospital, Bologna, Italy
** Department of Experimental and Diagnostic Medicine, Section of Medical Genetics, University of Ferrara, Italy
*** Experimental Institute of Zoo-prophylaxis, Emilia-Romagna and Lombardia Regions, Brescia, Italy
**** Department of Surgical Science, University of Rome "La Sapienza", Rome, Italy

Summary

***Aim*. Human tumour cell lines play an important rôle in basic and applied research. Recently, several cell banks have been created providing mutated phenotypes for the investigation of the biological and genetic bases of disease and for evaluating a potential use in cancer vaccine therapy. In fact cell lines produce factors that could interfere with biological functions so we decided to investigate some of them because they are taken into consideration as candidates for human anticancer vaccination procedures. Our attention was directed to the *in vitro* studies of cell-mediated mechanisms against tumour-associated antigens (TAA). *Materials and methods*. We characterized 28 ATCC tumour cell lines originated from different organs for the HLA-A,B and DR phenotypes and we investigated the expression of HLA-class I products at the cell membrane. Furthermore, we tested the cell lines for their capacity to modulate soluble classical HLA-class I and non-classical HLA-G1/HLA-G5 antigens, recently claimed to be tolerogenic molecules, and for the spontaneous production of cytokines. *Results*. The results identified specific HLA genotypes, with a significant increase in single allele detection at**

Received/Pervenuto 1.9.2004 - Accepted/Accettato 24.9.2004
Address/Indirizzo: Dr. Caterina De Vinci, Immunotherapy Module, Operative Unit of Urology, Department of Urology and Nephrology, S.Orsola-Malpighi Hospital, Via P. Palagi, 9, 40138 Bologna, Italia
Tel: 0039/051/6362478 - Fax: 0039/051/6362476
E-mail: caterina@med.unibo.it

[a] Financial support Italian Ministry Grant n. PRF 2000 201 and Foundation Asclepios, Switzerland.

Riassunto

***Scopo*. Le linee cellulari tumorali umane rivestono un importante ruolo nella ricerca di base ed applicata. Del tutto recentemente sono state allestite numerose banche cellulari che mettono a disposizione fenotipi, anche mutati, per ricerche genetiche e di vaccinoterapia dei tumori. Poiché tali linee producono fattori solubili che possono interferire con importanti funzioni biologiche si è deciso di caratterizzare quelle linee che potrebbero essere utilizzate in vaccinoterapia antineoplastica. Gli studi sono stati in particolare diretti sui meccanismi dell'immunità cellulo-mediata *in vitro* nei confronti degli antigeni tumore-associati (TAA). *Materiali e metodi*. Sono stati studiati i fenotipi HLA-A,B e valutata l'espressione, a livello della membrana cellulare, di antigeni HLA-classe I di 28 linee provenienti dalla collezione ATCC e originate da più organi. È stata inoltre saggiata la capacità di modulare antigeni solubili di istocompatibilità HLA-classe I e antigeni non classici HLA-G1/HLA-G5 che di recente sono stati considerati fattori tolerogenici. Si è inoltre valutata la produzione spontanea di citochine. *Risultati*. I risultati hanno mostrato un significativo aumento di genotipi HLA con allele singolo nei loci HLA-A,B e DR rispetto a cellule provenienti da soggetti normali. L'analisi citofluorimetrica ha rivelato l'espressione di HLA-classe I a livello di membrana su tutte le linee studiate. Gli studi con ELISA hanno evidenziato un polimorfismo nella produzione di antigeni solubili classici HLA-classe I (17/28), mentre una percentuale di queste linee (8/17) ha mostrato una contemporanea modulazione di molecole solubili di HLA-G non classico. *Conclusioni*. I dati osservati suggeriscono modulazione di molecole tolerogeniche in molte delle linee studiate**

the HLA-A,B and DR *loci* when compared to healthy subjects. Cytofluorimetric analysis detected HLA class I expression at the cell membrane level in all the cell lines investigated. ELISA investigation displayed a polymorphism in the production of soluble classical HLA-class I antigens (17/28), and furthermore a percentage of these cell lines (8/17) showed a contemporary modulation of soluble non-classical HLA-G molecules. *Conclusions*. These data suggest the modulation of tolerogenic molecules in a large percentage of the investigated cell lines. In addition, a spontaneous cytokine production able to interfere with the inflammation response was observed. These observations advocate for a careful analysis and characterization of cell lines candidates for *in vitro* or *in vivo* studies. Eur. J. Oncol., 9 (4), 231-236, 2004

***Key words*: vaccine, gene-modifications, cytokine, cell-line, HLA**

e produzione spontanea di citochine in grado di interferire con la risposta infiammatoria. Queste osservazioni devono indurre a caratterizzare con grande attenzione le linee cellulari candidate a studi *in vitro* o *in vivo*. Eur. J. Oncol., 9 (4), 231-236, 2004

***Parole chiave*: vaccino, modificazioni geniche, citochine, linee cellulari, HLA**

Introduction

More than thirty private companies and public organizations are currently involved in the production, characterization and cryopreservation of established cell lines that may be used as models for the understanding of the biological and genetic basis of diseases. In this context, human established cell lines have been the preferred approach for the investigation of the mechanisms of tumour transformation for several years. Furthermore, these cell lines are currently used as possible source of allogeneic antigens for vaccine therapy *in vitro* models, i.e., for exploring cellular immune mechanisms against tumour-associated antigens (TAA) in the perspective of therapeutic applications[1-6].

The concept of tumour vaccines is not new. At the beginning, tumour vaccines were composed of whole inactivated cancer cells, or tumour lysates administered together with immune adjuvants. The advances in gene transfer technology now suggest novel, more specific vaccine approaches. In fact, since the activation of cellular immunity requires synergistic signals, including presentation of specific tumour antigens, co-stimulatory signals, and propagation of the immune response via cytokine release[7], some investigators decided to insert specific cDNA that governs the release of some of the most important interleukins directly into the autologous tumour cells (ATC) to be used as a vaccine. However, transfection of ATC is difficult to carry out for each patient[8]. Consequently, another approach has been developed using established allogeneic tumour cell lines to produce, *inter alia*, IL2 by transfection with pertinent cDNA[1] and some encouraging results have been thus obtained in metastatic kidney cancer[2-4].

The main mechanisms involved in cancer immune evasion from host response[9] are the down modulation of HLA-class I products at cell membrane level and the loss of a single allele or of a complete haplotype. To assess this, we performed HLA class I and II genotyping of 28 human established cell lines and investigated the expression of the HLA-class I molecules on the cell membrane. Furthermore, we tested the capacity of the cell lines to modulate soluble HLA-class I and HLA-G molecules and cytokine production.

HLA-A,B,C antigens, defined as HLA-class I products, are characterized by an high polymorphism and a broad tissue expression. Furthermore, these antigens, found in soluble form (sHLA-class I) in the biological fluids of healthy individuals[10], showed an increased concentration in pregnancy, viral infections, autoimmune diseases and following bone marrow transplantation. The sHLA-class I serum levels evidenced a generalized decrease in patients with solid tumour diseases, excluding malignant melanoma, thus suggesting a relationship between soluble antigen concentrations and cell mediated activity[11-12].

HLA-G molecules are non-classical major histocompatibility class I antigens characterized by a limited polymorphism and an alternative translation of spliced mRNA that yields four membrane-bound proteins (HLA-G1 to -G4) and three soluble molecules (HLA-G5 to -G7)[13-15]. The HLA-G1 membrane bound antigen and the HLA-G5 soluble antigen isoform have been identified as the fundamental molecules in the induction of tolerance at the foeto-maternal interface. Their inhibitory effect influences the cytotoxicity of NK and CD8+ lymphocytes and the activity of CD4+ cell population[16, 17]. It has been suggested that the recently reported expression of HLA-G in some solid tumours could be an additional mechanism for the tumour cell phenotype to escape the host cellular response[18-20].

Considering the tolerogenic functions recently proposed for both these soluble molecules[21, 22], we tested by specific ELISA the presence of classical HLA-I class soluble molecules (sHLA-I) and the soluble HLA-G isoforms (sHLA-G1/HLA-G5) in the supernatants of each cell line.

The DNA genotyping suggests an increase of single specificities for the HLA -A,B and DR *loci*, when compared to healthy subjects. However, cytofluorimetric investigation showed the presence of HLA-class I products on the membrane in 28/28 cell lines. Furthermore, a polymorphism was reported in the production of sHLA-class I and sHLA-G1/HLA-G5 antigens in a large percentage of cell lines, without correlation to the tissue of origin.

Material and methods

The 28 ATCC (American Tumor Cell Culture) (Rockville, MD, USA) cell lines were obtained from the Institute of Experimental Zooprophylaxis of the Lombardy and Emilia Regions (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia), collected and cultured as suggested by the Brescia Centre for Cell

Substrates (Brescia Centro Substrati Cellulari). DNA from 200 bone marrow donors was used as control for the percentage of homozygosity at the HLA-A,B and DR *loci*. The supernatant of cell culture was obtained using confluent growing cells, and the amounts of products calibrated per million of cells during 24 hours.

HLA-A, B, C and DR typing

DNA extraction. Cell line DNA extraction was performed with a Nucleon BACC1 kit (Amersham Life Science, Buckinghamshire, UK). Briefly, 5-10 x 10^6 cultured cells were lysed and then purified by sodium perchlorate. After treatment with chloroform and Nucleon resin, the DNA was recovered, washed, measured by spectrophotometer and diluted to 100 ng for optimal amplification.

DNA typing. DNA typing of class I and class II HLA alleles was performed using micro polymerase chain reaction-single strand polymorphism (PCR-SSP) methodology, based on sequence-specific oligonucleotide primers for amplification of HLA alleles by the PCR. Pre-optimized primers were dried in different wells of a 96-well 0.2 ml thin-walled tube tray for PCR and made ready for addition of DNA samples, recombinant Taq polymerase (InVitrogen, Carlsbad, CA, USA) and dNTP buffer mix. Human beta-globins were used as internal control of PCR product (MICRO SSP HLA-ABDR Typing Trays OneLambda Inc, Canoga Park, CA, USA). After the PCR process was performed using Perkin Elmer 9700 Thermocycler, the amplified DNA samples were separated by agarose gel electrophoresis and visualized by staining with ethidium bromide and exposure to ultraviolet light. Interpretation of PCR-SSP results were based on the presence or absence of a specific amplified DNA fragment and was allowed using DNA/LMT OneLambda Software.

HLA-class I expression by flow cytometry (FACS)

Cell monolayers were harvested by conventional trypsin-EDTA treatment (InVitrogen) and washed two times with phosphate-buffered saline (PBS) 1x. Cells were incubated for 30 minutes at 4°C with the monoclonal antibody (MoAb) W6/32 that recognizes a framework determinant expressed on beta-2-microglobulin-associated HLA class I heavy chain, then washed two times with PBS 1x and stained with FITC-conjugated goat-anti-mouse IgG (Dako, Hamburg, Germany) for 30 minutes at 4°C. Flow cytometry was performed on FACS-Vantage flow cytometer (Becton Dickinson; Mountain View, CA, USA). Cell viability was determined by staining with propidium iodide.

sHLA-I determination

Quantitation of sHLA-I was carried out following the double determinant immunoassay previously described[23]. Briefly, ELISA Pro-Bind Falcon 3915 (Becton-Dickinson, Lincoln Park, NY, USA) assay plates were coated overnight at room temperature with 100 µl of anti-HLA class I MoAb W6/32, at a concentration of 25 µg/ml in carbonate-bicarbonate buffer (pH 9.5). Subsequently each well was washed four times with PBS containing 0.01% Tween-20 (Sigma, St. Louis, MO, USA) and blocked with 50 µl of PBS-Tween containing 3% bovine serum albumin (BSA) (Sigma) at 37°C for 60 min. Five washing steps followed, as described above. Undiluted culture supernatant samples were added to the wells in triplicate and were incubated for 60 min at 37°C. The quantity of bound sHLA was detected with 50 µl/well of a 1:1000 dilution of polyclonal beta-2-microglobulin specific antiserum conjugated with HRP (DAKO, Carpinteria, CA, USA). After a 60 min incubation at 37°C, wells were washed five times with PBS-T and eventually incubated with 100 µl of 2,2'-azinobis (3 ethyl-benzyl-thiazoline-sulphonic acid) (ABTS) substrate solution (DAKO) at room temperature in the dark. After 60 min, the colour development was read at 490 nm in an ELISA Microplate Reader 400 (Packard, Meridien, CT, USA). sHLA-I values were standardized using calibrated dilutions of lyophilized soluble HLA antigens. The limit of sensitivity of the assay was 1.0 ng/ml. We used the anti-HLA class I MoAb W6/32 that detects not only the classical sHLA-I (A, B, C), but also the non-classical sHLA-G. We calculated the difference between sHLA-I and sHLA-G concentrations to obtain the amount of classical sHLA-I.

sHLA-G1/ HLA-G5 determination

Soluble HLA-G levels in culture supernatants were assayed as previously reported[22]. Briefly, 96-microwell plates Nunc-Immuno Plate PolySorp were coated with the MoAb MEM-G9 (Exbio, Praha, Czech Republic) that recognizes HLA-G molecule in beta-2-microglobulin-associated form (sHLA-G1/HLA-G5) at the concentration of 20 µg/ml in 0.1 M carbonate buffer pH 9.5 for 1 h at 37°C and then overnight at 4°C. After three washes with PBS containing 0.05% Tween 20 (PBS-Tween), the plates were saturated with 100 µl PBS containing 4% BSA overnight at 4°C. Undiluted supernatant samples were added to each well (100 µl). After incubation for 2 h at 37°C, plates were washed three times with PBS-Tween, and incubated with 50 µl of biotinylated MoAb W6/32 that recognizes a framework determinant expressed on beta-2-microglobulin-associated HLA class I heavy chain, for 1 h at 37°C. Biotinylation of MoAb W6/32 was obtained using the EZ-Link Sulfo-NHS-LC Biotinylation Kit (Pierce, Rockford, IL, USA). After five washes with PBS-Tween, 50 µl of o-phenyldiamine peroxidase substrate (Sigma-Aldrich, Milan, Italy) was added to each well and the plates were incubated for 15 min at room temperature. The concentration of sHLA-G was estimated by absorbance at 405 nm on a microplate reader ELISA Microplate Reader 400 (Packard, Meridien, CT, USA). Standard supernatants of sHLA-G/.221 were utilized for the generation of standard calibration curves. The limit of sensitivity was 1.0 ng/ml.

Cytokines

The following cytokines were investigated: IL1-β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IFN-γ, GM-CSF, TGF-β1. The ELISA kits used for the determination were all obtained from Pierce Endogen (Rockford, IL, USA) (Codes: EH2-IL1β; EH2-IL2,4,6,8,10,12; EH2-IFNγ; EH-GMCSF) except for TGF (DRG-GmbH, Germany; code EIA-1864).

Results

HLA

The results obtained, expressed as pg/ml, are reported in Table 1. Each cell line was genotyped by DNA techniques for HLA-A,B

Table 1 – Cytokines[a] and HLA

Cell lines	Tissue derivation	IL1-β[a]	IL-2[a]	IL-4[a]	IL-6[a]	IL-8[a]	IL-10[a]	IL-12[a]	IFN-γ[a]	GM-CSF[a]	TGF-β1[a]	FACS[b]	sHLA-I[a]	sHLA-G[a]	HLA-A	HLA-B	HLA-DR
769-P	Primary renal cell adenocarcinoma	-	-	-	130	1900	-	-	-	1420	2400	+	-	-	*03; *24	*07; -	*15; -
786-O	Primary renal cell adenocarcinoma	-	-	-	1180	1660	-	-	-	500	1100	+	12	1	*03; -	*07; *44	*13; *15
A-172	Glioblastoma	-	-	-	80	160	-	-	-	60	-	+	13.5	-	*01; *03	*07; *08	*03; *11
A-427	Lung carcinoma	-	-	-	-	1360	-	-	-	-	500	+	-	-	*03; *33	*35; *47	*04; *13
A-431	Epidermoid carcinoma	-	-	-	-	320	-	-	-	140	750	+	28	-	*03; -	*07;-	*11;-
A-498	Kidney carcinoma	-	-	-	32	1220	-	-	-	40	7000	+	-	-	*02; -	*08; -	*03; -
A-549	Lung carcinoma	-	-	-	190	1700	-	-	-	100	9600	+	-	-	*25; *30	*18; *44	*07; *11
A-704	Kidney adenocarcinoma	-	-	-	30	1480	-	-	-	40	1100	+	-	-	*74; -	*35; *44	*15; -
ACHN	Renal adenocarcinoma	-	-	-	500	1650	-	-	-	240	650	+	10.7	-	*26; -	*49; -	*16: -
BXPC - 3	Pancreatic primary adenocarcinoma	-	-	-	10	1800	-	-	-	240	3200	+	-	-	*01; -	*37; -	*15; -
CAKI - 1	Kidney carcinoma	-	-	-	2960	1300	-	-	-	4600	-	+	17	-	*23; *24	*35; *44	*07; *11
CAKI - 2	Kidney carcinoma	-	-	-	880	1620	-	-	-	280	-	+	19	-	*01; *11	*08; *52	*03; *11
CALU - 1	Lung epidermoid carcinoma	-	-	-	660	1400	-	-	-	160	1650	+	-	-	*26;*29	*15; *44	*07; *14
CAPAN - 1	Pancreatic adenocarcinoma	-	-	-	180	1760	-	-	-	160	-	+	-	-	*01; *30	*13; *57	*07; *13
CAPAN - 2	Pancreatic adenocarcinoma	-	-	-	-	1450	-	-	44	820	1500	+	19	1.85	*29; -	*44; -	*07; -
CCF - STTG1	IV grade astrocytoma	-	-	-	200	1500	-	-	36	-	3500	+	-	-	*01; -	*08; *37	*07; *13
CFPAC - 1	Pancreatic adenocarcinoma	-	-	-	640	1800	380	-	-	440	-	+	21	2.8	02; *03	*35; 73	*11; -
DU 145	Prostate carcinoma	-	-	-	160	1560	-	-	-	50	3850	+	-	-	*03; *33	*50; *57	*01; *07
HEP G2	Hepatoblastoma	-	-	-	420	1420	-	-	-	320	-	+	-	-	*02; *24	*35; *51	*13; *16
HOS	Osteogenic sarcoma	-	-	-	-	150	-	-	-	-	-	+	13.5	1.45	*02; -	*51; -	*15; *16
HS 683	Glioma	50	-	-	2920	1200	-	-	-	4900	7600	+	19	1.55	*32; -	*07; *44	*08; *12
HT 1376	Bladder carcinoma	-	-	-	40	1500	-	-	-	30	1000	+	9.4	3	*24; -	*15; -	*13; -
MIA PACA - 2	Pancreatic carcinoma	-	-	-	-	1800	-	-	-	-	3200	+	14.5	-	*24; -	*14; -	*01; -
PANC - 1	Pancreatic epithelioid carcinoma	-	-	-	-	1240	-	-	-	-	1400	+	28	-	*02; *11	*38; -	*13; -
SK - LU1	Lung adenocarcinoma	-	-	-	860	1850	-	-	-	520	1850	+	12	2.5	*24; -	*35; *40	*13; -
SW 1088	Astrocytoma	-	-	-	2460	1650	-	-	-	60	2650	+	13.5	-	*01; *03	*08; *44	*04; *13
T98G	Glioblastoma	46	-	-	220	1020	-	-	-	10	4600	+	15.5	3.7	*02; -	*35; *39	*08; *12
U373 MG	Glioblastoma	-	-	10	320	1860	-	-	-	20	1200	+	13.5	-	*02; -	*18; -	*03; -

[a]Expressed as pg/24h per million cells; [b]Flow cytometry HLA-class I expression; * determined by molecular techniques

and DR alleles. The presence of a single specificity at the HLA-A *locus* was observed in 15/28 (53.5%) of the cell lines investigated, in 11/28 (39.2%) at the B *locus* and 13/28 (46.4%) at the DR *locus*. In 8/28 (28.5%) cell lines, only one haplotype was determined. We identified 10 subjects (5%) with a single allele identification at the HLA-A locus in the 200 healthy subjects, 4 (2%) at the HLA-B *locus* and 6 (3%) at the DR *locus*. No subjects were observed with a single HLA-A,B,DR haplotype. The FACS analysis by the MoAb W6/32 confirmed the presence of HLA-class I antigens on the cell surface membrane of all investigated cell lines. ELISA investigations showed the presence of discrete amounts of sHLA-class I antigens in 17/28 (60%) of the supernatants, with concentrations ranging between 9.4 to 28.0 ng/ml.

No correlation was observed between the number of HLA specificities identified for each cell line and the amount of sHLA-I detected in the culture medium. Furthermore 8 of the 17 sHLA-I positive cell lines also produced sHLA-G1/HLA-G5 molecules with a range from 1.0 to 3.7 ng/ml. It is worth noting that the capture antibody W6/32 of the sHLA-I ELISA recognizes a conserved epitope in the alpha-1 region of the HLA-G heavy chain so that it can also bind to the sHLA-G1/ HLA-G5 molecules. However, the comparison between sHLA-I and sHLA-G concentrations, revealed by the specific MoAb MEM-G9, clearly suggests the presence of both soluble isoforms in the supernatants.

Cytokines

As regards the cytokine production, a wide range and variable amounts of molecules carrying different biological activities were observed. All cell-lines produced a considerable amount of IL8, from 340 up to 1900 pg/ml/24h per million cells. Only one cell line produced IL-4 or IL-10, two produced IFN-γ or IL1-β, 22 produced IL-6 and GM-CSF and 21 TFG-β1. No cell lines produced IL-2.

Discussion

Human established cell lines are currently utilized to study the mechanisms inducing diseases and cancer transformation. Furthermore, established cell lines are used *in vitro* for the development of immunological approaches as well as in gene therapy against cancer.

It is well known that human tumour cells use different strategies to escape the recognition of effector cells. Among these, the most widely used appears to be the alteration of HLA-class I expression, reported in 20-50% of solid tumours, but only occasionally seen in haematological diseases and related to different mechanisms. In fact, the defects could be related to abnormalities in the assembly of the peptide heavy-chain-beta 2-microglobulin complex or to its transport to the cell membrane or be the consequence of abnormalities in the antigen processing/expressing machinery[24].

We characterized the cell lines for HLA- A,B and DR allele specificities by DNA analysis techniques. The frequencies of single *locus* allele assignment appeared significantly increased in the cell lines from ill patients when compared to those of healthy subjects. This suggests an elevated loss of single alleles or of complete HLA-A,B,DR haplotypes. However, all the 28 investigated cell lines showed, by FACS analysis, an HLA-class I antigen expression at the membrane level, that could develop a normal sensitivity to the CD8+ cells and cytotoxic activities.

It has been recently reported that sHLA-class I and sHLA-G1/HLA-G5 molecules are able to induce apoptosis in CD8+ activated cytotoxic T lymphocytes through CD8 ligation. Furthermore, soluble HLA-G antigens appear to be able to modify the cytokine production towards Th2 dominance[25], to inhibit NK and CD4+ cells activities[13-15], to abrogate trans-endothelial NK cell migration[26] and the maturation of immature dendritic cells to mature stimulatory cells. These functions suggest a generalized tolerogenic activity against cell-mediated immunity[27]. It is known that the relationship between HLA and tumour cells involves the modulation of HLA-class I molecules at the cell membrane levels. The results obtained in our investigations suggest that more than 50% (17/28) of tumour phenotypes obtained from primary tumour cells could produce sHLA-I molecules. Furthermore, 8 of these 17 sHLA-I positive cell lines appear to be able to modulate also sHLA-G1/HLA-G5 antigens that are fundamental in the induction of immune tolerance.

Contrasting results have been reported concerning mRNA and HLA-G molecules expression among fresh solid tumours[19, 28]. It has been suggested that micro-environmental content and transcriptional factors may be important for the maintenance of HLA-G expression and that HLA-G expression represents only an early event[29]. Our results suggest that a significant percentage of tumour established cell lines appear to express HLA-G products despite many *in vitro* passages, without correlation to the tissue of origin. The sHLA-class I and sHLA-G1/HLA-G5 production appears heterogeneous in tissue and cell lines phenotypes. This could be related to the tumour stage of the original tissue or to the presence of significant biological differences in similar tumour tissues.

Conclusion

If the percentage of tumour phenotypes (8/28, 28.5%) that produce both soluble classical HLA-class I and non classical HLA-G molecules is representative of an *in vivo* condition, it is possible that a significant number of tumours may be able to produce different biological molecules that help to escape the cell mediated immunity of the host.

The interest in the use of tumour cell lines for therapeutic purposes, both because they can be engineered to carry new genes for production of pertinent interleukins and/or because they could already share TAA cross-reacting with autologous tumours, is continuously growing. However, one must be aware of the need for accurate and strict selection of the cell line and carefully evaluate the spontaneous production of interleukins. In fact, cell lines, if not accurately selected, could spontaneously produce cytokines able to counteract the biological activity of the newly induced molecules, rendering thus vane the vaccine approach.

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Eur. J. Oncol., vol. 9, n. 4, pp. 237-243, 2004

La magnificazione d'immagine nella diagnosi endoscopica dei tumori epiteliali del colon. Criteri classificativi e indicazioni per il trattamento endoscopico (mucosectomia) dell'*early colon cancer*

Image magnification in endoscopic diagnosis of epithelial tumours of the colon. Classification criteria and indications for endoscopic treatment (mucosectomy) of early colon cancer

Luca Saragoni*, Ariele Saragoni*, Claudio Cortini**, Enrico Ricci**
* U.O. Anatomia Patologica, Ospedale G.B. Morgagni-L. Pierantoni, Forlì, Italia
** U.O. Gastroenterologia ed Endoscopia Digestiva, Ospedale G.B. Morgagni-L. Pierantoni, Forlì, Italia

Riassunto

L'introduzione delle nuove tecnologie in endoscopia ha contribuito a migliorare sensibilmente la accuratezza diagnostica dei tumori epiteliali precoci del colon (*early colon cancer*). In particolare, l'endoscopia con magnificazione consente di differenziare le lesioni neoplastiche (ad es. adenomatose) da quelle non neoplastiche (polipi iperplastici) e di identificare le lesioni intra-mucose e quelle che invadono la sottomucosa. La magnificazione può, inoltre, fornire un contributo rilevante nella scelta dell'intervento terapeutico più appropriato (resezione endoscopica o chirurgia). È comunque consigliabile un utilizzo moderato delle nuove tecnologie in endoscopia e, tuttavia, non prima di avere ottimizzato i percorsi diagnostico-terapeutici in endoscopia tradizionale. Eur. J. Oncol., 9 (4), 237-243, 2004

Parole chiave: **endoscopia, magnificazione d'immagine, cancro del colon**

Summary

The introduction of new technologies in endoscopy has contributed considerably improving the diagnostic accuracy in early epithelial tumours of the colon (early colon cancer). In particular, endoscopy with magnification allows us to distinguish neoplastic lesions (i.e. adenomas) from non neoplastic ones (hyperplastic polyps) and to identify intramucosal lesions from those invading the submucosa. Furthermore magnification may bring an important contribution to the choice of the most suitable therapeutic option (endoscopic resection or surgery). In any case a moderate use of new endoscopic technologies is advisable, though after optimisation of the diagnostic-therapeutic procedure by traditional endoscopy. Eur. J. Oncol., 9 (4), 237-243, 2004

Key words: **endoscopy, image magnification, colon cancer**

Introduzione

La diagnosi endoscopica delle patologie gastrointestinali dipende dalla capacità dell'endoscopista di riconoscere le alterazioni della mucosa e di valutare la loro natura patologica. Anche in mani esperte, però, i reperti riscontrabili mediante una endoscopia convenzionale non possono sempre essere correlati con i reperti anatomo-patologici. Allo scopo di migliorare l'accuratezza diagnostica, vari coloranti sono stati utilizzati da più di 40 anni sulla superficie mucosa per valutare meglio specifiche alterazioni.

Negli anni più recenti la qualità delle immagini endoscopiche è migliorata notevolmente grazie alla disponibilità di endoscopi dotati di alta risoluzione e di magnificazione di immagine[1,2]. I termini endoscopia ad alta risoluzione e magnificazione di immagine sono stati talora impiegati erroneamente come sinonimi. L'alta risoluzione di un'immagine endoscopica è una qualità distinta rispetto alla magnificazione, poiché essa consente solamente di discriminare meglio i dettagli dell'immagine, mentre la magnificazione consente di ingrandire l'immagine, così da poter visua-

Received/Pervenuto 2.9.2004 - Accepted/Accettato 29.9.2004
Address/Indirizzo: Dr. Luca Saragoni, U.O. Anatomia Patologica, Ospedale "G.B. Morgagni-L. Pierantoni", Via Forlanini 34, 47100 Forlì, Italia
Tel. 0039/0543/731833 - Fax 0039/0543/731797
E-mail: lsaragon@ausl.fo.it

lizzare minuti particolari del disegno mucoso. La maggior parte degli strumenti oggi disponibili sono forniti di un meccanismo azionabile dal pollice dell'endoscopista, in analogia a quanto viene effettuato per manovrare l'elevatore di un duodenoscopio durante una colangiografia retrograda endoscopica (ERCP), che modifica la posizione delle lenti situate nell'estremità distale dello strumento. Questi endoscopi permettono quindi di ingrandire l'immagine all'origine, prima che questa venga inviata al CCD (coupled cip device) elettronico (magnificazione ottica o *zoom endoscopy*), con una qualità migliore rispetto all'ingrandimento effettuato esclusivamente per via elettronica dal processore (magnificazione elettronica)[3]. Le specifiche tecniche degli endoscopi a magnificazione d'immagine sono riportate nella Tabella 1. Recentemente sono stati introdotti in commercio magnificatori ancora più potenti, ma preferiamo riportare i dati della valutazione tecnologica più recente effettuata dalla Associazione Americana di Endoscopia Digestiva (ASGE), piuttosto che le informazioni pubblicitarie delle ditte produttrici[4].

Le indicazioni

La magnificazione dell'immagine consente di differenziare le lesioni neoplastiche (p.e. adenomatose) da quelle non neoplastiche (iperplastiche) e di identificare le lesioni intramucose e quelle che invadono la sottomucosa[5].

L'endoscopista che si accinge ad utilizzare questa metodica deve preliminarmente acquisire, oltre alle capacità necessarie per l'esecuzione tecnica della procedura e l'interpretazione dell'immagine, anche le conoscenze relative all'inquadramento morfologico e alla differenziazione istopatologica della lesione e alla anatomia macro e microscopica della parete intestinale. Solo in questo modo le informazioni acquisite mediante la magnificazione d'immagine della lesione possono essere correlate in maniera appropriata con le decisioni terapeutiche che da questa scaturiscono. In altre parole è necessario modificare l'approccio finora utilizzato nell'esecuzione e nella valutazione diagnostica delle indagini endoscopiche sul grosso intestino fin dalla prima fase dell'esplorazione mediante l'endoscopia tradizionale.

La scuola giapponese da oltre un ventennio utilizza una classificazione macroscopica delle lesioni che può essere applicata in tutti i segmenti dell'apparato gastrointestinale. L'endoscopia a magnificazione d'immagine è nata e si è sviluppata in Giappone e il suddetto cambiamento di approccio in questo paese è già avvenuto da molti anni, così che oggi esiste una differenza importante tra l'atteggiamento occidentale e quello orientale. In estrema sintesi si può affermare che l'endoscopista occidentale, di fronte ad una lesione, affida prevalentemente al patologo il compito di formulare la diagnosi (mediante la biopsia o l'asportazione della lesione), mentre quello orientale si sforza preliminarmente di definire la lesione classificandola meticolosamente dal punto di vista macroscopico. Tale meticolosità ha lo scopo di migliorare l'accuratezza diagnostica e di guidare in maniera appropriata le successive scelte terapeutiche, correlando la valutazione macroscopica con quella microscopica. Anche i patologi orientali impiegano criteri interpretativi e classificativi diversi da quelli dei patologi occidentali, ma una dettagliata discussione di tale problematica esula dai fini di questa trattazione. Ricorderemo solamente che tali differenze sono state valutate e discusse in occasione di incontri di patologi occidentali ed orientali svoltisi a Vienna e Padova, che hanno consentito di elaborare criteri comuni oggi noti come "Classificazione di Vienna"[6,7]. Anche in campo endoscopico i criteri orientali ed occidentali sono stati valutati in incontri comuni ed è stata elaborata una classificazione comune riportata in un documento condiviso in una *Consensus Conference* che si è svolta nel dicembre 2002 a Parigi[5]. Oggi possiamo pertanto fare riferimento ad una "Classificazione di Vienna" per le problematiche isto-patologiche e ad una "Classificazione di Parigi" per quelle endoscopiche. Le modifiche proposte sono state inserite nella nuova classificazione delle neoplasie del tratto gastrointestinale della Organizzazione Mondiale della Sanità (WHO).

Secondo la Classificazione di Parigi[5] le lesioni neoplastiche del colon possono essere distinte macroscopicamente in polipoidi (tipo I) e non polipoidi (tipo II). Le lesioni polipoidi (o protrudenti) vengono ulteriormente differenziate in lesioni peduncolate (tipo Ip) e sessili (tipo Is)[8]. Le lesioni non polipoidi (tipo II) (o superficiali, o non protrudenti), a seconda della morfologia, possono essere ulteriormente suddivise in elevate (tipo IIa), piatte (tipo IIb) e depresse (tipo IIc). Le lesioni che hanno bordi rilevati e centro depresso vengono definite lesioni miste di tipo IIa-IIc.

Per differenziare le lesioni polipoidi sessili (tipo Is) da quelle non polipoidi superficiali elevate (tipo IIa), nelle classificazioni giapponesi viene valutato il rapporto tra l'altezza e il diametro della lesione: le lesioni polipoidi sessili (tipo Is) sono quelle nelle quali l'altezza è maggiore di un terzo o del doppio del diametro. Nella classificazione di Parigi più semplicemente è stato pro-

Tabella 1 - Descrizione degli endoscopi ad alta magnificazione ottica

	Olympus GIF-200Z	Olympus CF-200CL	Pentax EC-3430Z	Pentax EC-3830LZ	Fujinon EG-485ZH	Fujinon EC-485ZW
Lunghezza di lavoro (cm)	103	168	105	170	110	133 o 166
Angolazione della punta						
In alto	180°	180°	210°	180°	190°	180°
In basso	90°	180°	120°	180°	90°	180°
Destra/sinistra	100°	160°	120°	160°	100°	160°
Tubo di inserzione						
Diametro (mm)	11,9	13,7	11,4	12,8	10,7	13,6
Angolo di visione	120°	120°	120°	120°	120°	140°
Magnificazione[a]	105x	105x	105x	105x	70x	70x
Costi[b]	$ 20.151	$ 21.350	$ 19.650	$ 20.600	$ 25.650	$ 26.800
Costi del processore speciale	Non richiesto	Non richiesto	$ 18.950	$ 18.950	Non richiesto	Non richiesto

[a] Quando utilizzato con un monitor da 14 pollici
[b] Costi determinati 1/00

posto di utilizzare le branche di una pinza bioptica come parametro di paragone: avvicinando infatti una pinza chiusa alla lesione da esaminare, le lesioni polipoidi sessili (tipo Is) sono quelle che si estendono in altezza rispetto al piano della mucosa circostante oltre il livello di tali branche (approssimativamente di 2,5 mm), mentre le lesioni non polipoidi superficialmente elevate (tipo IIa) sono quelle che non superano tale limite[5]. Alcune lesioni elevate possono raggiungere un discreto diametro laterale (> 10 mm) senza incrementare la loro altezza sulla mucosa circostante. Esse sono denominate "lesioni a diffusione laterale" o "*lateral spreading tumours*" [5].

Per le lesioni polipoidi, il rischio di malignità è direttamente correlato con le dimensioni della lesione e l'espansione del peduncolo. Nella scelta tra un intervento endoscopico o chirurgico questi sono pertanto i parametri più importanti da considerare, mentre la valutazione della morfologia della superficie in questi casi non viene considerata rilevante.

Per le lesioni non polipoidi le dimensioni non costituiscono un fattore determinante. L'esame dettagliato della morfologia della lesione, e in particolare l'identificazione di una depressione, costituisce l'elemento più importante nella scelta terapeutica, evitando indicazioni inappropriate al trattamento endoscopico.

Queste indicazioni sono validate dagli studi di Kudo, eseguiti su 19.560 lesioni del colon in un periodo di 18 anni. La percentuale di invasione della sottomucosa aumenta per le lesioni polipoidi proporzionalmente con l'incremento delle dimensioni, raggiungendo il 30% nelle lesioni maggiori di 21 mm. Le lesioni non polipoidi di tipo depresso (IIc), anche di pochi millimetri di diametro possono presentare un rischio di invasione sottomucosa di gran lunga superiore, pari al 44% per quelle di 6-10 mm ed al 90% per quelle di 16-20 mm (Tabella 2)[5, 9].

Le lesioni che hanno la maggiore probabilità di invasione degli strati più profondi della sottomucosa sono pertanto le lesioni non polipoidi superficialmente depresse (tipo IIc), che dovranno essere valutate con la massima attenzione, anche con l'impiego di tecniche speciali come la magnificazione d'immagine o l'ecoendoscopia. La scelta tra l'una o l'altra di queste metodiche verrà fatta in base alle esperienze e alle disponibilità locali. Uno studio prospettico randomizzato[10] ha messo a confronto la accuratezza diagnostica dell'invasione sottomucosa tra l'endoscopia a magnificazione di immagine e l'ecoendoscopia, dimostrando che entrambe le metodiche sono parimenti efficaci nella valutazione delle lesioni non polipoidi.

Dalle considerazioni su esposte, scaturisce che le indicazioni appropriate alla magnificazione endoscopica nel colon sono costituite da:

– lesioni non polipoidi,
– lesioni di tipo IIc,
– lesioni a diffusione laterale o *lateral spreading tumours*,
– dopo una mucosectomia,
– nella sorveglianza dei pazienti affetti da malattie infiammatorie croniche intestinali.

Nel caso delle lesioni non polipoidi la valutazione della superficie mediante la magnificazione consente di differenziare le lesioni adenomatose da quelle iperplastiche, così da evitare il ricorso alla biopsia o alla polipectomia soprattutto per le lesioni "diminutive" minori di 0,5 centimetri[11-16].

Per le lesioni superficialmente depresse di tipo IIc è possibile valutare la probabilità di invasione profonda della sottomucosa e quindi, a seconda dei casi, decidere per una mucosectomia o per una biopsia mirata nell'area più sospetta[17].

Per i *lateral spreading tumours* la procedura è finalizzata alla ricerca, nel contesto della lesione talora anche di dimensioni cospicue, di eventuali aree di infiltrazione profonda, la cui presenza deve far considerare più appropriata una asportazione chirurgica piuttosto che una mucosectomia endoscopica[18, 19].

Dopo una mucosectomia endoscopica i margini e il fondo dell'area nella quale la lesione era localizzata devono essere esaminati per verificare la radicalità della asportazione, cioè l'assenza di ogni minuto residuo di tessuto adenomatoso[20].

Nelle malattie infiammatorie croniche sottoposte a sorveglianza oncologica, la cromoendoscopia e la magnificazione consentono di individuare e valutare minime alterazioni della superficie mucosa per la ricerca della displasia o del cancro (DALM). In questo modo il numero rilevante di biopsie random (circa 60) finora raccomandato può essere drasticamente ridotto a favore di prelievi mirati[21-30]. Ulteriori e più specifiche classificazioni saranno probabilmente necessarie per differenziare le aree di semplice rigenerazione dalle aree DALM[31].

Tabella 2 - Percentuale di invasione della sottomucosa in rapporto alla morfologia macroscopica delle lesioni ed alle dimensioni in una serie di 19.560 lesioni complete di valutazione istologica

Morfologia delle lesioni	Dimensioni delle lesioni				
	<5 mm	6-10 mm	11-15 mm	16-20 mm	>21 mm
Ip + Is	0	1,2	8	17	30
IIa + IIb	0,1	0,2	1,8	10	23
IIc	7	44	67	90	87
Totale	0,2	2	8	18	28

La tecnica

Materiali necessari

Sul carrello dell'endoscopia dovrebbero essere disponibili routinariamente le sostanze necessarie per eseguire la colorazione delle lesioni riscontrate all'endoscopia convenzionale. Esse sono costituite da acqua contenente simeticone, per la detersione della superficie e l'eliminazione delle bolle o della schiuma eventualmente presenti, e l'indaco di carminio allo 0,2%, per la colorazione di superficie della lesione.

Qualora debba essere eseguita la valutazione con magnificazione di una lesione, secondo le indicazioni su riportate, dovranno essere disponibili anche un mucolitico (acetilcisteina), un colorante vitale (cristalvioletto allo 0,05% o cresilvioletto allo 0,1%), un catetere (spray o normale a seconda delle preferenze), un cappuccio trasparente per mucosectomia.

La valutazione di una lesione

La cromoendoscopia con indaco di carminio costituisce il tempo preliminare per identificare e valutare minime alterazioni della mucosa, riscontrabili con l'endoscopia convenzionale[9], costituite da:

– discromia focale (eritema o pallore),
– spot emorragici,
– assenza o alterazione del disegno vascolare,

– granulosità o deformità della mucosa,
– convergenza plicale.

Le tappe della cromoendoscopia sono le seguenti[16]:
– lavaggio della lesione con 10-20 ml di acqua attraverso il canale dell'endoscopio,
– colorazione con 5-10 ml di indaco di carminio più 5-10 ml di aria attraverso il canale dell'endoscopio,
– classificazione macroscopica della lesione (morfologia, dimensioni da valutare comparativamente con una pinza bioptica, margini),
– rimozione dell'eccesso di colorante per aspirazione.

Se appropriata, secondo le indicazione precedentemente esposte, si può procedere alla magnificazione dell'immagine seguendo le seguenti tappe:
– lavaggio della lesione con 10-20 ml di acqua attraverso il canale dell'endoscopio,
– mucolisi con 5-10 ml di N-acetilcisteina (2 mg/ml) iniettata attraverso il canale dell'endoscopio,
– colorazione con 2-5 ml di indaco di carminio più 5-10 ml di aria attraverso il canale dell'endoscopio,
– magnificazione dell'immagine e classificazione del *pit pattern* della lesione.

In caso di lesioni depresse (IIc) (la depressione viene definita come una concavità costante nonostante l'insufflazione o l'aspirazione di aria[32]) o di *pit pattern* invasivo (IIIs – V)[17], le ulteriori tappe della magnificazione dell'immagine sono:
– lavaggio della lesione con 10-20 ml di acqua attraverso il canale dell'endoscopio,
– colorazione con poche gocce di cristalvioletto allo 0,05% o cresilvioletto allo 0,1% con un catetere normale, prestando molta attenzione a non produrre microtraumi della lesione con il getto di liquido (che possono poi ostacolare la valutazione del *pattern*),
– attesa di 2 minuti perché il colorante venga fissato dalla lesione,
– lavaggio della lesione con 10-20 ml di acqua attraverso il canale dell'endoscopio,
– magnificazione dell'immagine e valutazione del pattern della lesione.

Nella valutazione del *pit pattern* di lesioni localizzate dietro una plica o in corrispondenza di una flessura può essere difficile esaminare la lesione, per cui è opportuno variare il decubito del paziente o schiacciare la plica con un catetere o con un altro accessorio. Altre circostanze che possono creare difficoltà sono costituite dalla peristalsi vivace, che può essere controllata somministrando un antispastico, e dalla abbondante presenza di muco tenacemente adeso alla superficie, che oltre che con il mucolitico può essere rimosso effettuando lavaggi con acqua distillata o acqua calda. I movimenti respiratori del paziente o le pulsazioni trasmesse alla parete colica possono rendere difficile la messa a fuoco della lesione: è necessario invitare il paziente a fare alcuni secondi di apnea oppure, se la lesione è situata nel colon sinistro, si può utilizzare un cappuccio da mucosectomia. In questo modo lo strumento può essere delicatamente messo a contatto con la lesione e mantenere l'immagine a fuoco per il tempo necessario per la sua valutazione[14].

Una nota infine sull'impiego del catetere spray. Nella pratica clinica preferiamo iniettare il colorante o le altre soluzioni direttamente attraverso il canale bioptico dello strumento. Ciò consente di risparmiare tempo, di risparmiare sui costi del catetere e soprattutto di evitare microtraumi e microsanguinamenti della lesione che possono rendere poi difficile una buona valutazione della lesione.

Il catetere (nella nostra esperienza preferibilmente un catetere normale) si rende invece necessario quando bisogna colorare la lesione con il cristalvioletto o il cresilvioletto, poiché in questi casi è opportuno iniettare solo poche gocce di colorante, sufficienti per valutare l'area di interesse.

Valutazione dopo una mucosectomia

Dopo l'esecuzione di una mucosectomia, soprattutto in caso di lesione estesa e di asportazione con la tecnica in frammenti, è necessario valutare la radicalità della asportazione eseguendo una nuova colorazione e magnificazione[20], secondo le seguenti tappe:
– lavaggio con 10-20 ml di acqua, attraverso il canale dell'endoscopio, dell'area sottoposta a mucosectomia,
– colorazione con 5-10 ml di indaco di carminio più 5-10 ml di aria attraverso il canale dell'endoscopio,
– magnificazione e valutazione della lesione: l'identificazione di *pit pattern* tipo I nei margini orizzontali della mucosectomia, la mancata identificazione di un *pit pattern* riconoscibile nel cratere della mucosectomia vengono considerate un indicatore della radicalità dell'asportazione

Qualora le due situazioni precedenti non si verifichino, deve essere eseguita una nuova mucosectomia e se questa non è più possibile (incapacità dell'ansa di afferrare la lesione, mancato *lifting*, etc.) può essere eseguito un trattamento con Argon Plasma mirato sulle aree residue[33].

Interpretazione delle immagini e classificazione delle lesioni

La superficie mucosa del colon mostra un disegno costituito da numerosi orifizi che sono gli sbocchi delle cripte delle ghiandole di Lieberkuhn. Questo disegno, che prende il nome di *pit pattern*, può essere osservato *in vivo* mediante l'utilizzo di endoscopi a magnificazione d'immagine in grado di ingrandire l'immagine almeno fino a 35 volte. Il *pit pattern* è stato studiato per la prima volta dai patologi mediante l'impiego *in vitro* dello stereomicroscopio. Kato *et al*[14] hanno dimostrato che esiste una buona correlazione, che arriva fino al 97%, tra lo studio del *pit pattern* osservabile *in vitro* mediante la stereomicroscopia e quello realizzabile *in vivo* con l'uso di endoscopi dotati di magnificazione di immagine. La superficie mucosa del colon mostra diversi tipi di *pit pattern*. Sono state proposte varie classificazioni del *pit pattern*, ma quella oggi unanimemente utilizzata per il retto e il colon è quella descritta per la prima volta da Kudo *et al*[34], che divide i *pit pattern* in cinque gruppi, più due sottogruppi.

Il primo gruppo è caratteristico della mucosa normale e mostra un *pit pattern* costituito da orifizi rotondeggianti e regolari per forma, dimensioni e distribuzione.

Se i *pits* diventano asteroidi e più grandi del normale, ma conservano una distribuzione regolare, il *pit pattern* viene classificato di tipo II e questo è caratteristico delle lesioni iperplastiche.

Quando i *pits* diventano tubulari o rotondeggianti e sono più piccoli degli sbocchi ghiandolari normali costituiranno il *pit pattern* di tipo IIIs. Esso è riscontrabile più frequentemente nelle aree depresse ed è sospetto per infiltrazione profonda.

Se i *pits* diventano tubulari o rotondeggianti e sono più grandi degli sbocchi ghiandolari normali costituiscono il *pit pattern* di tipo III_L. Questo tipo è caratteristico delle lesioni adenomatose.

Se il *pit pattern* mostra orifici di forma dendritica o allungata, diventerà di tipo IV. Questo tipo è caratteristico delle lesioni adenomatose.

Quando la forma e la distribuzione dei *pits* sono irregolari per forma, dimensioni e distribuzione, il *pit pattern* verrà definito di tipo Vi (o irregolare).

Quando la superficie della lesione mostra la perdita del normale disegno del *pit pattern*, avremo il tipo Vn (o non strutturato).

In una singola lesione è possibile osservare contemporaneamente più di un *pit pattern*, poiché ciascuno di essi esprime le specifiche alterazioni istopatologiche della relativa area. In questi casi, più che focalizzare l'attenzione sul *pattern* prevalente, tenendo conto che l'osservazione serve ad indirizzare verso la scelta terapeutica più appropriata, è necessario fare riferimento al *pattern* che ha la prognosi peggiore[34, 35].

Differenziazione delle lesioni iperplastiche e adenomatose

È stata dimostrata una buona correlazione fra il tipo del *pit pattern*, osservabile sulla superficie mucosa (e quindi su di una superficie orizzontale) e la diagnosi istologica (ottenuta su una sezione verticale).

Konishi *et al*[11] in uno studio prospettico randomizzato su 660 pazienti con lesioni di diametro inferiore o uguale ad 1 cm, hanno valutato l'accuratezza diagnostica nel differenziare le lesioni adenomatose da quelle iperplastiche tra la colonscopia convenzionale e quella con magnificazione di immagine. Nelle lesioni piatte l'accuratezza è stata dell'86% e del 57% rispettivamente per l'endoscopia magnificata e quella convenzionale ($p < 0{,}0001$), con una sensibilità rispettivamente dell'83% e del 55% ($p < 0{,}0001$) e una specificità del 91% e del 60% ($p < 0{,}0001$). Il numero di lesioni neoplastiche non definite è stato di 10 con l'endoscopica magnificata e di 78 per quella convenzionale ($p < 0{,}0001$).

Kato *et al*[14] hanno valutato retrospettivamente 4.445 pazienti con 3.438 lesioni maggiori di 5 mm. Le lesioni incluse nello studio sono state asportate mediante mucosectomia e sono state escluse le lesioni solo biopsiate. L'accuratezza diagnostica per le lesioni non neoplastiche (*pit pattern* tipo I e II) è stata del 75%, per le lesioni adenomatose (*pit pattern* tipo III e IV) è stata del 94%, per le lesioni con carcinoma invasivo (*pit pattern* tipo V) è stata dell'85%. La sensibilità è stata rispettivamente del 42%, 98% e 82% e la specificità del 99%, 52% e 99%.

Tung *et al*[15], in uno studio prospettico su 175 lesioni, hanno ottenuto nelle lesioni non polipoidi una accuratezza diagnostica dell'83,7% nel differenziare le lesioni neoplastiche da quelle non neoplastiche, con una sensibilità del 100%, una specificità del 77,8%, PPV 61,9% e NPV del 100%.

Kiesslich *et al*[13], in uno studio prospettico su 100 pazienti, hanno riportato una sensibilità del 92% e una specificità del 93% nel differenziare le lesioni iperplastiche da quelle adenomatose.

Hurlstone *et al*[17], hanno valutato prospetticamente 1008 lesioni riscontrate in 33 mesi, con una sensibilità e specificità nel differenziare le lesioni neoplastiche da quelle non neoplastiche rispettivamente del 98% e del 92%.

L'accuratezza diagnostica della colonscopia con magnificazione nel differenziare le lesioni neoplastiche da quelle non neoplastiche appare quindi elevata, ma non raggiunge il 100% dei casi. Ulteriori studi sono necessari per migliorare i risultati e identificare le ragioni che sottendono a tali differenze. Recentemente è aumentato l'interesse per l'adenoma serrato, che rappresenta una limitazione nella valutazione del *pit pattern* con la classificazione di Kudo. Esso è una lesione nella quale coesistono aspetti iperplastici ed adenomatosi e rappresenta probabilmente una lesione con una istogenesi particolare, nella quale le alterazioni epiteliali non originano dalla superficie ma dal fondo delle cripte ghiandolari. Nell'adenoma serrato gli aspetti architetturali sono quelli di un polipo iperplastico, mentre quelli citologici sono quelli di un adenoma tubulare. Pochi studi hanno valutato gli aspetti endoscopici istopatologici di tali lesioni. Oka *et al*[36], recentemente hanno valutato una casistica di 367 adenomi serrati riscontrati in 11 anni ed hanno proposto la differenziazione di tali lesioni in polipoidi e superficiali. Il *pit pattern* di questi due tipi è infatti diverso: le lesioni polipoidi serrate hanno un *pattern* di tipo III_L o IV, mentre quelle superficiali un *pattern* di tipo II. La frequenza di displasia severa e carcinoma *in situ* è significativamente differente, pari al 9,2% e al 25,2%, rispettivamente.

Correlazione tra *pit pattern* e invasione sottomucosa

Il tipo di *pit pattern* può essere correlato con la frequenza di cancro invasivo nella sottomucosa. La Medicina è una scienza stocastica, e pertanto la valutazione del *pit pattern* ci fornirà la probabilità che tale evento sia presente. Infatti se per un *pit pattern* di tipo II o di III_L non è stata dimostrata la presenza di invasione della sottomucosa, nel *pit pattern* di tipo IIIs la probabilità di invasione della sottomucosa è del 3,9%, in quello di tipo IV del 3,8%, nel tipo V irregolare del 21,1%, nel tipo V non strutturato del 65,6%[34]. Nelle lesioni superficiali e depresse definibili secondo la classificazione di Parigi nel tipo macroscopico IIc, è stato dimostrato che la probabilità di invasione degli strati più profondi della sottomucosa (Sm 2 e Sm 3) è del 66,7% per il *pit pattern* di tipo Vi e del 100% nel *pit pattern* di tipo Vn[37] (Tabella 3).

Hurlstone *et al*[17], in uno studio prospettico eseguito su 1850 pazienti, pur con una sensibilità del 98% nel differenziare le lesioni iperplastiche da quelle adenomatose, hanno segnalato una diminuzione della sensibilità al 50% con una specificita' del 98% nella differenziazione delle lesioni neoplastiche non invasive da quelle neoplastiche invasive.

Saitoh *et al*[39], in uno studio su 64 *early colorectal cancer* con morfologia depressa, hanno valutato la probabilità di invasione profonda della sottomucosa con una accuratezza del 91%, combinando la valutazione videoendoscopica con la profondità della depressione, la presenza di irregolarità sul fondo della lesione e la convergenza plicale.

Nagata *et al*[39], in una serie di 75 casi di *early colorectal cancer*, per migliorare la probabilità di predire l'invasione profonda della

Tabella 3 - Probabilità di invasione dei vari strati della sottomucosa nelle lesioni superficialmente depresse (tipo IIc) in rapporto al tipo del *pit pattern*

Pit pattern	Invasione negli strati della sottomucosa	
	Sm 1	Sm 2 – 3
III – IV	6,7%	0
Vir	22,2%	66,7%
Vns		100%

sottomucosa (Sm 2-Sm 3) hanno proposto un'ulteriore differenziazione in sottotipi delle lesioni con *pit pattern* Vn. Il *pit pattern* Vn è stato riclassificato in 3 sottotipi, con grado A, B e C in base all'entità della destrutturazione e al disordine degli sbocchi ghiandolari. L'incidenza di carcinoma invasivo è stata significativamente più elevata nelle lesioni Vn con grado B e C rispetto a quelle Vn di grado A.

Questi dati dimostrano che è possibile migliorare l'accuratezza diagnostica migliorando ulteriormente la classificazione di Kudo ed utilizzando anche le informazioni disponibili dal punto di vista morfologico. La sensibilità nel riconoscimento di una lesione neoplastica invasiva può essere inoltre notevolmente migliorata con l'impiego, dopo la valutazione effettuata con l'indaco di carminio, del cristalvioletto che viene fissato selettivamente dagli sbocchi delle ghiandole di Lieberkuhn. Lo studio con tale colorante permette di differenziare con maggiore attendibilità i *pit pattern* di tipo invasivo (Vn e IIIs)[40].

Decisioni terapeutiche

La magnificazione può fornire un contributo rilevante nella scelta del più appropriato intervento terapeutico (EMR o chirurgia). Infatti le lesioni piatte e non depresse con *pit pattern* di tipo III_L o IV possono essere appropriatamente trattate mediante la mucosectomia endoscopica, mentre le lesioni depresse con un pattern di tipo V, dopo conferma bioptica mirata nelle sedi più sospette, possono essere trattate chirurgicamente[12]. Le lesioni diminutive (< 0,5 cm) con *pit pattern* di tipo II (iperplastico) non richiedono, secondo alcuni, l'asportazione o la biopsia[12, 41].

Casistica personale

Nel periodo gennaio 2003-ottobre 2004 sono state asportate 71 lesioni polipoidi su un totale di 60 pazienti con la tecnica di mucosectomia presso la U.O. di Gastroenterologia ed Endoscopia Digestiva del nostro ospedale. La distribuzione delle lesioni per sede era la seguente:

– 13 nel cieco,
– 6 nell'ascendente,
– 6 nel trasverso,
– 4 nel discendente,
– 16 nel sigma,
– 26 nel retto,

Le dimensioni variavano da 0,8 a 5 cm.

Le lesioni polipoidi sono state classificate dal punto di vista macroscopico come :

– *lateral spreading tumour* (17 casi),
– *creeping* (11 casi),
– lesione piatta (2 casi),
– lesione depressa (1 caso),
– lesione sessile (40 casi).

All'esame istologico, 27 erano adenomi tubulari, 11 adenomi villosi, 32 adenomi tubulo-villosi e 1 amartoma.

La displasia era di grado lieve in 11 casi, di grado moderato in 55 casi e di grado severo in 5 casi.

Il *follow-up* medio è di 10 mesi. Allo stato attuale si sono verificate 2 recidive a livello del cieco.

Conclusioni

L'endoscopia con magnificazione dell'immagine è una metodologia promettente in grado di migliorare l'accuratezza diagnostica ed influenzare le decisioni terapeutiche. In mani esperte essa consente di predire la diagnosi istopatologica in una percentuale molto elevata, ma non può discriminare il 100% delle lesioni, per cui al momento attuale essa non si sostituisce ma si integra con l'istologia tradizionale. Ancor prima di impegnarsi nella valutazione del *pit pattern*, ciascun endoscopista deve preliminarmente migliorare l'accuratezza diagnostica dell'endoscopia tradizionale, per imparare a riconoscere quelle alterazioni minime della mucosa meritevoli poi di un approfondimento diagnostico e acquisire un'adeguata conoscenza della morfologia e dell'istologia delle lesioni. La curva di apprendimento dell'endoscopia con magnificazione non è comunque lunga, poiché è stato calcolato che per raggiungere una adeguata accuratezza diagnostica è necessario eseguire almeno 50 procedure sotto la guida di un tutore[15]. La riproducibilità della diagnosi con lo strumento del *pit pattern* e la concordanza tra diversi osservatori sono elevate, purché la valutazione venga eseguita da un occhio esperto. Il gruppo di Kudo ha dimostrato un valore kappa medio di 0,810 e di 0,716 rispettivamente per la concordanza intra e inter-osservatore tra sei endoscopisti esperti, mentre un gruppo britannico solo del 49% tra 89 endoscopisti, tra cui 17 studenti[41].

L'endoscopia con magnificazione non prolunga significativamente i tempi dell'indagine diagnostica e può quindi essere proposta nei casi indicati come strumento routinario[11]. Ulteriori evoluzioni tecnologiche quali la microendoscopia confocale, l'endocitoscopia, la spettroscopia, etc. potranno fornire contributi ulteriori che al momento attuale è solo possibile ipotizzare. Anche se non ancora disponibili nella pratica clinica, l'entusiasmo per tali tecniche sta crescendo proporzionalmente con i progressi della ricerca nell'esplorazione della loro utilità clinica[2].

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Eur. J. Oncol., vol. 9, n. 4, pp. 245-248, 2004

Il carcinoma localmente avanzato della mammella. Indicazioni alla mastectomia

Locally advanced breast cancer. Indications for mastectomy

Gennaro Fabiano*, **, Angela Pezzolla*, Giovanni D'Eredità*, ***, Maria Alessandra Filograna*, Sara Tiziana Massa*, Filippo Ferrarese*

* Dipartimento di Scienze Chirurgiche Generali e Specialistiche - Sezione di Chirurgia Generale, U.O. di Chirurgia Generale Univ. "G. Marinaccio", Università degli Studi di Bari, Bari, Italia
** Cattedra di Chirurgia Generale
*** Cattedra di Chirurgia Generale

Riassunto

Nell'ambito del trattamento multimodale del carcinoma mammario localmente avanzato (CMLA) un ruolo fondamentale è ricoperto dal trattamento chirurgico. Anche se la terapia neoadiuvante porta ad una retrostadiazione clinica, non sempre a questa corrisponde una vera retrostadiazione biologica; la considerazione, inoltre, che il CMLA si riscontra spesso in donne in età avanzata, in cui il trattamento radioterapico postoperatorio può non essere agevole, porta a considerare la mastectomia come l'intervento di scelta in queste pazienti. Viene riportata l'esperienza degli ultimi 5 anni: 18 casi di CMLA su 223 pazienti con tumore mammario (8,07%), 8 IIIA, 10 IIIB. In tutti i casi è stata eseguita una mastectomia radicale modificata secondo Madden. Nove pazienti erano state sottoposte a chemioterapia neoadiuvante, in tutti i casi è stata eseguita una chemioterapia postoperatoria, in 5 la radioterapia. Dieci pazienti sono apparentemente libere da malattia, 5 presentano progressione di malattia; si sono verificati 2 decessi non correlati alla neoplasia, 1 decesso per neoplasia. Non sono state osservate recidive locali. Eur. J. Oncol., 9 (4), 245-248, 2004

Parole chiave: **carcinoma mammario localmente avanzato, mastectomia**

Summary

Surgical treatment plays an important rôle in the combined approach to locally advanced breast cancer (LABC). Neoadjuvant therapy causes a clinical downstaging, but not always a true biological downstaging. Furthermore LABC is often found in elderly women, for whom postoperative radiotherapy may not be practical. Hence mastectomy must be considered the surgical treatment of choice for these patients. We report our experience in the last five years: 18 LABC in 223 patients with breast cancer (8.07%), 8 IIIA, 10 IIIB. In all patients, we performed a Madden radical mastectomy. Nine of them underwent neoadjuvant chemotherapy, all patients had postoperative chemotherapy, and 5 radiotherapy. Ten patients are apparently disease-free, 5 have progressive disease, 2 died, but not from carcinoma, 1 died of cancer. We have observed no local recurrencies. Eur. J. Oncol., 9 (4), 245-248, 2004

Key words: **locally advanced breast cancer, mastectomy**

Introduzione

Sotto il termine Carcinoma Mammario Localmente Avanzato (CMLA) si raccolgono casi eterogenei di carcinoma mammario appartenenti agli stadi IIIA e IIIB della classificazione dell'AJCC[1]. In quest'ambito si distinguono il CMLA propriamente detto (o non infiammatorio) ed il carcinoma infiammatorio della mammella (o mastite carcinomatosa), T4d della classificazione TNM, che non prenderemo in considerazione in questa trattazione.

Definire qual è il ruolo della mastectomia nel trattamento del CMLA può sembrare all'apparenza molto semplice: la mastectomia è l'intervento di scelta. Se vogliamo, però, valutare l'argomento in maniera più approfondita possono essere poste delle obiezioni:

1) con il termine "localmente avanzato" indichiamo non solo neoplasie T4 o T3, ma anche neoplasie di dimensioni inferiori, che non hanno una diffusione nell'ambito della mam-

Received/Pervenuto 16.8.2004 - Accepted/Accettato 10.9.2004
Address/Indirizzo: Prof. Gennaro Fabiano, U.O. Chirurgia Generale Univ. "G. Marinaccio", Policlinico, P.zza G. Cesare, 70124 Bari, Italia
Tel. 0039/080/5592530 - Fax 0039/080/5478887
E-mail: gfabiano@chirges.uniba.it

mella tale da dover perseguire una demolizione ampia, ma hanno un importante coinvolgimento linfonodale;
2) il trattamento neoadiuvante può ricondurre neoplasie anche estese nei limiti di una chirurgia conservativa.

L'orientamento attuale, che vuole il trattamento chirurgico inserito in un più ampio approccio multidisciplinare e limitato nelle demolizioni, ci porterebbe a questo punto a dire che anche nelle forme localmente avanzate la mastectomia non dovrebbe avere più significato.

In realtà nelle forme localmente avanzate la mastectomia conserva tutto il suo significato terapeutico[2] e già la semplice *toilette* chirurgica anche se palliativa può portare evidenti benefici alla paziente[3].

Casistica e metodi

Presso la UO "G. Marinaccio" dell'Azienda Universitaria-Ospedaliera "Policlinico" di Bari, nel quinquennio dall'1/1/1999 al 31/12/2003 sono stati osservati 223 pazienti con neoplasia mammaria; di questi 18 (8,07%) presentavano un CMLA.

Di questi, 8 appartenevano allo stadio IIIA (T3 N1: 6, T3 N2: 2) con 6 < T < 8 cm, età media di 53,7 anni (31-81); 10 (9 femmine, 1 maschio) allo stadio IIIB (T4a: 2, T4b: 8), età media di 67,8 anni (52-87).

La chemioterapia neoadiuvante è stata praticata in 3 pazienti IIIA e 6 pazienti IIIB.

Tutti i pazienti sono stati sottoposti a mastectomia radicale modificata secondo Madden, con asportazione di una porzione di muscolo grande pettorale nei pazienti T4a.

Tutti i pazienti nel postoperatorio sono stati sottoposti a chemioterapia adiuvante, 5 a radioterapia (Tabella 1).

Risultati

Nei 9 pazienti sottoposti a chemioterapia neoadiuvante si è avuta una risposta clinica parziale con riduzione della massa neoplastica; non è stata osservata nessuna risposta clinica completa.

Il follow-up medio è stato di 24,5 mesi (6-54); non vi sono state perdite al follow-up.

Le 8 pazienti IIIA sono tutte viventi: 5 apparentemente libere da malattia, 2 con malattia metastatica in trattamento, in una è comparso dopo 20 mesi un carcinoma mammario controlaterale metacrono, trattato chirurgicamente.

Dei 10 pazienti IIIB 1 (maschio) è deceduto dopo 42 mesi per progressione di malattia; 2 pazienti, ultraottantenni, sono decedute per cause non neoplastiche, 5 sono apparentemente libere da malattia, 2 presentano progressione di malattia e sono in trattamento. In nessun caso abbiamo osservato recidive locali (Tabella 2).

Tabella 1 - Casistica

Paziente, sesso	Età	Stadio	Istologia	Stato linfonodale	Terapia neoadiuvante	Intervento	RT postoperatoria
GV, M	71	T4a	Duttale G3	N2	AC 3 cicli	Madden + res. m. pettorale	50 Gy
CM, F	65	T4b	Lobulare G2	N0		Madden	
PT, F	51	T3	Duttale G1	N2	AC 3 cicli	Madden	
QL, F	87	T4b	Duttale G2	N0		Madden	
CA, F	80	T4b	Duttale G2	N2	AC 3 cicli	Madden	
ML, F	44	T3	Duttale G3	N2	AC 4 cicli	Madden	
LC, F	83	T4a	Duttale G3	N2	AC 3 cicli	Madden + res. m. pettorale	45 Gy
SN, F	60	T3	Duttale G3	N1		Madden	
SP, F	50	T3	Lobulare G3	N1		Madden	
PD, F	52	T4b	Duttale G3	N2	AC 3 cicli	Madden	50 Gy
CA, F	53	T4b	Duttale G3	N3	AC 4 cicli	Madden	50 Gy
RS, F	81	T3	Duttale G2	N2		Madden	
DM, F	71	T3	Duttale G2	N2		Madden	
VA, F	42	T3	Duttale G2	N2		Madden	
FP, F	31	T3	Duttale G3	N2	AC 4 cicli	Madden	
CP, F	63	T4b	Duttale G2	N2	AC 3 cicli	Madden	45 Gy
DI, F	69	T4b	Duttale G3	N0		Madden	
DR, F	73	T4b	Lobulare G2	N0		Madden	

Tabella 2 - Risultati

Paziente, sesso	Mesi di follow-up	Risposta alla terapia neoadiuvante	Esito
GV, M	42	Discreta	Progressione malattia, exitus
CM, F	54		Libera da malattia
PT, F	49	Buona	Libera da malattia
QL, F	10		Exitus per causa non neoplastica
CA, F	41	Buona	Libera da malattia
ML, F	39	Discreta	Carcinoma mammario controlaterale
LC, F	13	Discreta	Exitus per causa non neoplastica
SN, F	36		Libera da malattia
SP, F	33		Libera da malattia
PD, F	30	Discreta	Progressione malattia
CA, F	25	Buona	Progressione malattia
RS, F	23		Libera da malattia
DM, F	17		Progressione malattia
VA, F	15		Progressione malattia
FP, F	10	Buona	Libera da malattia
CP, F	8	Discreta	Libera da malattia
DI, F	7		Libera da malattia
DR, F	6		Libera da malattia

Discussione

Diversi fattori intervengono nella scelta dell'intervento chirurgico nell'ambito del trattamento multimodale del CMLA.

Innanzitutto la risposta alla terapia neoadiuvante: Gioffrè Florio *et al*[4] riportano 86 casi di tumore mammario di dimensioni >2,5 cm (non propriamente localmente avanzati), in cui la terapia neoadiuvante ha permesso di limitare la mastectomia a soli 11 casi (12,9%); ma se prendiamo in considerazione i CMLA, i risultati sono meno incoraggianti; Cance *et al*[5], in uno studio su 62 pazienti con CMLA (includente anche 13 pazienti con carcinoma infiammatorio), riportano una risposta alla terapia neoadiuvante nell'84% dei casi, ma solo in 22 (45%) delle 49 pazienti con CMLA non infiammatorio è stato praticato un trattamento chirurgico conservativo per sufficiente *downstaging*.

Anche l'età gioca un ruolo nella scelta dell'intervento: Marrano e Taffurelli[6], in una revisione critica di alcuni anni fa, riportavano come nella fascia di età superiore ai 65 anni il più delle volte sia la paziente stessa, che ha spesso superato le problematiche psicologiche legate alla demolizione, a chiedere la mastectomia.

Brancato *et al*[7] riportano uno studio di 19 pazienti di età superiore a 65 anni con CMLA, tutte sottoposte a mastectomia. Hoff *et al*[8] riportano uno studio effettuato su 47 pazienti di età superiore a 75 anni con patologia associata, trattate con tamoxifene neoadiuvante: su 29 pazienti sottoposte a chirurgia curativa, solo 5 ebbero un trattamento conservativo.

Per converso anche le pazienti particolarmente giovani possono richiedere un trattamento locale aggressivo: Gaidos *et al*[9] riportano uno studio effettuato su 101 donne di età inferiore a 36 anni trattate per carcinoma mammario: di queste ben il 60% presentava alla diagnosi una malattia al II o III stadio con dimensione media dei tumori di 2 cm; pur essendo stato trattato il 59% con mastectomia si è avuto, nelle pazienti trattate con tecniche conservative, un alto tasso di recidive locali, imputato dagli autori ad un eccessivo ricorso alle metodiche conservative.

Questo porta a domandarsi se la chirurgia conservativa sia opportuna nei CMLA retrostadiati dalla terapia neoadiuvante. Sauven[10] riporta una risposta clinica completa (rcc) alla chemioterapia neodiuvante in 43 pazienti su 133 (32%); in 19 di queste la biopsia del sito primitivo, effettuata in corso di linfectomia ascellare, non ha mostrato evidenza di neoplasia e le pazienti sono state avviate al solo trattamento radioterapico; su 10 pazienti con rcc sottoposte comunque a mastectomia, in una sola paziente vi era una risposta completa istopatologica, in altre 2 pazienti la biopsia ha portato alla mastectomia ed in 12 alla resezione settoriale portando la risposta completa istopatologica al 18%.

Moneer *et al*[11] hanno studiato 41 casi di CMLA sottoposti a chemioterapia neoadivante e quindi a mastectomia: lo studio clinico e mammografico ha mostrato una risposta positiva dopo 3 cicli di chemioterapia nel 78% dei casi, con il 25% eligibile per un trattamento chirurgico conservativo; lo studio anatomopatologico delle mammelle asportate ha mostrato, però, un alto tasso di focolai peritumorali di carcinoma *in situ* e di multifocalità, come se la terapia neoadiuvante avesse determinato una sorta di frammentazione del tumore primitivo.

L'assenza di malattia nei margini di resezione negli interventi conservativi è importante al fine di prevenire recidive ed evitare mastectomie di salvataggio[12,13], che certamente sono peggio sopportate dalle pazienti rispetto alla mastectomia in prima istanza.

Il trattamento radioterapico postoperatorio si impone negli interventi conservativi; esso è utile, però, anche dopo interventi demolitivi[14,15] per prevenire le recidive locali, sempre possibili, anche se queste sembrano essere correlate in questi casi più alla positività linfonodale che non alle dimensioni della neoplasia[16], o alla cattiva risposta alla terapia neoadiuvante, con malattia neoplastica residua in sede linfonodale[17].

Il ruolo della radioterapia nel trattamento multimodale del CMLA è enfatizzato da alcuni autori che si domandano se la chirurgia abbia sempre un ruolo preminente in questi casi[18].

Conclusioni

Alla luce dei dati della letteratura e della nostra esperienza la mastectomia radicale, il più delle volte modificata, talvolta quella semplice, eccezionalmente la classica Halsted[2], costituisce il cardine del trattamento chirurgico inserito in un più ampio trattamento multimodale del CMLA.

Si tratta di solito di pazienti anziane, in cui la chemioterapia neoadiuvante dà spesso risposte brillanti, lasciando comunque un residuo di malattia, o anche di pazienti non in grado di sopportare prolungati trattamenti chemioterapici, o in cui, spesso, anche per motivi logistici, non è agevole accedere ad una radioterapia postoperatoria.

Pur volendo considerare il carcinoma mammario una "malattia sistemica", ciò non di meno un trattamento locoregionale adeguato rimane a nostro avviso il cardine della terapia.

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Eur. J. Oncol., vol. 9, n. 4, pp. 249-252, 2004

Resoconto di un caso di angioma a cellule littorali della milza e revisione della letteratura

Report of a case of splenic littoral cell angioma and review of the literature

Rosalia Ricco, Maria Giuseppina Lecce, Teresa Cipriani, Francesca Sanguedolce, Francesca Giotta, Anna Maria Dalena, Maria Carlucci

DAPEG – Dipartimento di Anatomia Patologica e di Genetica, Sezione di Anatomia Patologica, Università degli Studi, Bari, Italia

Riassunto

***Finalità*. Gli Autori riportano un nuovo caso di angioma a cellule littorali della milza, non associato a neoplasia maligna, insorto in una donna di 63 anni con trombocitopenia e splenomegalia evidenziabile all'esame obiettivo. La paziente soffriva di lesioni cutanee di natura non neoplastica da circa 20 anni. *Materiali e metodi*. Sono state eseguite indagini istologiche (ematossilina-eosina, colorazioni di Gomori e PAS) ed immunoistochimiche (anticorpi CD31, CD34, fattore VIII RA, CD68, lisozima, alfa-1-antichimotripsina, alfa-1-antitripsina, Ki-67/MIB1) sul campione operatorio della splenectomia, fissato in formalina ed incluso in paraffina. *Risultati*. All'esame istologico sono state individuate le caratteristiche architetturali e citologiche tipiche dell'angioma a cellule littorali. L'analisi immunoistochimica ha evidenziato l'immunoreattività delle cellule neoplastiche per i marcatori endoteliali (CD31, CD34, fattore VIII RA) ed istiocitari (CD68, lisozima, alfa-1-antichimotripsina, alfa-1-antitripsina); l'antigene Ki67/MIB1 era solo sporadicamente positivo. *Conclusioni*. L'angioma a cellule littorali della milza rappresenta una rara ma distinta entità nosologica, le cui caratteristiche cliniche, istologiche e biologiche sono discusse sulla base di una revisione della letteratura. Eur. J. Oncol., 9 (4), 249-252, 2004**

***Parole chiave*: angioma a cellule littorali, milza, tumori vascolari**

Summary

***Aim*. A new case of littoral cell angioma of the spleen not associated with malignancy in a 63-year-old woman with thrombocytopenia and palpable splenomegaly is reported. The patient presented with a 20-year history of cutaneous non-neoplastic lesions. *Materials and methods*. Histological (haematoxylin-eosin, Gomori and PAS stain) and immunohistochemical (CD31, CD34, factor VIII RA, CD68, lysozyme, alpha-1-antichymotrypsin, alpha-1-antitrypsin, Ki-67/MIB1) techniques were applied to the formalin-fixed paraffin-embedded surgical specimen from a splenectomy. *Results*. Typical architectural and cytological features of littoral cell angioma were histologically observed. Immunohistochemistry demonstrated immunoreactivity of the tumour cells for both endothelial (CD 31, CD34, factor VIII RA) and histiocytic markers (CD68, lysozyme, alpha-1-antichymotrypsin, alpha-1-antitrypsin); Ki-67/MIB1 was only sporadically positive. *Conclusions*. Splenic littoral cell angioma represents a rare but distinctive entity, whose clinical, histological and biological features are discussed on the basis of a review of the literature. Eur. J. Oncol., 9 (4), 249-252, 2004**

***Key words*: littoral cell angioma, spleen, vascular tumours**

Introduzione

L'angioma a cellule littorali (littoral cell angioma, LCA) è una rara neoplasia di recente descrizione[1]; nel capitolo dei tumori vascolari della milza, di cui fa parte, esso rappresenta una particolarità per via della sua origine dalle cellule che rivestono i seni della polpa rossa (cosiddette cellule littorali) anziché dagli endoteli vascolari. Ne sono affetti pazienti per lo più in età adulta o avanzata, ma è stato recentemente riportato un caso in età infantile[2]. Sono stati inoltre riportati in letteratura diversi casi di LCA in associazione a varie ed eterogenee neoplasie maligne: linfoma, seminoma, leiomiosarcoma gastrico, e carcinomi insorti in diversi organi[1, 3-9].

Received/Pervenuto 20.8.2004 - Accepted/Accettato 22.9.2004
Address/Indirizzo: Prof. Rosalia Ricco, Dipartimento di Anatomia Patologica e di Genetica, Sezione di Anatomia Patologica, Policlinico, Piazza G. Cesare 11, 70124 Bari, Italia - Tel. 0039/080/5478277
Fax. 0039/080/5593224 - E-mail: ricco@anatopat.uniba.it

Riportiamo qui un caso di angioma a cellule littorali in una paziente con una anamnesi positiva per lesioni cutanee di incerta origine, ed in trattamento con terapia corticosteroidea. Vengono inoltre discussi i più recenti dati della letteratura su tale patologia, tuttora rara e di difficile inquadramento diagnostico e prognostico.

Caso clinico

Il caso clinico è quello di una paziente di 63 anni, affetta per circa 20 anni da lesioni cutanee eritemato-desquamative; nel corso di tale periodo la paziente fu sottoposta a cinque biopsie con le seguenti diagnosi: granuloma annulare, istiocitoma eruttivo generalizzato, vasculite leucocitoclasica, dermatite granulomatosa, eritema *elevatum diutinum*. Inoltre, circa cinque anni prima dell'attuale ricovero le era stata riscontrata una trombocitopenia, con splenomegalia progressiva, per cui la paziente assumeva terapia corticosteroidea. L'esame TC dell'addome evidenziò multiple masse nodulari cistiche nel contesto di una splenomegalia, che vennero interpretate come lesioni angiomatose. A distanza di cinque anni da tale riscontro, la paziente fu sottoposta ad un intervento di splenectomia. Gli esami strumentali preoperatori non evidenziarono altre patologie. In seguito all'intervento si è assistito ad una normalizzazione della conta piastrinica; la paziente è attualmente in buona salute a distanza di 53 mesi dalla splenectomia.

Materiali e metodi

Il campione operatorio è stato processato con metodiche di istologia, istochimica ed immunoistochimica.

Il tessuto è stato fissato in formalina al 10% tamponata a pH 7,4; dopo adeguata disidratazione e diafanizzazione i campioni ottenuti dalla riduzione dell'organo sono stati inclusi in paraffina. Le sezioni allestite per la microscopia ottica, dello spessore di 5 micron, sono state colorate con le seguenti metodiche: ematossilina-eosina, Gomori, PAS.

Le sezioni allestite per l'analisi immunoistochimica sono state sottoposte alle seguenti marcature con la tecnica dell'avidina-biotina perossidasi: CD31 (anticorpo monoclonale, diluizione 1:300, Immunotech), CD34 (anticorpo monoclonale, diluizione 1:2000, Dakopatts), fattore VIII (anticorpo monoclonale, diluizione 1:10, Dakopatts), CD68 (anticorpo monoclonale KP-1, diluizione 1:400, Dakopatts; anticorpo monoclonale PGM-1, diluizione 1:200, Dakopatts), CD8 (anticorpo monoclonale, diluizione 1:25, Dakopatts), Ki-67/MIB1 (anticorpo monoclonale, diluizione 1:50, Dakopatts), alfa-1-antitripsina (anticorpo policlonale, diluizione 1:3000, Dakopatts), alfa-1-antichimotripsina (anticorpo policlonale, diluizione 1:8000, Dakopatts), lisozima (anticorpo policlonale, diluizione 1:2000, Dakopatts).

Reperto macroscopico

Il campione operatorio era rappresentato da una milza del peso di g 1450 e delle dimensioni di cm 25x14x10. Sulla superficie di taglio erano presenti noduli multipli, di colorito rosso-brunastro e di aspetto emorragico, del diametro variabile da cm 1 a cm 6.

Reperto istopatologico

All'esame al microscopio ottico l'aspetto dei vari noduli era sovrapponibile: tutti esibivano un caratteristico *pattern* angiomatoso, costituito da spazi vascolari ampi ed anastomizzati tra loro rivestiti da cellule tondeggianti, alte, protrudenti nel lume, e contenenti pigmento emosiderinico intracitoplasmatico (fig. 1). Il lume di tali spazi cisticamente dilatati era occupato da estroflessioni papillari e da singole cellule endoteliali esfoliate, con nucleo reniforme o indentato, citoplasma schiumoso e aspetti di eritrofagocitosi (fig. 2). In alcune aree erano presenti globuli citoplasmatici PAS-positivi (fig. 3), ed occasionali figure mitotiche.

Immunoistochimica

La valutazione immunoistochimica della lesione ha rilevato una marcata espressività dei marcatori endoteliali (CD34, CD31 e fattore VIII) e dei mar-

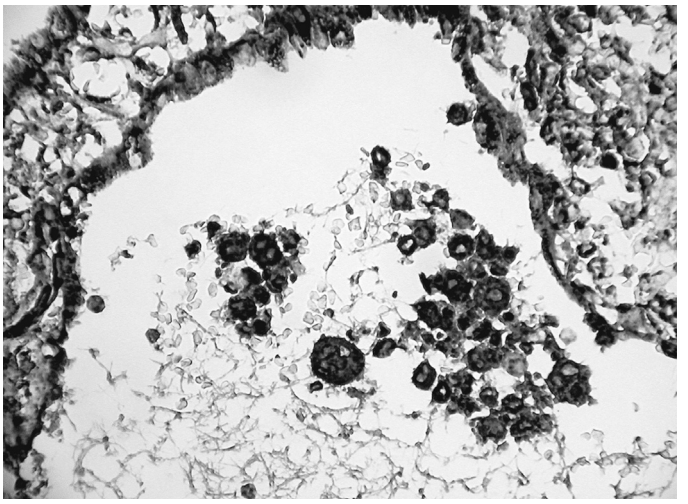


Fig. 1. Caratteristico *pattern* angiomatoso costituito da ampi spazi vascolari ematici rivestiti da cellule endoteliali rigonfie. E.-E., 600x.

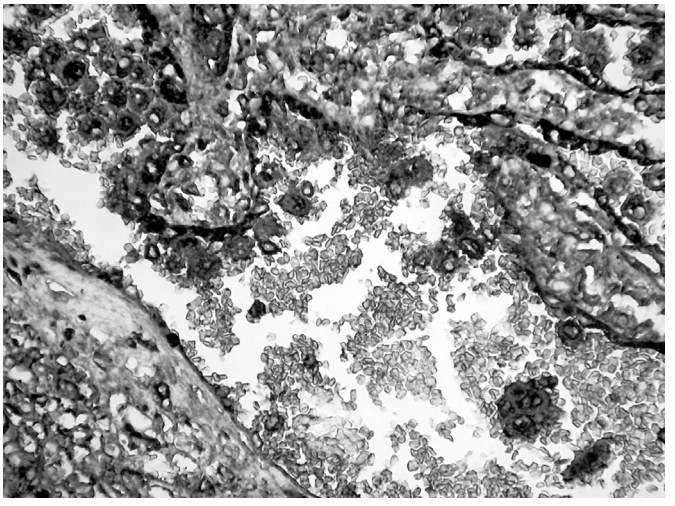


Fig. 2. I lumi degli spazi cistici sono occupati da fronde papillari e da isolate cellule di rivestimento esfoliate con citoplasma schiumoso. E.-E., 400x.

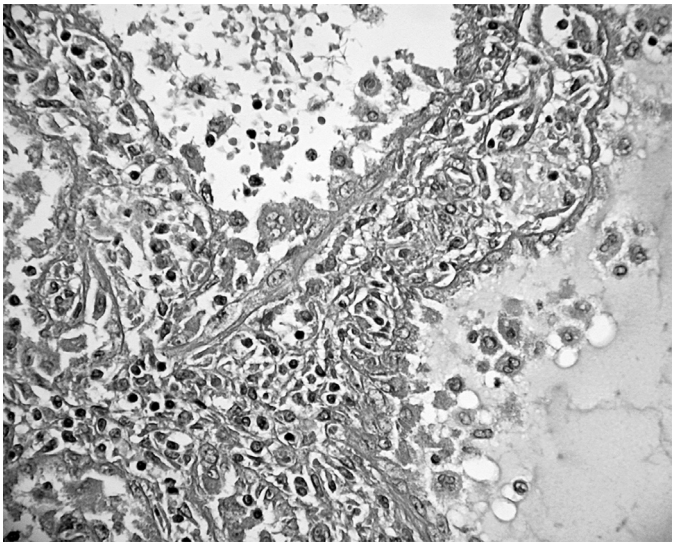
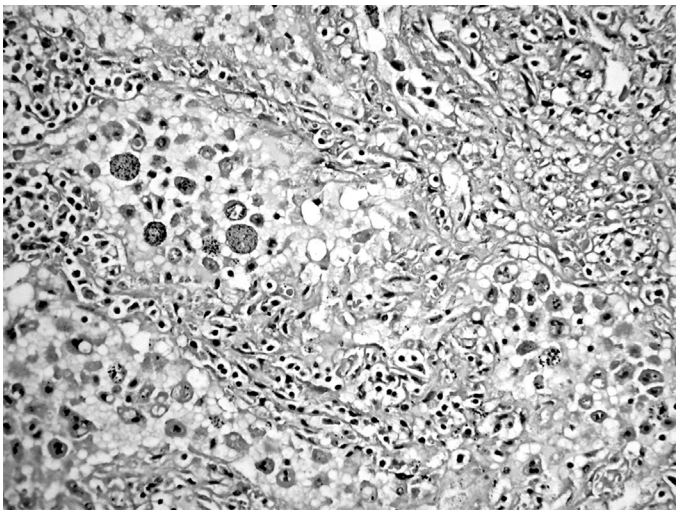
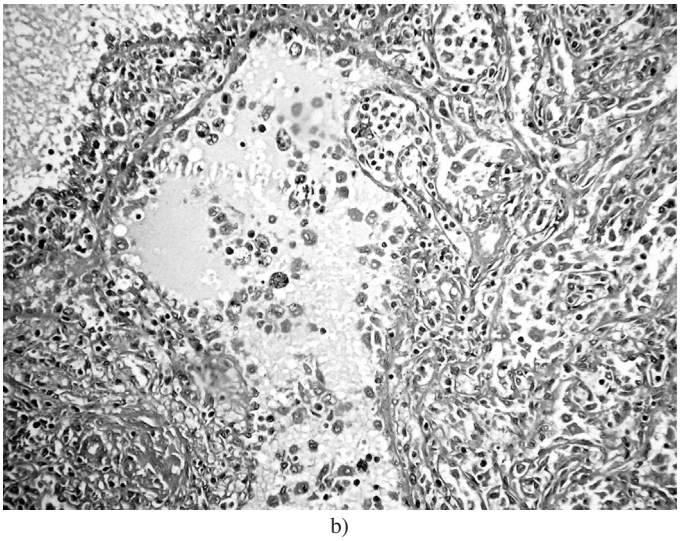


Fig. 3. In alcune aree sono presenti globuli citoplasmatici PAS-positivi. PAS, 400x.



a)



b)

Fig 4. Intensa immunoreattività per CD34 (a) e CD68 (b) nelle cellule littorali e nelle cellule disperse nel lume. CD34 e CD68, 400x.

catori istiocitari (CD68 KP-1 e PGM-1) a livello delle cellule di rivestimento. È inoltre apprezzabile una discreta positività del CD8 (fig. 4a, b).

L'antigene MIB1 è espresso solo localmente in sporadici elementi.

Discussione

L'angioma a cellule littorali è una neoplasia vascolare che origina dalle cellule littorali della polpa rossa splenica, e rappresenta una lesione tipica della milza. La caratteristica morfologica basilare di questo tumore è la presenza di spazi vascolari, simili ai seni splenici, con lumi irregolari, e delimitati da cellule alte che sporgono nei lumi e presentano i caratteri dell'emofagocitosi. Rispetto agli endoteli normali, tali cellule hanno proprietà di tipo istiocitario-macrofagico; all'analisi immunoistochimica infatti questi elementi tipicamente esprimono antigeni sia istiocitari che endoteliali, a riprova del duplice potenziale di differenziazione delle cellule reticoloendoteliali dei seni splenici[1]. Il dato che l'LCA non si riscontra nei linfonodi, in cui le cellule reticoloendoteliali sono anche ben rappresentate, ma solo nella milza dove le cellule littorali, identificate da marcatori endoteliali, possono acquisire un'attività fagocitaria dimostrata dalla presenza di attività lisosomiale alla microscopia elettronica e all'immunoistochimica, supporta fortemente tale ipotesi. L'immunopositività del CD68, costantemente esibita dalle cellule neoplastiche, conferma tale istogenesi; appare più controversa la dimostrazione della positività per CD8, considerato un marcatore delle cellule di rivestimento dei seni oltre che di un sottotipo di linfociti T, che non è stata riscontrata in tutte le casistiche[3, 10].

L'angioma a cellule littorali condivide le stesse manifestazioni cliniche di altri tumori vascolari splenici, ovvero splenomegalia e segni di ipersplenismo (anemia e trombocitopenia), e sintomi sistemici, come affaticamento e febbricola; in alcuni casi rappresenta un reperto occasionale in pazienti asintomatici[1, 11-14]. La diagnostica strumentale eseguita con ecografia e tomografia computerizzata mostra in genere il reperto non specifico di una milza ingrossata, di aspetto nodulare, con multiple masse cistiche ed una quantità variabile di parenchima splenico normale[4]; le alterazioni legate all'emosiderosi, evidenziate alla risonanza magnetica, rappresentano un dato non conclusivo[15, 16], pertanto la splenectomia con successivo esame istologico risulta indispensabile ai fini di una corretta diagnosi.

Diverse altre neoplasie vascolari spleniche devono essere prese in considerazione nella diagnosi differenziale dell'angioma a cellule littorali, tra cui l'emangioma cavernoso, l'emangiomatosi sinusoidale diffusa, il linfangioma cistico, l'emangioendotelioma epitelioide, l'angiosarcoma[1, 17-19], ma anche lesioni non neoplastiche come l'angiomatosi bacillare. Nel differenziare l'angioma a cellule littorali da altre lesioni vascolari bisogna prendere in considerazione le caratteristiche morfologiche ed immunoistochimiche delle cellule di rivestimento degli spazi vascolari, assieme alla presenza all'interno dei lumi di cellule fagocitarie. In questo caso l'analisi immunoistochimica riveste un ruolo fondamentale ai fini di una corretta diagnosi in quanto solo le cellule neoplastiche dell'LCA sono positive per entrambi i tipi di marcatori, endoteliale ed istiocitario, rivelando un fenotipo ibrido.

L'eziologia di questa neoplasia non è tuttora completamente chiarita. E' stato ipotizzato un meccanismo su base immunitaria, probabilmente scatenato da un'infezione cronica o da uno stato di immunosoppressione sistemica, indotto per esempio da terapie corticosteroidee prolungate, o nel post-trapianto[20]; l'associazione con neoplasie maligne aggressive in altre sedi potrebbe svolgere un ruolo importante nella patogenesi di tale lesione[5, 7, 21]. Nel nostro caso, la paziente assumeva terapia corticosteroidea e presentava una lunga storia di lesioni cutanee di incerta origine, eventualmente riconducibili ad una patologia di tipo immunitario, ma le indagini eseguite risultavano negative per altre patologie neoplastiche.

I numerosi casi di associazione dell'angioma a cellule littorali con varie neoplasie maligne rendono indispensabile un'attenta valutazione clinica e radiologica del paziente a cui è diagnosticato un LCA[3]. E' inoltre importante un accurato esame macroscopico e campionamento della milza asportata durante gli interventi chirurgici per tumori viscerali, poiché è stato recentemente riportato un caso di angioma a cellule littorali delle dimensioni di 1 mm in associazione con una neoplasia neuroendocrina maligna cistica del pancreas[8].

Attualmente si sa poco circa il potenziale di trasformazione maligna dell'LCA; generalmente è considerato una neoplasia benigna, anche se nella serie originale del 1991 era incluso un paziente con malattia disseminata al fegato ed all'encefalo, e con

presenza di aspetti solidi all'esame istologico, che però mostrava una buona prognosi in seguito al trattamento chemio e radioterapico. Successivamente è stato riportato un caso di "angiosarcoma a cellule littorali"[19], accidentalmente scoperto in un adulto in buone condizioni di salute. Da un punto di vista istologico ed immunoistochimico sembra che ci siano importanti differenze rispetto all'angioma: gli autori suggeriscono che la presenza di una neoplasia microscopicamente ben circoscritta e l'assenza di un *pattern* di crescita diffuso, di atipie citologiche, e di una elevata attività proliferativa possano essere considerati criteri validi per una diagnosi di benignità. Un ulteriore elemento discriminante è rappresentato dall'immunonegatività per CD68.

Il caso descritto riguarda un'entità nosologica che a nostro parere merita di essere segnalata poiché è di tuttora infrequente riscontro nella pratica clinica ed istopatologica. Inoltre, una revisione della letteratura ha evidenziato che gli aspetti eziologici e biologico-prognostici di questa patologia presentano ancora dei lati oscuri; è pertanto auspicabile la pubblicazione di studi, possibilmente su ampie casistiche, corredati dai dati anamnestici e di *follow-up* dei pazienti, al fine di delineare il corretto inquadramento clinico-patologico dell'LCA.

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Who uses alternative medicine: unconventional cancer treatment in Italy after the Di Bella scandal

Chi usa la medicina alternativa: terapie antitumorali non convenzionali in Italia dopo lo scandalo Di Bella

Stephan Tanneberger*, Achille Panetta**, Elena Strocchi*, Franco Pannuti*
* Foundation ANT Italia, Bologna, Italy
** Department of Oncology, Bentivoglio Hospital, Bentivoglio (BO), Italy

Summary

Every two or three years international oncology is shaken by someone claiming to have found the definitive treatment for cancer. In 1997 the Di Bella multitherapy was widely prescribed in Italy. However, 11 independent multicentric phase II trials failed to show efficacy in patients with advanced cancer. We have studied the situation of alternative cancer therapy in Italy in the post-Di Bella period with particular interest in the characteristics of alternative therapy users. In spite of the Di Bella therapy debacle, in the subsequent years 2000-2003, about 20% of our cancer patients used unconventional cancer treatment and invested enormous amounts of money in alternative therapy. The results make it evident that costly scientific studies have only limited value in alerting people against an unconventional treatment that is gaining widespread public acceptance in the absence of scientific evidence. Clinical studies on alternative cancer treatment without a sound pre-clinical, scientific basis, remain dubious from the ethical and economic point of view. Moreover, our results make it clear that the users of alternative cures do not always conform to the traditional stereotype of poorly educated people. Patients were well educated but attracted to therapeutic alternatives that reflect a social emphasis on personal responsibility and that move away from perceived deficiencies in conventional medical care. These findings suggest that other measures, besides better information, are called for to deter people from embracing misguided alternative cancer therapy. Eur. J. Oncol., 9 (4), 253-256, 2004

Key words: **cancer, therapy, Di Bella multitherapy**

Riassunto

Ogni due o tre anni l'oncologia internazionale è scossa da qualcuno che sostiene di aver trovato la cura definitiva per il cancro. Nel 1997 la multiterapia Di Bella è stata ampiamente prescritta in Italia. In realtà, 11 trials clinici di fase II, indipendenti e multicentrici, non ne dimostrarono l'efficiaca su pazienti con cancro in stadio avanzato. Abbiamo studiato la situazione della terapia alternativa anti-cancro in Italia nel periodo post-Di Bella, con particolare interesse alle caratteristiche degli utilizzatori della terapia alternativa. Malgrado il fallimento della cura Di Bella, negli anni successivi, dal 2000 al 2003, circa il 20% dei nostri pazienti ammalati di cancro utilizzava trattamenti non convenzionali e investiva enormi quantità di denaro nel trattamento alternativo. I risultati dimostrano che costosi studi scientifici hanno un valore limitato nel contrastare un trattamento non convenzionale che si sta guadagnando una vasta popolarità in assenza di prove scientifiche. Studi clinici su trattamenti alternativi anti-cancro privi di una ragionevole base scientifica e preclinica rimangono discutibili da un punto di vista etico ed economico. Inoltre i nostri risultati mettono in evidenza che gli utilizzatori di terapie alternative non sempre corrispondono allo stereotipo tradizionale di persone scarsamente istruite. I pazienti erano ben istruiti, ma erano attratti da terapie alternative che riflettono l'enfasi sociale sulla responsabilità individuale e che prendono origine dalle evidenti carenze della terapia medica convenzionale. Questi risultati indicano che altre misure, oltre ad una migliore informazione, sono necessarie per dissuadere la popolazione dall'accogliere terapie antitumorali alternative fuorvianti. Eur. J. Oncol., 9 (4), 253-256, 2004

Parole chiave: **cancro, terapia, multiterapia Di Bella**

Received/Pervenuto 25.8.2004 - Accepted/Accettato 8.9.2004
Address/Indirizzo: Prof. Stephan Tanneberger, Fondazione ANT Italia, Via Curiel 7, 40134 Bologna, Italia - Tel. 0039/348/3102797
Fax: 0039/051/6154122 - E-mail: stephan.tanneberger@antitalia.org

Introduction

In the second half of 1997 there was extensive international media coverage of the allegedly successful treatment in Italy of a number of malignant neoplasms with Di Bella multitherapy (MDB). Public pressure forced the decision to settle the matter by testing MDB in phase II clinical trials. Despite the lack of any real pre-clinical scientific evidence of its safety or efficacy, in February 1998, the Italian parliament passed an act authorising clinical studies to be conducted and making funds available for these MDB trials. By 1999 it became obvious that the somatostatin-based Di Bella cancer therapy was not the miracle cure its advocates had claimed[1-3].

The story led to two problems.

1) It remains to be explained whether cost-intensive clinical trials are the correct approach to managing such a situation. Silvio Garattini blamed the majority of cancer specialists who went along with clinical trials which had no scientific basis "for fear of being unpopular"[2].

2) It remains to be explained why, in an advanced society, it is possible to see medicine reduced to a sort of barter system. Umberto Veronesi shifted most of the blame on to the media and concluded that newspapers and television had treated the affair more like a soap opera than a serious question of medical research, ignoring the fact that their messages would confuse thousands of seriously ill patients and their families[2]. Passalacqua *et al*[4, 5] discussed patient opinions, feelings and attitudes after the emotional campaign promoting the Di Bella therapy was launched by the Italian media. It was reported that low education had a major impact on patients' choice of the Di Bella therapy and the authors concluded that less educated patients are less protected from the damage that such campaigns may cause, and more in need of help.

We have studied the situation of alternative cancer therapy in Italy in the post-Di Bella period with particular regard to the characteristics of alternative therapy users.

Patients and methods

The survey took place between November 1999 and January 2003. Alternative therapy was definitely used in the period after the day the Italian National Health Institute (Istituto Superiore di Sanità) announced that the MDB was not the miracle cure its advocates had claimed. Looking for the patient's characteristics, a questionnaire (Table 1) was distributed to collect the following information: (a) marital status; (b) living alone or with family; (c) place of residence; (d) degree of education; (e) profession; (f) outlook on life; (g) political position; (h) income; (i) use and experiences with alternative cancer treatment. Eligible patients were aged 40 years or older, had been diagnosed as having a malignant neoplasm, and did not suffer from any cognitive or physical impairment that could prevent them from completing the questionnaire. Patients had the possibility to complete the questionnaire anonymously or by aid of their assisting physician. A discriminant analysis was performed looking for independent variables to classify patients into two groups: alternative therapy and standard users.

Results

One hundred and ninety-two patients with primary (96) and advanced (96) cancer were enrolled in the trial. There were 70 male and 122 female patients. The mean age of the patients was 62 years (range 26-93 years). Thirty-six patients (18.9%) used alternative treatment (44.4% Di Bella multitherapy, 33.3% homeopathy, 22.2% other types such as aloe, high dosage vitamins, ayurveda and prayer). On average patients spent a total amount of 15,789 Euros on the Di Bella therapy, 1,515 Euros on homeopathy and 154 Euros on the other alternative therapy types.

As shown in fig.1, the results of the discriminant analysis show four independent variables with a significant impact on the choice of alternative treatment: advanced disease, living in a place with > 500,000 inhabitants, having a university level of education and a "left-green" political position. Discriminant classification afforded a reduction in classification error equal to 45.6%.

Discussion

We would like to draw two conclusions from these results.

1) Assuming that this approach was the best way to generate

Table 1 - Some of the most significant questions in the questionnaire proposed to the patients

Question	Categories/Options			
Place of residence (inhabitants)	>500,000	100-500,000	50-100,000	<50,000
Education	University	College	Secondary school	Primary school
Outlook on life	Trusting	Devout	Atheist	
Political position	Right	Left	Central	Green
Annual income	>50,000$	25-50,000$	20-25,000$	<20,000$
Source of information on alternative treatment?	Mass media	Friends	Doctor	Church
Have you met Prof. Di Bella?	Yes	No		
What costs have you had?	Drugs	Doctor	Other	Total
Was treatment paid by state/insurance?	Yes	No	In part	
What was the treatment result?	Feel better	Better quality of life	Nothing	
Do you know the difference between conventional and alternative treatment?	Yes	No		
Why did you use alternative treatment?	To be specified			
Would you choose alternative treatment again?	Yes	No		
Would you recommend alternative treatment to others?	Yes	No		
Do you think the government should stop alternative treatment?	Yes	No		
Do you think the Di Bella treatment was studied correctly?	Yes	No		

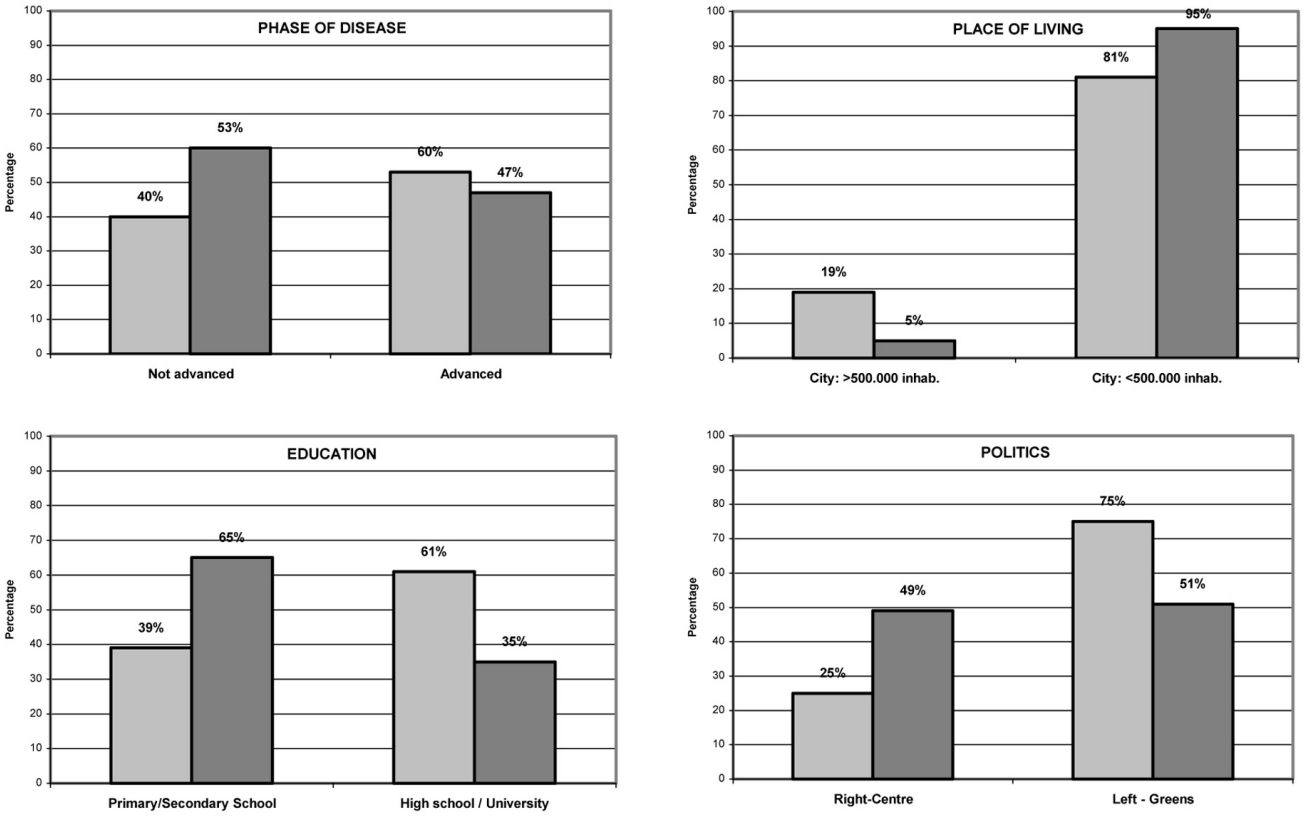

Fig. 1. Distribution of patients according to the significant discriminant factors (alternative therapy: light grey bars; no alternative therapy: dark grey bars). Chi-square statistics on single factors: phase of disease, p=0.177; place of residence, p=0.02; education, p=0.004; politics, p=0.008

evidence-based data and to put a damper on the enthusiasm for alternative medicine, the Italian parliament decided that the "Di Bella method" should undergo standard clinical evaluation, encompassing 300-400 patients. Obviously, this assumption was too optimistic. In spite of the Di Bella cure debacle[2], in the subsequent years 2000-2003, about 20% of our cancer patients used unconventional cancer treatments and invested enormous amounts of money in alternative treatments including Di Bella multitherapy. It was postulated that the scientific evaluation of unproven treatments may lead to new and useful cancer therapies and that negative test results could take away hopes from patients and doctors. Our results make it evident that costly scientific studies have only limited value to deter from unconventional treatment that may be gaining widespread public acceptance in the absence of scientific evidence. Instead it should be our first duty to fight consistently for science in medicine because unscientific medicine opens the door to inhumane medicine[6]. However, scientific medicine never means investigating unscientific theories in clinical trials. The scientific approach to studing unproven methods must first of all include collecting and screening scientific information about the method[7]. As proposed by the NCI BCS programme, before clinical research projects are supported meaningful data can and have to be generated[8]. Moreover, the label "unproven" is inappropriate for numerous alternative cancer therapies which have already been "disproved"[9]. Clinical studies without a sound pre-clinical, scientific basis are dubious from an ethical and economic point of view.

2) Passalacqua *et al*[4,5] reported that during the Di Bella period, educational attainment had a major impact. Better educated patients were more critical of Di Bella's therapy, communicated better with their oncologists and were less influenced by the media campaign. It was concluded that measures to reduce the negative impact of such campaigns for unconventional cancer treatment have to be directed mainly at patients in the less educated category, who are less protected from the damage that such campaigns may cause. Our study, in accordance with others[10,11], shows that the users of alternative care do not always conform to the traditional stereotype of poorly educated persons. Patients had a higher education but were attracted to therapeutic alternatives that reflect social emphasis on personal responsibility and that move away from perceived deficiencies in conventional medical care[12]. These findings suggest that other measures, besides better information, are called for to deter people from embracing misguided alternative cancer therapy.

Acknowledgement

The generous contributions of the Foundation ANT Italia are gratefully acknowledged. The authors wish to thank Drs. Maria Luisa Geminiani, Silvia Gambini and Paolo Mariano for their co-operation. We are grateful to the nurses, physicians and families who have cared for these patients for providing us with the data of this research.

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Eur. J. Oncol., vol. 9, n. 4, pp. 257-258, 2004

Professor Feng-sheng He
(1932-2004)

Professor Feng-sheng He, Fellow of the Collegium Ramazzini, member of the Chinese Academy of Engineering, Honorary Director of the National Institute of Occupational Health and Poison Control (NIOHPC), the Chinese Centre for Disease Control and Prevention (CCDCP), died in Beijing on November 16, 2004 of pancreatic cancer.

Professor He was an internationally renowned scholar in the field of occupational medicine and neurotoxicology. She made significant contributions to the study of a number of occupational nervous diseases and neurotoxic diseases. From the early-70s to the early-80s, she devoted herself to a series of epidemiological, clinical, toxicological and neuropathological studies, which for the first time systematically demonstrated the neurotoxicity of allyl chloride both in humans and in animal models. This led to the further development of diagnostic criteria for chronic occupational allyl chloride poisoning, as well as the establishment of occupational standards for allyl chloride. Her studies on the clinical features of mildewed sugarcane poisoning revealed for the first time that a mycotoxin (3-nitropropionic acid) induced dystonia with striatal CT lucencies in humans. These studies contributed to controlling this life-threatening brain disease with persistent sequelae in children throughout China. Professor He was the first to describe the clinical manifestations and the nerve excitability changes of acute pyrethroid insecticide poisoning in humans. Her work in acute carbon monoxide poisoning proved the significance of multiply-evoked potentials in the evaluation of brain function and advanced prediction of the onset of delayed encephalopathy. She and her collaborators first detected the novel exposure biomarker of acrylamide, acrylamide-haemoglobin adduct, as a yardstick of occupational risk of acrylamide. Her research on the intermediate myasthenia syndrome following acute organophosphate poisoning first provided evidence of a postsynaptic transmission block at the neuromuscular junction by single fibre stimulation electromyography both in humans and in experimental animals. Professor He was also an active leader in the development of occupational health and promotion of preventive medicine both nationally and internationally. In 1994, she co-chaired the Second Meeting of the Network of WHO Collaborating Centres in Occupational Health, which recommended the document Global Strategy on Occupational Health for All (The way to health at work) for consideration by the WHO. This important document was formally endorsed by the WHO in 1996 and became the framework of policy principles and strategies based on which the WHO addresses occupational health globally.

Professor He was born in Nanjing on June 26, 1932 and received her medical training at the Medical College of former Central University in Nanjing. After gaining her medical degree, she worked for six years as a neurologist at the Peace Hospital in Beijing. In 1962, she joined the Department of Occupational Medicine, Institute of Health, Chinese Academy of Medical Sciences as a Lecturer, and became an Associate Professor in 1978. She also chaired the Department of Occupational Medicine from 1978-1985. During 1979-1981, she was a Visiting Scholar at the Institute of Neurology of the University of London, England. From 1985 to 1991, she was Professor in Occupational Medicine, and Director of the Institute of Occupational Medicine (IOM) at the Chinese Academy of Preventive Medicine (CAPM). From 1991 to 1994, she worked as a Medical Officer in the WHO Office of Occupational Health in Geneva. She became Professor in Occupational Medicine at the IOM, CAPM and later NIOHPC and CCDCP in 1995. She was

also the Head of the WHO Collaborating Centre for Occupational Health (Beijing) from 1982 to 2003.

During her career, Professor He published more than 200 scientific papers. She was the principal author of two acknowledged premier reference textbooks "Chinese Occupational Medicine" and "Toxic and Metabolic Nervous Diseases". She also co-edited and contributed chapters to eleven other professional books. She received numerous honours and awards, including membership of the Chinese Academy of Engineering (from 1994), the Scipione Caccuri International Prize in 1984, the First Class Prize for Scientific Research from the Ministry of Public Health in China (1989), the National Medal of Labour in China (1986), the State Distinguished Scientist in China (1986), and the Second Class Prize of National Science and Technology Advances in China (1987). She was an Honorary Director of the IOM, CAPM and later NIOHPC, CCDCP from 1995; an Honorary Fellow of the Royal College of Physicians, Faculty of Occupational Medicine (London) from 1986 on; a member of the Council of Fellows, Collegium Ramazzini (from 1988); an Active Member of the European Academy of Sciences and Arts; and an Honorary Professor of seven universities and research institutes in China. Professor He was also very active in the professional field and served on many boards and committees, including Board Member of the International Commission on Occupational Health (1990-1993); President of the Asian Association of Occupational Health (1991-1994); Panel Member of the WHO Expert Advisory Panel on Occupational Health (from 1986); a member of the Board of Directors of the International Society of Complex Environmental Studies; a Council Fellow of the International Association for Indoor Air Quality; an Executive Board Member of the Chinese Association of Preventive Medicine; Vice President of the Chinese Association of Public Health; and Vice President of the Chinese Society of Occupational Health. Professor He was on the editorial board of 14 journals abroad and in China.

Friends, family, and colleagues who knew her agree that Professor He was most brave about her illness. Diagnosed a year ago with pancreatic cancer at its most advanced stage, she put up a battle with enormous energy and vitality. She participated in the diagnosis and therapy decision-making throughout her illness. Even in hospital, she continued her work on the promotion of occupational health. Those who knew her will always remember her generosity, her perseverance and her dedication to occupational medicine, to the working people and to her students.

Professor He is survived by her husband (these 47 years), Fangyi Qian, a cardiologist; her daughter Dayan of Beijing; her son Dapeng of Guilford, Connecticut; and 3 grandsons.

Yu-xin Zheng*
Zhao-lin Xia**
* National Institute of Occupational Health and Poison Control, Chinese Centre for Disease Control and Prevention, Beijing, China
** Department of Occupational Health and Toxicology, School of Public Health, Fudan University, Shanghai, China

NIVA, The Nordic Institute for Advanced Training in Occupational Health offers advanced courses and symposia for researchers and professionals in the field of occupational safety and health, and other work life matters.

During the last week of May 2005, NIVA organises a course on Ethical, Scientific, and Social Aspects of Predictive Testing in Occupational Health Practices.

New biotechnological and genetic methods applicable for medical screening and monitoring have been developed rapidly and may already be used to predict the future health and personal characteristics of employees and job applicants. These developments require proper legal and practical tools to handle new situations in an ethically acceptable way.

This course will give the necessary ethical tools for handling problems related to predictive testing in the work environment, in planning and conducting research projects in occupational health, and in developing codes of conduct and legislation in the field.

The main topics deal with testing practices and their scientific base, ethical aspects of predictive testing, legal developments and social consequences, and analyses of 'real-life' cases in work environments.

Lectures and discussions will be of equal importance, and also a role play and practical sessions are included in the programme.

The course will be held near the city of Turku, in the charming Baltic archipelago at the Hotel Strandbo, Nagu, Finland, starting Monday afternoon 23 May and ending by lunchtime on Friday 27 May 2005.

For futher information please contact course secretary Gunilla Rasi, e-mail gunilla.rasi@ttl.fi, or course leader Prof. Kirsti Husgafvel-Pursiainen, e-mail: kirsti.husgafvel-pursiainen@ttl.fi, or course coordinator Dr. Marja Sorsa, e-mail: marja.sorsa@transmix.fi. More information can also be found on NIVA's home page, www.niva.org.

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INDICE

The Precautionary Principle - Implications for Research and Prevention in Environmental and Occupational Health
P. Grandjean, M. Soffritti, F. Minardi, J.V. Brazier (Editors)

Ban on asbestos in Europe - Messa al bando dell'amianto in Europa
C. Bianchi, M. Soffritti, F. Minardi, J.V. Brazier (Editors)

Ramazzini Library

The Precautionary Principle Implications for Research and Prevention in Environmental and Occupational Health

P. Grandjean, M. Soffritti, F. Minardi, J.V. Brazier (Editors)

Secondo volume della collana "Ramazzini Library". Raccoglie gli atti del Congresso Internazionale "Il Principio di Precauzione: implicazioni per la ricerca e la prevenzione nella medicina ambientale del lavoro", svoltosi a Bologna il 23 e 24 ottobre 2002, promosso dal Collegium Ramazzini, dalla Regione Emilia-Romagna, dall'European Environmental Agency e dalla Fondazione Ramazzini.

Details	ISBN: 88-86235-80-1
Anno	2003
Pagine	248
Formato	volume in brossura stampato nel formato di cm 17 x 24
Prezzo	Euro 25,00 (Lire 48.407)
Collana	Ramazzini Library
Issue	2

Collegium Ramazzini - Final Draft Statement
The Precautionary Principle: implications for research and policy making

The Collegium Ramazzini endorses the use of the Precautionary Principle for protecting human health and sustainability of the environment. The Precautionary Principle embodies and operationalises the axiom that it is better to be safe than sorry. It brings foresight and transparency to situations with high stakes, uncertain scientific evidence, and disputed values, but where decisions on policy are needed before additional knowledge can be generated. The Precautionary Principle re-invigorates the public health tradition requiring that we do no harm.

Past successes of precaution include the introduction of safe drinking water to major cities in Western Europe and North America decades before elucidation of the germ theory of disease. These actions saved millions of lives. The more recent banning of lead additives in petrol before full appreciation of the health impacts resulted in reductions of up to 90% in paediatric blood concentrations of this toxic metal.

Failures to take precautionary action, despite early warnings, have resulted in severe harm to human health and the environment. Examples include asbestos, ionizing radiation, lead, mercury, some pesticides, polychlorinated biphenyls, tobacco, and the chlorofluorocarbons that damage the ozone layer. Public and occupational health practitioners have often applied preventive measures, but these are not necessarily precautionary. For example, stopping asbestos use and exposure in 2003 is preventive, but hardly precautionary, given that we have known for many decades about the impacts of asbestos on health. In contrast, restricting asbestos exposure in the early decades of the previous century would have been both preventive and precautionary. While precautionary actions are generally reversible, failure to take precautionary action may cause irreversible harm.

Current regulatory practice permits the marketing of many products and technologies on the assumption that they cause no unacceptable harm, thus placing the burden of proving harm on public authorities. Under the Precautionary Principle, by contrast, products and technologies must be assessed to show that they are acceptably safe before they are introduced for use, as is currently the case for most pharmaceuticals and pesticides. If already in use, safety may need to be reassessed, taking into account worst-case scenarios, emerging scientific knowledge and all potential direct and indirect impacts. This approach places the burden of demonstrating safety on those responsible for introducing products and technologies.

The Precautionary Principle uses the best available science as an input to public policy-making. However, sound policy depends not only on good science and technology, but also on other values such as the moral imperative to preserve health, life and the environment. The Precautionary Principle provides a framework for achieving transparent, democratic processes that take these dimensions into consideration in developing policies.

An impediment to precaution is that the scientific community typically requires strong evidence of an adverse finding before "crying wolf" about an agent or an exposure. The frequent insistence that a link between exposure and disease be established with strict statistical confidence presumes the innocence of hazards until there is very strong evidence of harm, and it creates a culture of scientific caution that is more highly focused on avoiding "false positives" than "false negatives". With this anti-precautionary attitude, science preserves its authority and enhances the impact of the alarms that it raises. However, because absence of proof is not a proof of absence, these positions need explicitly to be recognised and reconciled in light of the overall health and environmental issues at stake.

At a recent conference, the Collegium Ramazzini, in collaboration with the World Health Organization (WHO), the United States' National Institute for Environmental Health Sciences (NIEHS), and the European Environment Agency (EEA) explored the different methods and goals of science and policy-making and the implications of the Precautionary Principle for better research, training and prevention. An urgent need was demonstrated for striking a better balance between good science and the protection of public health.

Therefore, the Collegium Ramazzini calls for:

- Revision and expansion of the agendas of public health agencies at all levels to increase emphasis on precaution and primary prevention as tools for preventing disease.
- Increased allocation of resources to support research, training, education and policy analysis in primary prevention, with major investment in developing better instruments to assess the potential harms and benefits of products and technologies, both new and old.
- Dissemination of information about potential impacts of products and technologies, and development of better methods of two-way communication between scientists and decision-makers, including the public, who have the right to know about the potential hazards to which they may be exposed, about the uncertainties in science, and about how these uncertainties are managed.
- Increased incentives for the timely contribution, and penalties for the non-production, of adequate information about hazards and their prevention by those responsible for the products and technologies.
- Application of more sensitive health and environmental surveillance programs aimed at the early detection of any unwanted consequences from products and technologies.

Ban on asbestos in Europe

C. Bianchi, M. Soffritti, F. Minardi, J.V. Brazier (Editors)

Terzo volume della collana "Ramazzini Library". Raccoglie gli atti del Congresso Internazionale "Ban on asbestos in Europe - Messa al bando dell'amianto in Europa", svoltosi a Monfalcone nel febbraio del 2003, promosso dal Collegium Ramazzini, dalla Lega Italiana per la lotta contro i tumori (sezione di Gorizia), dal comune di Monfalcone e con il patrocinio della Regione Friuli Venezia Giulia.

Details	ISBN: 88-86235-89-5
Anno	2004
Pagine	104
Formato	volume in brossura stampato nel formato di cm 17 x 24
Prezzo	Euro 18,00 (Lire 34.853)
Collana	Ramazzini Library
Issue	3

Proceedings of the Conference "Ban on asbestos in Europe": presentation

Atti del Convegno "Ban on asbestos in Europe": presentazione

The Proceedings of the Conference on asbestos in Europe, held in Monfalcone in 2003, are published at a time of great relevance in European history. Starting in May 2004, the European Union will enlarge eastwards so that it will include a large part of our micro-continent. Huge problems, cultural as well as economic and political, will have to be to faced and resolved. The asbestos problem may be considered as a paradigm. Europe is affected by an epidemic of tumours induced by asbestos. Of such an epidemic it is not easy to predict the end. Asbestos consumption reached a peak in Europe in the 1970s. Since the mean latency periods of asbestos-related mesothelioma are about 50 years, a further worsening of the situation has to be expected over the next decades. Furthermore the diagnosis of mesothelioma is seldom timely and the results of treatment are still usually disappointing.

Asbestos legislation in Europe has been extremely heterogeneous. Once again the serious delays have demonstrated that scientific knowledge and political decisions run on parallel tracks. However, the most worrying factor is that the asbestos tragedy does not seem to have taught any lessons. The uncontrolled use of substances, the carcinogenic effect of which is well known continues, as does the use of substances whose long-term effects are completely unknown. Increasing risks are encountered in the sector of physical agents (e.g. electromagnetic fields), and the destruction of the environment continues.

Even in the case of asbestos, the European Union presents itself as a virtuous brother, whose example should be followed. However, there are some macroscopic exceptions. Still in the year 2000, Greece was the first among the "minor" asbestos producers, and the mineral was still used in the Iberian peninsula. Moreover, recent estimates show that, even in the virtuous European Union, great attention does not seem to be devoted to occupational cancer.

The contributions published in this book are not only a presentation and a discussion of scientific, historic, and legislative data. They are also a further call to change the course of events.

The asbestos tragedy has been the result of a series of mistakes. It is not by chance that the call comes from an area such as Monfalcone: an area, where a majority of families have a member struck by asbestos-related diseases. A large number of people exposed to asbestos over the last decades, now healthy, remain at high risk for asbestos disease. A campaign for the surveillance of this population has already been started. But the aim of the research is to identify ways of neutralizing the asbestos present in the tissues of a large number of people.

Gli Atti del Convegno sull'amianto in Europa, svoltosi a Monfalcone nel 2003, vengono pubblicati in un momento di grande portata storica. Dal maggio 2004 l'Unione Europea si ingrandirà verso est in modo da comprendere larga parte del nostro microcontinente. Immensi problemi culturali, economici e politici, dovranno essere affrontati e risolti. Il problema amianto può essere considerato un paradigma significativo. L'Europa è coinvolta da un'epidemia di tumori indotti dall'amianto, epidemia di cui non è facile prevedere la fine. I consumi di amianto hanno toccato il vertice in Europa negli anni '70 dello scorso secolo. Poiché i tempi medi di incubazione del mesotelioma da amianto si aggirano sui 50 anni, è da attendersi un peggioramento della situazione nei prossimi decenni. Inoltre per questo tumore la diagnosi non riesce ad essere tempestiva e la terapia rimane per lo più deludente.

La legislazione sull'amianto nel continente è stata estremamente disomogenea. Si sono verificati gravi ritardi, dimostrando ancora una volta che le conoscenze scientifiche e le decisioni politiche viaggiano su binari paralleli. Ma l'elemento più preoccupante è che la tragedia amianto sembra non aver insegnato niente. L'uso incontrollato di sostanze di cui si conosce l'effetto cancerogeno continua, come pure continua l'uso di sostanze di cui si ignorano del tutto gli effetti a lungo termine. Rischi sempre maggiori si incontrano nel campo degli agenti fisici (p.es. campi elettromagnetici) e continua lo scempio dell'ambiente.

Anche nel caso dell'amianto l'Unione Europea si presenta come un fratello virtuoso, del quale bisogna seguire l'esempio. Ma con qualche vistosa eccezione. Nel 2000 la Grecia era ancora il capofila tra i "piccoli" produttori di amianto e il minerale era ancora in uso nella penisola iberica. Inoltre anche nella virtuosa Unione Europea sembra non si presti molta attenzione ai tumori professionali, come recenti stime dimostrano.

I contributi riuniti in questo volume non sono solo un'esposizione e una discussione di dati scientifici, storici, legislativi. Sono anche un ulteriore richiamo ad invertire la rotta.

La tragedia amianto è stata il risultato di una sequenza di errori. Non è casuale che il richiamo venga da un'area come quella di Monfalcone: una zona dove la maggioranza delle famiglie ha avuto un proprio membro colpito da malattie asbesto-correlate. Un gran numero di persone esposte negli ultimi decenni e attualmente sane rimane ad alto rischio per malattie da amianto. Una campagna per la sorveglianza di tale popolazione è già stata avviata. Ma l'obiettivo della ricerca è individuare vie attraverso le quali neutralizzare l'amianto presente nei tessuti di un gran numero di persone.

Claudio Bianchi
Centre for the Study of Environmental Cancer, Italian League against Cancer,
Monfalcone (GO), Italy

EUROPEAN JOURNAL OF ONCOLOGY

GIORNALE EUROPEO DI ONCOLOGIA

The European Journal of Oncology (Eur. J. Oncol.), Official Organ of the Italian Society of Tumours (SIT)/Prevention, Diagnosis and Therapy, is promoted by the **European Foundation of Oncology and Environmental Sciences "B. Ramazzini"**
via Guerrazzi, 18 - 40125 Bologna, Italy
telephone: for Italy 051/237286; for abroad 0039/051/237286
fax: for Italy 051/2911679; for abroad 0039/051/2911679
and published by:
Mattioli 1885 - Casa Editrice
via Coduro, 1/B - 43036 Fidenza (PR) - Italy
telephone: for Italy 0524/84547; for abroad 0039/0524/84547
fax: for Italy 0524/84751 - for abroad 0039/0524/84751
All editorial mail must be addressed to the:
Editorial Office of the European Journal of Oncology
Cancer Research Centre,
Castle of Bentivoglio, Via Saliceto 3, 40010 Bentivoglio (Bologna), Italy
telephone: for Italy 051/6640650-6640143; for abroad 0039/051/6640650-6640143
fax: for Italy 051/6640223; for abroad 0039/051/6640223

All administrative mail must be sent to the publisher (Mattioli 1885 - Casa Editrice)
The European Journal of Oncology is sent free of charge to all paid-up members of the SIT.

The journal is a quarterly publication

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Abstract:

Abeloff MD, Gray R, Tarmey DC, *et al*. Randomized comparison of CMFPT versus CMFPT/VATHT and maintenance versus no maintenance tamoxifen in premenopausal, node positive breast cancer. An ECOG study. Proc Am Soc Clin Oncol 1991; 10, 43: abstr 47.

Supplement:

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Mattioli 1885 - Casa Editrice
via Coduro, 1/B - 43036 Fidenza (PR) - Italia
telefono: per l'Italia 0524/84547 - per l'estero 0039/0524/84547
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Supplemento:

Elison LO, Ekberg L. Ifosfamide, doxorubicin, vincristine, and etoposide in small cell lung cancer. Semin Oncol 1995; 22 suppl 2: 15-7.

Editoriale:

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Lettera all'Editore:

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