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Eur. J. Oncol., vol. 12, n. 2, pp. 69-73, 2007

Editorial/Editoriale

Bernardino Ramazzini: thoughts on his Treatise and linking exposure science and environmental/occupational medicine for prevention or intervention within environmental health

Bernardino Ramazzini: riflessioni sul suo Trattato e collegamenti fra ricerca sulle esposizioni e medicina ambientale/professionale per la prevenzione e gli interventi nell'ambito della medicina ambientale

Paul J. Lioy

Department of Environmental and Occupational Medicine, Robert Wood Johnson Medical School-UMDNJ, and Deputy Director of Government Relations and Director of the Exposure Science Division, The Environmental and Occupational Health Sciences Institute (EOHSI), Piscataway, NJ, USA - A Member of the Collegium Ramazzini

Summary

Bernardino Ramazzini was the Father of Occupational Medicine and his work has been widely discussed in the past. The importance of Ramazzini's work was that he identified the causes of disease and, as a second step, reconstructed exposure scenarios and pollutant doses. He was a careful observer of workplace exposure, thereby characterizing a wide range of pollutants and their sources. Furthermore, Ramazzini suggested remedies for occupational diseases and, in particular, stressed the need for their effective prevention. Eur. J. Oncol., 12 (2), 69-73, 2007

Key words: **Bernardino Ramazzini, occupational medicine, prevention**

Riassunto

Bernardino Ramazzini è stato il Padre della Medicina del Lavoro e la sua opera è stata ampiamente discussa in passato. L'importanza delle opere di Ramazzini è stata quella di identificare le cause delle malattie e, come seconda fase, di ricostruire gli scenari espositivi e la dose dei contaminanti. Egli è stato un attento osservatore delle esposizioni nei luoghi di lavoro, caratterizzando in questo modo un ampio spettro di contaminanti e la loro origine. Inoltre, Ramazzini ha suggerito rimedi per la malattie professionali e, in particolare, ha sottolineato la necessità di una efficace prevenzione. Eur. J. Oncol., 12 (2), 69-73, 2007

Parole chiave: **Bernardino Ramazzini, medicina del lavoro, prevenzione**

On 8th March 2007, I was provided an opportunity to deliver an invited talk as part of the Rutgers University *Italian Hours* Series, hosted by the Italian American Commission of New Jersey, and the Rutgers Italian Studies Committee. My topic was Bernardino Ramazzini (fig. 1)[1]. I was asked to talk about an Italian scientist or engineer, to provide a different flavour to the Series which had been primarily dealing with the Arts (including a talk by one of my wife, Jean's favourite chefs, Mario Batali). Because of my interest in Ramazzini's work, and my fond association with the Collegium Ramazzini, I developed the topic.

Some parts were derived from Dr. G. Franco's excellent article on Ramazzini[2] and my colleague, Michael

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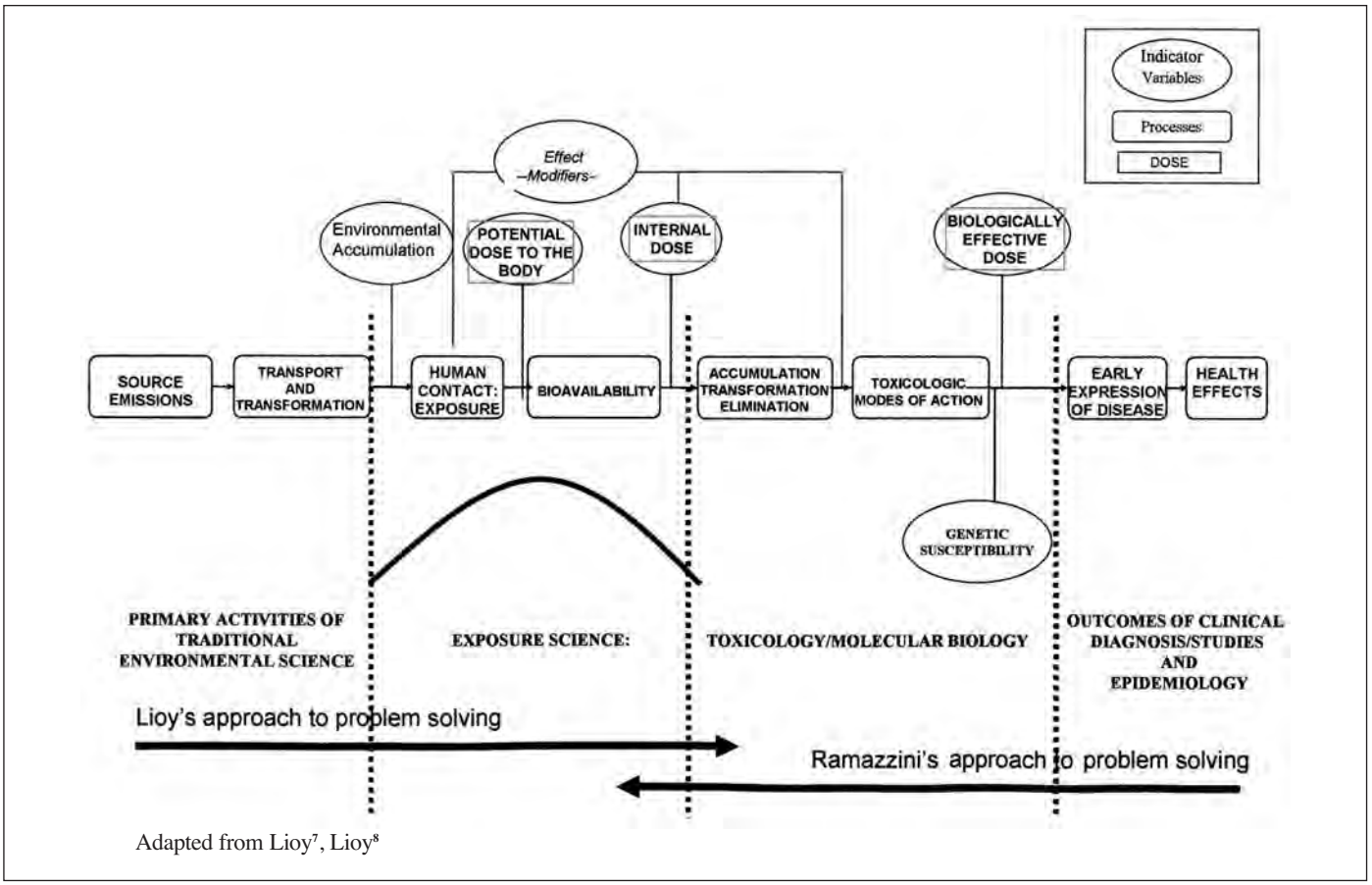
Fig. 1. Engraving by J.G. Sellier Schaffhusianus (Opera omnia, Geneva, Cramer and Perachon, 1716)

Gochfeld's review of occupational medicine[3]. However, most of the discussion was based upon Ramazzini's Treatise *De Morbis Artificum Diatriba* (1713), which was translated into English by Wilmer Cave Wright in 1940[4]. Further, as part of the talk, I reviewed Ramazzini's work from my perspective and experience within the field of exposure science[5, 6], and the more traditional areas of occupational hygiene and air pollution. In the past, some of my review articles on the field have suggested, and rightly so, that exposure science has evolved from those two disciplines, with the science of exposure being defined during the 1980s and early 1990s. However, it has become clear to me that Ramazzini was not only the Father of Occupational Medicine, but he actually understood and described parts of the conceptual framework that are used to define the field of exposure science today.

Since the late 1980s I have been writing and lecturing about exposure science within the context of a source to health effect continuum with the most recent version presented in fig. 2[7, 8]. As a physical scientist this seemed to be a reasonable approach because of the many studies and analyses that attempted to define the intensity and the distribution of a contaminant, and link it to a human exposure and then a fractional dose that can lead to a health effect. During my analysis of the structure of Ramazzini's Treatise, it became clear that he too had conceptualized and implemented the same process continuum, however, *in the reverse order* (fig. 2). He started with disease outcomes within the lung, nervous system, and other organ systems, and went on to reconstruct the dose and exposure. The brilliance of his approach was that Ramazzini did not stop at treatments for the affected individuals, but attempted to find the source of the agent that led to an exposure and eventually the observed health effects, and he suggested remedies! Clearly, Ramazzini had only rudimentary tools available to work with, but the most important were his powers of observation and smell. He conducted detailed interviews of workers, and visited workplaces and community environments, now called "walk through" surveys. Using these tactics he was able to adequately characterize many sources of contaminants that led to disease. Further, these activities were not just limited to the analysis of the diseases of the industrial and commercial trades: Ramazzini also examined the "sedentary" worker and the "learned men", who could be considered the modern day office worker or "home office small business person". In both cases he worried about inactivity, and poor eating and drinking habits as part of the disease cycle. He even considered the conditions observed in workers as "biomarkers", but recognized that the potential for exposure needed to be linked to the presence of the "biomarker" to fully understand the association between exposure and disease caused by occupational toxicants. A lesson that we may need to relearn today.

By starting with the disease end point illustrated in fig. 2, Ramazzini was able to examine the stresses on the individual's life and habits in relation to toxicant exposures, to help him better understand the potential individual or synergistic causes of the disease. In this way the nature of the activities participated in by individuals would not be ignored, such as drinking, etc, or the location of a home near an outdoor source of air pollution, as mentioned by Franco[2]. Today many of the research projects within the field of exposure science detail the activities and behaviours of individuals potentially at risk as a necessary part of reducing uncertainties. Our modern day exposure scientists, however, should never lose the power of "observation" because I think far too many people tend to rely on computers and computer software, i.e. canned activity and human characteristic profiles, to provide them with answers. In reality they help to effectively frame the problems and guide us towards answer. The computer and computational analyses are very valuable tools in science/engineering and the field of exposure science. Their value is in helping interpret information obtained



Adapted from Lioy[7], Lioy[8]

Fig. 2. A continuum from emission of a contaminant to a health effect

through multiple measurements and observation, and/or defining the boundaries, complexity or priorities of the issues through simulations uncertainty analysis. However, these efforts must not replace the observation of individual behaviour or activities, as a core component of the field. In any case, understanding the nature of human contact with toxicants is a critical part of the continuum, and the goals of a scientist, engineer or physician should use all available tools to eliminate or mitigate the source and prevent associated health outcomes.

Before continuing, I must make note of an important fact: Ramazzini went to the next step, suggesting remedies that could intervene or prevent more disease or at least exposures. This concept of prevention is and has been a part of the environmental health sciences, but it has not always been used effectively. We still spend most of our time treating disease and not eliminating the major cause. One growing success story in the US has been the severe restriction placed on exposure to environmental tobacco smoke; however, smoking cigarettes is still a major cause of lung cancer around the world, and its effects on non-smokers continue to be documented.

Ramazzini also appeared to have a good sense of the world around him, for example, one anecdote in the section about diseases of "sulphur-workers". He discussed the use of acid sulphur as a cleaning agent for clothes, and the effects of exposure when in close contact. The translated section reads as follows for the case of "...an unfaithful wife who, when her husband came home, hid her lover under the bed; to cover up her crime she threw a garment that had been cleaned with sulphur, but by this she betrayed herself, for the lover choked by the smell of fresh sulfur and could not help coughing and sneezing violently". Ramazzini could have even been a good forensic detective, but it is clear that he understood that there can be a wide range of sources that must be considered in preventing or reducing the severity of a health outcome. In my view, this point, as well as the many workplace examples, does contain a deeper message, in that he was totally aware of the fact that it is "contact" with the agent emitted by any source that makes the exposure, which is fundamental to the well-known concept of "the dose making the poison". Over the centuries we sort of lost these fundamental linkages. A good example of the disconnect were statements made in the US National Research Council Risk Assessment "Red Book" in 1983[9], suggesting that exposure was the weakest link in the risk assessment process and that it

Fig. 3. Participants at the Italian Hours meeting
From left to right on photograph:
1. Alessandro Vettorio, Professor of Italian, Rutgers University (member of RU Italian Studies)
2. Mary Ann Puccio, Program Advisor to the New Jersey Italian-American Commission
3. Paul J. Lioy, also a Program Advisor to the New Jersey Italian-American Commission
4. Mary Anne Re, Executive Director of the New Jersey Italian-American Commission
Photograph: Courtesy of Mary Anne Re

would be very difficult to complete multi-route exposure assessments. As described in the US National Research Council 1991 "White Book" on exposure assessment[10], and the subsequent fifteen years of research, the Red Book was wrong. However, the resources necessary to achieve the same level of in-depth analyses, such as toxicological studies, still remains illusive. It is a pity that Ramazzini was not translated into English until 1940. His work was completed less than 200 years after Paracelsus' treatise in the 1500s.

As you read Ramazzini, it is clear that not all members of a trade or occupation contracted the typical diseases or close variants. It depended in part upon their job. In fact he cautioned about assuming that merely hearing that a person was a "potter" should not lead to a prescribed set of "… remedies to correct disorders contracted from minerals". This also fits closely with his observation of the various activities and the lifestyle issues participated in by the various trades (60 in all) that Ramazzini studied in his attempt to characterize the link from disease to source! As mentioned above, these are clearly associated with the exposure science concept of contact with the agent or vector of concern during an event that could yield exposures.

I was also struck by Ramazzini's ability to differentiate between the consequences of short-term and long-term exposures. Until 9/11/2001, the successes that had occurred since the 1970s to reduce emissions in the USA had been moving more and more to looking at the consequences of long-term low-dose exposures on diseases, such as cancer, and reducing our attention to acute exposure situations[6]. The disease outcomes experienced by those present or working during the first 24 to 48 hours post-9/11 brought the need to evaluate acute exposure issues back into focus from the stand point of both acute effects and long-term health effects. For example, the work of the National Research Council AEGL committee has improved our understanding of the health effects that could result from community exposures to highly toxic gases[11].

A reader will not learn much new information on occupational disease from the 300+ year-old Treatise, but you will gain some important insights: 1) on the times and the severity of worker and general health problems, and 2) on how to go about examining information that is available

along the source to health effect continuum, – *no matter which end of the continuum you use to start approaching a problem*. Thus, in closing, I would like to recommend, before this august audience (fig. 3), that members of the exposure and generally the environmental health community and their students read the Treatise *De Morbis Artificum Diatriba*.

Acknowledgements

A thank you, to Michael Gochfeld, MD, PhD, Professor of Environmental and Occupational Medicine, Robert Wood Johnson Medical School, UMDNJ and EOHSI, for discussions on this Editorial, and to Giuliano Franco, MD, Chair of Occupational Medicine, School of Medicine, University of Modena, Italy, for the generous use of some of his slides and lithographs which were part of my Lecture – both are members of the Collegium Ramazzini, Carpi, Italy – also to Dr. Clifford Weisel, PhD, Professor of Environmental and Occupational Medicine, Robert Wood Johnson Medical School, UMDNJ and EOHSI, for his insights and comments and finally, to T. Cory Brennan, and Alessandro Vettorio, Rutgers University, for inviting me.

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Eur. J. Oncol., vol. 12, n. 2, pp. 75-80, 2007

Commentary/Commentario

Update on the devil facial tumour in Tasmania

Aggiornamento sul tumore facciale dei diavoli in Tasmania

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Summary

A forum of some eighty research scientists, all with concern about the infectious, malignant disease affecting Tasmanian devils (devil facial tumour = DFT) was held in Hobart in February 2007. Wide agreement was expressed about the extreme impact of the condition, with likelihood of the devil becoming critically endangered or even facing extinction in the wild within 10 to 15 years. The gradual diffusion of DFT into western parts of Tasmania, where it has not yet been recognised to date, now seems inevitable. Definitive transmission experiments that corroborate the allograft cell transfer theory have been attempted but the results have not been published. No early diagnostic test for DFT has yet been developed so that visibly obvious tumours are the sole criterion for recognising the condition which has proved fatal in every known case. Nor, according to Tasmanian Government field biologists, is there any sign of recovery or natural immunity in residual devil communities in which DFT has now been known for over a decade. Approaches to retaining disease-free populations of wild devils are continuing. A field experiment is underway on Forestier Peninsula where all visibly DFT-affected devils are being euthanased with the aim of managing the spread of DFT and maintaining a depopulated 'buffer' between diseased and currently disease-free devil populations on the linked Tasman peninsula. Another approach being explored is the placement of populations of wild

Riassunto

Un convegno con un'ottantina di scienziati ricercatori, preoccupati della malattia infettiva maligna che colpisce i diavoli della Tasmania (devil facial tumour = DFT) è stato tenuto ad Hobart nel febbraio 2007. È stato espresso un ampio accordo sulle gravissime conseguenza della malattia, con una probabile seria minaccia per i diavoli o addirittura rischio di estinzione per gli animali selvatici entro 10-15 anni. La graduale diffusione del DFT nella parte occidentale della Tasmania, dove finora non è stato osservato, sembra ormai inevitabile. Sono stati condotti esperimenti decisivi sulla trasmissione, che confermano la teoria del trasferimento cellulare eterologo, ma i risultati non sono stati pubblicati. Nessun test per la diagnosi precoce del DFT è stato finora sviluppato, per cui i tumori manifesti sono il solo criterio per riconoscere la malattia che si è rivelata fatale in tutti i casi noti. Non ci sono nemmeno, secondo i biologi del Governo della Tasmania, segni di recupero o immunità naturale nelle residue comunità di diavoli in cui il DFT è noto ormai da oltre un decennio. Continuano i tentativi di preservare popolazioni di diavoli selvatici libere da malattia. Un esperimento sul campo è in corso nella penisola di Forestier dove tutti i diavoli visibilmente affetti da DFT vengono sacrificati allo scopo di controllare la diffusione del DFT e mantenere una "zona cuscinetto" spopolata tra le popolazioni di diavoli malate e quelle attualmente libere da malattia sulla collegata penisola della Tasmania. Un altro approccio esplorato è il trasferimento di popolazio-

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disease-free devils on one or more suitably-sized offshore islands. Difficulties with this programme of rendition of disease-free devils to these islands or to other biosecure sites are discussed. The development of a protective vaccine against DFT is being considered to ensure in the long-term the future of the Tasmanian devil. Eur. J. Oncol., 12 (2), 75-80, 2007

Key words: **Tasmanian devil facial tumour (DFT), infectious malignancy, disease control measures**

ni di diavoli selvatici libere da malattia su una o più isole al largo di dimensioni adeguate. Vengono discusse le difficoltà di questo programma di cattura e trasferimento di diavoli liberi da malattia in queste isole o in altre sedi sicure. Viene presa in considerazione la preparazione di un vaccino protettivo contro il DFT per assicurare nel lungo periodo il futuro del diavolo di Tasmania. Eur. J. Oncol., 12 (2), 75-80, 2007

Parole chiave: **tumore facciale del diavolo di Tasmania (DFT), tumore maligno infettivo, misure di controllo della malattia**

Introduction

Current understandings and research directions of the Devil Facial Tumour (DFT) situation in Tasmania were examined at a forum of some eighty scientists held at the University of Tasmania in Hobart from 20 to 22 February 2007[1]. Research findings from ongoing field and laboratory studies were presented for open debate and critical discussion of the many aspects of the ongoing management of the Tasmanian devil in the face of this disease.

The present update supplements that of 2006[2] and highlights the ongoing plight that the largest living marsupial carnivore still faces and the greater urgency for international participation in the research programme.

Although the definition of this unusual cancer is somewhat clarified, there are still critical gaps in the understanding of its biology and, more importantly, an absence of effective means to detect, monitor and manage the disease in free-living devil populations. In particular, the absence as yet of any diagnostic test for DFT at a stage earlier than the development of visible tumours is a major hindrance to many other aspects of research, but was a topic barely touched upon.

Forum participants shared some significant findings about this newly emerging disease entity in Tasmanian devils and the existing constraints to effective management. Several important research objectives have yet to be met. In addition the overall programme still suffers from capacity constraints, both in terms of multidisciplinary scientific collaboration and funding.

At the conclusion of the forum the senior scientist to the Tasmanian DFT programme, Professor Hamish McCallum, assessed the major consensus conclusions and the challenges still faced both by the disease research and the species conservation components of the programme.

Consensus Conclusions of the forum

- DFT is recognised as a new form of a transmissible neoplasm; its closest analogue being the canine transmissible venereal tumour[3].
- The natural mode of transmission is thought to be via direct devil-to-devil facial biting with the deposition of viable neoplastic cells into sub-dermal and subepithelial tissues of the head and oral cavity.
- At present the detection of DFT within devil populations relies on clinical visual assessments backed with histopathology and cytogenetics. No diagnostic test is currently available to assist in the monitoring or management of DFT in wild devils.
- There is a real likelihood of DFT spreading to all devil populations in all parts of the island within 5 or 6 years and a belief amongst Tasmanian Government biologists that the species faces further declines in numbers and the potential of becoming extinct in the wild within a period of perhaps 10-15 years[4].
- Based on data sets from several long-term monitoring sites where the disease has been endemic for 7 to 10 years, bio-mathematical modellers and forecasters[5-7] have recognised no signs of a recovery in devil populations. This has been corroborated in part by the number and diversity of close intra-species interactions which individual devils have in their normal life[8]. This may explain the low levels of DFT in wild populations, where very few animals have mature facial tumours, in that transmission may be influenced by the number of adversarial conflicts and direct facial biting contacts between DFT individuals and unaffected cohorts[8].
- Attempts to control the spread by active culling of affected animals[9] and field surveillance of the disease in wild devil populations, currently tracking a spread to the south, west and north-west[10,11], now imply that

the opportunity to collect wild disease-free devils for species survival purposes may now be closing.

Laboratory research findings

Direct horizontal transmission of viable DFT cells has been experimentally attempted, but the results are preliminary and not yet published[12]. Such a study would test the proposed allograft rogue cell spread hypothesis[13,14] between animals by biting causing deep wounds in the facial area. Such direct intra-species biting interactions occur particularly during feeding and mating[8].

Unlike the canine transmissible venereal tumour, DFT does not appear to undergo spontaneous regression and, by all field observations, is invariably fatal with no reports of devils recovering after developing DFT. The possibility of some level of host resistance to DFT should not however be overlooked. Whilst infectious diseases are unlikely to be agents of extinction due to the density-dependent behaviour of transmission[15,16], this depends on the presence or absence of possible reservoir hosts that could maintain the pathogen even at low devil densities[17,18]. Large reduction in host density may also lead to greater probability of reproductive failure, due to lack of genetic diversity[10, 17, 18].

The rôle of the genes controlling histocompatibility in the devil as a species is now under investigation[19]. If experimental studies can definitively demonstrate that allografted DFT-cells are the means of natural transmission of this disease[20], then the rôle of Major Histocompatibility Complex (MHC) genes, including their regulation and the functionality of the devil's immunobiology, become relevant to an understanding of the pathology and will be necessary prerequisites for any planning to counter the infection.

All DFT tumour cells so far evaluated have been found satisfactorily to express all classes of MHC molecules[21] and, as a species, the devil is known to lack genetic diversity (Belov K., personal communication). Genetic and immunological research has begun to explore the functionality of histocompatibility and immune competency in devils[22-24]. Understanding of the biology of DFT as a cancer remains a puzzle and its solving could contribute to the wider understanding generally of neoplastic processes (Harris J., personal communication).

Exposure of devil populations to man-made chemicals used in agricultural and forestry land-management was identified as a priority research activity in 2004[2]. Toxicology studies to assess residues in liver and fatty tissues have effectively been shelved on the grounds that such research is deemed irrelevant to saving the devil today[25]. DFT triggers, such as exposure to environmental chemicals and the presence of activated oncogenes or endogenous viral genes, have not yet been determined and no data were presented at the forum on the background levels of a range of potentially genotoxic or carcinogenic chemicals found in Tasmanian devil tissues[25].

Regarding the likely genesis of DFT as a new disease of devils, it is assumed that the disease emerged spontaneously in high-density populations in north-east Tasmania in the mid-1990s. Interestingly one disease modeller raised the possibility that, based on the field data available, DFT could have been independently initiated at three separate locations: one in the far north-east of Tasmania; one north of the Freycinet peninsula on the central east coast (fig. 1) and the last at a location in the central south of the island[6]. This conclusion raises the potential for future spontaneous eruptions of DFT in wild devil populations, and genomic search for the presence of endogenous retroviruses in the Tasmanian devil genome is continuing.

After protracted delays, linked to concerns about intellectual property rights accruing to research results, the Tasmanian government finally agreed in January 2007 to allow Tasmanian devil material to be sent overseas for genetic and epigenetic research. For instance, devil genetic materials have now been supplied, in March

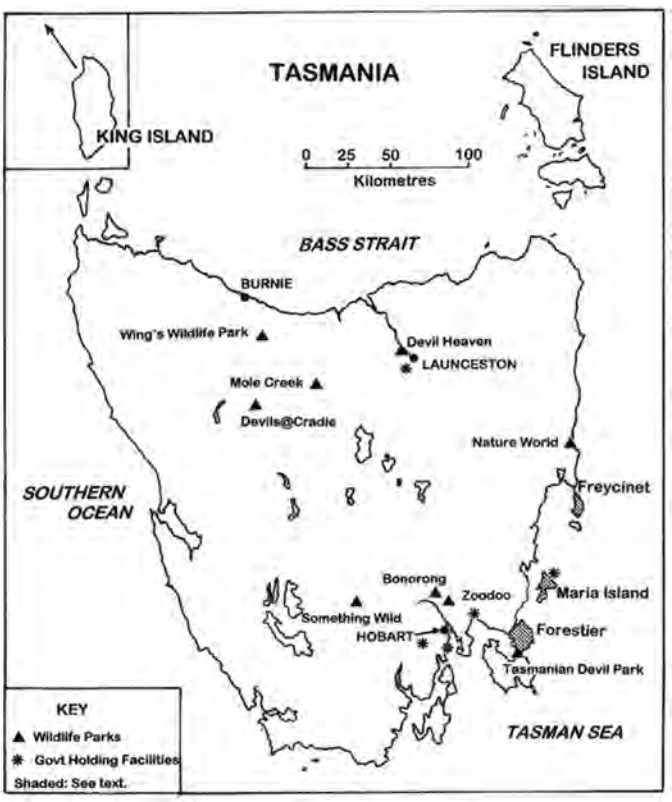

Fig. 1. Captive Tasmanian devil facilities

2007, to scientists at Cold Spring Harbor Laboratories in New York, and the CSIRO (Commonwealth Scientific and Industrial Research Organisation) Australian Animal Health Laboratory in Geelong, Victoria, is also assisting with virology and genetic studies. A protocol to allow for biological material transfers has been prepared so as to improve the accessibility of devil material to other institutions, both within Australia and overseas.

The chromosomal rearrangement of DFT cells within a tumour mass and between affected devils was a stable and recognisable feature of these tumorous cells when DFT was first reported[20]. Up to four clonal karyotypes of DFT have now been identified[13]. However, the aneuploidy of DFT cells with the presence of distinct marker chromosomes may be the basis for future important discoveries.

Managing the Tasmanian devil in the wild

Within Tasmania two approaches to retaining self-sustaining populations of devils in the wild are being actively considered. An existing programme in the Forestier peninsula (fig. 1) has trapped and culled (by euthanasia) a large number of diseased devils (over 115 animals by February 2007) with the intention of leaving only healthy animals[9]. Full demographic results of this trial, which commenced in January 2006, are not yet clear, but there is some indication that the remaining population still retains representatives of all age classes. This strategy may be slowing the spread of disease within the population, but there is evidence that animals are breeding at a younger age (2 years old)[9]. Several disease control professionals attending the forum commented that the Forestier study highlighted the value of taking interventionist action against the disease and monitoring the outcome epizoologically. Critical and available data about the progression of the disease to assist in predictive modelling is still far too limited; also data on the survival period of DFT-affected devils is lacking and more precise data on the interval during which DFT-carriers are potentially 'infectious' is needed in order to calculate a hypothetical transmission rate (R_o) assuming "the bite transfer" of the neoplastic cells.

The forum reached general agreement that currently there is no effective management tool or strategy to mitigate the impact of DFT on wild devils.

A second means for maintaining wild, disease-free devils has been proposed; this involves the release of disease-free devils to one or more of Tasmania's off-shore islands[26]. This strategy is parallel to the transfer of devils to four Australian parks[a] and the ongoing rôle of Tasmanian wildlife parks in breeding devils in captivity (fig. 1). There are already difficulties in these parks becoming overstocked with assumedly disease-free stock. To retain these animals in captivity will necessitate a major and financially assisted increase in the building of holding spaces within existing Tasmanian wildlife parks. A corollary too is a decision needed about what to do with captive animals past breeding age and continuing to take up caring time, space and food.

There is a recognised sense of urgency in obtaining wild, disease-free devils before the disease spreads into the remote west coast areas of Tasmania. Some thirty young devils from the western population were trapped from several sites and transferred into the biosecure holding facilities operated by the government near Hobart. It is envisaged that these devils will be maintained as an insurance stop-gap until the offshore island release option has been properly assessed[26]. This strategy is complicated however by the serious impact which introduced devils are expected to have on the existing island ecosystems[27]. Environmental risk assessments of several potential islands have been prepared; these include the effects of devils on threatened fauna, particularly ground-nesting birds[b] and on overall island biodiversity. Maria Island National Park off Tasmania's east coast (fig. 1) is still mooted as the most likely island of devil rendition[2] and, with the Tasmanian devil now listed as a threatened species under national legislation, such releases are subject to Federal Government approval processes. One aspect to assess will be the sustainability of island ecologies faced with the artificial introduction of a terrestrial, carnivorous predator. Despite recognised difficulties the policy of translocation was considered at the forum to be currently the best strategy for devil survival in the wild and is now adopted as a key policy[28].

The urgency of planning to transfer the once populous Tasmanian devil to off-shore islands for its continued survival merely highlights the very serious plight the species currently faces in Tasmania. It is unclear whether the establishment of free-ranging devil populations on one or more off-shore islands is a short or a long-term species survival strategy[28, 29]. If the island rendition strategy is adopted it may offer a safekeeping option

[a] Australian wildlife parks with captive Tasmanian devils are situated at Currumbin, in south east Queensland; Healesville Sanctuary, near Melbourne, Victoria; Monarto, east of Adelaide, South Australia and at Gosford Park, north of Sydney, New South Wales. The park at Currumbin has recently reported successful breeding in captivity.

[b] Amongst other avian species these would include the hooded plover, masked lapwing and fairy penguin at risk of depredation by devils

whilst the impact of the disease on devil populations in the endemic areas is monitored and whilst the true aetiology and pathogenesis of this new disease are studied in more detail.

A further and different approach (but one not yet employed) would be to explore some identifying feature in the residual devil population of areas, such as north-east Tasmania, to establish in what manner they differ from those which have already died. These survivors would then be devils frequently exposed to DFT which have apparently remained resistant or immune to the disease.

In late 2006, three captive devils at a Tasmanian wildlife park developed DFT lesions, the first reported instance of DFT in devils held in captivity. The most probable explanation for this outbreak was the entry of a wild diseased devil into a captive devil enclosure during the breeding season[30]. This incident emphasises the importance of physical barrier separation in preventing DFT spread by devil-to-devil contact and the practical difficulties in maintaining DFT-free populations. The likely incursion of a DFT-affected devil into an ostensibly biosecure captive facility also demonstrated the high risks of attempting to maintain free-ranging devils within large surface area enclosures in close proximity to diseased devils. It is important to record that this same wildlife park provided four devils to the Copenhagen Zoo in April 2006 before a DFT risk categorisation was developed.

The feasibility of maintaining disease-free wild devils on the Tasmanian mainland was not discussed. This may in part be due to the cost of constructing and operating biosecure sanctuaries of sufficient size for the long-term maintenance of wild devils.

One beneficial outcome from the incident was the drafting of a DFT risk categorisation document. It describes the DFT-biosecurity and quarantine compliance under which all Tasmanian wildlife parks that maintain devils must assess their animals[31]. This will provide a useful guide to permitted movements of devils for intrastate, interstate or international transfer and as a basis for controlled breeding.

To anticipate the effectiveness of any overall strategy for the long-term survival of the devil in the natural environment is at present premature. As with other species' survival programmes requiring captive maintenance and breeding or geographic translocations to new environments, there is a risk that the species may either acquire or inadvertently transfer other pathogens and parasites to their new surroundings (Harris J., personal communication).

In contrast and as a long-term opportunity to intervene in the occurrence of DFT in Tasmania, the development of a vaccine against the tumour is also being actively pursued[32] although the limited genetic diversity of devils and the lack of immune response to the tumour militate against vaccination. Anti-cancer vaccine for humans has been developed in only two cases, both virus-related. The most successful is that to prevent hepatocellular cancer which results from chronic infection with hepatitis-B virus (HBV); the second being that which protects women against cervical cancer by directly targeting the papilloma virus infection responsible for the disease[33].

Conclusions

The forum underscored the sombre fact that Devil Facial Tumour remains a significant scientific and policy challenge for Australia's smallest state. The ultimate goal of the State Government Programme is to ensure that healthy and self-sustaining Tasmanian devil populations survive in the wild.

There is an increasing sense of urgency to take meaningful action and to make bold priority investment decisions. Ensuring the devil's survival in the face of uncertainty about this new disease continues to be Tasmania's ongoing dilemma.

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Frequenza di tumori ed esposizione ad amianto nell'industria del cemento-amianto

Cancer frequency and asbestos exposure in the asbestos-cement industry

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Riassunto

L'industria del cemento-amianto è stata la maggior utilizzatrice di fibre in amianto, in Italia come nel resto dei paesi del mondo. In questa rassegna presentiamo una sintesi degli studi sul rischio di neoplasia maligna tra i lavoratori del cemento-amianto e sul rischio associato all'esposizione ambientale o domestica ad amianto conseguente a tale prodotto. La maggiore azienda produttrice di cemento-amianto in Italia si trovava a Casale Monferrato, dove è stata attiva dal 1907 al 1986. Lo studio di coorte dei dipendenti ha incluso 2.657 uomini e 777 donne. Il follow-up è stato esteso al 2003. La mortalità è stata superiore all'atteso in entrambi i sessi per tutte le cause di morte (complessivamente 1809 osservati *vs* 1330,4 attesi; p<0,01), per le neoplasie della pleura (105 *vs* 3,6; p<0,01), del peritoneo (52 *vs* 2,6; p<0,01) e del polmone (249 *vs* 103,6; p<0,01) e per l'asbestosi (186 *vs* 0,4; p<0,01). Risultati simili sono stati osservati in numerosi altri studi sui lavoratori nella industria del cemento-amianto, sia in Italia che in altri paesi. A Casale Monferrato abbiamo anche indagato il rischio di mesotelioma maligno della pleura dopo esposizione domestica o ambientale. In uno studio di coorte si sono osservati 6 decessi per mesotelioma maligno tra le mogli dei lavoratori del cemento-amianto nel 1950-1993, con un RSM pari a 1200. Uno studio caso-controllo sul mesotelioma maligno della pleura ha evidenziato una riduzione del rischio con l'aumento della distanza dell'abitazione dallo stabilimento Eternit. L'evidenza scientifica è basata

Summary

The asbestos-cement industry has been the largest user of asbestos fibres in Italy, as elsewhere in the world. In this review we present a summary of studies on cancer occurrence in asbestos-cement workers and on cancer risk associated with domestic and environmental exposure. The largest Italian asbestos-cement factory was located in Casale Monferrato and was in operation between 1907 and 1986. The cohort study included 2,657 men and 777 women. Follow-up was extended until 2003. Mortality was increased in both sexes for all causes (overall 1809 observed *vs* 1330.4 expected; p<0.01), pleural (105 *vs* 3.6; p<0.01) and peritoneal (52 *vs* 2.6; p<0.01) malignancies, lung cancer (249 *vs* 103.6; p<0.01) and asbestosis (186 *vs* 0.4; p<0.01). Similar results on workers in the asbestos-cement industry have been observed in several studies in Italy, as all over the world. In Casale Monferrato we also investigated the risk of pleural mesothelioma after domestic and environmental exposure. The SMR for pleural malignancy in wives of asbestos-cement workers was 1200, based on 6 cases occurring in 1950-1993. A case-control study showed strong inverse association between distance of dwelling from the factory and risk of mesothelioma. Evidence from the literature is limited to a few studies; there is however a general consensus on the increased risk of mesothelioma for subjects with domestic and environmental exposure. We wish to emphasize that Casale Monferrato is not a unique

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su un numero ancora limitato di studi, comunque esiste consenso sull'aumento del rischio di mesotelioma per le persone che hanno subito esposizione domestica od ambientale a fibre di amianto. Sottolineiamo che quella di Casale Monferrato non è una situazione unica, ma che in Italia esistono altre aree dove si osserva un aumento della frequenza di mesotelioma a seguito di esposizione sia occupazionale, sia domestica o ambientale. Tali situazioni dovrebbero essere studiate con lo stesso approfondimento messo in atto a Casale Monferrato. Eur. J. Oncol., 12 (2), 81-88, 2007

Parole chiave: **amianto, cancro, mesotelioma, pleura, peritoneo, polmone**

situation, and that other areas in Italy show increased occurrence of mesothelioma following occupational, domestic and environmental exposure. These areas ought to be studied as thoroughly as Casale Monferrato. Eur. J. Oncol., 12 (2), 81-88, 2007

Key words: **asbestos, cancer, mesothelioma, pleura, peritoneum, lung**

Introduzione

Le aziende produttrici di cemento amianto sono state le maggiori utilizzatrici di amianto, in Italia come nella gran parte dei paesi del mondo[1,2].

Una delle maggiori aziende produttrici di materiali in cemento-amianto in Italia, di proprietà del gruppo Eternit, è stata attiva a Casale Monferrato dal 1907 al 1986. Casale Monferrato è una città di circa 40.000 abitanti, lontana dalle altre aziende o settori produttivi che tradizionalmente hanno fatto largo uso di amianto, ma è uno dei maggiori centri di produzione di cemento nel Nord Italia. Proprio per questa ragione la città venne scelta nel 1907 dalla Eternit, che aveva da poco brevettato il prodotto, per avviare il primo stabilimento italiano. Tale stabilimento è rimasto il maggiore in Italia, basti pensare che nel 1980 circa il 10% dell'amianto utilizzato in Italia è stato utilizzato a Casale.

Studio sui lavoratori di Casale Monferrato

I problemi sanitari legati all'esposizione ad amianto dei lavoratori dello stabilimento Eternit di Casale Monferrato sono stati portati all'attenzione dei ricercatori e della comunità in generale dal Patronato Sindacale e da alcuni medici del locale ospedale a partire dall'inizio degli anni '80[3]. Vorremmo quindi ringraziarli pubblicamente per il loro ruolo. Il nostro ruolo come ricercatori è iniziato poco dopo, nel 1983-85, con l'avvio dello studio di coorte sulla mortalità tra i dipendenti dello stabilimento[4-6]. La coorte dei dipendenti Eternit attivi al 1/1/1950, o assunti successivamente, include 2.657 uomini e 777 donne.

La Tabella 1 presenta i risultati principali dell'aggiornamento dello studio al 2003. Tra gli uomini si è osservato un aumento statisticamente significativo della mortalità per tutte le cause, per tutti i tumori, per i tumori dell'apparato respiratorio (polmone e pleura) e del peritoneo oltre, ovviamente, ad un incremento della mortalità per asbestosi. Occorre ricordare che i lavoratori di questo stabilimento sono una popolazione particolarmente selezionata per le buone condizioni fisiche di cui godevano al momento dell'assunzione in azienda[4-6]. A Casale non abbiamo osservato aumenti della mortalità né per i tumori della laringe, né per quelli dell'apparato digerente. Si osservano sostanzialmente gli stessi aumenti di rischio sia per gli uomini che per le donne (Tabella 1). Tra queste ultime è evidente anche un aumento dei tumori dell'ovaio e un aumento, non spiegabile dal punto di vista eziologico per quanto conosciamo ma che è importante segnalare, dei tumori dell'utero[6].

Tabella 1 - Coorte dei lavoratori dello stabilimento Eternit di Casale Monferrato. Mortalità nel periodo 1965-2003

Uomini	OSS	RSM	IC 95%
Tutte le cause	1438	1,34	1,27-1,41
Tumori maligni	628	1,87	1,73-2,03
peritoneo	36	19,45	13,62-26,93
polmone	237	2,41	2,12-2,74
pleura	96	32,39	26,23-39,55
Asbestosi	162	0,3 attesi	
Donne	**OSS**	**RSM**	**IC 95%**
Tutte le cause	371	1,46	1,32-1,62
Tumori maligni	169	2,27	1,95-2,65
peritoneo	16	20,88	11,94-33,91
polmone	12	2,23	1,15-3,90
pleura	39	64,14	45,61-87,68
Asbestosi	24	0,1 attesi	

Da Magnani *et al*[6]

Indagini epidemiologiche in Italia e all'estero

La relazione tra esposizione ad amianto nel settore industriale del cemento-amianto ed insorgenza del mesotelioma maligno e del tumore polmonare è ampiamente documentata da numerose altre indagini epidemiologiche, condotte sia in Italia sia in ambito internazionale (Tabella 2). Il riscontro di una mortalità elevata per tumori del-

Tabella 2 - Sintesi degli studi epidemiologici di coorte sui lavoratori esposti a cemento amianto. I lavori sono ordinati per anno di pubblicazione

Autori	Nazione in cui lo studio è stato effettuato	Numero dei lavoratori della coorte	MM (numero di casi di mesotelioma maligno pleurico o peritoneale e relativi tassi)	Numero di tumori polmonari (TP) e relativi tassi (SMR)	Periodo di *follow-up*	Esposizione a fibre: tipo e concentrazioni	Annotazioni
Lacquet LM *et al*, 1980[7]	Belgio	N=1963	No	No	1963-1977	-	Solo eccesso di tumori gastrointestinali
Sarto F *et al*, 1982[8]	Italia (Padova)	N = 176 (M =132 F = 44)	No	TP = 7 (M) SMR 5,38 (IC 95%: 2,16-11,10)	1961-1980	Crisotilo, crocidolite 1-8 f/cc (1977)	-
Thomas HF, *et al*, 1982[9]	Gran Bretagna (Galles)	N = 1592 (M) + 378 (F)	MM = 2 (SMR=93)	TP = 27+1 (SMR = 0,93)	1936-1977	Solo crisotilo 0.1-20 f/ml ('60) <2 f/ml ('70-'80)	-
Finkelstein MM, 1983[10]	Canada (Ontario)	N = 339 (186 operai ad esp. elevata; 58 manutentori; 87 operai a esposizione bassa)	MM = 11 (5 pleurici, 5 peritoneali, 1 non specificato)	TP = 20	1948-1980	Crisotilo e crocidolite in percentuale indeterminata 3 gruppi di esp. cumulativa media (almeno 12 mesi): A: 44 f-y/ml B: 92 f-y/ml C: 180 f-y/ml (Concentrazioni tra 40 e 0.3 f/ml)	Assunti da almeno 9 anni e prima del 1960
Alies Patin AM *et al*, 1985[11]	Francia	N = 1506	MM = 3 (>20 aa da prima esp.) e 1 peritoneale (<20 aa da prima esp.)	TP = 9 (>20 aa da prima esp.) + 3 (<20 aa da prima esp.)	1940-1982	-	Tempo dalla prima esposizione almeno di 20 anni (N = 941) di cui impiegati per 5-20 anni N = 357 e più di 20 anni N = 584
Ohlson CG *et al*, 1985[12]	Svezia	N = 1176	0	TP = 11 (SMR = 1,28)	-	1970: 10 mg/m^3 1976: 5 mg/m^3 (1 ff/ml) Anfiboli 1% dell'asbesto totale utilizzato	-
Gardner MJ *et al*, 1986[13]	Gran Bretagna (Inghilterra)	N = 2167	MM = 1	TP = 41 (SMR = 0,92; IC 95%: 0,64-1,27)	1941-1984	Solo crisotilo, no crocidolite	No relazione dei TP e degli MM con l'esposizione
Hughes JM *et al*, 1987[14]	USA	N = 5492	MM = 10	TP = 155 SMR 2,3-2,9 (x100)	1940-1969	70-100 ff/anno (1-2 f/ml) – 3% crocidolite, 1% amosite	Confronto tra due stabilimenti

(continua)

Tabella 2 - *(segue)*

Autori	Nazione in cui lo studio è stato effettuato	Numero dei lavoratori della coorte	MM (numero di casi di mesotelioma maligno pleurico o peritoneale e relativi tassi)	Numero di tumori polmonari (TP) e relativi tassi (SMR)	Periodo di *follow-up*	Esposizione a fibre: tipo e concentrazioni	Annotazioni
Raffn E *et al*, 1989[15]	Danimarca	N = 7996 (M) + 584 (F)	MM = 10 (O/E = 5.46 IC 95%: 2.62-10.05)	TP = 162 (SMR = 1,80 IC 95%: 1,54-2,10)	1928-1984	Crisotilo 89% Amosite 10% ('50-'80) Crocidolite 1% ('50-'60) 50-800 f/ml ('48) 10-100 f/ml ('57) >2 f/ml (1973)	-
Albin M *et al*, 1990[16]	Svezia	N = 2898	MM = 13	TP = 27	1907-1986	Crisotilo > 95% Crocidolite <3-4% (1966) Amosite <18% ('60) 1.5-6.3 f/ml ('50) 0.3-5 f/ml ('60) 0.9-1.7 f/ml('70) esp. mediana: 1,2 f/ml 2,3 f-y/ml	Valutata relazione dose-risposta con esposizione cumulativa e periodo intercorso dalla prima esposizione
Neuberger M, *et al,* 1990[17]	Austria	N = 2816	MM = 4	TP = 52 SMR = 1,72, che diventa 1,04 non significativo dopo aggiustamento per consumo di sigarette, anche per esp. cum. >25 ff/ml/anno	1950-1981	Crisotilo prevalentemente; crocidolite dal 1920 al 1977 (produzione di tubi), amosite dal 1970 al 1986	Mesoteliomi con alta esposizione a crocidolite
Pettinari A *et al*, 1994[18]	Italia (Senigallia)	N = 561	No	23 (IC 95%: 1,75-4,15)	1948-1984	Crisotilo, amosite, crocidolite fino al 50%; 1979-80: <2 ff/cc	Incremento della mortalità per tumori vescicali. Relazione dose-risposta per tumore polmonare
Magnani C *et al*, 1996[5]	Italia (Casale M.)	N = 3367 M = 2605 F = 762	MM (M) = 53 obs *vs* 1.7 exp MM (F) = 21 obs *vs* 0.4 exp	TP = 162(M) (SMR = 2,48; IC 95%: 2,11-2,89) + 9 (F) (SMR = 2,82; IC 95%: 1,29-5,36)	1950-1980	Crisotilo Crocidolite 20-200 f/cc (1971) 0.15-2.09 f/cc (1978-79)	-
Tulchinsky T *et al*, 1999[19]	Israele	N = 3057	MM = 26; SMR >5,676 (3,242-8,088)	TP = 28; SMR = 1,35 (0,85-1,85)	1953-1992	Crisotilo 90% Crocidolite 10% 1.5-14 f/cc ('60-'70) 0.3-40 f/cc; <0.4 f/cc ('80)	-
Szeszenia-Dabrowska N *et al*, 2000[20]	Polonia	N = 3220 - 2616 (M)	SMR (M) = 2,846; MM = 5 SMR (F) = 11,275; MM = 2	No	1960-1980	Crisotilo e crocidolite (dal 1985)	-

(continua)

Tabella 2 - *(segue)*

Autori	Nazione in cui lo studio è stato effettuato	Numero dei lavoratori della coorte	MM (numero di casi di mesotelioma maligno pleurico o peritoneale e relativi tassi)	Numero di tumori polmonari (TP) e relativi tassi (SMR)	Periodo di *follow-up*	Esposizione a fibre: tipo e concentrazioni	Annotazioni
Ulvestad B *et al,* 2002[21]	Norvegia	N = 545	MM = 18 (SMR: 52.5; IC 95%: 31.1-83.0)	TP=33 (SMR: 3,1; IC 95%: 2,1-4,3)	1953-1999	Crisotilo, anfiboli in piccolissima percentuale; 1964: 100-1900 ff/ml 1973: < 5 ff/ml	Impiegati per almeno un anno dal 1941 al 1976
Coviello V *et al*, 2002[22]	Italia (Bari)	N = 417	MM = 3pl-SMR = 1,560 con IC 95%: 431-4,081 + 2 per-SMR = 1,705 (con IC 95% = 303-5,367)	TP = 20 (SMR = 1,91 IC 95%: 1,26-2,77)	1972-1995	Crisotilo 70-80%, crocidolite15-20% e amosite; Esp.: 4-19 f/cc	-
Luberto F *et al*, 2004[23]	Italia (Reggio Emilia)	N = 3358 (M = 2712 F = 646)	MM = 18	TP = 90 SMR = 1,57	1952/73-1987	-	Relazione dose-risposta positiva per tempo di induzione, latenza e durata. Confronto tra diversi stabilimenti nella provincia di Reggio Emilia
Smailyte G *et al*, 2004[24]	Lituania	N = 1887 – 1282 (M)	MM = 1 (Att. 0.3)	TP = 30 (M = SMR: 0,9; IC 95%: 0,7-1,3)	1978-2000	Crisotilo	Aziende in funzione dal 1956

esp. = esposizione

la pleura e del polmone è comune a quasi tutti gli studi. Hanno evidenziato un eccesso di mesoteliomi e di tumori polmonari in particolare le indagini condotte in Canada[10], Stati Uniti[14], Svezia[16], Norvegia[21], Danimarca[15, 25], Francia[11], Polonia[20], Israele[19] e presso i maggiori poli industriali italiani, oltre a Casale Monferrato, a Reggio Emilia[23] e Bari[22].

Rischi inferiori sono emersi dalle indagini su esposti al solo crisotilo: l'indagine eseguita da Thomas *et al*[9] in Galles ha potuto riscontrare solo due casi di mesotelioma (uno deceduto nel 1962 e l'altro nel 1974) e nessun eccesso di mortalità per tumore pleurico o polmonare. Anche dall'indagine condotta da Gardner *et al*[13] in due stabilimenti in Gran Bretagna non emerge alcun aumento della frequenza di neoplasie dell'apparato respiratorio.

Diversa è la situazione di alcuni studi condotti in Italia che non rilevano alcun eccesso di queste neoplasie. Nel caso dello studio condotto da Sarto *et al*[8] in provincia di Padova il tempo di osservazione delle coorte era troppo breve. In altri casi, come nello studio di Pettinari *et al*[18] tra i lavoratori dello stabilimento di Senigallia, emerge una mortalità aumentata solo per tumore polmonare (SMR: 2,76; 23 casi osservati) con una relazione dose-risposta positiva con il tempo di induzione, latenza e con la durata dell'esposizione.

Effetti dell'esposizione non professionale

Oltre che per gli operai sono stati documentati nella letteratura scientifica anche gli effetti dell'esposizione non occupazionale: domestica (o paraoccupazionale) e ambientale (o residenziale). È stata cioè segnalata una relazione tra insorgenza di neoplasie pleuriche e amianto anche nei familiari dei dipendenti di stabilimenti per la produzione del cemento-amianto e per i residenti nelle vicinanze degli impianti. L'esposizione domestica o paraoccupazionale si verifica quando per qualche motivo le fibre si liberano in ambiente domestico, generalmente dagli indumenti di persone esposte a livello lavorativo, oppure dall'uso di materiali contenenti amianto nelle abitazioni.

L'esposizione para-occupazionale è stata documentata in Italia, da Magnani *et al*[26], in una indagine svolta sulla mortalità per tumore maligno della pleura in una coorte di 1964 mogli di operai impiegati in uno stabilimento per la produzione di cemento-amianto (Tabella 3). L'eccesso di mortalità per tumori pleurici è stato attribuito alle fibre che si liberavano dagli indumenti che gli uomini riportavano a casa per il lavaggio, poichè nel luogo di lavoro non era presente una lavanderia.

L'inquinamento ambientale da amianto si poteva verificare, sia a causa della dispersione diretta delle fibre nell'ambiente, sia attraverso l'uso improprio dei materiali di risulta delle lavorazioni, in forma umida o secca, utilizzati per la pavimentazione di strade, sentieri, cortili, campi sportivi e come materiali da costruzione in genere da parte delle popolazioni che risiedevano nelle zone limitrofe degli stabilimenti per la produzione di manufatti in cemento-amianto.

Non si conoscono dalla letteratura scientifica internazionale molte stime dei livelli ambientali di fibre di amianto disperse. Marconi *et al*[27] hanno riportato valori di concentrazione di fibre (con lunghezza superiore a 5 μm) di 11 f/l nelle vicinanze di uno stabilimento per la produzione di manufatti in cemento-amianto e 1 f/l nelle aree più lontane della città dove quest'ultimo era collocato (il 15-30% erano fibre non di serpentino). Altre indagini sono state condotte successivamente ed hanno sempre documentato nell'aria di Casale un livello di fibre non particolarmente elevato, ma con una proporzione elevata di anfiboli e di fibre lunghe (Tabella 4).

Un'indagine effettuata tra i residenti di una cittadina del New Jersey, sede di un grande impianto industriale del Nord America, ha documentato il carico di mortalità per mesoteliomi derivante dall'esposizione non occupazionale ad amianto[31]. Una volta rilevati tutti i casi occorsi tra il 1979 e il 1990, attraverso il registro tumori operante nell'area, sono stati rintracciati ed esclusi i casi di origine professionale. Il numero di casi osservati non professionalmente esposti eccedeva in maniera significativa il numero di attesi calcolati in base ai tassi di incidenza per la contea per entrambi i sessi (rispettivamente SIR = 10,1; IC 95% = 5,8-16,4 e SIR = 22,4; IC 95% = 9,7-44,2).

In Italia, con un approccio metodologico simile, è stato riscontrato un elevato numero di casi non ascrivibili ad esposizione occupazionale o para-occupazionale a Casale Monferrato. L'incidenza dei mesoteliomi maligni con diagnosi confermata istologicamente tra i residenti era di 4,2 x 100.000 p-a (26 casi) negli uomini e 2,6 x 100.000 p-a (18 casi) nelle donne per il periodo 1980-1991 e i tassi del periodo 1985-1989 apparivano aumentati rispetto al quinquennio precedente[32]. In uno studio successivo sempre nella stessa zona è stato dimostrato un rischio elevato tra i soggetti residenti nell'area compresa entro i 1000 m di distanza dalla fabbrica non esposti a livello professionale[28] (Tabella 5). Lo stesso studio ha anche confermato il ruolo dell'esposizione domestica (Tabella 6).Verosimilmente il ruolo dell'esposizione per via aerea e quello derivato dall'uso del materiale in amianto e degli scarti di lavorazione si mescolano. In una coorte di lavoratori polacchi dove 4 casi di mesotelioma osservati tra il 1987 e

Tabella 3 - Mortalità tra le mogli dei lavoratori del cemento-amianto

	Oss	SMR	IC 95%
Tutte le cause	210	0,92	0,80-1,05
Tumori maligni pleura	6	12,00	4,04-26,16
Tumori maligni polmone	6	1,50	0,55-3,26

Da Magnani *et al*[26]

Tabella 4 - Misurazioni della concentrazione ambientale di fibre di amianto a Casale Monferrato

- Marconi *et al* (1989)[29]: 1-11 ff/l
- ASL (1990-91): media annuale 1 ff/l (valore superato nel 12% dei campioni)
- Chiappino *et al* (1991)[30]: 2,2-7,4 ff/l
- % anfiboli nei 3 sets: 15-50% sul totale

Da Magnani *et al*[28]

Tabella 5 - Studio caso controllo sul mesotelioma pleurico a Casale Monferrato, 1987-93. Rischio di mesotelioma pleurico in relazione all'esposizione lavorativa ed ambientale ad amianto a Casale Monferrato

		ORc	IC 95%
Occupazione nel cemento-amianto		52,5	12,5-220,0
Casale	<500	27,7	3,1-247,7
	500-1499	22,0	6,3-76,5
	1500-2499	21,0	4,9-91,8
	> 2500	11,1	1,8-67,2
Comuni limitrofi		8,3	2,1-32,6
Nessuna delle precedenti		1	

Da Magnani *et al*[28]

Tabella 6 - Studio caso controllo sul mesotelioma pleurico a Casale Monferrato, 1987-93. Rischio di mesotelioma pleurico in relazione all'esposizione occupazionale dei parenti

Soggetti senza esposizione occupazionale	ORc	IC 95%
Padre e/o madre	7,4	1,9-28,1
Coniuge	3,1	0,6-17,7
Altri parenti	3,4	1,0-11,8
Almeno 1 congiunto	4,5	1,8-11,1

Da Magnani *et al*[28]

il 1998 si sono verificati con tempo di latenza molto breve, inferiore a 15 anni, il sospetto di fonti di esposizione misconosciute ha indotto i ricercatori ad una analisi più approfondita di questi casi, permettendo di documentare l'associazione della patologia con l'esposizione a fibre derivante anche dall'utilizzo massivo che era stato fatto del materiale di scarto della fabbrica in attività improprie, come la pavimentazione stradale[33].

L'epidemiologia degli effetti sanitari conseguenti all'esposizione ad amianto associata al cemento-amianto in Italia è ben lungi però dall'essere completamente conosciuta. Un esempio di conoscenza solo parziale è quello dell'area costituita dal comune di Broni e dai comuni limitrofi, in Provincia di Pavia. A Broni è stato in funzione, dal 1930 al 1990 circa, uno stabilimento di produzione di materiali in cemento-amianto di dimensioni analoghe allo stabilimento Eternit di Casale Monferrato. Una indagine epidemiologica sulla mortalità per tumore maligno della pleura in Italia, per il periodo 1980-1987, ha segnalato un elevato carico di mortalità specifica, non solo per la popolazione residente a Broni, ma anche per quella di alcuni comuni limitrofi[34]. Un'analoga indagine, condotta per il periodo 1988-1994, ha rilevato un eccesso di mesoteliomi statisticamente significativo, rispetto alla regione Lombardia, sia a Broni (SMR = 16,40; IC 95% = 6,57-33,80) che nel vicino comune di Voghera (SMR = 2,47; IC 95% = 1,35-4,14)[35]. La stessa patologia, oggetto di studio in termini di incidenza, ha un tasso per il periodo 1980-1989, standardizzato per età, di 16,2 (x100.000 p-a) per gli uomini e di 9,2 (x100.000 p-a) per le donne[36].

Un'indagine più recente[37] conferma che la mortalità per tumore maligno della pleura rimane elevata nel comune di Broni, dove è stato riscontrato uno scostamento tra casi osservati e attesi particolarmente elevato, soprattutto negli anni successivi al 1990, in entrambi i sessi e in modo relativamente più marcato per le classi di età più giovani (sotto i 50 anni di età) così come nei comuni limitrofi. Peraltro mancano ancora indagini di epidemiologia analitica riguardanti quest'area che affrontino in modo sistematico la valutazione degli effetti dell'esposizione lavorativa e dell'esposizione ambientale. Analoghi risultati sono stati osservati a Bari in relazione al locale stabilimento Fibronit, di dimensioni minori rispetto a quelli citati in precedenza, ma collocato in prossimità del centro cittadino ed in funzione dal 1934 al 1989[38].

Conclusioni

Alla luce dei dati epidemiologici riteniamo che l'esposizione a cemento-amianto, non solo nella produzione ma anche nell'esposizione ambientale, sia una delle maggiori cause della patologia asbesto-correlata in Italia. In particolare, per quanto riguarda l'esposizione non lavorativa, riteniamo sia opportuno valutare la possibilità di affiancare alle procedure di risarcimento del danno conseguente all'esposizione lavorativa anche quelle di risarcimento del danno conseguente all'esposizione ambientale o di "prossimità", in particolare nel caso del mesotelioma della pleura e di popolazioni come quelle di Casale, Broni o Bari dove il contributo di una singola industria, quella del cemento-amianto, è stato così decisivo e preminente rispetto alle altre fonti di esposizione.

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Targeted therapies in gastrointestinal stromal tumours (GIST): results and promises

Terapie mirate nei tumori stromali gastrointestinali (GIST): risultati e prospettive

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Summary

Gastrointestinal stromal tumours (GIST) are rare soft tissue sarcomas arising primarily from mesenchymal tissue in the gastrointestinal tract and abdomen. GISTs are diagnosed by strong and diffuse positive immunohistochemical staining of the proto-oncogene c-kit, a type III tyrosine kinase receptor. Approximately 80% of cases have mutated KIT codifying gene, and 5% have mutated platelet-derived growth factor receptor A (PDGFRA). The identification of these mutations as a key factor in the pathogenesis of GIST has substantially altered the diagnosis and treatment of GIST. Imatinib, a molecularly targeted drug that inhibits the kinase activity of KIT and PDGFRs, has been shown to be highly efficacious in patients with advanced GIST. However, early or late tumour resistance to imatinib is an increasing clinical problem. Sunitinib, a multitargeted tyrosine-kinase inhibitor with antiangiogenic activity, is an effective therapeutic option for patients with advanced disease after failure of imatinib treatment. Eur. J. Oncol., 12 (2), 89-92, 2007

Key words: **GIST, targeted therapies, imatinib, sunitinib**

Riassunto

I tumori gastrointestinali stromali (GIST) sono tumori rari dei tessuti molli, che insorgono dal tessuto mesenchimale del tratto gastrointestinale e dell'addome. I GIST sono caratterizzati da intensa e diffusa positività immunoistochimica per il prodotto del proto-oncogene c-kit, recettore tirosin-chinasico di tipo III. Nell'80% dei casi si riscontra una mutazione attivante del gene che codifica per KIT; nel 5% la mutazione è a carico del recettore PDGFRA (*platelet-derived growth factor receptor A*). L'identificazione di tali mutazioni, che giocano un ruolo chiave nella patogenesi dei GIST, ne ha radicalmente cambiato l'approccio non solo diagnostico, ma anche terapeutico. Imatinib, un farmaco a bersaglio molecolare rivolto contro i recettori tirosin-chinasici KIT e PDGFR, ha dimostrato una forte efficacia nei pazienti con GIST avanzato. Tuttavia il fenomeno della resistenza, precoce o tardiva, ad imatinib rappresenta un rilevante problema clinico. Sunitinib, un inibitore tirosin-chinasico a bersaglio multiplo ad attività antiangiogenetica, rappresenta una valida opzione terapeutica per i pazienti con malattia avanzata dopo fallimento del trattamento con imatinib. Eur. J. Oncol., 12 (2), 89-92, 2007

Parole chiave: **GIST, terapie mirate, imatinib, sunitinib**

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Background

Gastrointestinal stromal tumours (GISTs) are very rare neoplasms, representing only 0.2% of all gastrointestinal (GI) tumours, nevertheless they are the most common mesenchymal neoplasia of the GI tract. Their incidence is 14.5 new cases per 1 million people per year, without significant differences between men and women. They can arise along the entire length of the GI tract, but the principal site of origin is the stomach, followed by the small intestine, while they are rare in the colon, rectum and oesophagus. Occasionally they originate in the omentum, mesentery or retroperitoneum. The peak age of incidence is 40-60 years.

Recently, thanks to the improvement of biologic and molecular techniques, GISTs have been distinguished from other mesenchymal and neural crest tumours (principally from leiomyosarcoma) and have become a distinct clinical-pathological entity. GISTs appear to be related to the interstitial cells of Cajal, the intestinal pacemaker cells with which they share certain differentiation markers. In particular, at immunohistochemistry, in contrast to smooth-muscle tumours, GISTs are positive for expression of the KIT receptor tyrosine kinase (TK) (detected as CD117 antigen).

The normal ligand for KIT is a cytokine known as stem cell factor (SCF) or Steel factor, that binds the cell-surface transmembrane receptor KIT and triggers a cascade of intracellular signals that stimulate proliferation and enhance cellular survival. There are frequent gain-of-function c-kit mutations in GISTs. These mutations result in the constitutive ligand-independent activation of KIT signalling, which leads to uncontrolled cell proliferation and resistance to apoptosis. Complete surgical resection (without lymph node dissection, given the lack of lymphatic spread of these tumours) has been the mainstay of therapy for localized GISTs. However, many patients (27-42%) develop recurrent GISTs, predominantly in the peritoneum surface and in the liver. Conventional chemotherapeutic agents (such as doxorubicin or ifosfamide), used in the treatment of other sarcomas, are inadequate in this case[1]. The response rate of GISTs to standard chemotherapy is extremely low (less than 10%).

Also radiotherapy, essential in the local therapy of soft tissue sarcomas of the extremities, has a minimal rôle in GISTs.

Targeted therapy with imatinib

The advent of imatinib mesylate has radically changed the clinical approach to GISTs, marking a new era of rational and targeted molecular cancer inhibition. Imatinib is a small molecule, initially used only against Philadelphia chromosome-positive chronic myeloid leukaemia (CML), because it selectively inhibits the characteristic BCR-ABL fusion protein expressed in CML. Subsequently it has shown the property to inhibit also other TKs, including KIT-TK. The clinical employment of imatinib to treat metastatic and unresectable GISTs has given exceptional results**.**

An open-label, randomized, multicentre, clinical phase II trial was conducted to test the efficacy and safety of imatinib in patients with unresectable or metastatic, histologically confirmed GIST[2]: 147 patients were randomly assigned to receive a daily dose of either 400 mg or 600 mg of imatinib orally. The crossover to 600 mg/*die* was provided for patients in progression after treatment with 400 mg/*die*: 81.6% of patients had a partial response or stable disease with a median time of objective response of 13 weeks, and with no significant differences in the rate of response between the two arms. The estimated 1-year survival rate for all patients was 88%, while median survival had not been reached after a median follow-up of 24 weeks after the onset of response. Treatment with imatinib was generally well tolerated; only mild or moderate adverse events (AEs) were observed: periorbital and widespread oedema, nausea, diarrhoea, musculoskeletal pain, fatigue, skin rash, abdominal pain; while serious AEs (GI or intraabdominal haemorrhages) occurred in 5% of patients. This study confirmed the clinical activity of imatinib and its considerable antiproliferative and proapoptotic effects on GISTs; it has therefore been approved worldwide for use in unresectable or metastatic GISTs, with a usual recommended dose of 400 mg daily, while surgery remains the principal treatment for primary disease.

Concerning the dose/progression free survival (PFS) relation, Verweij *et al*[3] conducted a randomized trial to test whether the highest feasible daily dose yields a higher initial response rate or a better PFS than the recommended dose. So 946 patients, with advanced or metastatic GISTs, were randomized to receive imatinib 400 mg either once or twice a day. For patients who had progression at 400 mg daily dose, the option of crossover was offered. At a median follow-up of 760 days, 56% of patients allocated imatinib once a day had progressed, compared with 50% of those who were assigned treatment twice a day (p=0.026). This study has shown that there is no significant difference between therapy with 400 mg once a day and 400 mg twice a day, in terms of response induction and overall survival (OS), while there is a significant benefit in terms of median PFS with an initial treatment at 400 mg twice a day;

therefore this dose might be preferred as initial treatment dose in patients with metastatic and symptomatic disease.

Although most patients affected by GIST respond dramatically to imatinib mesylate, there is a little subset of patients (5%) that exhibits primary resistance to imatinib, and there are also many patients (14%) who acquire this resistance after several months (with a median of about 2 years) of drug administration (secondary resistance). Molecular mechanisms seems to be responsible for these events.

Heinrich *et al*[4] conducted a multicentre, open-label, randomized phase II study to examine the relationship between tumour genotype and clinical response to imatinib in 127 patients with advanced GIST. Patients whose tumour expressed an exon 11 mutation were much more likely to have a partial response (PR) with imatinib (83.5%) than patients with exon 9 mutant isoform protein (47.8% of PR, p=0.0006), and also in comparison to patients with no detectable KIT mutation (0.0% of PR, p<0.0001). The PFS was also significantly better for patients with exon 11 mutation than for patients with exon 9 mutation (p<0.0001), and also in comparison to patients without KIT mutation (p<0.0001). OS for the entire population at 17 months was 85%, but patients with exon 11 mutant KIT isoform had improved survival, compared to patients with mutation in the exon 9 (p=0.0034), and to patients with no KIT mutation (p<0.0001).

In conclusion, the type of KIT mutation in advanced GISTs is predictive of response to imatinib therapy and the findings of this study highlight the importance of molecular mechanisms in the differentiation between patients who are responsive to imatinib and patients who are at high risk for early treatment failure.

Another cause of resistance can be represented by gene amplification and consequently receptor overexpression. In this case, it is possible to obtain a response to treatment, by using higher doses of imatinib, while, when the resistance is due to mutations in the enzymatic site, new treatments with different drugs are required.

Targeted therapy with sunitinib

Considering the primary and secondary resistance to imatinib, a second generation of TK inhibitors has been developed.

A randomized, double-blind, placebo-controlled, multicentre phase III clinical trial has been recently conducted to assess the efficacy and safety of sunitinib, an oral multitargeted TK inhibitor with antiangiogenetic properties, in patients with advanced GIST after failure of imatinib because of resistance or intolerance[5]. In this clinical trial, 312 patients were randomized in a 2:1 ratio to receive blinded study drug (sunitinib or placebo), at a starting dose of 50 mg daily for 4 consecutive weeks, followed by 2 weeks off-drug. Crossover option was available for patients assigned initially to placebo in case of progression. The study was unblinded early, in January 2005, when a planned interim analysis showed significantly longer time to tumour progression (TTP) in patients initially treated with sunitinib than in those on placebo (>4 times longer for sunitinib: 27.3 weeks *vs* 6.4 weeks; p<0.0001), so all patients were allowed to crossover to open-label sunitinib. In the sunitinib group, 16% of patients were progression-free for at least 26 weeks, compared with 1% in the placebo group. OS obtained with initial sunitinib treatment was better than that obtained with placebo (p=0.007). The median time to tumour response on sunitinib was 10.4 weeks, with a median duration of response of 6 months. Therapy was reasonably well tolerated; AEs (fatigue, diarrhoea, rash and skin discolouration, stomatitis, hand-foot syndrome, nausea and hypertension) were generally mild to moderate in intensity and easily managed by dose reduction, dose interruption or standard supportive medical treatments.

This study shows that sunitinib is an effective therapeutic option for patients with GIST resistant to or intolerant of previous treatment with imatinib. In particular, sunitinib administered to patients with GIST associated to KIT mutation in exon 9, that are typically resistant to imatinib, showed relatively higher rates of antitumour response and clinical benefit. New studies are necessary in order to further investigate the molecular mechanisms by which sunitinib affects disease control after imatinib failure, so that an adequate and rational algorithm could be defined.

Assessing response

Another relevant aspect in the management of patients with GIST is the evaluation of tumour response to treatment. Traditionally, the response to cancer treatment in solid tumours is evaluated on the basis of decrease in measurable tumour dimensions, but in GISTs this is difficult because the reduction in the viable tumour cell fraction does not always result in a reduction of volume (that tends to occur later on, during the treatment), since tumour tissue can be replaced by necrotic or fibrotic tissue and morphological images are unable to differentiate between these different tissue types.

The new drugs lead to a stabilisation of tumour growth rather than tumour shrinkage, so dramatic changes in volume are not to be expected. New criteria need to be defined to evaluate treatment efficacy: fluorodeoxyglucose-positron emission tomography (^{18}FDG-PET) may represent an early and sensitive method for such evaluation[6].

Conclusions

GISTs are rare tumours, but they are a very interesting pathology. New knowledge about the critical pathogenetic mechanisms responsible for GIST has represented a major advance in the treatment of this disease, and the information gained from the successful application of imatinib in this tumour has represented a valid model of target strategies for other cancers.

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Ruolo del *brain natriuretic peptide* (BNP) nella gestione della cardiotossicità da farmaci antiblastici

The rôle of brain natriuretic peptide (BNP) in the management of chemotherapy-induced cardiotoxicity

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Riassunto

Razionale*. Il monitoraggio della cardiotossicità da chemioterapia è una problematica affrontata quotidianamente in oncologia. Il peptide natriuretico cerebrale (*Brain Natriuretic Peptide* = BNP) si sta imponendo in letteratura come marcatore sensibile di danno miocardico, predittivo dell'evoluzione verso lo scompenso di circolo in diverse patologie cardiache. *Metodi*. Il frammento N-Terminale (NT) del proBNP è il frammento inattivo dell'ormone BNP, prodotto dai cardiomiociti umani in uno stadio precoce dello scompenso cardiaco congestizio; è stato dosato con elettrochemi-luminescenza (ECLIA) in 110 pazienti prima e dopo il trattamento con schemi chemioterapici potenzialmente cardiotossici. In 31 soggetti è stata valutata anche la correlazione tra i livelli del marcatore ed i reperti ecocardiografici ed EcoColor Doppler di disfunzione cardiaca. Erano esclusi soggetti con insufficienza renale, ipertensione arteriosa, ascite e diabete. *Risultati*. L'andamento di NT-proBNP risulta significativamente in crescita nei pazienti sottoposti a trattamenti antiblastici contenenti 5-fluorouracile ed antracicline. Il 13% dei pazienti passa da valori normali di marcatore all'inizio della terapia a valori oltre il *cut-

Summary

***Background*. The monitoring of chemotherapy-induced cardiotoxicity is a problem faced daily in oncology. The Brain Natriuretic Peptide (BNP) is increasingly reported in the literature as a sensitive marker of myocardial damage, predictive of the evolution towards circulatory failure in several cardiac pathologies. *Methods*. The N-terminal (NT) fragment of proBNP is the inactive fragment of the BNP, produced by human cardiomyocytes in an early stage of congestive heart failure; it has been evaluated by electrochemiluminescence (ECLIA) in 110 patients before and after treatment with potentially cardiotoxic chemotherapy schedules. In 31 subjects the relationship between marker levels and echocardiographic and EcoColor Doppler signs of cardiac dysfunction has also been evaluated. Patients with renal failure, arterial hypertension, ascites and diabetes were excluded. *Results*. The trend of NT-proBNP increases significantly in patients submitted to antitumour treatments containing 5-fluorouracil and anthracyclines: 13% of patients with normal marker values at the start of therapy reach values over cut-off level by the end of treatment. In patients submitted to echocardiographic**

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***off* dello stesso al termine del trattamento. Nei pazienti sottoposti a monitoraggio ecocardiografico e Color Doppler si è osservata una lieve correlazione tra incremento del peptide e disfunzione cardiaca diastolica, valutata attraverso il rapporto E/A (rapporto tra picco diastolico precoce e tardivo) e DT (tempo di decelerazione dell'onda E). Nessun soggetto ha sviluppato sintomatologia da scompenso cardiaco. *Conclusioni*. Nella nostra esperienza, l'NT-proBNP aumenta significativamente in relazione a terapie potenzialmente cardiotossiche, quali antracicline e 5-fluorouracile, sottolineando un suo possibile ruolo nell'identificazione precoce del danno cardiaco e come metodica aggiuntiva di selezione dei pazienti a rischio cardiologico, da sottoporre a successive indagini ecocardiografiche periodiche. Eur. J. Oncol., 12 (2), 93-99, 2007**

Parole chiave: **BNP, chemioterapia, cardiotossicità**

and Color Doppler monitoring, a slight relationship has been observed between peptide increase and diastolic cardiac dysfunction, evaluated by the E/A ratio (ratio between early and late diastolic peak), and the DT (deceleration time of E wave). No subjects presented symptoms of heart failure. *Conclusions*. In our experience NT-proBNP increases significantly during potentially cardiotoxic therapies, such as anthracyclines and 5-fluorouracil, so emphasizing its possible rôle in the early identification of cardiac damage and, furthermore, as an additional method for the selection of patients at cardiac risk, to be submitted to subsequent periodic echocardiographic controls. Eur. J. Oncol., 12 (2), 93-99, 2007

Key words: **BNP, chemotherapy, cardiotoxicity**

Introduzione

La gestione della chemioterapia è condizionata dai potenziali effetti tossici dei farmaci antiblastici, tra i quali la cardiotossicità è una problematica non frequente ma invalidante.

Numerosi sono i farmaci antitumorali di cui è nota la cardiotossicità, come le antracicline, in particolare doxorubicina e daunorubicina, il 5-fluorouracile, alcuni agenti alchilanti come ciclofosfamide e ifosfamide, la mitomicina C, i taxani ed anche molecole di più recente introduzione come il trastuzumab (Tabella 1). Gli effetti cardiotossici, in particolare delle antracicline, sono noti da tempo, tuttavia, ad oggi, non esiste un protocollo di stratificazione del rischio di sviluppare danno cardiaco, nè uno schema codificato di cardioprotezione[1]. La tossicità delle antracicline è legata alla dose cumulativa ed al picco di concentrazione del farmaco; è, quindi, preferibile una somministrazione prolungata, l'associazione con cardioprotettori come il dexrazoxano, e il mantenimento della dose cumulativa sotto il livello soglia[2]. Per gli altri farmaci potenzialmente cardiotossici, l'unica modalità di profilassi è evitarne la somministrazione o monitorare il paziente, al fine di sospendere il trattamento qualora si manifestino effetti indesiderati[3].

Diverse sono le metodiche per il monitoraggio della funzione cardiaca, ognuna delle quali presenta vantaggi e limiti. La biopsia miocardia, ad esempio, è il metodo più sensibile e specifico per l'identificazione precoce del danno miocardico, ma è una manovra molto invasiva; l'eco-

Tabella 1 - Cardiotossicità da antiblastici e patogenesi

Antracicline/Antracenedioni	- Effetti cardiotossici acuti (reversibili) - Scompenso cardiaco ritardato (dose-correlato) - Possibile disfunzione sistolica tardiva
Fluorouracile	- Angine, alterazioni ECG - Ischemia - Aritmia
Taxani	- Alterazioni reversibili del ritmo (bradicardia) - Ipotensione ortostatica
Bleomicina	- Rari eventi di dolore acuto - Pericardite
Cisplatino	- Alterazioni ECG - Ischemia coronarica transitoria
Vincristina	- Rari eventi di infarto miocardio acuto
Agenti alchilanti	- Sindrome miocardite-pericardite (possibile evoluzione in scompenso cardiaco acuto)
Mitomicina C	- Miocardiopatia dilatativa - Favorisce insorgenza di miocardiopatia da doxorubicina
Trastuzumab	- Miocardiopatia dilatativa? - Favorisce insorgenza di miocardiopatia da doxorubicina

cardiografia può essere una valida alternativa, poiché permette una valutazione completa e non traumatica della *performance* cardiaca, e dei parametri di funzione sistolica e diastolica del ventricolo sinistro e del ventricolo destro. Il limite di tale metodica è l'identificazione tardiva del danno miocardico, mentre sarebbe utile avere a disposizione un indicatore precoce[4]. A tale scopo sono stati proposti nuovi metodi di monitoraggio, come la scintigrafia con antimiosina o il dosaggio di parametri biochimici, come le troponine e i peptidi natriuretici, la cui sensibilità e specificità sono in corso di valutazione[5]. Il peptide natriuretico cerebrale (*Brain Natriuretic Peptide* = BNP), in particolare, è un piccolo peptide prodotto e rilasciato dai miociti cardiaci in risposta ad un sovraccarico di lavoro che determini uno stress cardiaco in assenza di necrosi cellulare.

Abbiamo valutato le concentrazioni di NT-proBNP (frazione N-terminale del proBNP) in un campione di pazienti ambulatoriali affetti da neoplasia e candidati a chemioterapia con la finalità di studiare:

– l'andamento temporale in relazione a diversi schemi terapeutici, e
– la possibile correlazione con gli indici funzionali ecocardiografici,

nell'intento di utilizzare in futuro tale peptide in qualità di indicatore biologico di danno miocardico precoce indotto da farmaci antiblastici.

Materiali e metodi

La popolazione oggetto dello studio è stata selezionata tra i pazienti afferenti all'Ambulatorio di Oncologia dell'Azienda Ospedaliera "Luigi Sacco" di Milano. I criteri di inclusione erano: età compresa tra 18 e 75 anni, assenza di cardiopatia nota e di diabete mellito scompensato, diagnosi di neoplasia che richieda trattamento con terapia antiblastica e consenso informato orale; mentre erano esclusi i soggetti con ipertensione arteriosa non controllata dalla terapia, con insufficienza renale moderata o grave, con ascite, ed i pazienti che negavano il consenso.

Sono stati arruolati pazienti candidati a differenti trattamenti chemioterapici. Sono stati considerati schemi potenzialmente cardiotossici quelli contenenti antracicline, 5-fluorouracile, taxani, trastuzumab e agenti alchilanti. Tra i trattamenti non cardiotossici sono stati inclusi schemi con gemcitabina, alcaloidi della vinca, carboplatino, oxaliplatino, irinotecan, etoposide e rituximab.

Sono riportate nella Tabella 2 le caratteristiche della popolazione in base al sesso, all'età e al tipo di chemioterapia somministrata. In tutti i pazienti è stata raccolta un'anamnesi, focalizzata sui fattori di rischio cardiovascolare, seguita da una visita medica per escludere la presenza di eventuali segni e sintomi di scompenso cardiaco; sono stati poi effettuati prelievi venosi con raccolta del sangue, in provette da 3 ml contenenti EDTA-sodio, per prevenire la degradazione del peptide, all'inizio della chemioterapia ed ad ogni ciclo successivo per sei mesi. Il dosaggio del marcatore è stato eseguito presso il Laboratorio di Endocrinologia con la metodica di *ImmunoAssay* in ElettroChemiLuminescenza[6].

Tabella 2 - Caratteristiche dei pazienti

Sesso	Maschi	51	(47%)	
	Femmine	59	(53%)	
Età	62 anni media (*range*: 33-75)			
Stadio di malattia	Localizzata	76	(69%)	
	Metastatica	34	(31%)	
Chemioterapie	1ª Linea	72		
	2ª Linea	38		
1ª Linea			Adiuvante	33
			Elettiva	39
Adiuvante			Mammella	17
			Gastro-enterico	13
			Polmone	3
Cardiotossicità	Certa	86		
	Dubbia	24		
Totale N. pazienti		110		

Secondo le indicazioni del *Consensus Panel* 2004 sul BNP[7], il *range* di normalità è stato definito in rapporto all'età e al sesso del singolo paziente, come riportato nella Tabella 3.

In 31 soggetti, in cui l'attesa per gli esami non ritardava l'inizio della chemioterapia, è stata inoltre esaminata la funzione cardiaca mediante esame ecocardiografico e Color Doppler, all'inizio ed alla fine del trattamento.

La funzione sistolica ventricolare sinistra è stata indagata, rispettivamente, per escludere anomalie della cinesi segmentaria e per valutare la cinesi globale, ed è stata calcolata la frazione d'eiezione (FE) mediante metodo Simpson biplano, che prevede la misurazione dei volumi telediastolico e telesistolico in due diverse proiezioni (api-

Tabella 3 - *Range* di normalità dei valori di NT-proBNP

Sesso	Età (anni)	Valori (pg/ml)
Maschi	<50	<88
	>50	<227
Femmine	<50	<153
	>50	<334

cale 4 camere e apicale 2 camere); per valutare la funzione diastolica del ventricolo sinistro sono stati utilizzati diversi indici: il picco diastolico precoce di velocità di flusso transmitralico (E); il picco diastolico tardivo di velocità di flusso transmitralico (A); il rapporto tra picco precoce e tardivo (E/A *ratio*) ed il tempo di decelerazione dell'onda E (DT).

Per l'analisi dei dati ottenuti è stato applicato il test t di Student per campioni appaiati e l'analisi della varianza per misure ripetute (test ANOVA), utilizzando il *software* per analisi statistiche SPSS.

Risultati

Dal settembre 2005 al giugno 2006 sono stati arruolati complessivamente 110 soggetti, di cui 51 maschi (47%) e 59 femmine (53%), l'età è risultata compresa tra un minimo di 33 anni ed un massimo di 75, con una media di circa 62 anni.

La distribuzione per sede delle neoplasie è stata la seguente: mammella 37, colon-retto 31, polmone (NSCLC) 23, linfoma non-Hodgkin 9, e 10 tumori vari. Il 69% è rappresentato da soggetti con malattia locale ed il 31% da soggetti con malattia metastatica; 72 pazienti hanno ricevuto solo una prima linea di terapia, di cui 33 in regime adiuvante e in 20 casi associata a radioterapia mammaria tangenziale senza irradiazione del miocardio, mentre 38 pazienti sono stati sottoposti a linee di trattamento successive alla prima.

Per valutare le relazioni dei diversi farmaci con l'andamento del NT-proBNP, si è scelto di suddividere gli schemi chemioterapici in quattro gruppi: a) schemi contenenti antracicline (30 pz), b) schemi contenenti 5-fluorouracile (39 pz), c) schemi con taxani, trastuzumab e agenti alchilanti (14 pz), e d) farmaci a cardiotossicità non dimostrata (27 pz). La terapia più frequentemente utilizzata (36,8%) è stata quella del gruppo *b*; quella del gruppo *a* è rappresentata per il 27,6%, quella del gruppo *c* per il 12%, mentre gli schemi a cardiotossicità non dimostrata rappresentano il 24,6%. Da notare che le antracicline vengono utilizzate soprattutto in prima linea, mentre nelle linee successive sono predominanti i taxani. Sono stati presi in considerazione i risultati del dosaggio dell'NT-proBNP e sono stati messi in relazione con le caratteristiche della popolazione e con gli schemi chemioterapici. Sono stati considerati i valori basali, cioè quelli ottenuti da prelievi effettuati prima del primo ciclo di terapia, i valori finali, cioè quelli effettuati al termine dell'ultimo ciclo e un valore intermedio, cioè quello più vicino alla metà dei cicli previsti. Sono state considerate le medie per i tre gruppi, con i seguenti risultati:

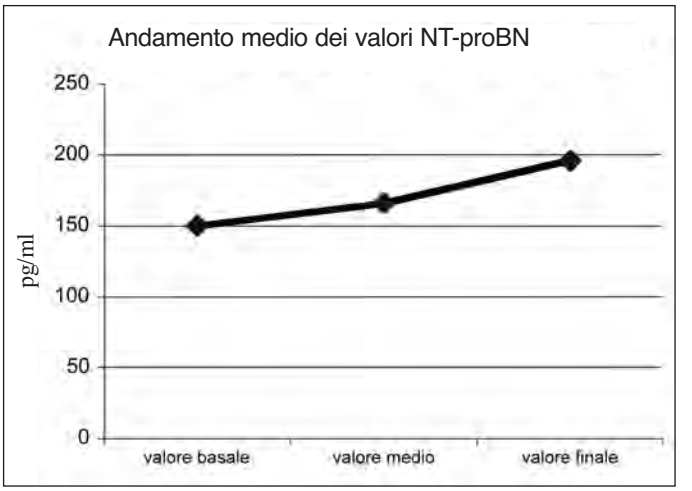

Fig. 1. Valori di BNP durante chemioterapia

– valore basale: media 150 pg/ml;
– valore centrale: media 166 pg/ml;
– valore finale: media 196 pg/ml.

La fig.1 presenta i valori di BNP in grafico in rapporto alla chemioterapia.

L'andamento medio dell'NT-proBNP durante la terapia è in salita, anche se la distribuzione dei valori rispetto alla media è piuttosto ampia.

L'analisi con test t di Student indica una differenza significativa tra i livelli medi di NT-proBNP basale e finale (p=0,004). Complessivamente 96 pazienti hanno mostrato valori normali sia basali che al termine della terapia (87%), mentre 14 hanno evidenziato valori patologici al termine del trattamento (Tabella 4). Suddividendo la popolazione in classi di età, si osserva che l'aumento del marcatore è più accentuato per i soggetti con età maggiore di 60 anni, mentre è sostanzialmente lineare per i soggetti di età inferiore. Considerando la tipologia di trattamento si osserva che il gruppo *a* (antracicline) e il gruppo *b* (5-fluorouracile) determinano un incremento del peptide, soprattutto per i trattamenti con 5-fluorouracile, mentre nei gruppi *c* (taxani, alchilanti, trastuzumab) e *d* (non cardiotossici) l'NT-proBNP segue un andamento sostanzialmente lineare (fig. 2).

In 31 pazienti è stata effettuata anche una valutazione ecocardiografica della funzione cardiaca, pre- e post-chemioterapia. Per quanto riguarda la percentuale della frazione di eiezione (FE%), si osserva che non vi sono variazioni prima e dopo la chemioterapia, sia nei soggetti

Tabella 4 - NT-proBNP: andamento complessivo

Valore basale	Valore finale	N. pazienti	%
BNP < cut off	BNP < cut off	96	87,3%
BNP < cut off	BNP > cut off	14	12,7%

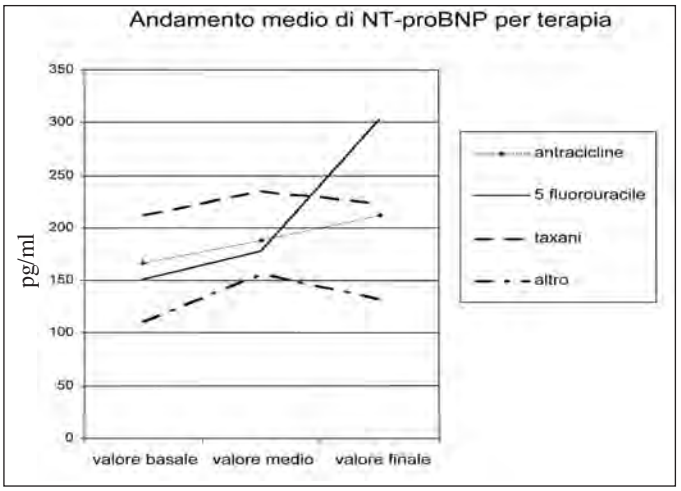

Fig. 2. Valori di BNP per tipo di chemioterapia

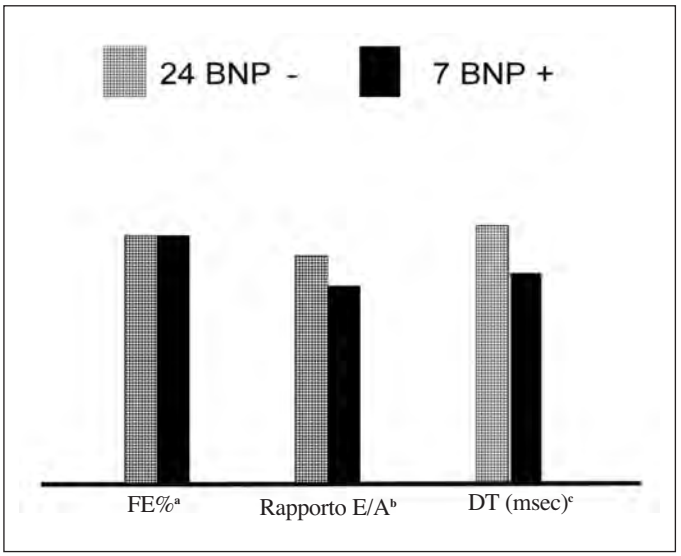

Fig. 3. Indici ecocardiografici dopo chemioterapia
[a] Percentuale della frazione di eiezione
[b] Rapporto tra picco diastolico precoce e tardivo
[c] Tempo di decelerazione dell'onda

che mantengono valori di NT-proBNP normali, che in quelli in cui essi diventano patologici. Nell'analisi della funzione diastolica, il rapporto E/A risulta più basso nei soggetti con valori patologici di BNP dopo chemioterapia rispetto a quelli con valori normali anche se, considerata l'età dei pazienti, tali modificazioni rientrano ancora nei limiti di normalità. Più accentuate sono le modificazioni a carico del DT: il DT è costantemente più basso nei soggetti con un livello di marcatore oltre i limiti di *cut off* dopo la terapia, anche se l'indice di correlazione non raggiunge valori significativi (fig. 3). Nessuno dei pazienti reclutati nello studio ha manifestato sintomi clinici di disfunzione cardiaca durante il periodo di osservazione.

Discussione

Molti agenti chemioterapici possono indurre effetti tossici a livello cardiaco. Spesso si tratta di un evento dose-correlato e può determinare una severa compromissione funzionale cardiaca, soprattutto tra i pazienti che sopravvivono a lungo dopo il trattamento. Tra questi farmaci, la cardiotossicità da antracicline è stata a lungo studiata, ma l'incidenza e le caratteristiche dell'effetto tossico cardiaco indotto da altri agenti, come 5-fluorouracile, capecitabina, mitoxantrone, cisplatino, paclitaxel e trastuzumab, sono state descritte solo di recente e non sono ancora ben conosciute.

La possibilità che gli eventi cardiotossici abbiano inizio anche dopo anni dal trattamento apre il capitolo del monitoraggio a lungo termine, che presenta attualmente molte limitazioni.

Il problema del *follow-up* appare ancora più rilevante se si considera che i metodi disponibili per la cardioprotezione sono ancora molto limitati.

Per queste ragioni, negli ultimi anni, oncologi e cardiologi, partendo da punti di vista sostanzialmente diversi, ma in fondo convergenti, si sono mossi alla ricerca di nuove modalità di indagine, con l'obiettivo di trovare quella più adatta e affidabile per l'identificazione dei soggetti a rischio, meritevoli di ulteriori indagini, per la diagnosi precoce di danno cardiaco.

Tra i candidati a questo ruolo, il BNP, rispetto agli altri peptidi della stessa famiglia, ha mostrato caratteristiche adatte ed, insieme alla troponina I, è oggetto di molti lavori sviluppati in questo periodo. In letteratura troviamo dati interessanti che riguardano l'utilizzo dell'NT-proBNP, come marcatore di tossicità cardiaca da antiblastici.

Una delle prime pubblicazioni riguarda l'identificazione della cardiomiopatia indotta da doxorubicina nelle cavie. Lo studio giapponese[8] ha osservato un incremento della concentrazione plasmatica del BNP in relazione alla somministrazione di cicli successivi di chemioterapia ed una correlazione tra i livelli del peptide e le alterazioni funzionali associate al danno miocardico, identificate con ecocardiografia; tali alterazioni, in particolare una riduzione del *fractional shortening*, sono risultate successive, in ordine temporale, al rialzo del marcatore.

Successivamente sono stati esaminati prelievi seriati di BNP ed altri marcatori in pazienti trattati con antracicline, in relazione ai reperti ecocardiografici[9]. I risultati hanno mostrato un aumento significativo ma transitorio dei livelli di BNP, con un picco massimo entro 3-7 giorni dopo la somministrazione del farmaco; coloro che evidenziavano un'alterazione persistente nel tempo dei valori di BNP hanno poi sviluppato patologie cardio-circolatorie conclamate.

Studi più recenti offrono una visione contrastante. Nel 2005 Pichon *et al*[10], in uno studio su 12 pazienti trattate con antracicline e monitorate per 3 anni circa, sostengono il ruolo predittivo del BNP, indicando che un aumento del marcatore durante chemioterapia con antracicline è un evento frequente ma reversibile; solo chi ha mostrato un incremento costante e persistente nel tempo ha sviluppato successivamente scompenso cardiaco.

Nella valutazione di 107 pazienti trattati con antracicline, invece, Daugaard *et al*[11] non hanno dimostrato alcuna correlazione tra i livelli di BNP e la riduzione della FE: né i valori basali del peptide, né le sue successive modificazioni durante il trattamento si sono dimostrati in grado di predire la successiva riduzione della FE.

All'Istituto Europeo di Oncologia sono stati valutati 52 pazienti sottoposti a terapia ad alte dosi con antracicline: un aumento persistente dell'NT-proBNP è risultato correlato a reperti ecocardiografici di disfunzione diastolica, permettendo così di identificare i pazienti a rischio di sviluppo di scompenso cardiaco sistolico, che sono stati monitorati nel tempo e trattati con successo[12].

Nonostante la carenza di pubblicazioni riguardo alle variazioni del BNP in relazione a terapie antiblastiche diverse dalle antracicline, in alcune segnalazioni si rileva come l'NT-proBNP sia un potenziale *marker* di mortalità durante terapia a lungo termine con trastuzumab in donne con carcinoma mammario metastatico[13, 14].

Le incertezze che emergono dalla letteratura[15] riguardo all'affidabilità del NT-proBNP ci hanno portato ad iniziare questo studio con l'obiettivo di osservare l'andamento del peptide durante la somministrazione di trattamenti chemioterapici in un ampio campione di pazienti; inoltre, su una popolazione più ristretta, abbiamo cercato di correlare l'andamento riscontrato con alcuni indici ecocardiografici.

I risultati indicano che l'NT-proBNP tende ad aumentare in relazione soprattutto a schemi terapeutici contenenti 5-fluorouracile, o un suo profarmaco (la capecitabina); per i pazienti trattati con schemi contenenti antracicline si è osservato un incremento più moderato ed i valori di BNP restano entro valori normali durante i cicli di chemioterapia. Tale osservazione sembra essere in linea con le acquisizioni della letteratura[16, 17], dato che la cardiotossicità da antracicline è per lo più tardiva e poiché anche il BNP tende ad aumentare più tardivamente, durante i periodi di *follow-up* che vanno da 6 mesi a 3 anni nei diversi studi. Poiché la nostra analisi copre per ciascun paziente un periodo che va da 8 settimane a 6 mesi circa, è possibile che nel nostro campione non si siano resi manifesti gli effetti di tale tossicità. Le alterazioni più importanti di BNP si sono verificate in corrispondenza dei diversi schemi chemioterapici contenenti derivati del fluorouracile.

Le conoscenze sulle modalità con cui si esplica la cardiotossicità di questo farmaco confermano i nostri risultati. Il 5-fluorouracile, infatti, determina un'alterazione cardiaca abbastanza precoce, sostenuta da un vasospasmo delle arterie coronariche mediato da elevati livelli di endotelina-1, testimoni di un possibile danno endoteliale[18], durante i primi cicli di trattamento, che persiste e si accentua all'accumularsi delle dosi. Questo spiegherebbe perché il BNP tende ad aumentare prevalentemente nei pazienti che usano la infusione continua, modalità che, come sottolinea la letteratura[19], è associata ad un incremento della cardiotossicità. È anche da aggiungere che tale effetto tende a regredire con la sospensione del farmaco e potrebbe quindi associarsi ad una successiva riduzione dei livelli di BNP.

Da queste acquisizioni emerge l'importanza di monitorare il BNP a distanza di tempo per rivalutare i livelli del marcatore, sia nei pazienti trattati con antracicline, sia per quelli in terapia con 5-fluorouracile. Per i primi ci aspettiamo di rilevare un incremento tardivo del BNP, in alcuni pazienti, che ci indichi l'eventuale insorgenza di cardiopatia, avvalorando il ruolo predittivo del BNP nella cardiotossicità da antracicline; per i secondi ci attendiamo invece un ritorno dei valori entro la norma, esclusi ovviamente quei pazienti che avranno nel frattempo sviluppato una cardiopatia conclamata.

Dove è stato possibile effettuare una valutazione combinata ecocardiografica, i risultati suggeriscono una modesta correlazione ($r = 0,35$) tra incremento del BNP e segni di iniziale disfunzione cardiaca diastolica, soprattutto se si considera il DT.

È possibile, quindi, che il BNP si comporti come un indicatore precoce di disfunzione cardiaca, utile a selezionare i pazienti a rischio di sviluppare un'alterazione più grave, che sfoci, a lungo termine, in un quadro di scompenso cardiaco. Tuttavia, dato il numero limitato di pazienti reclutati per la valutazione ecocardiografica e per il limitato *follow-up*, non è possibile trarre delle conclusioni definitive.

È ipotizzabile un ruolo futuro dell'NT-proBNP nel monitoraggio della cardiotossicità da farmaci antiblastici, come metodica semplice e rapida per valutare la popolazione dei soggetti sottoposti a trattamenti chemioterapici e indirizzare verso il controllo ecocardiografico solo i soggetti più a rischio. L'ecocardiogramma è infatti una metodica certamente molto valida, ma che necessita tempo per l'esecuzione e abilità specifica dell'operatore; inoltre l'indagine ecografica andrebbe focalizzata non solo sulla funzione sistolica, ma soprattutto sulla funzione diastolica del ventricolo sinistro, che, come indica la let-

teratura[20,21], è la componente che si deteriora più precocemente, ma può essere monitorata nel tempo per impedire l'evoluzione verso lo scompenso cardiaco.

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Il mesotelioma da amianto tra i lavoratori degli zuccherifici: la casistica della Fondazione Ramazzini

Mesothelioma following asbestos exposure in workers of sugar refinery plants: the Ramazzini Foundation case series

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Riassunto

Vengono riportati 18 casi italiani di mesotelioma (di cui 16 osservati in Emilia Romagna), insorti a seguito di esposizione ad amianto presente negli zuccherifici. Diciassette casi riguardano lavoratori esposti professionalmente, mentre uno è dovuto ad esposizione familiare. Si tratta di mesoteliomi localizzati alla pleura eccetto uno al peritoneo. Il tempo medio di latenza calcolato è di 37,2 anni e l'età media di insorgenza è di 62 anni. Questo studio dimostra come l'industria saccarifera sia un settore a rischio per quanto riguarda la esposizione ad amianto e coinvolge, considerando anche il consistente *turn-over* in questa categoria, approssimativamente 28.000 persone, tra lavoratori stabili e stagionali, a cui vanno aggiunti quelli con potenziale esposizione familiare. Per valutare l'entità di questo rischio sono necessari studi epidemiologici sistematici, scientificamente ed eticamente non più procrastinabili. Eur. J. Oncol., 12 (2), 101-107, 2007

Parole chiave: **mesotelioma, amianto, zuccherifici**

Summary

Eighteen Italian cases of mesothelioma (16 of which observed in the Emilia-Romagna Region), following exposure to asbestos present in sugar refinery plants, are reported. Seventeen cases arose in workers occupationally exposed, and one is due to family contact. All were cases of pleural mesothelioma, except one, which was peritoneal. The average latency time was 37.2 years and the average age at onset was 62 years. This study demonstrates the risk of asbestos exposure in the sugar refinery industry, which involves about 28,000 people, also in consideration of the substantial turnover in this category, including full-time and seasonal employees, to whom we must add potential family members. To assess this risk, further epidemiologic researches are necessary and, from both a scientific and ethical standpoint, may no longer be delayed. Eur. J. Oncol., 12 (2), 101-107, 2007

Key words: **mesothelioma, asbestos, sugar refineries**

Introduzione

Il contesto

L'industria saccarifera italiana nasce nel 1811, quando a Fidenza, in provincia di Parma, sorge il primo zuccherificio per volere di Napoleone I. In seguito, all'inizio del XX secolo, il settore ha avuto una rapida espansione, facendo però registrare fasi di crescita alternate a fasi di crisi fino agli anni '50, quando il regime protezionistico applicato dallo Stato italiano, che non poteva rinunciare ai notevoli introiti provenienti da questo settore, lo ha defi-

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nitivamente consolidato. Nel 1957 esistevano 32 società saccarifere e gli zuccherifici attivi erano 82, dislocati 33 in Emilia Romagna, 27 in Veneto, 6 tra Piemonte e Lombardia, 11 nel Centro Italia e 5 nel Mezzogiorno. In totale si producevano 9.500 tonnellate di zucchero. Molte di queste aziende, per effetto delle ristrutturazioni e delle crisi degli ultimi quarant'anni, sono state chiuse, ma nella quasi totalità dei casi sono ancora presenti gli impianti dismessi[1].

La filiera saccarifera è un settore che ormai da anni è in declino in Italia come nel resto d'Europa. Le cause principali sono l'assenza di investimenti, la moneta unica con la conseguente mancanza di svalutazioni limitanti la competitività, la diminuita professionalità bieticola, ma, soprattutto, la concorrenza sul libero mercato di paesi emergenti e forti produttori di zucchero, come il Brasile, oltre che l'impiego sempre crescente di dolcificanti artificiali. A tutto questo va aggiunto che nel 2006 è stata varata la riforma dell'Organizzazione Comune di Mercato (OCM) riguardante il regime di quote per la produzione di zucchero a livello della fase agricola e a livello della fase di trasformazione industriale. Tale riforma prevede un taglio drastico delle quote e una riduzione dei prezzi del 33%, quindi un ridimensionamento della politica protezionistica che da sempre è stata garantita a tutela di questo settore produttivo.

La filiera italiana dello zucchero contava fino al 2005 circa 46.000 aziende bieticole con decine di migliaia di addetti, 5 società saccarifere, 19 zuccherifici che davano lavoro a 7.000 dipendenti, e un indotto industriale che coinvolgeva oltre 20.000 unità lavorative[2].

Nel 2005 gli agricoltori italiani hanno destinato alla coltivazione della bietola il 30% di ettari di terreno in più, passando dai circa 185.000 del 2004 a 240-245.000. La coltivazione è stata incentivata dalla crisi del settore cerealicolo e dagli accordi di settore. La produzione di zucchero prevista alla fine della campagna di raccolta delle bietole era di 14-15.000 tonnellate[2].

L'attività industriale degli zuccherifici, se pur non destinata ad espandersi, è comunque una realtà forte e presente sul territorio nazionale.

Lo scenario espositivo

Soprattutto tra gli anni '50 e gli anni '80, come in altri settori quali l'edilizia, le ferrovie, i cantieri navali, le aziende tessili, l'industria della ceramica, dei laterizi e le fonderie, anche negli zuccherifici è stato fatto largo uso di amianto, nonostante i suoi effetti nocivi sulla salute fossero conosciuti fin dall'inizio del '900, quando si descrissero i primi casi di fibrosi polmonare da amianto, denominata poi asbestosi[3].

L'utilizzo dell'amianto era motivato dalle molteplici proprietà di questa fibra, dotata di eccezionale resistenza, incombustibilità, coibenza, durata e basso costo.

Negli zuccherifici l'amianto è stato impiegato per la coibentazione delle tubazioni, caldaie, serbatoi di liquidi ed aria, e per la centrale termica di cui ogni stabilimento è dotato, dato che la maggior parte delle fasi del ciclo di lavorazione della barbabietola avviene a caldo (diffusione, filtrazione, evaporazione, cottura, centrifugazione). Le forme minerali più usate erano il crisotilo e l'amosite che costituivano parte integrante di impasti gessosi di silicati di calcio o carbonato di magnesio, oppure di nastri, tele e cartoni.

Gli impianti venivano spesso isolati con cemento-amianto; le guarnizioni delle caldaie delle centrali termiche e delle tubazioni, soggette al passaggio di liquidi caldi, dovevano resistere all'alta pressione e temperatura, e per questo gli impasti di gomme, resine o leganti plastici contenevano amianto crisotilo ad una concentrazione variante fra il 5-10%. Infine l'amianto era presente spesso nelle strutture di copertura (tetti in cemento-amianto). Nella maggior parte dei casi, il materiale contenente amianto era poi ricoperto da un rivestimento protettivo di tipo cementizio, di lamiera (lamierino), stoffa o nastro telato, destinati nel tempo a deteriorarsi e quindi consentire la fuoriuscita delle fibre nell'ambiente.

Se si considera il fatto che la costruzione degli zuccherifici attivi in Italia risale per lo più agli anni '60-'70, è realistico pensare che gli impianti industriali, per allestire i quali sono stati utilizzati molti materiali contenenti amianto, risultino piuttosto datati e quindi probabilmente danneggiati dal tempo e dall'usura, e che inoltre gli interventi manutentivi siano stati eseguiti spesso senza adeguati sistemi di protezione per gli addetti. Molti di questi stabilimenti sono stati anche in parte o completamente demoliti, e di frequente tali operazioni sono state svolte senza le adeguate misure di sicurezza e da personale non addestrato, con conseguenti gravi rischi per la salute dei lavoratori coinvolti oltre che di un forte inquinamento ambientale.

Un esempio paradigmatico è rappresentato dalla dismissione dello zuccherificio di Crevalcore, nel bolognese. Nel 1986 vennero avviati i lavori di smantellamento, inizialmente affidati ad un'azienda addetta al recupero di materiali ferrosi che, sia pur per breve tempo, ha eseguito la demolizione dell'impianto senza seguire nessuna procedura a tutela dell'ambiente e della salute degli addetti. Solo successivamente, per imposizione dell'USL locale e del Presidio Multizonale di Prevenzione di Bologna, i lavori vennero affidati ad un'azienda specializzata che bonificò un'area di circa 10.000 m^2, interna e circostante l'edificio (fig. 1)[4].

Fig. 1. Lo zuccherificio di Crevalcore: lavori di decontaminazione
Da Acquafresca[4]

Gli zuccherifici rappresentano quindi uno scenario espositivo per l'amianto molto importante.

La dimensione della popolazione esposta in Italia

Il comparto saccarifero in Italia coinvolge circa 3.000 lavoratori fissi e 5.000 stagionali (fig. 2)[5], con una durata media di presenza nel settore di circa 5 anni. Si può quindi supporre un completo *turn-over* del personale stagionale ogni 5 anni. Questi dati ci permettono di stimare che il totale dei lavoratori potenzialmente esposti negli ultimi 25 anni possa essere di circa 28.000 persone (fig. 3).

I soggetti a maggior rischio espositivo negli zuccherifici sono gli installatori, i manutentori, i riparatori di forni, caldaie e tubi, gli operai che in genere lavorano in ambienti dove l'amianto è presente, e ovviamente gli addetti alla decoibentazione e alla demolizione degli impianti. Ad essi vanno aggiunti i familiari che vengono a contatto con le fibre di amianto attraverso gli abiti, la pelle, i capelli dei lavoratori.

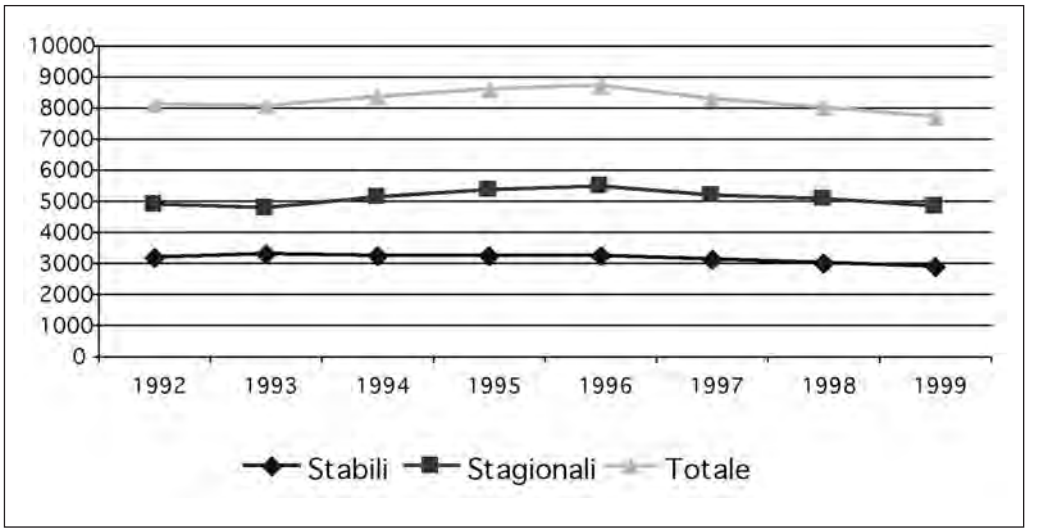

Fig. 2. Addetti al comparto saccarifero in Italia[5]

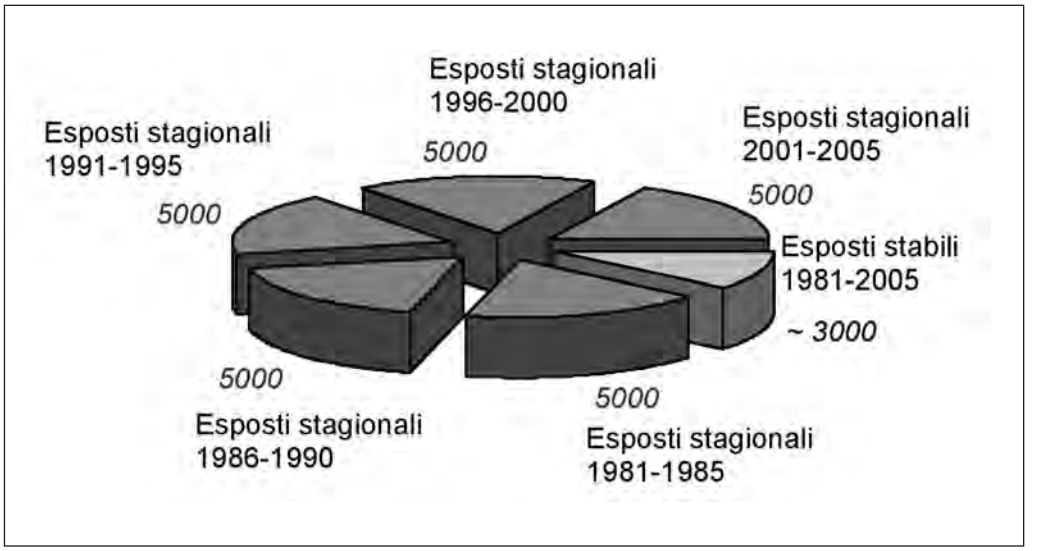

Fig. 3. Proiezione dei lavoratori stabili e stagionali degli zuccherifici italiani (1981-2005) sulla base dei dati forniti dall'Osservatorio CGIL Zuccherifici, che valuta un *turn-over* medio di stagionali ogni 5 anni pari a 5.000 (comunicazione personale, M. Paseschi, 2005)

Mesoteliomi insorti in lavoratori del comparto saccarifero: la casistica della Fondazione Ramazzini

Tutti i casi di mesotelioma descritti nel presente resoconto provengono dalla casistica della Fondazione Europea di Oncologia e Scienze Ambientali "B. Ramazzini" e sono stati in gran parte pubblicati singolarmente[6-22].

I 18 casi riportati sono stati raccolti tra il 1986 e il 2000. Di ciascuno vengono indicati i dati relativi al paziente (sesso, sede della neoplasia, latenza, anno ed età alla diagnosi, anno ed età alla morte) e allo scenario espositivo (sede dello stabilimento, periodo di esposizione, mansioni del lavoratore e condizione familiare) (Tabella 1).

Essi sono geograficamente circoscritti all'Emilia Romagna tranne due: un lavoratore dello zuccherificio di Bottrighe (RO) e un addetto alle caldaie dello zuccherificio di Montecosaro Scalo (MC).

Dei 18 mesoteliomi descritti, 17 sono insorti dalla pleura e 1 dal peritoneo. I casi riguardano 17 lavoratori, impiegati con diverse mansioni ad accertato rischio espositivo; di questi, 6 erano stagionali per tempi variabili. Un caso si riferisce a un familiare.

La latenza (tempo che intercorre tra l'inizio dell'esposizione e l'insorgenza dei primi sintomi della malattia) è compresa tra 19 e 56 anni, con una media di circa 37,2 anni. L'età media in cui è stata fatta la diagnosi è di circa 62 anni e l'età media alla morte è di 66,3 anni.

Interessante è il caso che riguarda la figlia di un lavoratore, dipendente prima dello zuccherificio di Mirandola (MO) e poi di quello di Crevalcore (BO). La donna è stata esposta fin dalla nascita esclusivamente attraverso gli abiti da lavoro sporchi del padre, dato che la sua anamnesi lavorativa non fa supporre alcuna altra forma di contatto con l'amianto. La paziente ha manifestato la patologia all'età di 37 anni.

Tabella 1 - Casi di mesotelioma in lavoratori degli zuccherifici e loro familiari[a]

N. casi	Iniziali del paziente	Sede dello zuccherificio	Sesso	Mansione	Periodo di esposizione (anni)	Sede della neoplasia	Anno della diagnosi (età)	Latenza (anni)[b]	Anno della morte (età)
1	MA	Bologna	M	Capo centrale termica	1946-1970 (stabile)	Pleura	1994 (82)	48	1994 (82)
2	FG	Bondeno (FE)	M	Varie mansioni	1961-1962 (stagionale)	Pleura	1989 (44)	28	1994 (49)
3	GB	Bondeno (FE)	M	Manutentore impianto termo-idraulico	1957-1974 (stabile)	Pleura	1991 (54)	34	1993 (56)
4	MA	Bondeno (FE)	M	Manutentore impianti termo-idraulici	1958-1995 (stabile)	Pleura	1995 (62)	37	Sconosciuto
5	SR	Bottrighe (RO)	M	Addetto alle turbine	1977-1987 (stagionale)	Pleura	1998 (41)	21	Sconosciuto
6	DU	Classe (RA)	M	Manutentore meccanico, tubista, capo centrale termica	1950-1963 (stabile)	Pleura	1992 (62)	41	1993 (63)
7	LT	Codigoro (FE)	M	Varie mansioni	1955-1958 (stagionale)	Pleura	1986 (49)	31	1986 (49)
8	LS	Crevalcore (BO)	M	Conduttore di centrifughe, manutentore di impianti di raffinazione	1957-1983 (stabile)	Pleura	1986 (61)	29	1987 (62)
9	BA	Crevalcore (BO)	M	Muratore per manutenzione e riparazione dei forni	1956-1977 1978-1990 (stagionale)	Pleura	1993 (77)	37	1994 (78)
10	BG	Migliarino (FE)	M	Manutentore impianti termo-idraulici	1941-1944 1946-1952 (stabile)	Pleura	1997 (71)	56	1997 (71)
11	BD	Molinella (BO)	M	Manutentore impianto termo-idraulico	1950-1980 (stabile)	Pleura	1991 (65)	41	1992 (66)
12	CG	Molinella (BO)	M	Verniciatore dei tubi delle caldaie, addetto alla cottura del sugo delle bietole	1949-1975 (stabile)	Pleura	1993 (78)	43	1993 (78)
13	RF	Molinella (BO)	M	Facchino	1953 (stabile)	Peritoneo	1994 (80)	41	1994 (80)
14	EG	Molinella (BO)	M	Manutentore impianti termo-idraulici	1963-1967 (stabile)	Pleura	1997 (78)	34	1998 (79)
15	CM	Molinella (BO)	M	Addetto alle turbine	1950-1958 (stabile)	Pleura	1998 (84)	48	Sconosciuto
16	GA	Montecosaro Scalo (MC)	M	Addetto alle caldaie	1972 (stagionale)	Pleura	1995 (45)	23	Sconosciuto
17	DP	Mezzano, Classe (RA); Forlì; Cesena; Forlimpopoli (FO)	M	Facchino	1975-1994 (stagionale)	Pleura	1994 (37)	19	1997 (40)
18	NF	Mirandola (MO) e Crevalcore (BO)	F	Figlia di lavoratore degli zuccherifici	1951-1982	Pleura	1988 (37)	37	1995 (43)

[a] Elencati secondo la sede territoriale degli zuccherifici

[b] Periodo di tempo intercorso tra l'inizio dell'esposizione e la comparsa dei primi sintomi e segni della neoplasia

Discussione e conclusioni

In tutta Europa e specialmente in Gran Bretagna, Francia, Svezia ed Italia, è stato registrato un forte incremento dei casi di mesotelioma pleurico a seguito del largo impiego dell'amianto in ambito industriale e nell'edilizia pubblica e privata. In Italia tra il 1969 e il 1994 la mortalità dovuta a questa neoplasia è aumentata del 15% ogni 5 anni, con 500-900 morti ogni anno[23]. Studi condotti da Peto e*t al* nel 1999 prospettano un picco di 940 casi annui tra il 2015 e il 2019, dopo di che il numero dei mesoteliomi dovrebbe diminuire e questo perché si ritiene che la popolazione italiana maggiormente esposta sia quella dei nati negli anni '40-'50[24].

I casi riportati in questo resoconto dimostrano che i lavoratori degli zuccherifici e i loro familiari rappresentano una categoria esposta all'azione cancerogena dell'amianto.

Per quanto riguarda le osservazioni di casi di mesotelioma in lavoratori di zuccherifici, la prima segnalazione è avvenuta negli anni '70 in India, quando furono descritti 4 casi di mesotelioma pleurico e 1 caso di mesotelioma pericardico in 3 operai di 35 anni d'età, la moglie di uno di questi, ed un chimico[25]. Data la latenza così breve, i mesoteliomi furono correlati all'inalazione di fibre vegetali derivanti dalle procedure di lavorazione della canna da zucchero, trascurando il fatto che in queste aree gli zuccherifici vengono costruiti e demoliti quasi annualmente e che si fa ampio sfruttamento del lavoro minorile, esponendo gli addetti fin da bambini.

In uno studio epidemiologico condotto in Louisiana (USA) su lavoratori esposti ad amianto presente in vari tipi di ambienti lavorativi, su 815 deceduti per cancro polmonare o mesotelioma, ben 58 erano impiegati nel comparto saccarifero[26].

Nel 1983 Malker pubblicò i dati di uno studio epidemiologico effettuato su una coorte di lavoratori degli zuccherifici svedesi in servizio nel 1960[27]. In questo studio furono riportati 7 casi di mesotelioma in lavoratori deceduti tra il 1960 e il 1979, dei quali 3 erano addetti alle caldaie, 2 erano stagionali addetti alla manutenzione di tubi e caldaie, oltre a un caposquadra che spesso partecipava allo smantellamento degli impianti, e un operaio specializzato nella demolizione. Il rischio relativo risultava aumentato di ben 11,3 volte.

Il caso di mesotelioma contratto nell'ambiente domestico, riportato nella nostra casistica, conferma i dati di studi condotti da altri autori[28-31]. Ciò mette in luce il pericolo insito nell'esposizione non lavorativa, ed evidenzia la necessità di applicare norme di prevenzione primaria non solo per ridurre i rischi negli ambienti di lavoro, ma anche per impedire che si verifichino contaminazioni dell'ambiente, in particolare di quello familiare che il più delle volte coinvolge bambini.

Come riportato precedentemente il bacino di lavoratori italiani coinvolti negli ultimi vent'anni nel comparto saccarifero è di circa 28.000 persone. Se però si considerano fra le persone potenzialmente esposte anche i familiari e i residenti in prossimità degli zuccherifici, spesso dismessi e non adeguatamente decontaminati, la dimensione della popolazione esposta è certamente più ampia.

Nell'immediato, oltre alle iniziative di prevenzione primaria, le azioni da intraprendere, nell'ambito di una strategia di controllo delle neoplasie da amianto, devono prevedere accurati studi di sorveglianza oncologica degli esposti, al fine di effettuare diagnosi precoci che permettano di avere ampi margini di intervento clinico, per quelle forme tumorali dove la precocità dell'intervento può prolungare di molto o addirittura salvare la vita del paziente. Questo può essere il caso dei carcinomi del cavo orale, della laringe, del polmone, del tratto gastroenterico e del rene.

Un approfondimento degli studi epidemiologici riguardanti il comparto saccarifero potrebbe inoltre permettere a un maggior numero di lavoratori esposti l'accesso al riconoscimento di malattia professionale e al conseguente risarcimento.

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Pathobiological features of breast tumours in the State of Kuwait: a comprehensive analysis

Caratteristiche biopatologiche dei tumori mammari nello Stato del Kuwait: un'analisi complessiva

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Summary

***Objectives*. Breast cancer accounts for 30.3% of all cancer types in Kuwaiti women. Death occurs in approximately 43% of these patients. Our goal was to conduct a comprehensive analysis of the pathobiological characteristics of the tumours in an attempt to determine any particular trend that could be present. *Patients and methods*. One hundred and sixty-six cases were included in this study. All the pathology reports and paraffin blocks pertaining to these cases were collected. Four micrometer sections were taken from each block, and immunostaining against Her-2, ER, and PgR was performed. Both the proportion and intensity of immunostaining were scored according to the Allred's method, and typing of the tumour was done according the WHO criteria regarding tumour classification. Grading of invasive carcinomas was done according to the modified Bloom-Richardson-Elston's method, and tumour stage was determined according to the criteria set by the American Joint Committee on Cancer. *Results*. The mean age of the patients below 55 years was 40, as compared to 68 for those above 55 (p <0.0001). More than half of the cases were in the right breast, and were surgically treated by total mastectomy with axillary clearance. The majority**

Riassunto

***Finalità*. I tumori della mammella costituiscono il 30,3% di tutti i tipi di cancro nelle donne del Kuwait. La morte avviene nel 43% circa di queste pazienti. Il nostro scopo è stato quello di condurre un'analisi esauriente delle caratteristiche biopatologiche dei tumori in modo da individuare qualsiasi particolare tendenza potesse essere presente. *Pazienti e metodi*. Sono stati inclusi nello studio 166 casi. Sono stati recuperati tutti i dati patologici ed i blocchetti di paraffina riguardanti questi casi. Da ciascun blocchetto sono state ricavate sezioni di 4 μm ed è stata eseguita una colorazione immunoistochimica contro Her-2, ER e PgR. La proporzione e l'intensità della colorazione sono state valutate secondo il metodo di Allred, e la tipizzazione dei tumori è stata fatta secondo i criteri del WHO riguardanti la classificazione dei tumori. Il grado dei carcinomi invasivi è stato stabilito secondo il metodo di Bloom-Richardson-Elston modificato, e lo stadio dei tumori è stato determinato secondo i criteri fissati dall'American Joint Committee on Cancer. *Risultati*. L'età media delle pazienti sotto i 55 anni era di 40 anni, rispetto ai 68 delle pazienti sopra i 55 anni (p <0,0001). Più della metà dei casi erano nella mammella destra, e furono trattati chirurgicamente con mastectomia totale e**

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of the tumours had irregular (stellate) margins, was invasive, and had a surrounding breast tissue of adenosis or fibrocystic type. Their mitotic index was 10-20 or >20 with a marked to moderate nuclear pleomorphism. They were mostly grade II or III, sized 2-5 or >5 cm, had absent or scanty tumour lymphocytes, and were stage II or III. The *in situ* tumours were mainly ductal carcinoma (DCIS) of which comedo and cribriform were the major histological subtypes. The major histological subtypes of the invasive tumours were ductal-not otherwise specified, lobular, and tubular/cribriform. In this study, we also found a significant ($p < 0.05$) association between overexpression of Her-2, lack of expression of ER and some of the characteristics mentioned above. *Conclusions*. Breast cancer in Kuwait seems to be more aggressive than what is currently seen in Europe, North America, Australia, and parts of Asia. Further investigations regarding the features observed in this study need to be performed. Eur. J. Oncol., 12 (2), 109-121, 2007

Key words: **breast cancer, pathobiological features, Kuwait**

svuotamento ascellare. La maggior parte dei tumori aveva margini irregolari (stellati), era invasiva, ed aveva un tessuto mammario circostante di tipo adenosico o fibrocistico. L'indice mitotico era 10-20 o >20 con un pleomorfismo nucleare da marcato a moderato. Erano per lo più di grado II o III, delle dimensioni di 2-5 o >5 cm, avevano linfociti tumorali scarsi o assenti, ed erano di stadio II o III. I tumori *in situ* erano soprattutto tumori duttali (DCIS), dei quali i sottotipi istologici più comuni erano il comedo ed il cribriforme. I sottotipi istologici più frequenti fra i tumori invasivi erano il duttale non altrimenti specificato, il lobulare ed il tubulare/cribriforme. In questo studio abbiamo trovato anche un'associazione significativa ($p < 0,05$) tra sovraespressione di Her-2, mancanza di espressione di ER e alcune delle caratteristich summenzionate. *Conclusioni:* I tumori della mammella nel Kuwait sembrano più aggressivi di quanto si vede abitualmente in Europa, Nord America, Australia e parti dell'Asia. Sono necessari ulteriori studi sugli aspetti osservati in questa ricerca. Eur. J. Oncol., 12 (2), 109-121, 2007

Parole chiave: **carcinoma mammario, caratteristiche biopatologiche, Kuwait**

Introduction

The Kuwaiti population is a growing one, and so is the incidence of cancer among the Kuwaitis. In females, the most common types of malignancies include breast cancer, followed by thyroid cancer, cervical uteri and colorectal carcinomas, and ovarian cancer. Breast cancer accounts for 30.3% of all cancer types, and death occurs in approximately 43% of patients[1].

The amount of research addressing breast carcinoma in Kuwait is still minimal, and nation-wide studies are still lacking. Accordingly, little is known so far about the disease. Based on our clinical encounters with breast cancer patients at Mubarak Al-Kabeer Hospital, which is one of the five major public hospitals in the State of Kuwait, we have noticed that these patients seem to be presenting at a relatively young age, with the disease at an advanced stage. Therefore, we sought to conduct a comprehensive analysis of the pathobiological characteristics of the tumours, in an attempt to provide the clinicians with a better picture about the biological behaviour of the tumours including any particular trend that could be present. This could hopefully help the clinicians better understand the disease manifestation in the Kuwaiti population, and, accordingly, help them develop a platform on which further disease investigation and clinical management strategies could be based.

In this study, we put much emphasis on carefully examining the pathobiological characteristics of the tumours. These characteristics included tumour margin, histological types and subtypes, tumour-surrounding breast tissue type, tumour grade, mitotic index, tumour size, nuclear pleomorphism, stage of the disease, lymphocyte involvement, breast laterality, nature of surgical management (operation performed), and degree of expression of the prognostic markers Herceptin-2 (HER-2, also known as C-erb B-2 or Her-2/neu), oestrogen receptor (ER), and progesterone receptor (PgR). We also investigated any possible significant association between each of the above characteristics and the degree of expression of Her-2, ER, and PgR.

Materials and methods

One hundred and sixty-six breast cancer cases, seen at Mubarak Al-Kabeer Hospital, were reviewed. All the pathology reports and the H-E slides pertaining to these

cases were collected. We selected the cases where complete information regarding the patient and the tumour were available. The Mubarak Al-Kabeer Hospital provides annual health care to around 750,000 people living in the Mubarak Al-Kabeer, Hawally, Salmiya and Jabriya districts of Safat. The study was approved by the Human Ethics Committee at the Faculty of Medicine, Health Science Centre, Kuwait University, and it conformed to the provisions of the Declaration of Helsinki.

For immunohistochemical staining, the following standard procedure was followed[2]. Sections were cut at 4 μm thickness, mounted onto silane-coated slides (S21.1910.110, Novocastra, Newcastle upon Tyne, UK), and left to dry overnight at 37°C. They were then deparaffinized, re-hydrated, and underwent antigen retrieval (Epitope Retrieval PH6, RE7115, Novocastra) by microwaving (Daewoo KOR-161G, 1000W, 2450 MHz, 10 power levels, Seoul, South Korea) for 20 minutes. After cooling down to room temperature, the sections were incubated for 15 minutes with 3% hydrogen peroxide to block any endogenous peroxidase activity, washed with TBS, and incubated with goat serum (NCL-G-SERUM, Novocastra) for 20 minutes to block any non-specific staining. They were then washed with TBS, and incubated with 200 μl of primary antibodies for 1 hour at 37°C in a humidified rotator. The dilution of the primary antibody against Her-2 (Clone 10A7, NCL-CBE-356, Novocastra) was 1:40, while those of ER (Clone 6F11, NCL-ER-6F11, Novocastra) and PgR (Clone 16, NCL-PgR-312, Novocastra) were 1:40 and 1:100, respectively. The sections were then washed with TBS, and incubated with the secondary link antibody (Novolink Max RE7280-K, Novocastra) for 30 minutes. This was followed by washing with TBS and incubation with the tertiary antibody (Novolink Max RE7280-K, Novocastra) for 30 minutes. Finally, the sections were washed with TBS and incubated for 10 minutes with DAB (Novolink Max RE7280-K, Novocastra) chromogen contrasted with haematoxylin counterstain. Positive and negative control slides were used in each staining experiment. The positive controls were breast carcinomas known to be positive for Her-2, ER, and PgR. The negative controls included sections of breast carcinomas known to be negative for Her-2, ER, and PgR, and sections taken from the same tissue block but incubated with antibody diluent instead of the primary antibody.

The scoring system developed by Allred *et al*[2] in relation to ER, PgR, and Her-2 staining was followed. Accordingly, both the proportion and intensity of staining are taken into consideration. A proportion score indicated the proportion of positive tumour cells on the entire slide, and it ranged from 0 to 5. An intensity score indicated the average staining intensity of positive tumour cells, and it ranged from 0 to 3. Both scores were then added, to obtain a total score ranging from 0 to 8. This scoring system has been followed in numerous experiments and has resulted in an inter- and intra- observer reproducibility of more than 90%[2]. Based on this scoring system, any total score between 6 and 8 was considered overexpression in our study. If the total score was 0, the tumour was considered lacking expression.

The tumours were typed according to the WHO classification system[3]. Although it is beyond the scope of this manuscript to provide details of such classification, we will summarize the major points. To start with, such classification includes invasive *versus* non-invasive breast carcinomas, based on the infiltration of the basal membrane by the tumour cells. The non-invasive carcinomas are classified as ductal carcinoma *in situ* (DCIS) or lobular carcinoma *in situ* (LCIS), based on the involvement of the ducts or the lobules forming the breast tissue. The DCIS tumours include the following subtypes based on the morphology: comedo, cribriform, solid, papillary, micropapillary, and apocrine. The invasive tumours are further classified as ductal-not otherwise specified, lobular, tubular/cribriform, colloid (mucinous), medullary, papillary, comedo, Paget's disease, adenoid, and apocrine.

The modified Bloom-Richardson-Elston histological system was used for grading the invasive carcinomas[4]. This aims at examining the growth pattern as well as the biological characteristics of differentiation of these carcinomas. Accordingly, the following criteria were used:

1) Formation of the gland tubules and acini
   a) characteristic formation of tubules (>75%) = 1 point
   b) moderate formation of tubules (10-75%) = 2 points
   c) little or without tubules at all (<10%) = 3 points;
2) Pleomorphism of cancer cell nuclei (abnormalities in size, structure, and shape)
   a) isomorphism of nuclei or small variability in shape, size, and structure of nuclei = 1 point
   b) moderate variability in shape, size, and structure of nucleus = 2 points
   c) marked characteristic polymorphism = 3 points;
3) Mitosis per 10 high power fields
   a) <10 mitosis = 1 point
   b) 10-20 mitosis = 2 points
   c) >20 mitosis = 3 points.

By adding up the points of the above criteria, the level of tumour differentiation could then be calculated as follows: grade I (well differentiated; 3-5 points), grade II

(moderately differentiated; 6-7 points), grade III (poorly differentiated; 8-9 points).

As far as staging the carcinomas is concerned, we followed the criteria set by the American Joint Committee on Cancer[5] and which include briefly:

Stage 0: LCIS or DCIS;

Stage I: invasive carcinoma ≤2 cm in diameter without nodal involvement;

Stage II: invasive carcinoma ≤5 cm in diameter with up to three involved axillary nodes, or invasive carcinoma >5 cm without nodal involvement;

Stage III: invasive carcinoma ≤5 cm in diameter with four or more involved axillary nodes, or invasive carcinoma >5 cm in diameter with nodal involvement, or invasive carcinoma with ≥10 involved axillary nodes, or invasive carcinoma with involvement of the ipsilateral internal mammary lymph nodes, or invasive carcinoma with skin involvement, chest wall fixation, or clinical inflammatory carcinoma;

Stage IV: any distant metastases.

The common standard international approach among pathologists for measuring tumour size was followed in this study. Accordingly, we used the largest diameter of the cut surface of the tumour, and we made much of the diameter of the invasive component. Therefore, we made use of the largest size of the invasive component on the cut surface.

Statistical analysis

The Student's t-test was used for comparison of patient age. The chi square (χ^2) test was used to compare the association of expression of Her-2, ER, and PgR and the macroscopic and microscopic characteristics of the tumours. The results were considered statistically significant if the p value was <0.05.

Results

Age and association with Her-2, ER, and PgR expression (Tables 1, 2)

The age-world-standardized incidence rate of breast cancer in Kuwaitis is 31.8, which is higher than that of neighbouring Gulf countries such as the United Arab Emirates (24.1) and Saudi Arabia (24.7)[1]. Our results showed that 68.1% of the patients were between the age of 30 to 55 years, 29.5% above 55 years, and 2.4% below 30 years. The mean age of the patients below 55 years was 40, as compared to 68 for those above 55 (p <0.0001).

Table 1 - Age-world-standardized incidence rate (ASR(W)) of breast cancer in Kuwait as compared to neighbouring Gulf countries[a], and age distribution of breast cancer in Kuwait according to our study

Incidence (ASR(W)) in neighbouring Gulf countries				
Bahrain	Qatar	Kuwait	UAE	Saudi Arabia
40.2	33.3	31.8	24.1	24.7

Age distribution in Kuwait cases (N. 166 patients)		
Age distribution	N.	% frequency
30-55 years	113	68.1
>55 years	49	29.5
<30 years	4	2.4
Age distribution	Mean age	p value
< 55 years	40 years	<0.0001
> 5 years	68 years	

[a] From Ferlay *et al*[1]

There was a significant association between overexpression of Her-2 and age of the patients, whereby 78.8% of the patients aged 30-55 years and 75% of the patients aged less than 30 years were Her-2 positive (p = 0.0003). A similar age trend but with different expression was observed with ER expression, since 81.4% of the patients aged 30-55 years and 75% of those aged less than 30 years were ER negative (p <0.0001). Patients aged above 55 years were more ER positive (61.2%). On the other hand, no significant association between the different age groups and PgR expression was observed (p = 0.36).

Pathobiological characteristics and association with Her-2, ER, and PgR expression (Tables 2, 3)

The percentage distribution of cancer lesions did not differ between the right (53.6%) and left (42.2%) breasts, but 87.6% of the ones in the right breast were significantly associated with overexpression of Her-2 (p <0.0001). A similar association was seen with lesions present in both the right and left breasts, but the number was too small (n = 7) to deduce any conclusions. Also, six out of seven cases where the cancer lesion was present in both breasts were ER negative, but, again, the number was too small to make any comments.

Various types of surgical management were seen in our study, but the most common ones were total mastectomy with axillary clearance (53.6%) and total lumpectomy without axillary clearance (29.5%). The majority of the former were Her-2 positive (94.4%), ER negative (87.6%), and PgR negative (84.3%), and a significant association was found (p <0.0001). A similar trend was seen in patients who underwent total lumpectomy with axillary

Table 2 - Association of Her-2, ER, and PgR expression and age of the patients and the pathobiological characteristics of the breast tumours[a]

	Her-2+ve		Her-2-ve		ER+ve		ER-ve		PgR+ve		PgR-ve	
	N.	%	N.	%	N.	%	N.	%	N.	%	N.	%
Age of the patients												
< 30	3*	75	1	25	1	25	3*	75	2	50	2	50
30-55	89*	78.8	24	21.2	21	18.6	92*	81.4	53	46.9	60	53.1
> 55	23	46.9	26	53.1	30	61.2	19	38.8	29	59.1	20	40.8
Pathobiological characteristics of the tumours												
Tumour location												
Right breast	78*	87.6	11	12.4	45	50.6	44	49.4	40	44.9	49	55.1
Left breast	32	45.7	38	54.3	34	48.6	36	51.4	33	47.1	37	52.9
Both	7	100	0	0	1	14.3	6	85.7	3	42.9	4	57.1
Margins												
Irregular (stellate)	130*	89	16	11	26	17.8	120*	82.2	70	47.9	76	52
Defined (demarked)	9	45	11	55	10	50	10	50	11	55	9	45
Operation performed												
Total mastectomy with axillary clearance	84*	94.4	5	5.6	11	12.4	78*	87.6	14	15.7	75*	84.3
Total lumpectomy without axillary clearance	25	51.0	24	49.0	27	55.1	22	44.9	26	53.1	23	46.9
Total mastectomy without axillary clearance	10	58.8	7	41.2	8	47.1	9	52.9	9	52.9	8	47.1
Total lumpectomy with axillary clearance	7*	100	0	0	2	28.6	5*	71.4	1	14.3	6*	85.7
Total quadrantectomy	2	50	2	50	2	50	2	50	2	50	2	50
Type												
Invasive carcinoma	120*	87	18	13	28	20.3	110*	79.7	40	29	98*	71
Non-invasive carcinoma	2	7.1	26*	92.9	24*	85.7	4	14.3	20*	71.4	8	28.6
Surrounding breast tissue												
Adenosis	40	47.1	45	52.9	42	49.4	43	50.6	46	54.1	39	45.9
Fibrocystic	65*	89.0	8	11.0	13	17.8	60*	82.2	31	42.5	42	57.5
Normal	0	0	6	100	1	16.7	5	83.3	3	50	3	50
Papillomatous	1	50	1	50	1	50	1	50	1	50	1	50
Mitotic index												
<10	6	21.4	22*	78.6	19*	67.9	9	32.1	20*	71.4	8	28.6
10-20	79*	86.8	12	13.2	14	15.4	77*	84.6	51	56.0	40	44.0
>20	42*	89.4	5	10.6	7	14.9	40*	85.1	26	55.3	21	44.7
Nuclear pleomorphism												
Marked	76*	90.5	8	9.5	9	10.7	75*	89.3	44	52.4	40	47.6
Moderate	30	46.9	34	53.1	36	56.2	28	43.7	33	51.6	31	48.4
Small	4	22.2	14*	77.8	12*	66.7	6	33.3	11*	61.1	7	38.9
Grade												
I	1	5.9	16*	94.1	14*	82.3	3	17.6	10	58.8	7	41.2
II	78*	92.9	6	7.1	4	4.8	80*	95.2	40	47.6	44	52.4
III	57*	87.7	8	12.3	10	15.4	55*	84.6	33	50.8	32	49.2
Size (cm)												
<2	7	28	18*	72	20*	80	5	20	14	56	11	44
2-5	75*	84.3	14	15.7	17	19.1	72*	80.9	29	32.6	60*	67.4
>5	48*	92.3	4	7.7	6	11.5	46*	88.5	12	23.1	40*	76.9

(footnotes next page)

(to be continued on the next page)

Table 2 - continued

	Her-2+ve		Her-2-ve		ER+ve		ER-ve		PgR+ve		PgR-ve	
	N.	%	N.	%	N.	%	N.	%	N.	%	N.	%
Tumour lymphocytes												
Absent	26	45.6	31	54.4	29	50.9	28	49.1	35	61.4	22	38.6
Scanty	20	46.5	23	53.5	25	58.1	18	41.9	24	55.8	19	44.2
Multifocal outside the tumour	8	44.4	10	55.6	9	50	9	50	11	61.1	7	38.9
Band outside the tumour	6	46.1	7	53.8	8	61.5	5	38.5	9	69.2	4	30.8
Multifocal within the tumour	5	45.4	6	54.5	7	63.6	4	36.4	6	54.5	5	45.4
Diffuse outside the tumour	7	63.6	4	36.4	6	54.5	5	45.4	7	63.6	4	36.4
Diffuse within the tumour	3	42.9	4	57.1	3	42.9	4	57.1	5	71.4	2	28.6
Band within the tumour	5	83.3	1	16.7	1	16.7	5	83.3	3	50	3	50
Stage												
I	6	21.4	22*	78.6	19*	67.9	9	32.1	16	57.1	12	42.9
II	34	51.5	32	48.5	30	45.4	36	54.5	28	42.4	38	57.6
III	39*	81.2	9	18.7	7	14.6	41*	85.4	22	45.8	26	54.2
IV	21*	87.5	3	12.5	6	25	18*	75	14	58.3	10	41.7
Histological types of non-invasive carcinoma												
Ductal carcinoma *in situ* (DCIS)	18*	78.3	5	21.7	7	30.4	16*	69.6	11	47.8	12	52.2
Lobular carcinoma *in situ* (LCIS)	3	60	2	40	2	40	3	60	3	60	2	40
Histological subtypes of ductal carcinoma in situ (DCIS)												
Comedo	9*	81.8	2	18.2	3	27.3	8*	72.7	6	54.5	5	45.4
Cribriform	5*	83.3	1	16.7	1	16.7	5*	83.3	3	50	3	50
Solid	1	50	1	50	1	50	1	50	2	100	0	0
Papillary	0	0	2	100	2	100	0	0	1	50	1	50
Micropapillary	0	0	1	100	1	100	0	0	0	0	1	100
Apocrine	0	0	1	100	1	100	0	0	1	100	0	0
Histological subtypes of invasive carcinoma												
Ductal- not otherwise specified	89*	89.9	10	10.1	13	13.1	86*	86.9	44	44.4	55	55.6
Lobular	13*	92.9	1	7.1	2	14.3	12*	85.7	8	57.1	6	42.9
Tubular/cribriform	3	23.1	10*	76.9	11*	84.6	2	15.4	5	38.5	8*	61.5
Colloid (mucinous)	0	0	2	100	1	50	1	50	1	50	1	50
Medullary	2	100	0	0	0	0	2	100	1	50	1	50
Papillary	1	50	1	50	1	50	1	50	2	100	0	0
Comedo	2	100	0	0	0	0	2	100	1	50	1	50
Paget's disease	0	0	1	100	0	0	1	100	0	0	1	100
Adenoid	0	0	1	100	1	100	0	0	1	100	0	0
Apocrine	0	0	1	100	1	100	0	0	1	100	0	0

[a] Total number of cases = 166

* p-value <0.05

clearance, but the total number in this group did not exceed seven cases. No significant association was observed in patients who underwent total lumpectomy without axillary clearance and Her-2, ER, and PgR expression.

The tumour margins studied were irregular (stellate), and defined (demarked). The former accounted for 87.9% of all the breast cancer cases, and it was associated with overexpression (89%) of Her-2 ($p < 0.0001$) and with negative expression (82.2%) of ER ($p = 0.0011$). No association was seen with PgR expression ($p = 0.55$).

Our results showed that 83.1% of the tumours were invasive as compared to 16.9% of *in situ* tumours. The invasive tumours were predominantly Her-2 positive (87%), and ER (79.7%) and PgR negative (71%) ($p < 0.0001$). On the other hand, the *in situ* tumours were predominantly Her-2 negative (92.9%), and ER (85.7%) and PgR (71.4%) positive. The *in situ* tumours were either ductal or lobular. The ductal ones significantly overexpressed Her-2 and were ER negative ($p < 0.001$).

Table 3 - Pathobiological characteristics of the tumours with data arranged by decreasing order of percentage[a]

Pathobiological characteristics	Cases	
	N.	(% frequency)
Tumour location		
Right breast	89	53.6
Left breast	70	42.2
Both	7	4.2
Margins		
Irregular (stellate)	146	87.9
Defined (demarked)	20	12.0
Operation performed		
Total mastectomy with axillary clearance	89	53.6
Total lumpectomy without axillary clearance	49	29.5
Total mastectomy without axillary clearance	17	10.2
Total lumpectomy with axillary clearance	7	4.2
Total quadrantectomy	4	2.4
Type		
Invasive carcinoma	138	83.1
Non-invasive carcinoma	28	16.9
Surrounding breast tissue		
Adenosis	85	51.2
Fibrocystic	73	44.0
Normal	6	3.6
Papillomatous	2	1.2
Mitotic index		
10- 20	91	54.8
>20	47	28.3
<10	28	16.9
Nuclear pleomorphism		
Marked	84	50.6
Moderate	64	38.5
Small	18	10.8
Grade		
II	84	50.6
III	65	39.2
I	17	10.2
Size (cm)		
2-5	89	53.6
>5	52	31.3
<2	25	15.1
Tumour lymphocytes		
Absent	57	34.3
Scanty	43	25.9
Multifocal outside the tumour	18	10.8
Band outside the tumour	13	7.8
Multifocal within the tumour	11	6.6
Diffuse outside the tumour	11	6.6
Diffuse within the tumour	7	4.2
Band within the tumour	6	3.6
Stage		
II	66	39.8
III	48	28.9
I	28	16.9
IV	24	14.5
Histological type of non-invasive carcinoma		
Ductal carcinoma *in situ* (DCIS)	23	82.1
Lobular carcinoma *in situ* (LCIS)	5	17.9
Histological subtype of ductal carcinoma in situ (DCIS)		
Comedo	11	47.8
Cribriform	6	26.1
Solid	2	8.7
Papillary	2	8.7
Micropapillary	1	4.3
Apocrine	1	4.3
Histological subtype of invasive carcinoma		
Ductal- not otherwise specified	99	71.7
Lobular	14	10.1
Tubular/cribriform	13	9.4
Colloid (mucinous)	2	1.4
Medullary	2	1.4
Papillary	2	1.4
Comedo	2	1.4
Paget's disease	2	1.4
Adenoid	1	0.7
Apocrine	1	0.7

[a] Total number of cases = 166

The breast tissue type surrounding the tumour was either adenosis (51.2%), fibrocystic (44%), normal (3.6%), or papillomatous (1.2%). Eighty nine percent of the tumours which were surrounded by fibrocystic tissue overexpressed Her-2 ($p < 0.0001$) and were ER negative ($p = 0.0004$). On the other hand, 100% and 83.3% of the normal type were Her-2 and ER negative respectively. No significant association was observed with PgR expression ($p = 0.54$).

Our results showed that 54.8% of the tumours had a mitotic index of between 10 and 20, 28.3% above 20, and 16.9% below 10. Most of those with a mitotic index between 10 and 20 (86.8%) and above 20 (89.4%) over-expressed Her-2 ($p<0.0001$), and 84.6% and 85.1% were ER negative respectively ($p<0.0001$). Most of the ones with a mitotic index below 10 were Her-2 negative (78.6%) and ER positive (67.9%). No significant association was observed between the mitotic index characteristic

and the degree of expression of PgR by the tumours ($p = 0.31$).

A marked nuclear pleomorphism was seen in 50.6% of the cases we examined. Such pleomorphism was either moderate or small in the remaining cases (38.5% and 10.8% respectively). The marked nuclear pleomorphism was significantly associated with overexpression of Her-2 (90.5%; $p<0.0001$) and with negative ER expression (89.3%; $p<0.0001$). No significant association was noticed with PgR expression ($p = 0.76$). As for the cases where nuclear pleomorphism was small, 77.8% were Her-2 negative and 66.7% were ER positive.

As far as tumour grade is concerned, 50.6% of the tumours were grade II, 39.2% grade III, and 10.2% grade I. The grade II and III tumours predominantly (92.9% and 87.7% respectively) overexpressed Her-2 and were ER negative (95.2% and 84.6% respectively). Grade I tumours were mostly Her-2 negative (94.1%) and ER positive (82.3%). A significant association between tumour grade and expression of Her-2 ($p <0.0001$) and ER ($p <0.0001$) but not PgR ($p = 0.69$) was observed.

The size of the tumour was between 2 and 5 cm in 53.6% of the cases studied. The remaining cases had a tumour size of more than 5 cm (31.3%) and less than 2 cm (15.1%). Similarly to the tumour grade and mitotic index indicated above, most of the cases with a tumour size between 2 and 5 cm (84.3%) and more than 5 cm (92.3%) overexpressed Her-2 ($p <0.0001$) and were ER negative (80.9% and 88.5% respectively; $p <0.0001$). In contrast to tumour grade and mitotic index, most of these cases were also PgR negative (67.4% and 76.9% respectively; $p = 0.016$). Tumours smaller than 2 cm were mostly Her-2 negative (72%) and ER positive (80%).

We examined the lymphocytes in the vicinity of, or inside the tumour, in an attempt to associate their presence/absence with Her-2, ER, or PgR expression. We classified the lymphocytes as follows: absent, scanty, multifocal outside the tumour, multifocal inside the tumour, band outside the tumour, band inside the tumour, diffuse outside the tumour, and diffuse inside the tumour. Most of the tumours had lymphocytes either absent (34.3%), scanty (25.9%), or multifocal outside the tumour (10.8%). Our results did not show any significant association between the above classification and Her-2 ($p = 0.73$), ER ($p = 0.66$), and PgR ($p = 0.98$) expression.

The most common tumour stage was stage II (39.8%), followed by stage III (28.9%), stage I (16.9%), and stage IV (14.5%). Our results showed that overexpression of Her-2 and lack of ER expression were mostly seen with stages III and IV, whereby 81.2% and 85.4% of stage III tumours overexpressed Her-2 and were ER negative respectively ($p <0.0001$), and 87.5% and 75% of stage IV tumours overexpressed Her-2 and were ER negative respectively ($p <0.0001$). No significant association was observed with PgR expression ($p = 0.41$). Stage I tumours were mostly Her-2 negative (78.6%) and ER positive (67.9%).

The histological subtypes of *in situ* ductal tumours included comedo (47.8%), cribriform (26.1%), solid (8.7%), papillary (8.7%), micropapillary (4.3%), and apocrine (4.3%). Most of the comedo (81.8%) and cribriform (83.3%) subtypes overexpressed Her-2, and a significant association was found ($p = 0.004$). A similar trend was seen with ER expression, whereby 72.7% of the comedo and 83.3% of the cribriform subtypes were ER negative ($p = 0.013$). No significant association was found between the above histological subtypes and PgR expression ($p = 0.80$).

The histological subtypes of invasive tumours included ductal-not otherwise specified, lobular, tubular/cribriform, colloid (mucinous), medullary, papillary, comedo, Paget's disease, adenoid, and apocrine. The most common histological subtypes were ductal-not otherwise specified (71.7%), followed by lobular (10.1%), and tubular/cribriform (9.4%). The ductal-not otherwise specified and lobular tumours were predominantly Her-2 positive (89.9% and 92.9% respectively) and ER negative (86.9% and 85.7% respectively). The tubular/cribriform tumours were predominantly Her-2 negative (76.9%), ER positive (84.6%), and PgR negative (61.5%). The χ^2 test showed a significant association between the most common histological subtypes mentioned above and the expression of Her-2 and ER ($p <0.0001$, and $p = 0.0005$ respectively). No significant association was found with PgR expression ($p = 0.23$).

Discussion

The current trend in analyzing the clinical outcome of a patient with breast cancer is to examine predictive and prognostic factors related to the patient and her tumour. The former are related to the degree to which the patient could respond to a specific therapy, while the latter are related to the metastatic potential of the tumour. Several studies have examined predictive and prognostic factors, such as the age of the patient, tumour size, grade, proliferation, hormonal status, histological type of the tumour, and lymph node involvement, to name a few[6-11]. With the advancement in science and technology, molecular markers have been added to the above list, in an attempt to help clinicians better monitor the course of the disease and predict its outcome[10]. In this study, we conducted a comprehensive analysis of breast carcinomas taken from

166 patients in Kuwait. We limited our study to a sample size of 166 because these were the only cases for which we had complete information about the patient and the tumour. Also, these were the only cases whose paraffin blocks had enough tissue in them so that taking extra sections from these blocks for our study did not jeopardize the amount of tissue remaining in them in case of future examination.

In contrast to what is commonly known about a rising incidence of breast cancer with age, our results showed that 70.5% of the patients we examined were young with an age not exceeding 55 years. The mean age of these patients was 40, and the majority of them were between the ages of 30 and 55. This age distribution is significantly younger than what is currently seen in Western and Arab countries[1, 11], and requires further careful examination to determine the nature of the predisposing factor(s). One possible explanation is that traditional marriages among first-degree relatives in Kuwait are very common, and, accordingly, hereditary factors could play a major rôle. Another factor could be the degree of obesity associated with a diet high in fat, carbohydrate, and protein, and lack of exercise, which have been prevalent in Kuwait for the past 20 years. We are in the process of conducting a comprehensive study at the Mubarak Al-Kabeer Hospital, in order to examine the above and some other predisposing factors. An interesting finding in this study is that the carcinomas from the patients in the above age category predominantly overexpress Her-2. These results confirm those obtained from studies where an association between age of breast cancer patients and their tumour overexpression of Her-2 was found[12-15]. Other studies, however, did not find any significant association between the former and the latter[16, 17]. We also found that there is a significant association between the nature of the tumours' expression of ER and the age of the patients. Patients with ER negative tumours were mostly young (< 30 years and between 30 and 55 years), as compared to positive ER expression in patients aged above 55 years.

Studies where an association between the nature of breast carcinomas' expression of ER and age of the patients was found have been documented. A recent study conducted by Jalava *et al*[18] reported a significant association between the former and the latter. Ferno *et al*[19] reported a lower expression of ER in patients below 50 years old, and Quong *et al*[20] found that the expression of ER by breast carcinomas increases with age. Similar findings to Quong were observed by Holdaway and Mountjoy[21], Clark *et al*[22], Wilking *et al*[23], Gaskell *et al*[24], Rhodes *et al*[25], and Tominaga *et al*[26]. Other studies have, however, found no association between the age of the patient and the degree of expression of ER by the tumour[27].

In our study, we did not find any significant association between the age of the patients and their tumour expression of PgR. Similar findings were reported by Holdaway and Mountjoy[21], Clark *et al*[22] and Wilking *et al*[23]. Other studies, however, have reported a higher tumour expression of PgR in patients older than 59 years, as compared to those between 50 and 59 years[19]. The overexpression of Her-2 and lack of ER expression by the tumours of the patients aged below 55 years in our study might explain the high mortality rate reported earlier[1] among these patients, since such tumours often become resistant to adjuvant and hormonal therapies.

Other characteristics that we examined were the margins of the tumours, laterality (right *versus* left breast), and the type of surgical management. Tumour margins often represent a reliable source of positive or negative disease outcome. In our study, the margins of the tumours were mostly irregular (stellate). Having an irregular (stellate) margin means that the tumour is not confined and there is a potential for metastasis. This is further confirmed by the degree of malignancy of the tumours, which was significantly associated in our study with bad prognostic markers such as overexpression of Her-2 and negative expression of ER. Irregular (stellate) margins did not associate with PgR expression. An association between the tumour margin status and Her-2, ER, or PgR expression has been investigated in several studies[28-42]. Putti *et al*[29] recently demonstrated that breast tumours with a pushing margin (another terminology for irregular or stellate margin) were found to be ER negative and overexpressing Her-2. A similar association with Her-2 overexpression was reported by Miller *et al*[38]. The circumscription of the tumour margin was significantly associated with negative PgR expression in a study conducted on 281 women with breast cancer in Finland[30]. In another study conducted on 980 patients with breast cancer, and in which the patients were divided into three age categories (≤35 years, 36-50 years, and >50 years), Fowble *et al*[42] reported that young patients had significantly more association between tumour margin status and negative ER expression. Unlike our results, Kim *et al*[37] reported an association between tumour margin and positive rather than negative ER expression: moreover, the authors found an association with positive PgR expression. Lack of an association between the tumour margin and hormone receptor status was reported in a study conducted on 254 patients undergoing partial mastectomy[35]. Similarly, Horiguchi *et al*[31, 33] found no association with tumour expression of ER.

We also took breast laterality into consideration. In our

study, the number of carcinomas present in the right breast was slightly more than in the left one (53.6% *versus* 42.2%). Such tumour laterality was significantly associated with Her-2 overexpression, but not with ER or PgR expression. However, since the difference in the location of the carcinomas (right *versus* left breast) was not significant, we prefer not to deduce, at this stage, any conclusions in relation to the expression of the above markers. The small marginal difference in relation to the location of the tumour in the right *versus* left breast in our study was similar to the one reported by Largent *et al*[43]. When analyzing the demographic and tumour characteristics of early breast cancer patients, the authors found that 52% of the carcinomas were present in the right breast as compared to 48% in the left one. The only studies that we found in the literature, which attempted to find an association between breast laterality and hormone receptor expression, were the ones conducted by Tominaga *et al*[26] and by Borisenkov and Bazhenov[44]. Borisenkov and Bazhenov reported that the degree of expression of hormone receptors by breast carcinomas taken from Russian patients significantly depended on whether the tumour was present in the right or left breast[44]. On the other hand, such an association was lacking in the study conducted by Tominaga *et al*[26] on Japanese women with breast cancer. In our study, the percentage of patients who underwent total mastectomy with axillary clearance was the highest. Interestingly, there was a significant association between this type of surgical management and the tumour expression of Her-2, ER, and PgR. This association was also seen in the patients who underwent total lumpectomy with axillary clearance. The carcinomas of the patients in both surgical categories predominantly overexpressed Her-2, and were mostly ER and PgR negative. According to our knowledge, this is the first time where such an association has been reported.

The histological characteristics of breast carcinomas have been investigated in several studies trying to correlate the histological type/subtype of the tumour with the disease outcome such as local recurrence, site of recurrence (ipsilateral *versus* bilateral), metastasis (regional *versus* distant), and response to therapy. For instance, even earlier reports have shown that the 30-year survival rate of women with certain histological types of breast cancer such as tubular or lobular is greater than 60%, as compared with less than 20% for women with breast cancer of no special type[45]. Chen *et al*[46] have demonstrated that breast cancer of lobular histological type is more often bilateral when compared to other types.

In our study, we conducted a comprehensive histological analysis of the breast tumours. Our results showed that 83.1% of the carcinomas were invasive. Eighty two percent of the *in situ* carcinomas were ductal, and they were predominantly comedo or cribriform. The invasive carcinomas were mostly ductal-not otherwise specified, lobular, or tubular/cribriform, and the breast tissue type surrounding the carcinoma was predominantly adenosis or fibrocystic. Our results are similar to those reported by Andersson *et al*[47], where the incidence of *in situ* breast carcinoma was 16%, in contrast to a higher incidence (26%) reported by May *et al*[48]. When we analyzed the above parameters in relation to the tumour expression of Her-2, ER, and PgR, we found that the invasive carcinomas predominantly overexpressed Her-2 and were mostly ER and PgR negative. On the other hand, the *in situ* tumours were mostly Her-2 negative, and ER and PgR positive. This provides more evidence to the hypothesis that aggressive tumours seem to lack hormonal receptors and to overexpress Her-2. Our results are similar to those reported by Zafrani *et al*[49], where 81% of the *in situ* tumours the authors examined were ER positive and 73% were PgR positive. We also found an association between some histological subtypes of the *in situ* ductal tumours and Her-2, ER, and PgR expression, whereby the comedo and cribriform subtypes significantly overexpressed Her-2 and were ER negative. This is similar to the findings reported by Janssens *et al*[50] and by Provenzano *et al*[51], and confirms previous reports that showed lack of ER expression in comedo histological subtype of ductal carcinoma *in situ*[27]. As far as the histological subtypes of invasive carcinoma are concerned, we found that the most common subtypes were ductal-not otherwise specified, lobular, and tubular/cribriform. This confirms the WHO classification of invasive breast carcinomas in relation to the percentage occurrence of these subtypes[3]. The ductal-not otherwise specified carcinomas overexpressed Her-2 and were predominantly ER negative. This again shows the association between the nature of the biological expression of Her-2 and ER by the tumour and its degree of malignancy, since it has been argued that ductal-not otherwise specified carcinomas are the most aggressive type of breast cancer. This is based on the fact that its tumour cells are often seen infiltrating into the surrounding tissue, including perivascular and perineural spaces, as well as lymphatic and blood vessels. We also found a significant association between Her-2 overexpression and lack of ER expression and the lobular histological subtype. This could imply that, although this histological subtype of invasive breast carcinoma is less common than the ductal-not otherwise specified one, lobular carcinomas could be equally aggressive. Various and sometimes contradictory reports have been published in the literature regarding the association between the

expression of Her-2 and hormonal receptors and the various histological subtypes of invasive breast carcinomas. In a recent study conducted by Jalava *et al*[18], the breast carcinomas' ER expression was found to be greater in lobular than in ductal tumours. Other authors have reported that the mucinous type is associated with an increase in the expression of ER and with a decrease in the expression of Her-2[52]. Similarly, Her-2 was found to be inversely associated with ER status based on the histological type in a study conducted by Coradini *et al*[53]. Still, other studies confirmed a lack of an association between the histological type of breast tumour and its hormone receptor status[54].

As far as other tumour characteristics are concerned, we have noticed that tumours with a surrounding breast tissue that is fibrocystic in nature commonly overexpressed Her-2. Whether there is a direct biological association between the former and the latter remains to be investigated.

Conclusions

Our above findings showed so far that tumour characteristics in Kuwaiti patients are rather aggressive. In order to further analyze the profile of these tumours, we examined some important tumour-related prognostic factors, such as grade, mitotic index, size, nuclear pleomorphism, stage, and lymphocytes. The majority of our patients presented with grade II or III tumours. These two grades were Her-2 positive and ER negative, in contrast to grade I tumours which were Her-2 negative and ER positive. This confirms the results of the study conducted by Keshgegian[15], where Her-2 overexpression was found to correlate with higher tumour grade. Our results are also similar to those reported by Armes *et al*[55, 56], where tumour grade was found to correlate with overexpression of Her-2 and with negative ER expression. However, our finding is not in agreement with the studies where a weak or no association was found between the grade of the tumour and its expression of Her-2, ER and/or PgR[15, 22, 27, 54]. Interestingly, the same significant trend that we observed in grade II and III tumours, in relation to association with overexpression of Her-2 and negative ER expression, was also seen in tumours with a mitotic index of between 10 and 20 and above 20, and in tumours sized between 2 and 5 cm and above 5 cm. Similarly, tumours with marked nuclear pleomorphism and those in stages III and IV overexpressed Her-2 and were ER negative. We did not find significant association between Her-2, ER, and PgR expression and the nature of presence or absence of tumour lymphocytes. The literature has cited controversial findings in relation to a possible association between Her-2, ER, and PgR tumour expression and some of the above prognostic parameters. Some studies have reported that the expression of Her-2 and ER does not associate with the size of the tumour[15, 54], whereas others reported a significant association with ER expression[21, 22, 27]. For instance, the expression of ER was found to be higher in small breast tumours, and to significantly decrease in tumours above 5 cm[21, 27]. Our study showed that tumours which were above 5 cm in size were ER negative, and so were those sized between 2 and 5 cm. The high mitotic index that we observed, and which was associated with both Her-2 overexpression and negative ER, reflected a poor prognosis in our patients. Several studies have reported similar association[15, 18, 27]. Finally, our results do not confirm earlier studies in which the expression of ER was associated with the nature of the presence of lymphocytes in the vicinity of the tumour[57, 58]. We chose to examine the nature of the presence of tumour lymphocytes as one of the tumour characteristics that we examined in this study, since such a characteristic could provide an insight into the immunological status of the tumour. The observations reported in some types of tumours such as melanomas, which share with breast carcinoma their epithelial origin, have shown that tumours infiltrated by lymphocytes respond better to therapy, as compared to those where the lymphocytes are present outside the tumour (being in clusters or as a band), and as compared to those where the lymphocytes are absent[59, 60]. According to our study, the expression or lack of expression of Her-2, ER, and PgR does not seem to have a direct effect on the nature of the location of lymphocytes in relation to the breast carcinomas that we examined. This is a new finding according to our knowledge.

In conclusion, breast cancer is currently affecting women at a young age in Kuwait. The characteristics of the tumours that we studied showed that they were more aggressive than those reported in neighbouring and regional countries. Our results seem to confirm the observation that overexpression of Her-2 and lack of ER expression correlate with a degree of malignancy of breast tumours which leads to poor prognosis and resistance to therapy. Our results also argue for the necessity of establishing awareness of the disease in Kuwaiti society, along with further investigating its molecular pathogenesis.

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Invasive cribriform carcinomas in patients with grade 1 and stage IIA (T2 N0 M0) breast cancer strongly express the v3 and v6 but not the v4 isoforms of the metastatic marker CD44

I carcinomi cribriformi invasivi in pazienti con carcinoma mammario di grado 1 e stadio IIA (T2 N0 M0) esprimono fortemente le isoforme v3 e v6 ma non le v4 del marcatore metastatico CD44

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Summary

Patients with breast carcinomas of the invasive cribriform (IC) histological type often have excellent prognosis. Nevertheless, prognostic markers such as CD44v3, v4, and v6 isoforms have never been evaluated in this histological type. Cases seen between 1996 and 2006 at two major public hospitals in Kuwait were reviewed. We selected the cases which still had enough tissue in the paraffin blocks, had pure rather than mixed typical histological type, did not receive hormonal or any other type of therapy prior to or at the time the tumour was excised, and which were grade 1 and stage IIA (T2 N0 M0). This is to control for confounding factors that could affect the degree of tumour expression of the above isoforms. Sections were immunostained using a highly sensitive peroxidase-anti-peroxidase kit, and scoring of immunostaining was performed in a semi-quantitative manner as established in the literature. An extensive expression of the CD44v3 and v6 isoforms was seen in 83.3% of the IC tumours, while 83.3% lacked the expression of the v4 isoform. A significant association between the histological type and degree of expression of CD44 isoforms was only found with the v3 isoform.

Riassunto

I pazienti con carcinoma mammario di tipo istologico cribriforme invasivo (CI) hanno spesso una prognosi eccellente. Ciononostante i marcatori prognostici come le isoforme v3, v4 e v6 del CD44 non sono mai stati valutati in questo tipo istologico. Sono stati riesaminati i casi visti tra il 1996 ed il 2006 in due grossi ospedali pubblici nel Kuwit. Abbiamo selezionato i casi che avevano ancora abbastanza tessuto nei blocchetti di paraffina, avevano un tipo istologico puro piuttosto che misto, non avevano ricevuto terapie ormonali o di qualunque tipo prima o al momento dell'escissione del tumore, ed erano di grado 1 e di stadio IIA (T2 N0 M0). Questo per controllare i fattori confondenti che avrebbero potuto influenzare l'espressione del grado tumorale nelle forme suddette. Le sezioni sono state colorate immunoistochimicamente con un metodo perossidasi-anti-perossidasi altamente sensibile, e la valutazione è stata eseguita con un metodo semi-quantitativo, come previsto dalla letteratura. Una notevole espressione delle isoforme v3 e v6 del CD44 è stata vista nell'83,3% dei tumori CI, mentre l'83,3% non presentava l'espressione dell'isoforma v4. Un'associazione significativa tra tipo istologico e grado di espressione delle isoforme del

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The degree of expression of the v3 isoform was significantly different in the IC tumours as compared to the papillary, invasive lobular, and invasive ductal (NOS) ones. There was a significant negative correlation (r_s= - 0.201) between the expression of the v4 and v6 isoforms. In conclusion, IC tumours seem to have a strong expression of the prognostic markers v3 and v6 isoforms of the transmembrane molecule CD44, and to lack the expression of the v4 isoform. Eur. J. Oncol., 12 (2), 123-134, 2007

Key words: **invasive cribriform breast carcinoma, CD44v3, v4, and v6 isoforms, Kuwait**

CD44 è stata trovata solo con l'isoforma v3. Il grado di espressione dell'isoforma v3 era significativamente diverso nei tumori CI rispetto alle forme papillari, lobulari invasive e duttali invasive (NOS). C'era una correlazione negativa significativa (r_s=-0,201) tra l'espressione delle isoforme v4 e v6. In conclusione i tumori CI sembrano avere una forte espressione dei marcatori prognostici nelle isoforme v3 e v6 della molecola transmembranosa CD44 ed essere privi dell'espressione dell'isoforma v4. Eur. J. Oncol., 12 (2), 123-134, 2007

Parole chiave: **carcinoma mammario cribriforme invasivo, isoforme v3, v4 e v6 del CD44, Kuwait**

Introduction

Human breast carcinomas of the invasive cribriform (IC) histological type often have a remarkably favourable outcome, with the 10-year overall survival being between 90% and 100%[1-3]. These carcinomas account for up to 3.5% of all breast carcinomas, and the mean age of the patients presenting with such a tumour is between 53 and 58 years[1, 2, 4]. The tumour may present as a mass, but it is often occult clinically. Histologically, it is arranged as invasive, often angulated islands in which well-defined spaces are formed by arches of the cells. Apical snouts are regular features, and the tumour cells are often small, with a low to moderate nuclear pleomorphism. It is rare to find mitoses in these cells, and a prominent reactive-appearing fibroblastic stroma is often seen with these tumours. The incidence of axillary lymph node metastases in IC carcinomas can be as much as 14.3%, and the tumours are 100% and 69% positive for oestrogen and progesterone receptors, respectively[1, 2].

The expression pattern of CD44 in breast cancer is still not clear, and its rôle as a reliable prognostic marker is still controversial[5-10]. CD44 is an integral transmembrane molecule that is predominantly located extracellularly. It was originally described as a lymphocyte-homing receptor which enables lymphocytes to adhere to high endothelial venules[11]. The carboxyterminal end is intracellular, while the extracellular part consists of the middle variable and the aminoterminal (hyaluronan or HA binding) domains. The variable domain is where differing isoforms express their characteristic variant protein, encoded by the variant exons v2-v10. The most prolific isoform of CD44 is the standard isoform being referred to as CD44s. Variant CD44 can contain one or more variant regions, such as CD44v6 or CD44v3-v7. It has been argued that CD44 may be involved in cancer metastasis through hyaluronate degradation, cellular adhesion and migration, angiogenesis, lymph node homing, and release of cytokines[12]. Moreover, an association was found between CD44 and the matrix metalloproteinase 9 (MMP-9), which is known to facilitate tumour cell invasion and metastasis[13]. Binding of hyaluronic acid (HA) of the extracellular part of the CD44 molecule to MDA-MB-231 metastatic breast cancer cells resulted in up-regulation of the growth, survival, and invasion of these cells in a study conducted by Bourguignon *et al* [14]. The authors also recorded, in a separate study, the expression of the CD44v3 isoform on the SP1 metastatic breast tumour cells, and reported that binding of HA to this isoform resulted in cytoskeleton-mediated tumour cell migration[15].

Several studies have investigated the CD44v3, v4, and v6 isoforms as possible prognostic markers in breast cancer, and the degree of expression of these isoforms has been examined in some histological types of the disease, but not in the IC type[7, 16-38]. Accordingly, this study aims at examining the degree of expression of CD44v3, v4, and v6 isoforms in IC breast carcinomas, in an attempt to compare the latter with other histological types of the disease. Moreover, the possible existence of an association between these isoforms and the IC histological type is investigated.

Materials and methods

Patients and histopathological examination

The study was approved by the Human Ethics Committee of the Faculty of Medicine, Health Science

Centre, Kuwait University, and it conformed to the provisions of the Declaration of Helsinki.

The clinical and pathology reports and the H-E slides of breast carcinoma cases seen between 1996 and 2006 at two major public hospitals in Kuwait, namely Al-Amiri and Al-Farwaniya hospitals, were reviewed. Cases were selected where the patients' records showed that they did not receive hormonal or any other type of therapy prior to or at the time the tumour was excised. The cases were also restricted to those which were grade 1 and stage IIA (T2 N0 M0), and which had pure rather than mixed typical histological type. These selection and restriction criteria in our study protocol were mainly to control for some factors that could directly or indirectly affect the degree of expression by the tumours of the CD44v3, v4, and v6 isoforms, such as tumour grade, stage, size, hormones, and mixed histological types. The total number of cases which matched these selection and restriction criteria, and which had enough tumour tissue remaining in the paraffin blocks was 106. These included 75 invasive ductal (NOS), 6 invasive cribriform, 6 papillary, 3 mucinous, and 16 invasive lobular. We could not find pure tubular cases. The histological classification of the tumours as well as tumour grading and staging were determined based on the World Health Organization (WHO) guidelines and on the Elston and Ellis method[39, 40].

Immunohistochemical staining against the CD44v3, v4, and v6 isoforms

Immunohistochemical staining was performed using a highly-sensitive immunostaining kit (NovoLink Max Polymer DetSys RE7280-K, Novocastra, Newcastle upon Tyne, UK), and the steps followed were according to the manufacturer's guidelines. Sections were cut at 4 μm thickness, mounted onto silane-coated slides (S21.1910.110, Novocastra), and left to dry overnight at 37°C. They were then deparaffinized, re-hydrated, and underwent antigen retrieval (Epitope Retrieval pH6, RE7115, Novocastra) by microwaving (Daewoo KOR-161G, 1000W, 2450 MHz, 10 power levels, Seoul, South Korea) for 30 minutes. After cooling down to room temperature, the sections were incubated for 15 minutes with 3% hydrogen peroxide (Peroxidase Block RE7157, Novocastra) to block any endogenous peroxidase activity, washed with TBS, and incubated with goat serum (Protein Block RE7158, Novocastra) for 20 minutes. They were then washed with TBS, and incubated with 200 μl of primary antibody for 1 hour at 37°C in a humidified rotator. The manufacturer's recommended dilution of the primary antibody against CD44v3 (Clone VFF-327v3, Novocastra) and CD44v4 (Clone VFF-11, Novocastra) was 1:100, while that against CD44v6 (Clone VFF-7, Novocastra) was 1:50. The sections were then washed with TBS, and incubated with the post primary antibody (RE7159, Novocastra) for 30 minutes. This was followed by washing with TBS and incubation with the tertiary antibody (NovoLink Polymer RE7161, Novocastra) for 30 minutes. Finally, the sections were washed with TBS, and incubated for 10 minutes with the DAB chromogen (RE7162, Novocastra) followed by haematoxylin counterstain. Positive and negative control slides were used in each staining run. The positive controls were normal lymphocytes and samples of breast carcinomas known to be positive for CD44v3, v4, and v6. The negative controls included samples of breast carcinomas known to be negative for CD44v3, v4, and v6, and sections taken from the same tissue block but incubated with the antibody diluent instead of the primary antibody.

Scoring of immunohistochemical staining against the CD44v3, v4, and v6 isoforms

The literature has cited several studies in which scoring of immunostaining of various CD44 isoforms in human breast cancer tissues was performed. We followed the methodology recently published by Auvinen *et al*[36], which reproduced that of Schumacher *et al*[17]. The proportion of CD44v3, v4, and v6-positively-stained tumour cells was estimated and classified as negative (0% of cells positive), weak (<10% of cells positive), moderate (10-50% of cells positive), and extensive (>50% of cells positive). Such classification was then recorded as 0-3 for practical and statistical purposes as follows: 0 (0% of cells positive), 1 (<10% of cells positive), 2 (10-50% of cells positive), and 3 (>50% of cells positive).

Statistical analysis

We performed all statistical analyses using STATA (SE 8.2, StataCorp, College Station, TX, USA). Five percent was used as the threshold for statistical significance. The Fisher's exact test was used to assess any possible association between the degree of expression of CD44v3, v4, and v6 isoforms and histological type. The Kruskal-Wallis analysis of variance test was used to compare the actual median values of CD44v3, v4, and v6 isoform expression among the various histological types, while the Mann-Whitney test was used to compare such values between two histological types at a time. The Spearman's rank correlation coefficient, r_s was used to determine any possible correlation among the v3, v4, and v6 isoforms.

Results

CD44v3 isoform (figs. 1A, 2)

A significant association was found between the degree of expression of the CD44v3 isoform and the histological type variable. The majority (83.3%) of the IC tumours showed extensive (>50% of the cells positive) expression of this isoform, while negative expression (0% of the cells positive) was only seen in 16.7% of the tumours. On the other hand, negative expression of the isoform was seen in 100% of each of the mucinous and papillary types, and in 92% and 87.5% of the invasive ductal (NOS) and invasive lobular types, respectively. The comparison of the actual median values of CD44v3 isoform expression revealed a significant difference among the various histological types. The median value in the IC tumours was 95 (range 0-100) as compared to 0 in the other histological types. In particular, a significant difference was found between the IC and papillary histological types, IC and invasive lobular histological types, and IC and invasive ductal (NOS) histological types. No significant difference was found between the IC and mucinous histological types, and this could be due to the small sample size in both groups.

CD44v4 isoform (figs. 1B, 3)

There was no significant association between the degree of expression of the CD44v4 isoform and the histological type variable, despite the fact that negative expression of this isoform was seen in 83.3% of the IC tumours, 100% of the mucinous tumours, 93.8% of the invasive lobular tumours, 100% of the papillary tumours, and 94.7% of the invasive ductal (NOS) tumours. Similarly, no significant difference was found among the actual median values of CD44v4 isoform expression of the various histological types studied.

CD44v6 isoform (figs. 1C, 4)

Similarly to the CD44v4 isoform, no significant association was found between the degree of expression of the CD44v6 isoform and the histological type variable, despite the fact that such expression was extensive (>50% of the cells positive) in 83.3%, 100%, 81.3%, 83.3%, and 89.3% of the IC, mucinous, invasive lobular, papillary, and invasive ductal (NOS) tumours, respectively. Moreover, comparison of the actual median values of CD44v6 isoform expression revealed no significant difference among the various histological types. The Spearman's rank correlation test confirmed the different trend in the expression of the isoforms v4 and v6 in our study, whereby there was a significant negative correlation between these isoforms (r_s= -0.201).

Discussion

Some pure typical histological types of breast carcinoma reflect a more favourable prognosis than others[40]. The 10-year overall survival rate in patients with IC carcinomas ranges between 90% and 100%, and the long-term prognosis for patients with breast carcinoma of the tubular histological type is similar to age-matched women without breast cancer[1-3, 41]. Patients with mucinous breast carcinomas have a 10-year survival rate ranging between 80% and 100%[42-44]. Some studies have reported a better disease outcome for invasive lobular breast carcinomas as compared to invasive ductal (NOS), while others have reported a worse prognosis[45-47]. Patients with medullary breast carcinomas have an overall 10-year survival rate of 50%, not exceeding 90% in the best case scenario[3, 48-52]. Moreover, the outcome of patients with medullary carcinoma in the presence of more than three positive axillary lymph nodes has been reported to be poor[3, 48-53].

Various prognostic markers have been examined in different histological types of breast cancer, and some of these markers were found to be associated with some of these types[54-56]. In a recent study conducted by Jalava *et al*[56], the expression of oestrogen receptors was found to be significantly higher in lobular than in ductal tumours. Other studies have reported that the mucinous type was associated with an increase in the expression of oestrogen receptors, and with a decrease in the expression of HER-2 (also known as C-erb B-2 or Her-2/neu) protein[55]. Similarly, Her-2/neu was found to be inversely associated with oestrogen receptor status, based on the breast cancer histological type, in a study conducted by Coradini *et al*[54]. In addition to hormone receptors and Her-2/neu, the transmembrane molecule CD44 has emerged as another possible prognostic marker in breast cancer. In a recent study conducted by Sheridan *et al*[57], breast cancer cells expressing CD44 were found to have both high levels of proinvasive genes and invasive properties. Similarly to the studies trying to correlate the degree of expression of some prognostic markers such as hormone receptors and Her-2/neu with the histological type in breast cancer, a number of studies have attempted to establish a possible correlation between the degree of expression of various CD44 isoforms and some breast cancer histological types, but not the IC type[7, 17, 19, 21, 25, 28, 29, 31, 33, 35-37]. Bassarova *et al*[37] examined the expression of the CD44v3, v4, and

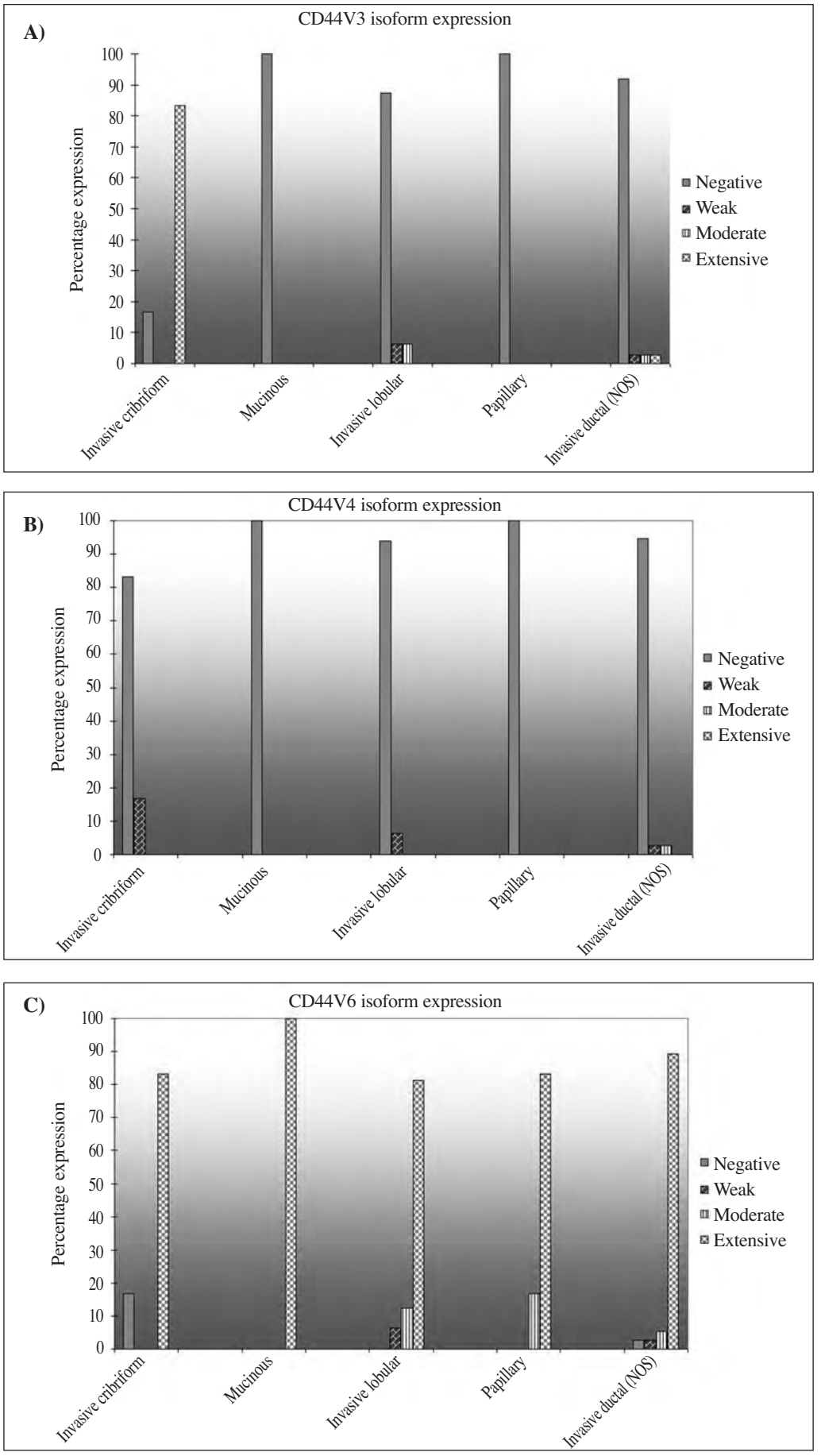

Fig. 1. Percentage expression of the CD44 isoforms in the various histological types examined.
A. Percentage expression of the CD44v3 isoform.
B. Percentage expression of the CD44v4 isoform.
C. Percentage expression of the CD44v6 isoform

Negative = 0% of cells positive
Weak ≤10% of cells positive
Moderate = 10-50% of cells positive
Extensive ≥50% of cells positive

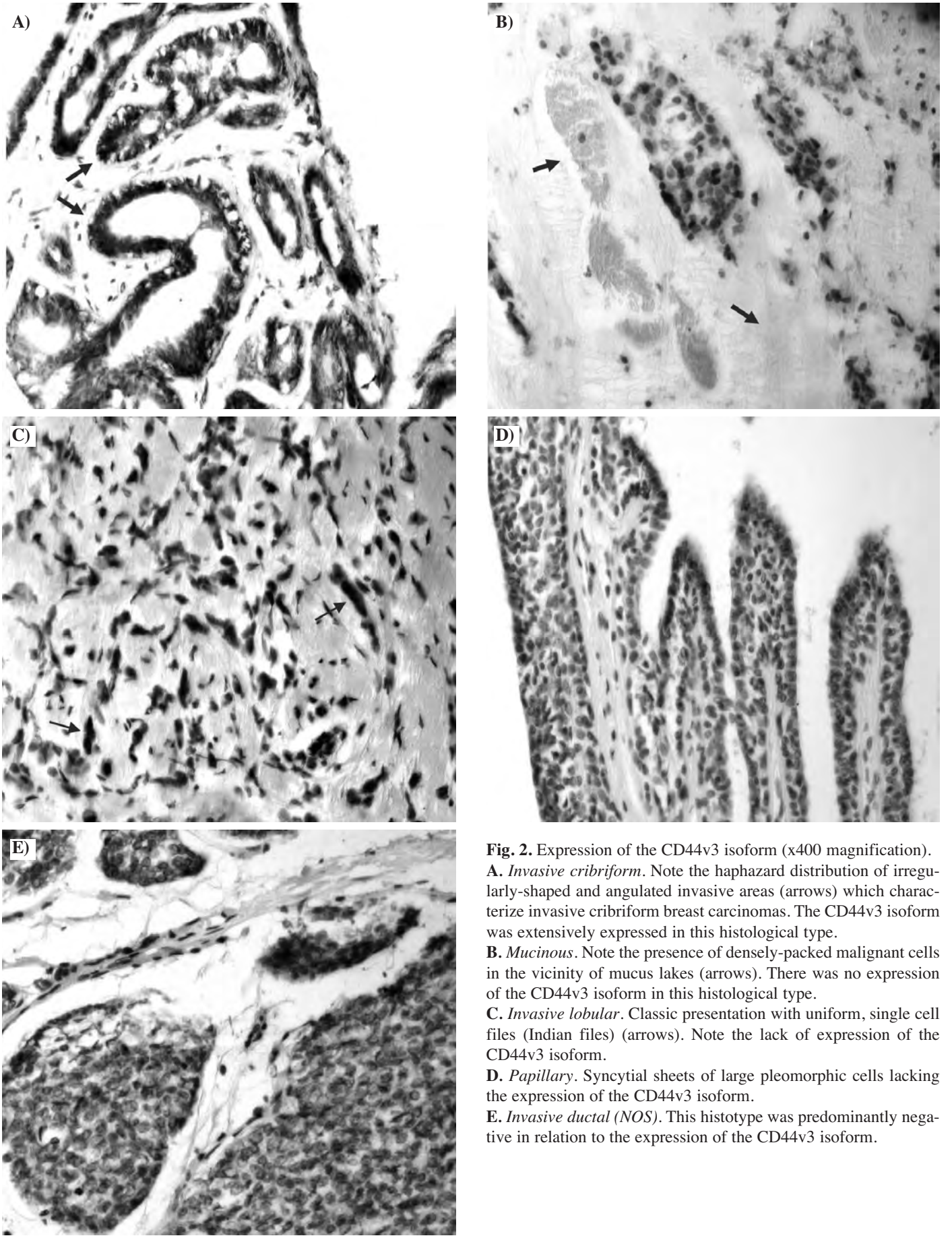

Fig. 2. Expression of the CD44v3 isoform (x400 magnification).
A. *Invasive cribriform*. Note the haphazard distribution of irregularly-shaped and angulated invasive areas (arrows) which characterize invasive cribriform breast carcinomas. The CD44v3 isoform was extensively expressed in this histological type.
B. *Mucinous*. Note the presence of densely-packed malignant cells in the vicinity of mucus lakes (arrows). There was no expression of the CD44v3 isoform in this histological type.
C. *Invasive lobular*. Classic presentation with uniform, single cell files (Indian files) (arrows). Note the lack of expression of the CD44v3 isoform.
D. *Papillary*. Syncytial sheets of large pleomorphic cells lacking the expression of the CD44v3 isoform.
E. *Invasive ductal (NOS)*. This histotype was predominantly negative in relation to the expression of the CD44v3 isoform.

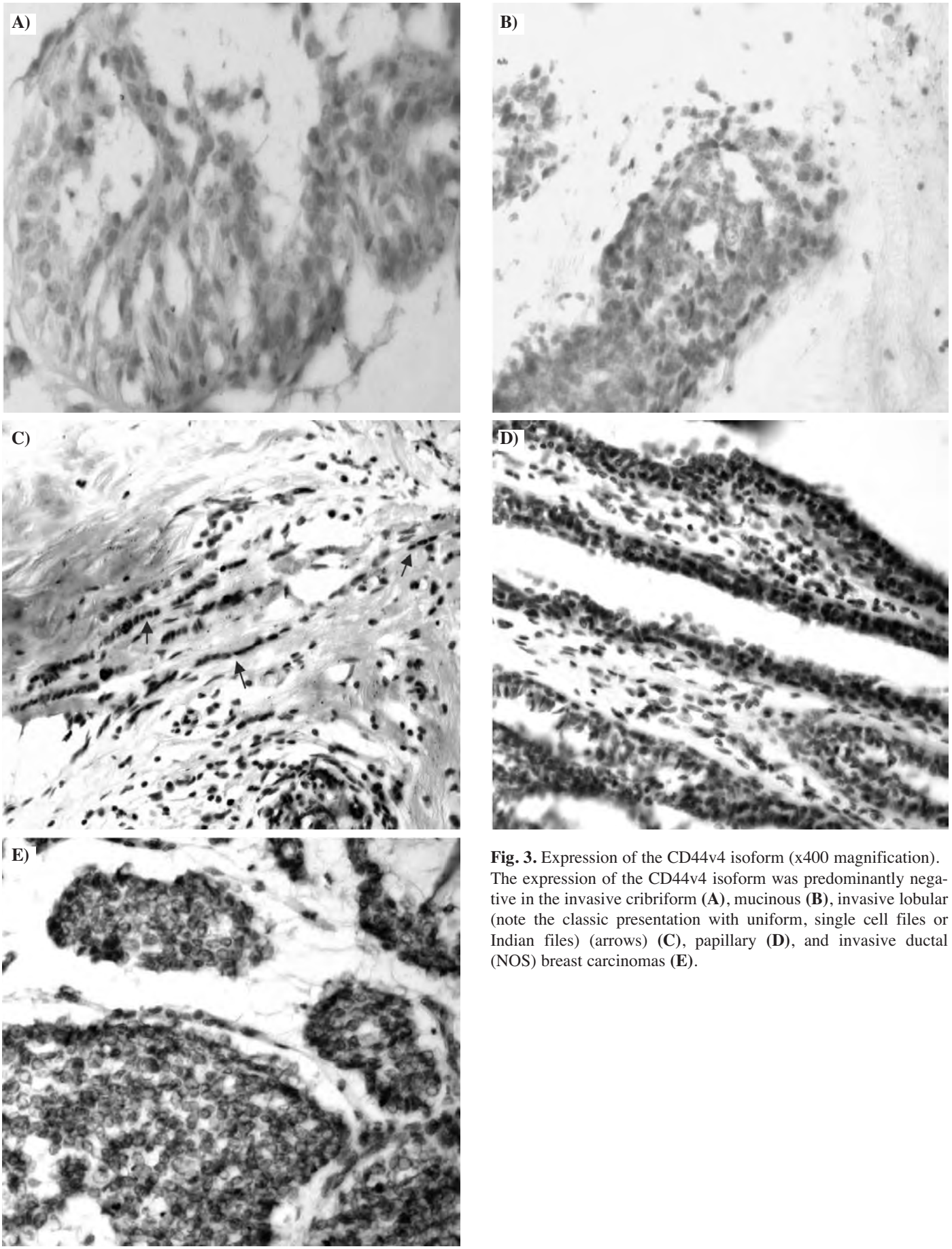

Fig. 3. Expression of the CD44v4 isoform (x400 magnification). The expression of the CD44v4 isoform was predominantly negative in the invasive cribriform **(A)**, mucinous **(B)**, invasive lobular (note the classic presentation with uniform, single cell files or Indian files) (arrows) **(C)**, papillary **(D)**, and invasive ductal (NOS) breast carcinomas **(E)**.

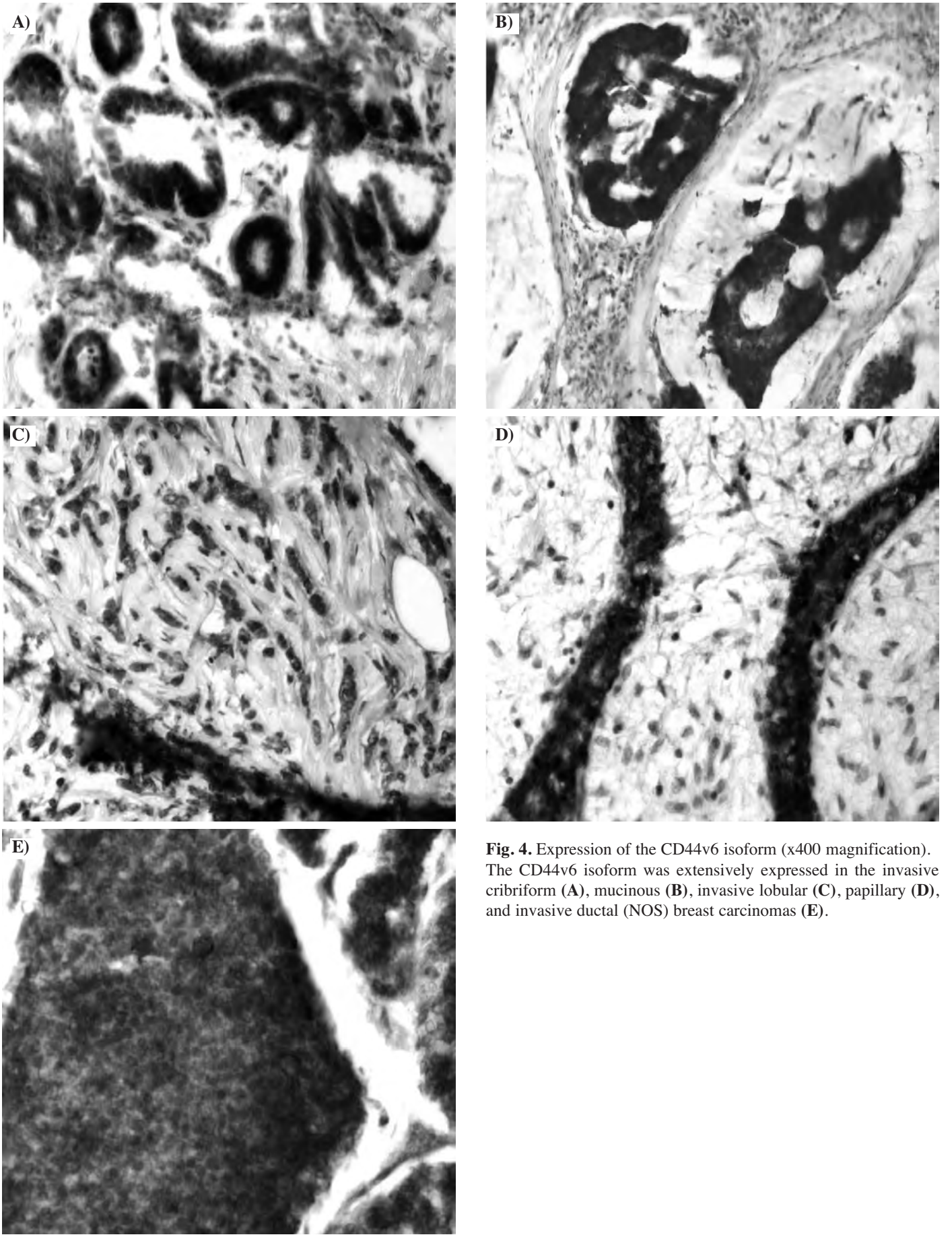

Fig. 4. Expression of the CD44v6 isoform (x400 magnification). The CD44v6 isoform was extensively expressed in the invasive cribriform **(A)**, mucinous **(B)**, invasive lobular **(C)**, papillary **(D)**, and invasive ductal (NOS) breast carcinomas **(E)**.

v6 isoforms in breast carcinomas having the following histological types: invasive ductal (NOS), mucinous, lobular, and medullary. The authors found that the expression of CD44v3 isoform was negative in 50% of the invasive ductal carcinomas (NOS) studied, in 100% of the mucinous and medullary types, and in 60% of the lobular type. The expression of CD44v4 isoform was negative in 100% of the invasive ductal (NOS), mucinous, and medullary carcinomas, and in 80% of the lobular histological type. In contrast, the expression of CD44v6 isoform was positive in 100% of the above various histological types[37].

Our results showed that IC breast carcinomas seem to have extensive expression of the CD44v3 isoform, which was weak or lacking in the mucinous, invasive lobular, papillary, and invasive ductal (NOS) histological types. Moreover, there was a significant association with the degree of expression of this isoform. In a study conducted by Auvinen *et al*[36] in relation to the expression of the CD44v3 isoform, the authors reported no significant difference among invasive ductal (NOS), mucinous, lobular, medullary, ductal *in situ*, and lobular *in situ* carcinomas. Berner *et al*[29] reported a close finding to that reported by Bassarova *et al*[37] whereby the percentage expression of the CD44v3 isoform was 59% in lobular breast carcinomas in the former study and 40% in the latter. Similarly, the percentage expression of this isoform in lobular carcinomas was 52% in a study conducted by Kaufmann *et al*[7]. Since patients with IC breast cancer often have excellent prognosis, one could argue that the extensive expression of the CD44v3 isoform in the IC tumours in our study may, therefore, reflect good rather than bad prognosis. This possible argument is supported by the study conducted by Bassarova *et al*[37] where strong expression of the CD44v3 isoform was not associated with metastases. It is also supported by Auvinen *et al*[36], who reported lack of an association between this isoform and tumour grade. Other studies, however, have shown that the expression of the v3 isoform correlates with poor overall survival, increased tumour grade, and presence of metastases to lymph nodes[7, 16, 25, 26, 34, 58].

As far as the degree of expression of the CD44v4 isoform is concerned, our study revealed that the IC histological type did not significantly differ from the other histological types which we examined. The IC tumours were mostly negative for the v4 isoform, and so were the mucinous, invasive lobular, papillary, and invasive ductal (NOS) tumours. However, the p value for an association between lack of expression and histotype did not reach significance. Our results confirm those published by Bassarova *et al*[37] whereby the mucinous, invasive lobular, papillary, and invasive ductal (NOS) tumours were mostly negative for this isoform. Bankfalvi *et al*[25] also reported no significant difference between the lobular and ductal breast carcinomas in relation to the expression of the v4 isoform. Based on our results and those reported by Bassarova and Bankfalvi, the CD44v4 isoform may not be a prognostic marker which could be used to distinguish the biological behaviour of various histological types in breast cancer. In fact, Regidor *et al*[59] reported no significant correlation between the expression of the v4 isoform and lymph node involvement or tumour grade. Similarly, Thanakit *et al*[13] reported no significant difference in the expression of this isoform in high grade node positive and node negative breast carcinomas. In contrast, Sinn *et al*[16] reported that the degree of expression of the v4 isoform correlated with increased tumour grade.

Our results showed that the degree of expression of the CD44v6 isoform in the IC histological type was not significantly different from that of the other histological types that we examined. Such expression was extensive in most (81.3% to 100%) of the IC and other histological types. Nevertheless, no significant association was found between v6 isoform expression and histotype. Extensive expression of the v6 isoform was seen in 100% of mucinous tumours, 60% of lobular tumours, and 50% of invasive ductal (NOS) and papillary tumours in a study conducted by Bassarova *et al*[37]. Another study revealed that extensive expression of this isoform was seen in 57% of mucinous tumours, 20% of lobular tumours, 38.1% of invasive ductal (NOS) tumours, and 100% of papillary tumours[28]. Still, other studies demonstrated no significant difference in the expression of the v6 isoform among the various breast cancer histological types, as well as no significant association between such expression and histotype[17, 19, 21, 25, 33, 35, 36]. Similarly to the v3 isoform, our results could possibly imply that expression of the v6 isoform may not reflect bad prognosis, since it was extensively expressed in the IC histological type, which is known to have excellent prognosis. The fact that in our study similar extensive expression of the v6 isoform seen in the IC tumours was also observed in other histological types, which are known to have a worse prognosis than the IC histotype, could not be explained at this stage of the study. Nevertheless, our results confirm those reported by other studies in which the expression of the v6 isoform did not reflect a bad prognosis, since it was found not to correlate with tumour grade, stage, size, lymph node involvement, and clinical outcome[21, 35, 36, 59-61], to be associated with less aggressive tumours and with prolonged disease-free survival[6], and to be expressed to an equal degree in both node-positive and node-negative non-palpable T1a and T1b invasive ductal (NOS) carci-

nomas[62]. Other studies, however, reported that increased v6 isoform expression in breast cancer was associated with metastasis[20, 22, 24, 27, 32], and that such expression correlated with poor overall survival and tumour grade[7, 16, 18, 25, 30]. In a recent study conducted by Ma *et al* [38], the authors reported a sequential increase in the expression of the v6 isoform in women with metastases in the axillary lymph nodes, tumour size above 2 cm, advanced pTNM stage, and a survival period of less than five years[38]. Kopp *et al* [30] reported that the degree of expression of the v6 isoform was significantly associated with the number of metastasized organs, presence of hepatic metastases, and poor response to chemo- and hormonal therapy.

In our study, we observed a significant negative correlation between the v4 and v6 isoforms, which confirms our results whereby there was negative expression of the former in the IC as well as other histological types as compared to extensive expression of the latter.

Conclusions

Our results have demonstrated, for the first time in the literature, that IC tumours significantly differ from other histological types of breast cancer in relation to the degree of expression of the CD44v3 isoform, but not of the v4 or v6 ones. Having controlled, in our study, for confounding variables (tumour grade, stage, size, and lymph node involvement) which could affect the degree of expression of these isoforms by analyzing tumours which were all grade 1 and stage IIA (T2 N0 M0), our results may therefore reflect the actual biological behaviour of the IC histological type. We acknowledge the fact that we have a small sample size in some of the histological groups which we studied. This is due to two main reasons. First, it is known that the occurrence of some histological types of breast cancer is rarer than other histological types. Second, we faced tremendous difficulties trying to recruit specimens which could match our strict selection criteria. The clinical relevance of our observations remains to be investigated in further studies by our group.

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Short congress report
Breve resoconto di congresso

International Meeting on Environmental Mutagens, held in Antalya, Turkey, 20th-24th May 2007, attended by several Collegium Ramazzini Fellows

Convegno Internazionale sugli Agenti Mutagenici, tenutosi ad Antalya, Turchia, 20-24 maggio 2007, al quale hanno partecipato alcuni soci del Collegium Ramazzini

Under the direction of one of the chief organizers, William Au, a Collegium Ramazzini Fellow, along with two Turkish Colleagues, organized the 5th International Conference on Environmental Mutagens held May 20th-24th in Antalya, Turkey. In attendance were, in total, seven Fellows. In addition to Dr. Au were present Drs. Myron Mehlman, Wagida Anwar, William Suk, Chris DeRosa, Ruchirawat Mathuros, and Arthur Frank. Drs. Mehlman, Anwar, DeRosa and Frank all presented papers and/or chaired sessions and Dr. Suk gave one of the keynote addresses.

Bringing together scientists from some 40 countries, this was the fifth in a series of meetings held each four years and followed meetings in Cairo (organized by Dr. Anwar), Prague, Thailand and Brazil. Dr. Au has been the chief organizer of each of these excellent meetings.

Dr. Mehlman gave an excellent talk about the history and hazards of benzene. Dr. DeRosa spoke of his work on eating contaminated fish from the Great Lakes. Dr. Anwar, who heads the Aim Shams University Center for Genetic Engineering and Biotechnology in Cairo, presented some of her work on aflatoxin and liver cancer and also on pesticides at a poster session. Dr. Suk addressed the issue of global environmental health concerns. Dr. Frank spoke on social and ethical issues regarding hazardous exposures. He was also asked by Dr. Au to serve as one of four judges for the poster session awards.

The main topics of the meeting had to do with the molecular changes induced by various chemicals in a variety of populations. Most studies were on cell cultures or from whole animal studies, but some human epidemiologic data was presented as well. The main keynote address was by Professor Doctor Her Royal Highness Princess Chulaborne from Thailand. Her own scientific work is in the area of environmental mutagens. The Collegium Ramazzini is planning a satellite meeting in Bangkok in November to coincide with a meeting hosted by the Princess. Dr. Au serves on the international advisory board for that meeting.

While it is always a pleasure to be with diverse scientific colleagues, being together with other Fellows always adds something special.

Arthur L. Frank
Fellow of the Collegium Ramazzini
Professor of Public Health
Drexel University School of Public Health
Philadelphia, PA, USA

European NanOSH Conference
Nanotechnologies: a critical area in occupational safety and health
Helsinki, Finland, 3rd-5th December 2007

Convegno Europeo NanOSH
Nanotecnologie: un'area critica nella sicurezza negli ambienti di lavoro e nella salute occupazionale
Helsinki, Finlandia, 3-5 dicembre 2007

The growth rate of nanoparticle research, the rapidity of nanotechnology development, and the speed of new industrial and consumer products is dramatic. We need to identify the next steps toward assuring the safe research of nanoparticles, and their safe use in occupational environments and consumer products.

The EuroNanoOSH Conference will discuss global safety issues surrounding nanoparticles and nanotechnologies, in occupational safety and health in particular; and will provide insight into future actions for assuring the safety, and thereby the future success of nanotechnologies. Safety is the key for future success of nanotechnologies.

The Conference is arranged by the Finnish Institute of Occupational Health in collaboration with the Tekes – Finnish Funding Agency for Technology and Innovation and VTT Technical Research Center in Finland. In addition, the Conference is supported by the ISPESL – National Institute for Occupational Safety and Prevention in Italy, and the National Institute for Occupational Safety and Health in USA.

Programme and other information are available at http://www.ttl.fi/euronanosh

- *Deadline for abstract submission on 30 August 2007*
- *Deadline for early registration on 15 October 2007*

Information on this conference has been provided by the Secretariat of Professor Harri Vainio, Fellow of the Collegium Ramazzini, Director General of the Finnish Institute of Occupational Health, Helsinki, Finland