EUROPEAN JOURNAL OF ONCOLOGY
GIORNALE EUROPEO DI ONCOLOGIA

Official Organ of the Italian Society of Tumours
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Associato all'Unione Stampa Periodica Italiana

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

Publisher
Mattioli 1885 - Spa
Casa Editrice

Autorizzazione del Tribunale di Parma n. 14/97 del 11/6/1997 - ISSN 1128-6598
La testata fruisce dei Contributi Statali diretti di cui alla legge 7 agosto 1990, n. 250

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

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notes (note)

Issn	
1128-6598	
Issues/year	*Periodicità*
4	Trimestrale
Language	*Lingua*
Italian-English	Italiano-Inglese

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EUROPEAN JOURNAL OF ONCOLOGY
GIORNALE EUROPEO DI ONCOLOGIA

CONTENTS/INDICE

VOLUME 12 - N. 3 - 2007

Eur. J. Oncol., vol. 12, n. 3, pp. 149-154, 2007

Editorial/Editoriale

The global asbestos struggle today

La lotta globale contro l'amianto oggi

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Summary

Global asbestos use dropped by half in the 1990s but has remained over 2 million metric tons per year in the new century. Most of the people in the world still live in countries where asbestos is widely used, with few safeguards, despite bans in over 40 countries around the world and the virtual elimination of asbestos in the leading industrial nations. For countries experiencing rapid industrialization, the use of asbestos in the coming generation of construction materials would have dire public health consequences. The case of India, where asbestos use is still rapidly expanding even in the face of growing public health opposition, is illustrative. The recent commitment of the World Health Organization, the International Labour Organization, and the World Bank Group to take action on asbestos offers new hope that the *impasse* in lowering global asbestos use will be overcome. Progress so far has depended on the dedicated efforts of many individuals and institutions of civil society, including doctors, unionists, environmentalists, lawyers, politicians, government officials, public health workers, journalists, and asbestos victims' groups. Working together worldwide, civil society has pushed back powerful interests and created conditions for improved development and health in one country after another in the global asbestos struggle. Eur. J. Oncol., 12 (3), 149-154, 2007

Key words: **asbestos, public health, international organisations**

Riassunto

L'utilizzo mondiale dell'amianto è diminuito della metà negli anni '90, ma è rimasto di oltre 2 milioni di tonnellate per anno nel nuovo secolo. La maggior parte delle persone nel mondo vive ancora in paesi dove l'amianto viene usato su larga scala, con poche precauzioni, a dispetto di divieti in oltre 40 paesi in tutto il mondo e l'eliminazione virtuale dell'amianto nelle principali nazioni industrializzate. Per i paesi in rapida crescita industriale, l'impiego dell'amianto nei materiali di costruzione di nuova generazione potrebbe portare a conseguenze gravissime di salute pubblica. Il caso dell'India, dove l'uso dell'amianto è ancora in rapida espansione, a dispetto della crescente opposizione da parte degli addetti della sanità pubblica, è significativo. Il recente impegno dell'Organizzazione Mondiale della Sanità, dell'*International Labour Organization* e del Gruppo della Banca Mondiale di prendere una posizione riguardo all'amianto offre una nuova speranza di superare l'*impasse* nella diminuzione dell'utilizzo globale dell'amianto. Finora il progresso è dipeso dall'impegno di molti individui ed istituzioni della società civile, tra cui medici, sindacalisti, ambientalisti, avvocati, politici, funzionari, lavoratori della sanità pubblica, giornalisti e gruppi di vittime dell'amianto. Lavorando insieme a livello mondiale, la società civile ha respinto potenti interessi e ha creato le condizioni per migliorare lo sviluppo e la salute, in un paese dopo l'altro, nella lotta globale contro l'amianto. Eur. J. Oncol., 12 (3), 149-154, 2007

Parole chiave: **amianto, sanità pubblica, organizzazioni internazionali**

Received/Pervenuto 30.5.2007 - Accepted/Accettato 28.6.2007
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Introduction

Over 90% of world asbestos use is in asbestos-cement pipe, flat sheet, and corrugated roofing sheet. Most of the rest is used in brake linings and pads. Smaller amounts are used in industrial gloves and gaskets, etc. Asbestos dust exposures from the use, disposal and replacement of these products can be quite significant. The major asbestos mining countries are Russia, Canada, Kazakhstan, China, Brazil and Zimbabwe.

"Controlled use" of asbestos was soundly rejected by the World Trade Organization (WTO) in 2001, in a decision upholding the asbestos ban in France and, in effect, all national asbestos bans. Global asbestos consumption dropped by half in the 1990s but has levelled off since then (fig. 1). Over 40 countries have asbestos bans in place, including the 27 countries in the European Union. Asbestos is also now banned in Chile, Argentina, Uruguay, Honduras, Kuwait, Saudi Arabia, Jordan, Australia, Japan, the Seychelles, New Caledonia and Gabon. Egypt, Croatia, Vietnam, Peru, South Korea and South Africa are moving to end their consumption of asbestos products. Following the 2006 elections, the United States Congress is expected to enact legislation to ban asbestos in 2007, mainly to halt the importation of asbestos brake linings and asbestos-cement sheet products.

At the same time, asbestos use is increasing by 9% per year in India, and new asbestos plants are being built. Asbestos use is also increasing in other countries, primarily China, Ukraine, Indonesia, Kazakhstan, Iran, Kyrgyzstan and Thailand. Most of the people in the world still live in countries where asbestos products continue to be used, under poorly controlled conditions.

An analysis of the experience of 33 countries has shown that national asbestos consumption, after a latent period of 30-40 years, was proportional to the number of deaths from mesothelioma and asbestosis. These deaths were accompanied by probably even more numerous asbestos-related deaths from lung cancer, laryngeal cancer, and gastrointestinal cancers. The accompanying costs for health care, lost productivity, human suffering, and the management of asbestos hazards in buildings and waste disposal are enormous. Such burdens are still largely preventable for countries that have not used that much asbestos in the past and move to ban asbestos rather than go on using it for years to come[1,2].

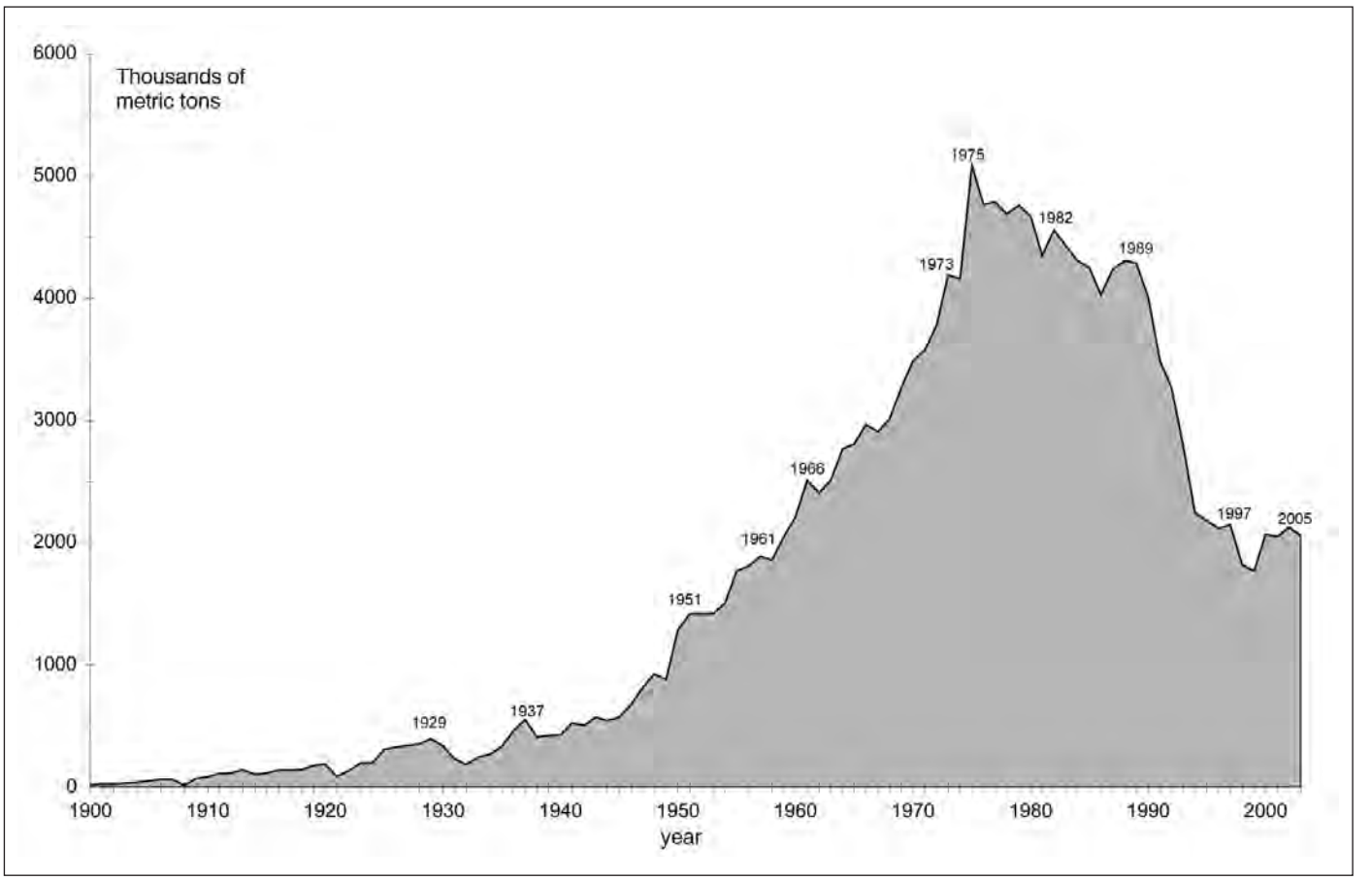

Fig. 1. World production of asbestos, 1900-2006
Graph designed by Mr Stephen Berger, on the basis of data supplied by Mr Robert Virta of the US Geological Survey

Canada's rôle and the asbestos industry in the 21st Century

With almost all of its asbestos exported to poor countries, the government of Canada remains the major obstacle to progress on asbestos. In 2006, Canada threatened South Africa with a trade challenge at the WTO, joining the government of Zimbabwe in pressing South Africa to allow the continued sale of asbestos and asbestos products. More seriously, Canada led other asbestos producing and consuming countries to take the unprecedented step of blocking the inclusion of chrysotile asbestos under the UN Rotterdam Convention. This convention has until now required pre-export notification that a substance is banned as a hazard in multiple parts of the world. Pesticides still involved in international trade have been so designated without dispute, and the precedent of chrysotile asbestos being exempted raises serious concerns about the future of this minimally burdensome instrument of international public health. Obtaining prior informed consent before exportation amounts to little more than placing warning labelling on the products. Canada and other asbestos mining and manufacturing countries have blocked inclusion of chrysotile asbestos under the Rotterdam Convention since 2004, and the next time this will come up for consideration will be in 2008.

Canada's rôle has been criticized by Canadian health scientists Colin L. Soskolne and David V. Bates†, and members of Canada's Parliament, including Pat Martin, have called for Canada to close the asbestos mines and pension off the remaining miners, who number less than 1000. The Canadian press has disclosed internal government memos that acknowledge that competing asbestos-mining countries could easily put Canada's mines out of business, but this is not done because Canada plays such a unique rôle in defending asbestos. Canada has for years supported a "Canadian Chrysotile School" of researchers who blame asbestos deaths mainly on the historic use of other varieties of asbestos (together amounting to about 5% of global consumption). These scientists travel to India, Brazil, Indonesia, South Africa and other countries where controversy has been raised over asbestos, making the case for the "magic mineral" at medical meetings and arranging media interviews. In March 2006, the Canadian Embassy and the Indonesian asbestos industry arranged a conference in Jakarta. Dr. Zulmiar Yanri, Indonesia's Director of Occupational Health, boycotted the conference after being excluded from a rôle in its planning[3].

No more multinational asbestos corporations remain, only national enterprises. Close relations with government and media ownership ensure their profitability, through minimization of the costs of prevention and compensation. These business interests are also intimidating to trade unionists and public health workers who have called for protection and compensation of asbestos-exposed workers, public health campaigns on asbestos, and banning asbestos[4].

The asbestos situation in India

According to the World Health Organization (WHO), the developing South East Asian countries now have the largest number of workers directly exposed to asbestos. The WHO believes asbestos to be the most important occupational carcinogen, causing 54% of all deaths from occupational cancers[5].

The asbestos exposures in India are significant and will result in an increase in related malignant illnesses in the future. According to studies conducted by the Indian National Institute of Occupational Health (NIOH) in the 1980s and 1990s, there were 18 asbestos-cement factories located in different parts of the country. The NIOH carried out environmental epidemiological studies in four asbestos- cement factories located in Ahmedabad, Hyderabad, Coimbatore and Mumbai. The reported prevalence of asbestosis in these factories varied from 3% to 5%. The levels of asbestos fibres were found to be higher than the permissible levels of 2 fibres/ml in two of the factories. In the asbestos textile industry, the average levels of airborne asbestos fibres varied from 216 to 418 fibres/ml. This is so far above the permissible level that one would expect a very high eventual incidence of asbestosis in the exposed workers. The prevalence of asbestosis reported was 9%[6]. In 2005, the number of asbestos-cement units stood at 32 (fig. 2)[7].

The Indian asbestos-cement manufacturers have formed a powerful trade association, the Asbestos Cement Products Manufacturers Association (ACPMA), which works in close concert with the Montreal-based Chrysotile Institute. ACPMA currently has 12 members having 38 manufacturing units located in various states and having a gross annual turnover of approximately US $500 million. They spearhead the propaganda to claim chrysotile asbestos is harmless and can be safely used under controlled conditions. The figures of imports and exports they provide are much higher than the ones provided by the government of India for the corresponding period[8].

The plight of Indian asbestos workers was placed before the Supreme Court of India through a writ of petition filed by the Consumer Education and Research Centre (CERC), Gujarat. The judges directed the Union and state governments *"to review the standards of*

Name of the States	Nr. of chrysotile plants
Assam	1
Andhra Pradesh	3
Gujarat	1
Jharkhand	1
Haryana	1
Karnataka	1
Kerala	1
Madhya Pradesh	2
Maharasthra	9
Orrisa	1
Tamil Nadu	6
Uttar Pradesh	1
West Bengal	2
Rajasthan	1
Union territory of Dadra and Nagar Haveli	1
Total	32

Fig. 2. State-wise distribution of asbestos-cement plants in India
Members Reference Service. Lok Sabha Secretariat, Parliament library and reference and research, documentation and information service, Government of India[7]

permissible exposure limit value of fibre... in tune with the international standards reducing the permissible limit". The court directed the NIOH to examine employees in the asbestos industries and to certify cases of disability. Ten years later, less than 30 had been compensated for occupational disease from asbestos, out of an estimated workforce of 100,000 people exposed to asbestos in India[9].

The government of India remains ambivalent on chrysotile asbestos use. The Ministry of Environment and Forests sponsored an international conference on Environmental Health in New Delhi in 2002, and in its final communiqué stated: *"Environmental epidemiological studies are required to be carried out near to industrial estates and hazardous waste disposal sites to estimate the extent of health risks including from asbestos. Alternatives to asbestos may be used to the extent possible and use of asbestos may be phased out"*. But in his reply to a question raised in the upper house of Indian Parliament in the year 2004 on banning all asbestos use in India, the Minister for Environment and Forests said that: as *"no scientific study establishing that the use of white asbestos causing lung cancer is available, it is not considered as desirable to ban the use of white asbestos"*. India has ratified only 41 international labour standards accepted by International Labour Organization (ILO). This does not include the Convention Nr. 155 on Occupational Safety and Health, 1981, or Nr. 162, the Asbestos Convention, 1986.

An increasing number of scientists, trade unionists, and members of civil society are joining the anti-asbestos campaign. It was demonstrations from such people which prevented the French ship Clemenceau from docking in India for shipbreaking in Gujarat, because of the presence of asbestos and other hazardous materials that had not first been removed from the old ship[10].

It is hoped that in India the next generation of construction materials will not contain asbestos, as it becomes more widely accepted that this is a hazardous, discredited technology.

Positive developments at international organizations

The year 2006 brought major new initiatives from international bodies. The ILO passed a resolution in June

2006 explicitly supporting national asbestos bans for the first time. Introduced at the initiative of the Workers' Group, the Committee on Safety and Health proposed a resolution on asbestos which was adopted by the ILO at the International Labour Conference in 2006. The resolution calls on the ILO to *"promote the elimination of future use of all forms of asbestos and asbestos-containing materials in all member States"*[11].

The WHO concluded in 2006 that "*the most efficient way to eliminate asbestos-related diseases is to stop the use of all types of asbestos"*. The WHO has inaugurated an asbestos action programme and is now working with the ILO to help countries around the world develop national plans to eliminate asbestos use and minimize the hazards from in-place asbestos materials. The only opposition statements received on the WHO policy paper on asbestos came from asbestos-mining countries Kazakhstan and Zimbabwe[12].

The World Bank is avoiding the use of asbestos-cement materials in tsunami reconstruction in Indonesia. The World Bank is drafting a best-practices guidance note to help project officers select safer materials in new construction projects and minimize asbestos hazards in infrastructure renovation: this is undergoing internal review and will be finalized this year.

The international development banks have been moving against asbestos in new building and industrial projects. In 2005, the World Bank specified that asbestos-cement materials should not be used in replacing roofing in a Ukraine schools renovation project. The International Finance Corporation (IFC), the arm of the World Bank Group that lends to the private sector, financed a non-asbestos brake pad manufacturing plant in China in 2005. IFC performance standards revised in 2006 urge avoiding use of hazardous materials where hazards to workers and the community under normal conditions of use and disposal cannot easily be prevented, such as the use of asbestos in building materials.

The new initiatives from the international organizations are a hopeful sign that progress in lowering world asbestos consumption can be resumed. Global asbestos use declined by half in the 1990s but has since then stabilized at over 2 million metric tons per year, following the Asian economic crisis of 1998. The United Nations agencies and development banks may be able to provide the critical impetus to overcome obstacles to the change to safer alternative technologies. Efforts to ban asbestos, regulate exposure, and obtain compensation for workers disabled by asbestos are coordinated by the non-governmental organization, International Ban Asbestos Secretariat (IBAS), in London. The IBAS organizes international conferences (such as Tokyo in November 2004, and Bangkok in July 2006) to gather together government officials, scientists, doctors, lawyers, unionists, politicians, journalists, and others concerned about the effects of asbestos on public health around the world[13].

Concluding thoughts about compensation

Dr. Irving J. Selikoff, renowned for his life's work on asbestos, concluded that the asbestos catastrophe resulted in part from human failure to anticipate its scale.

The situation he described in a paper published after his death refers to the industrial nations where asbestos companies and their insurers have had to bear substantial financial responsibility for the toll of asbestos disease.

Dr. Selikoff said:

> *"The asbestos disaster did not result from superficial miscalculations. Rather, it resulted from very careful calculations, many of which were wrong. They were made not only by scientists but by individuals who were skilled in making estimates (e.g., auditors and actuaries for insurance companies that provided policies to companies making asbestos products). They were wrong in their predictions and are now liable for huge sums of money. These are troubling reflections, particularly when we remember that "statistics are human beings with the tears wiped away"*[14].

The toll in human suffering is increased where the responsible parties escape, with impunity, liability for the tragic human consequences of their actions, as is the case in countries where there are still thriving asbestos industries. It seems that one of the essential requirements of the asbestos business in the world today is that few, if any, of the workers harmed can obtain compensation. National laws and policies that allow such a situation to be perpetuated obstruct the progress of public health, thus permitting much preventable human suffering to occur.

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Eur. J. Oncol., vol. 12, n. 3, pp. 155-170, 2007

Genetic heterogeneity and its effect on susceptibility to environmental factors

L'eterogeneicità genetica e suoi effetti sulla suscettibilità ai fattori ambientali

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Summary

For many years, human susceptibility to xenobiotics has been known to show wide variations, often on a geographic basis. These insights can be used to identify potentially sensitive populations and provide early prediction of adverse outcomes. A proposed framework to guide the use of such data in risk assessment for vulnerable populations is also presented. Genetic differences play an important rôle in susceptibility to certain exposures. In the biotransformation process, a xenobiotic undergoes a two-phase process. In the phase I reaction, enzymes from the cytochrome P450 (CYP) family oxidize foreign substances to form high-energy, reactive intermediates. In the phase II reaction, the reactive metabolites are conjugated to form non-reactive, water-soluble molecules that can be more easily transported and excreted from the body. Mutations in the CYP genes may result in an altered metabolism of the xenobiotic substances. Mutations in genes coding for phase II enzymes, such as glutathione S-transferases and N-acetyltransferases, may also lead to decreased catalytic efficiency for the detoxification of a particular xenobiotic and thus increase its toxicity. Several polymorphisms of these enzymes have been implicated for susceptibility to potential chemical carcinogens. These polymorphisms differ in frequencies and in prevalence by geographic distribution. Given its biological significance, genetic hetero-

Riassunto

Per molti anni si è ritenuto che la suscettibilità umana agli xenobiotici mostrasse ampie variazioni, spesso su base geografica. Queste intuizioni possono essere usate per identificare potenziali popolazioni sensibili e predire precocemente eventi sfavorevoli. Viene anche presentata una proposta per utilizzare questi dati nell'accertamento del rischio per le popolazioni vulnerabili. Le differenze genetiche giocano un ruolo importante nella suscettibilità a certe esposizioni. Nel processo di biotrasformazione, un agente xenobiotico subisce un cambiamento in due fasi. Nella reazione di fase I, gli enzimi della famiglia del citocromo P450 (CYP) ossidano le sostanze estranee per formare degli intermedi reattivi ad alta energia. Nella reazione di fase II, i metaboliti reattivi sono coniugati per formare molecole non reattive idrosolubili che possono essere più facilmente trasportate ed escrete dall'organismo. Le mutazioni nei geni CYP possono risultare in un alterato metabolismo delle sostanze xenobiotiche. Le mutazioni nei geni che codificano per gli enzimi di fase II, come la glutatione S-transferasi e la N-acetiltransferasi, possono anche portare ad una diminuzione della efficienza catalitica per la detossificazione di particolari xenobiotici e quindi all'aumento della loro tossicità. Diversi polimorfismi di questi enzimi sono stati implicati nella suscettibilità a potenziali cancerogeni chimici. Le frequenze di questi polimorfismi

Received/Pervenuto 17.5.2007 - Accepted/Accettato 26.6.2007
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geneity and its global variation should be explicitly addressed in conducting risk assessments for vulnerable populations in different regions of the world. Eur. J. Oncol., 12 (3), 155-170, 2007

differiscono tra loro e nella prevalenza sulla distribuzione geografica. Considerato il suo significato biologico, la eterogeneicità genetica e la sua variazione globale possono essere esplicitamente valutate nella conduzione di accertamenti del rischio per le popolazioni vulnerabili nelle diverse regioni del mondo. Eur. J. Oncol., 12 (3), 155-170, 2007

Key words: **genetic polymorphism, xenobiotic, risk assessment**

Parole chiave: **polimorfismo genetico, xenobiotico, accertamento del rischio**

Introduction

Several issues affect health disparities. These include socioeconomic factors, access to health care, biologic risk factors, and factors inherent to genetic variations in the population. Genetic differences play an important rôle in susceptibility to certain exposures. For example, studies in African children with severe malaria have shown that heterozygous females and hemizygous males for glucose-6-phosphatase dehydrogenase (G6PD) deficiency are underrepresented, thus suggesting that inheriting the enzyme deficiency gene protects against malaria[1]. For many years, it has become evident that inter-individual variations in susceptibility to environmental toxicants result from polymorphisms in a variety of genes that metabolize toxicants, repair their damage, or transduce intracellular signals.

Environmental genomics seeks to understand how individual responses to environmental factors are influenced by genetic variability. The assumption is that high-risk genotypes are subject to more damage and, accordingly, are at greater risk of developing exposure-related diseases. Therefore, "omics" (i.e., genomics, transcriptomics, proteinomics, and metabolomics) have led to the development of predictive biomarkers that identify potentially sensitive populations and allow earlier prediction of adverse outcomes, ultimately resulting in better intervention strategies[2].

Xenobiotics go through one and/or two biotransformation phase processes: 1) in the phase I reaction, enzymes from the cytochrome P450 (*CYP*) family oxidize foreign substances to form high energy, reactive intermediates; 2) in the phase II reaction, the reactive metabolites are conjugated to form non-reactive, water-soluble molecules that can be more easily transported and excreted from the body. Many of these enzymes are polymorphically distributed in the human population. The term *genetic polymorphism* is defined as the existence of at least two different alleles, with allele frequencies of at least 1%, at a particular genetic locus[3]. The allelic variants include point mutations, which may or may not lead to an amino acid shift, as well as deletions and/or insertions of nucleotides. Mutations in the phase I *CYP* genes may result in altered metabolism of the xenobiotic substances, thus increasing the toxic effect of a xenobiotic. Mutations in genes coding for phase II enzymes, such as glutathione S-transferases and N-acetyltransferases, may also lead to decreased catalytic efficiency for the detoxification of a particular xenobiotic and thus increase its toxicity.

The effects of genetic polymorphisms are dependent on the compound being metabolized and the enzyme involved, as well as the target organ/disease and the population being investigated. For example, the enzyme *GSTT1* has two opposite effects: it detoxifies ethylene oxide[4], a component of cigarette smoke, but it activates the solvent methyl chloride[5]. Furthermore, a particular genetic variant may be associated with an increased risk of one effect and a decreased risk of another effect of the same compound. The lack of *GSTT1* allele, on one hand, seems to increase the formation of protein adduct and, on the other hand, appears to decrease the neurotoxic effects caused by high exposure to methyl bromide[6]. *CYP* isoenzymes, as well as phase II and other enzymes of major interest for human environmental and occupational toxicology, are identified in Table 1.

This paper addresses some examples of gene polymorphisms in the population and their rôle in modifying the health outcome of chemical substances.

PCBs and *CYP1A1* polymorphism

The human cytochrome P450 isozyme 1A1 (*CYP1A1*) is a well conserved phase I enzyme in many epithelial tissues. *CYP1A1* contributes to aryl hydrocarbon hydrolase activity, catalyzing the first step in the metabolism of

Table 1 - Substances that are substrates of polymorphic enzymes and health outcome

Gene	Exposure	Outcome
CYP1A1	Organochlorine compounds (PCBs, TCDD), PAHs, tobacco smoke	Breast cancer, lung cancer, PAH metabolite in urine, DNA adducts
CYP1A2	Aflatoxin B1, tobacco smoke, organophosphate compounds	Aflatoxin-albumin adducts, bladder cancer
CYP2E1	Benzene	Hematoxicity
CYP3A4	Afltoxin B1, organophosphate pesticides	Aflatoxin-albumin adducts
GSTM1	Arsenic, aflatoxin B1, organophosphate, airborne PAHs, tobacco smoke	Arsenic metabolites in urine. hepatocellular carcinoma, lung cancer, bladder cancer
GSTT1	Benzene, halogenated solvents (TCE), organophosphate pesticides	Sister chromatid exchange in lymphocytes, renal cell carcinoma
NAT1	Tobacco smoke	Lung cancer
NAT2	Heterocyclic amines, aromatic amines (dye industry), tobacco smoke	Colon cancer, breast cancer, bladder cancer, lung cancer, genotoxic effect in respiratory tract
ALDH2	Alcohol	Upper aerodigestive cancer
ALAD	Lead	Blood lead level, bone lead level
VDR	Lead	Bone lead level

Adapted from Kelada *et al*[2]

a number of polycyclic aromatic hydrocarbons (PAHs), such as the carcinogen benzo(a)pyrene in tobacco and other combustion by-products, to their ultimate DNA-binding forms. Induction of *CYP1A1* by xenobiotics such as PAH is associated with the cell's capacity to generate reactive metabolites from incorporated PAH pollutants[7]. The half-life and concentration of PAH metabolites that may subsequently cause adverse effects depend on the metabolic capacities of both phase I-enzymes (oxidative) generating these metabolites (such as *CYP1A1*) and detoxifying phase II-enzymes (conjugation), such as glutathione S-transferases (GST). Fig. 1 illustrates the rôle of *CYP450* and *GSTM1* in the detoxification of benzo(a)pyrene.

Several studies have investigated, with inconsistent results, the relationship between different *CYP1A1* variants and multiple forms of cancer of the lung, head and neck, oesophagus, urinary tract[8], and breast[9].

The *CYP1A1* isozyme is also involved in oestrogen metabolism by catalyzing the hydroxylation of 17 β-estradiol at the C-2 position and thus has been proposed to play a rôle in the aetiology of breast cancer[9].

Several *CYP1A1* alleles have been described[9], and four polymorphisms have been studied in relation to breast cancer: 1) *m1* (differentiated by a T to C substitution at nucleotide 3801 in the 3'-noncoding region); 2) *m2* or *CYP1A1*2C*, characterized by an amino acid change of isoleucine to valine at codon 462 caused by an A to G substitution at nucleotide 2455; 3) *m3* or *CYP1A1*3*, caused by a T to C substitution at nucleotide 3205 in the 3'-noncoding region; and 4) *m4*, characterized by an amino acid change of threonine to asparagine at codon

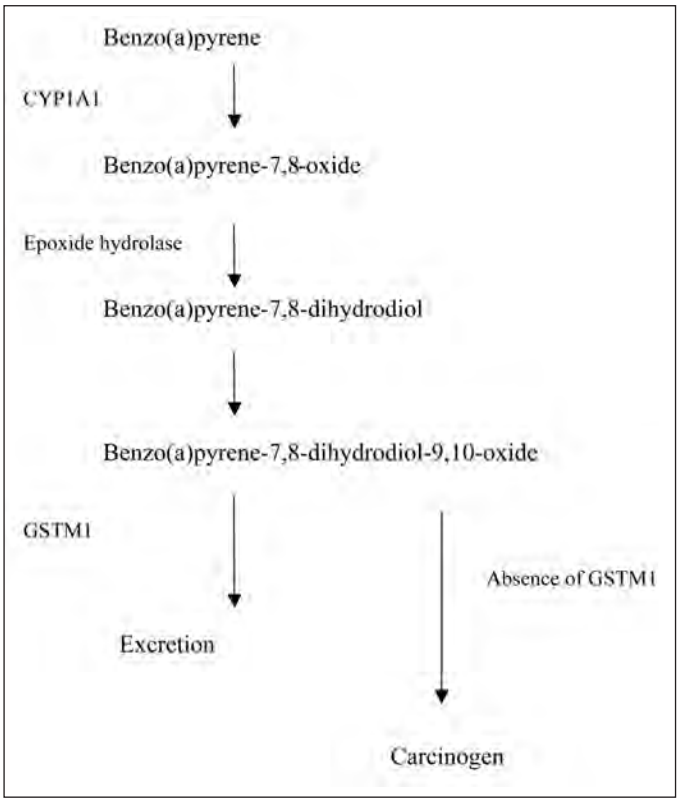

Fig. 1. Detoxification of benzo(a)pyrene by CYP (cytochrome P450) and GSTM1 (glutathione S-transferase M1)

461. The variant Ile462Val (*CYP1A1*2C*) is most common among Asians, where 1-8% are Val/Val and 15-46% are Ile/Val.

In Europeans and American Caucasian, at most 3% are Val/Val and as many as 15% are Ile/Val. The Val variant is less common among African Americans than among Caucasians[9]. In studies of African Americans, no subjects had the Val/Val genotype, but up to 6% were Ile/Val. On the other hand, the 3205T/C (*CYP1A1*3*) variant is found solely in African Americans (fewer than 1% had the C/C genotype, while 14–24% were heterozygous)[9].

Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) induce the *CYP* isozymes to varying degrees and specificities. The non-*ortho* PCB congeners have been shown to induce *CYP1A1/1A2* through aryl hydrocarbon receptor (Ah-receptor)-dependent mechanisms, whereas di-*ortho* PCBs, which have low affinity for the Ah-receptor, mainly induce the *CYP2B* monooxygenases. The mono-*ortho* PCB congeners, which exhibit dioxin-like properties, are often mixed inducers of *CYP1A* and *CYP2B* isozymes[10].

Observation that PCBs may mimic endogenous hormones has raised concern about whether they increase the risk of breast cancer. However, the lack of a consistent association between PCBs and breast cancer risk in earlier studies may indicate that only a portion of the study population, such as persons with the *CYP1A1*2C* variant of the *CYP1A1* gene, are susceptible to the adverse effects of PCB exposure. A meta-analysis that included a total of 1,269 cases (postmenopausal women n=910) and 1,323 controls (postmenopausal women n=936) was conducted by Scinicariello *et al*[11]. The total frequency of the *CYP1A1*2C* carriers (that is, those with at least one variant allele) was 11.3% in the cases (12.7% among postmenopausal women) and 10.1% in the controls (9.3% among postmenopausal women). An increased risk of breast cancer (OR=2.13; 95% CI: 1.38-3.28) was found in postmenopausal women who carry the *CYP1A1*2C* allele and have high PCB exposure, and no risk (OR= 1.01; 95% CI: 0.66-1.53) among women who have low PCB exposure (fig. 2). Conversely, there was a significant decreased risk associated with the *CYP1A1*2C* genotype in premenopausal women (OR=0.57). These discrepancies in cancer risk associated with the *CYP1A1*2C* genotype may be explained in part by differences in frequency of the genotypic polymorphism, with the frequency of the variant allele much lower in premenopausal women than in postmenopausal women. Also, the rôle of other environmental factors (i.e., tobacco smoking) and other host factors among these subgroups must be addressed.

The *CYP1A1*2C* variant has been demonstrated to have a higher activity than the wild type. *CYP1A1*2C* alleles were associated with increased mRNA levels and a three-fold increase in aryl hydrocarbon hydrolase (AhR) enzyme activity[12]. PCBs can act as endocrine-disrupting agents, and both oestrogenic and anti-oestrogenic effects of PCBs have been reported in various *in vitro* and *in vivo* models. Non-planar PCBs have been reported to have weak oestrogenic activity[13, 14], whereas anti-oestrogenic activity has been frequently reported in coplanar dioxin-like PCBs through AhR-dependent mechanisms[15, 16].

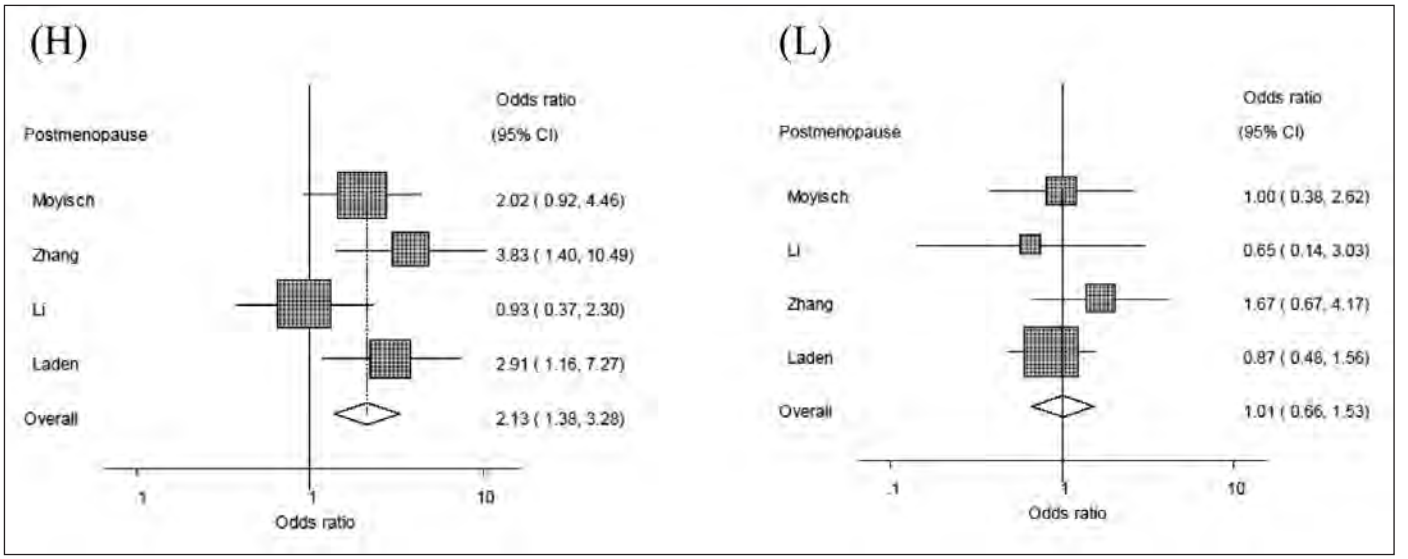

Fig. 2. Stratified analysis of OR for breast cancer in CYP1A1*2C carriers vs individuals homozygous for CYP1A1 in high (H) and low (L) level PCB exposures in postmenopausal women

Several mechanisms for the anti-oestrogenic action of AhR agonists might include repression of 17β-estradiol (E_2)-dependent gene expression by interaction of activated AhR with DNA regions of E_2 responsive gene promoters[16, 17] or by proteasomal degradation of the ER protein[18]. Ohtake *et al*[19], in a study in human MCF-7 breast cancer cells, showed that AhR-mediated regulation of oestrogenic effect depends on the concentration of oestrogens. In the presence of oestrogen, liganded AhR exhibits anti-oestrogenic effects, whereas in the absence of oestrogen, the liganded AhR associates directly with ER-α and ER-β, with a resulting increase of E2-mediated transcription. This mechanism may explain the reported weak oestrogenic effects of the coplanar PCB 126, a known coplanar PCB-congener with anti-oestrogenic activity, in oestrogen-deprived tissues of ovariectomized rats[20]. Therefore, it is possible that in the presence of low endogenous oestrogens, the anti-oestrogenic activities of coplanar PCB-congeners will generate a weak oestrogenic effect, and thus have a rôle in sustaining the growth of breast cancer in postmenopausal women carrying the *CYP1A1*2C* variant.

Genes for glutathione S-transferases (*GST*)

The glutathione S-transferases (*GST*) are a family of enzymes involved in detoxification; they mediate the conjugation of a large number of electrophilic compounds with reduced glutathione (*GSH*), in a typical phase II metabolic reaction[21]. These conjugation reactions facilitate the excretion of many xenobiotics, including carcinogens, toxins, and drugs in the form of mercapturic acids. The *GST* family comprises at least eight classes of *GST* isoenzymes: alpha, mu, pi, sigma, theta, kappa, omega, and zeta[21]. The *GSTM1* and the *GSTT1* genes are polymorphic, and they are the most investigated *GST* genes. One polymorphism is a deletion that results in a lack of functional gene product (*GSTM1 null*, and *GSTT1 null*). The frequency of the *GSTM1 null* and *GSTT1 null* alleles in populations, adapted from Cotton *et al*[22] and from Geisler and Olshan[23], is shown in Table 2.

Impaired *GSTM1* or *GSTT1* enzyme activity has been associated with increased induction of sister chromatid exchanges in lymphocytes exposed to specific mutagenic substrates[24] and among smokers[25]. Therefore, the absence of *GSTM1* or *GSTT1* enzyme activity could increase the risk for DNA damage from genotoxic substrates. *GSTT1* enzyme activity does not always result in detoxification; it can also yield mutagenic metabolites, such as conjugated metabolites of dihalomethanes (e.g., dichloromethane) and dihaloethanes (e.g., ethylene dibromide)[5].

Table 2 - Frequency of the GSTM1 Null and GSTT1 Null allele in various populations

Population	GSTM1 Null[a]	GSTT1 Null[a]
Africa	-	15-26%
North America USA		
Afro-American	23-41%	22-29%
Asian	32-53%	-
Hispanic	40-53%	10-12%
European ancestor	35-57%	15-31%
Caucasian	48-57%	-
South American		
Chile	21%	-
Brasil: Caucasian	55%	19%
Brasil: Black	33%	19%
Brasil: Amazonian	20%	11%
Asia		
Japan	48-50%	-
Korea	53-56%	42-46%
China	35-63%	46-58%
Malaysia	>62%	38%
Europe		
Italy	53%	10-21%
Hungary	44%	21%
Slovak Republic	50%	28%
Oceania		
Australia	-	9-19%

Adapted from Cotton *et al*[22] and Geisler and Olshan[23]
[a]GSTM1 Null and GSTT1 Null identify the lack of functional gene product

GSTT1

In humans, the *GSTT1* polymorphism is represented by two alleles: a functional or wild allele (*GSTT1*1*) and a non-functional or null allele (*GSTT1*0*, or *GSTT1 null*). The *GSTT1 null* allele corresponds to a total or partial deletion of the gene, causing a deficiency in enzymatic activity[26]. Therefore, two phenotypes are possible: "*GSTT1 null*", the homozygote for the deleted allele, and "*GSTT1-positive*", the phenotype with at least one copy of the gene. The frequency of the *GSTT1 null* genotype in Caucasian populations is approximately 20%, whereas this frequency is different in other ethnic groups, with higher frequencies in Chinese (46-58%) and Koreans (42-46%). The frequency of the genotype is 22-29% in African Americans (Table 2).

The *GSTT1* enzyme is involved in the biotransformation of various low-molecular-weight toxins, like ethylene oxide, mono- and dihalomethanes, and other substrates, many of which are known or suspected carcinogens. However, this enzyme has the dual properties of detoxifying and activating many environmental

pollutants. For example, the *GSTT1 null* genotype was associated with chromosomal aberrations in workers exposed to 1,3-butadiene[27] and in smokers[28]. On the other hand, *GSTT1*-catalyzed reactions can also increase the toxicity of some compounds, such as dichloromethane[5]. *GSTs* also conjugate isothiocyanates, which are potent inducers of enzymes that detoxify environmental mutagens[29]. The conjugation process diverts the isothiocyanates from the enzyme induction pathway into excretion, leading to elimination of these anti-carcinogenic substances and thus decreasing their potential chemopreventive effect[29].

GSTM1

The *GSTM1* gene is polymorphic and represented by a non-functional null allele, *GSTM1*0* or *GSTM1 null*[30], and by two other active alleles, *GSTM1*A* and *GSTM1*B*, which differ by a single base pair in exon 7, introducing a restriction site for *Hae II* in the gene sequence[30]. The enzyme catalyzes the detoxification of alkyl and polycyclic aromatic hydrocarbons that are intermediary forms of many carcinogens. It is involved in reducing some superoxides and the products of oxidative stress, such as the DNA hydroperoxides[31]. The frequencies of the *GSTM1 null* allele in individuals is quite variable in different racial groups, ranging from 20 to 60%. The frequency of the *GSTM1 null* genotype in Caucasian populations is approximately 50%, ranging from 35–63% in Chinese and 53-56% in Koreans, whereas it is lower in African Americans (23-41%) (Table 2).

Several studies have suggested that *GSTM1* deficiency may be a risk factor for cancer, causing greater sensitivity to given chemical carcinogens[31]. Increased risks in individuals homozygous for *GSTM1 null* have been reported for bladder cancer, lung cancer, adenocarcinoma, and colorectal cancer. The association between the *GSTM1* polymorphism and bladder cancer is one of the most investigated. Incidence and mortality rates of bladder cancer vary about 10-fold worldwide. Rates are highest in North America and Europe, but they are lower in many parts of Asia[32]. Bladder cancer is the fourth most-frequent cancer diagnosed in men, and the ninth most-frequent cancer in women. The American Cancer Society estimated that 61,420 new cases of bladder cancer were diagnosed in the United States in 2006 (44,690 men and 16,730 women). In the United States, bladder cancer incidence varies markedly by race. African Americans and Hispanic Whites have incidence rates approximately half those of non-Hispanic Whites. Rates are even lower among Asian Americans[33].

Environmental exposures to tobacco as well as occupational carcinogens are the primary risk factors for bladder cancer. It is estimated that 66% of bladder cancers in western countries is attributable to tobacco smoking. The urinary bladder cancer incidence is two to three times higher among cigarette smokers than for those who have never smoked[34]. A dose-response relationship has been observed for both the intensity (i.e., number of cigarettes smoked per day) and the duration of smoking, although the relation for intensity appears to level off at higher exposures. The increased risk is probably due to the presence in tobacco smoke of PAHs, nitrosamines, and aromatic amines. These environmental factors can interact with genetic factors to place one individual at a greater risk of bladder cancer than another. Alcohol consumption, dietary factors, and the use of hair dyes have also been suggested as risk factors for bladder cancer[35-38].

Several genetic susceptibility factors involved in the phase II detoxification of polycyclic aromatic hydrocarbons, such as *GSTM1* and *NAT2* slow genes, have been studied in relation to bladder cancer. A majority of the studies suggest that the null genotypes of *GSTM1* are significantly associated with increased risk of bladder cancer. However, several studies did not identify a significant association between *GSTM1 null* polymorphism and bladder cancer risk[39].

Engel *et al*[39] performed meta- and pooled analyses of published and unpublished case-control, genotype-based studies (17 studies, 2,149 cases, 3,646 controls) that examined the association between *GSTM1 null* genotypes and bladder cancer risk, obtaining a summary odds ratio of 1.44 (95% CI: 1.23-1.68) for *GSTM1 null* status, with all studies included. Results from studies with at least 100 cases and 100 controls produced a summary odds ratio of 1.42 (95% CI: 1.26-1.60). There was a suggestion of additive interaction (additive interaction = 0.45, 95% CI: -0.03-0.93), but no evidence of multiplicative interaction between the *GSTM1 null* genotype and ever smoking in relation to bladder cancer.

Recently, Garcia-Closas *et al*[40] updated the previous meta-analysis with their Spanish hospital-based case-control study and an additional 10 studies (28 studies, 5,108 cases, 6,483 controls). The summary odds ratio for *GSTM1 null versus* present genotype for all populations combined was 1.5 (95% CI 1.3-1.6). Moreover, the relative risk for *GSTM1 null* genotype and bladder cancer was similar for smokers and never-smokers within population subgroups, suggesting that the *GSTM1* activity protects equally against tobacco-related and non tobacco-related bladder cancers. The authors indicate that *GSTM1* may reduce the risk of bladder cancer through mechanisms that are not specific to the detoxification of poly-

cyclic aromatic hydrocarbons (PAHs) in tobacco smoke. Other mechanisms of action for *GSTM1* could be protection from oxidative damage through metabolism of reactive oxygen species. It has been hypothesized that persons homozygous for the *GSTM1* deletion should have higher PAH(BPDE)–DNA adduct levels and therefore may be more susceptible to carcinogenesis[31].

Lead poisoning and *ALAD* polymorphism

Lead poisoning is a complex disorder affecting many organs in the body, including developing red blood cells, the kidneys, and the nervous system. Young children are most susceptible to the toxic effects of lead. Major concerns are the cognitive and neurobehavioural deficits resulting from lead exposure levels that were previously considered safe. High levels of exposure can cause encephalopathy and death[41].

Lead is a potent inhibitor of the δ-aminolevulinic acid dehydratase (*ALAD*) enzyme, which catalyzes the second steps in the biosynthesis of heme. *ALAD*, an octameric zinc-containing enzyme, catalyzes the condensation of two molecules of 5-aminolevulinic acid (ALA) into one molecule of monopyrrole porphobilinogen (PBG). Lead displaces zinc from the enzyme's active site, and the inactivation of *ALAD* has been implicated in the pathogenesis of lead poisoning. The resulting accumulation of its substrate, ALA has been shown to have a neuropathogenic effect, possibly by acting as a γ-aminobutyric acid receptor agonist in the nervous system[42].

Human *ALAD*, encoded by a single gene localized to the chromosome 9q34 region, is a polymorphic enzyme with two alleles, *ALAD1* and *ALAD2*. The difference between the two alleles lies in a single G→C transversion mutation of nucleotide 177 in *ALAD2*; the allozyme resulting from the *ALAD2* allele contains the substitution of a neutral asparagine for a positively charged lysine at residue 59[43]. Three differently charged allozymes, *ALAD1-1, 1-2*, and *2-2*, result from the expression of the *ALAD1* and *ALAD2* genes.

Table 3 shows the frequency of the *ALAD* polymorphism in different populations[44-69]. In several Caucasian populations, the frequencies of the *ALAD1* and *ALAD2* genes have been estimated to be 0.9 and 0.1, respectively. Asian and African populations have lower frequencies of the *ALAD2* allele.

Several epidemiologic studies have attempted to correlate the *ALAD* allelic variations with a differential susceptibility to lead poisoning. The biologic plausibility for a differential rôle of the two alleles lies in the fact that the lysine substitution at residue 59 changes the electrical charge of the enzyme[62]; the more electronegative *ALAD2* enzyme may thus have a higher affinity/stability for the lead cation than *ALAD1*[43]. This could result in an alteration of lead toxicokinetics and susceptibility to lead toxicity.

The first studies comparing blood lead level (BLL) and *ALAD* polymorphism were conducted on a chronically exposed population of 202 male lead workers in a German factory[60] and in an environmentally exposed population of 1,051 children with elevated free erythrocyte protoporphyrin[46]. These studies showed that individuals carrying one or two copies of the *ALAD2* allele exhibited higher blood lead levels (BLLs) than homozygous individuals with only the *ALAD1* allele. These findings led to the suggestion that *ALAD2* may be a determinant for increased susceptibility to lead toxicity. However, some studies have reported either no difference among individuals homozygous for *ALAD1* and individuals carrying the *ALAD2* allele, or else that the differences among the two groups were not statistically significant. The extreme variability in the published data is due to several factors: relatively small numbers of subjects, different frequencies of the *ALAD2* allele in various populations, and different levels of lead exposure, as determined by BLLs in the populations studied. A meta-analysis by Scinicariello *et al*[70] indicated that individuals carrying the *ALAD2* allele had generally higher blood lead levels than those homozygous for *ALAD1*. There was a statistically significant association between *ALAD2* carriers and higher BLL in lead-exposed workers (weighted mean differences of 1.93 μg/dL). There was no association with *ALAD* carrier status among environmentally exposed adults with BLLs <10 μg/dL. There was a suggestion that *ALAD2* carriers were potentially protected against adverse haemopoietic effects (ZPP and haemoglobin levels), perhaps due to decreased lead bioavailability to heme pathway enzymes. There was no statistically significant association of *ALAD* status with bone lead, serum creatinine, or blood pressure. The rôle of the polymorphism on those effects was not well defined, partly due to the small number of subjects studied and potential modifications caused by other proteins in target tissues or by other polymorphic genes, such as VDR and eNOS. Therefore, more studies are needed to address the rôle of gene-gene interactions with environmental exposure to lead.

Metabolism of alcohol and aldehyde dehydrogenase 2 (*ALDH2*)

Excessive chronic alcohol consumption is a risk factor for the development of oesophageal and head and neck

Table 3 - Frequencies of ALAD genotypes

Country	ALAD 1/1	ALAD1/2	ALAD 2/2	N. Subject	Reference
North America					
Canada	0.817	0.175	0.008	382	Fleming *et al*, 1998[44]
Canada	0.851	0.149	0	134	Alexander *et al*, 1998[45]
United States	0.786	0.198	0.016	1,074	Astrin *et al*, 1987[46]
United States	0.86	0.137	0.003	691	Smith *et al*, 1995[47]
United States	0.889	0.1	0.011	1,278	Wetmur *et al*, 1991[48]
South America					
Brazil-White	0.848	0.142	0.009	112	Sousa *et al*, 1991[49]
Brazil-Mulatto	0.914	0.086	0	359	Sousa *et al*, 1991[49]
Brazil-Black	1	0	0	48	Sousa *et al*, 1991[49]
Chile	0.903	0.086	0.011	93	Perez-Bravo *et al*, 2004[50]
Asia					
Japan	0.901	0.083	0.017	121	Benkmann *et al*, 1983[51]
Japan	0.836	0.155	0.009	317	Sakai *et al*, 2000[52]
South Korea	0.889	0.11	0	307	Schwartz *et al*, 1995[53]
South Korea	0.901	0.099	0	795	Schwartz *et al*, 2000[54]
China	0.921	0.079	0	229	Shen *et al*, 2001[55]
Taiwan	0.955	0.044	0.002	660	Hsieh *et al*, 2000[56]
Taiwan	0.759	0.241	0	87	Wu *et al*, 2004[57]
Singapore-Chinese	0.964	0.036	0	56	Chia *et al*, 2004[58]
Singapore-Malay	0.833	0.167	0	36	Chia *et al*, 2004[58]
Singapore- Indian	0.821	0.143	0.036	28	Chia *et al*, 2004[58]
Europe					
Sweden	0.862	0.13	0.008	339	Bergdahl *et al*, 1997[59]
Germany	0.813	0.153	0.035	144	Benkmann *et al*, 1983[51]
Germany	0.792	0.158	0.05	144	Ziemsen *et al*, 1986[60]
Poland	0.887	0.11	0.003	300	Raczek *et al*, 1994[61]
Italy	0.803	0.182	0.015	798	Battistuzzi *et al*, 1981[62]
Italy	0.814	0.167	0.02	762	Petrucci *et al*, 1982[63]
Spain	0.85	0.145	0.006	339	Garcia-Orad *et al*, 1987[64]
Spain	0.842	0.15	0.008	500	Caeiro and Rey, 1985[65]
Portugal	0.827	0.165	0.008	1,043	Amorim *et al*, 1994[66]
Greece	0.919	0.071	0.01	508	Kapotis *et al*, 1998[67]
Turkey	0.783	0.209	0.009	230	Suzen *et al*, 2004[68]
Africa					
Israel-Ashkenazis	0.645	0.306	0.049	386	Ben-Ezzer *et al*, 1987[69]
Israel-Arabs	0.779	0.2	0.021	95	Ben-Ezzer *et al*, 1987[69]
Liberia	1	0	0	296	Benkmann *et al*, 1983[51]

cancers, based on data from epidemiological studies[71]. Although ethanol in its pure form does not act as a carcinogen in experimental models, several possible mechanistic pathways through which drinking alcohol may cause cancer have been hypothesized: alcohol acts as a solvent for tobacco carcinogens, impurities in alcoholic drinks are the carcinogenic agents[72], or exposure to acetaldehyde, the first metabolite formed during the breakdown of the ethanol, are three such possible pathways[73]. On the basis of animal carcinogenicity studies, the International Agency for Research on Cancer (IARC) classifies acetaldehyde as "possibly carcinogenic to humans"[73]. The inhalation of acetaldehyde causes nasopharyngeal carcinoma in rats and laryngeal carcinoma in hamsters[50]. Acetaldehyde has been shown to induce chromosomal aberrations, micronuclei and sister chromatid exchanges in cultured mammalian cells[74], and a covalent interaction with DNA to form DNA adducts, which may be a critical initiating event in the multistage process of chemical carcinogenesis[75].

Ethanol is oxidized to acetaldehyde by the enzyme alcohol dehydrogenase (*ADH*). Acetaldehyde is then

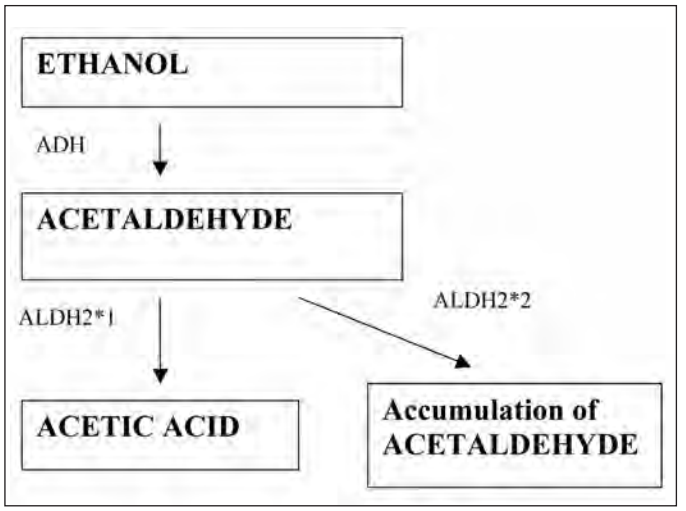

Fig. 3. Metabolism of ethanol

converted to acetate by the mitochondrial enzyme aldehyde dehydrogenase 2 (*ALDH2*) (fig. 3). In humans, the gene for the homotetrameric enzyme *ALDH2* has a polymorphism[76]. The mutant allele *ALDH2*2* is the result of a single point mutation causing an amino acid substitution from glutamic acid (glutamate) to lysine at residue 487, producing an inactive subunit. Therefore, in the presence of *ALDH2*2*, after intake of alcohol, there will be no production of acetate, with resulting accumulation of acetaldehyde. Individuals with heterozygous *ALDH2*1/2*2* genotype should have only 6.25% of normal ALDH2*1 protein[77]. Aldehyde dehydrogenase 2 detoxifies other aldehydes such as benzaldeheyde, a metabolic product of the solvent toluene[78], and chloroacetaldehyde, a toxic metabolite of vinyl chloride[79].

The *ALDH2*2* genotype has been associated with psycho-physiological responses such as facial flushing, chest palpitation, or dysphoria after the intake of even a small amount of alcohol[80].The genotype has also been found to have a substantial inhibitory effect on alcohol consumption, thus preventing the likelihood of alcoholism[81]. However, the preventive effect of heterozygous *ALDH2*1/2*2* is incomplete, and it is influenced by socio-cultural factors. For example, there has been a dramatic increase in the rate of Japanese heavy drinkers who have inactive heterozygous *ALDH2*, increasing from 2.5% of Japanese alcoholics in 1979 to 8.0% in 1986 to 13.0% in 1992[82].

Approximately 40% of Japanese have the inactive mutant *ALDH2*2* allele[81]. Distribution of the *ALDH2*2* allele varies by race: nearly all Caucasians carried the functional *ALDH2*1/1* genotype[80]. Similar patterns were seen among populations from Southeast Asia and Mexico. In contrast, the *ALDH2*2 null* allele was frequently observed in East Asian populations, typically with 30% *ALDH2*1/2* heterozygous and 5-10% *ALDH2*2/2* homozygous. No information on African populations was available (fig. 4).

The optimal situation for risk assessment would be to have a detailed understanding of the biological processes underlying the effects of any chemical and of how these effects may lead to an adverse health outcome in humans. At the moment, for most compounds there is a general lack of the underlying biological toxicokinetics and tokicodynamics processes, so that a risk characterization

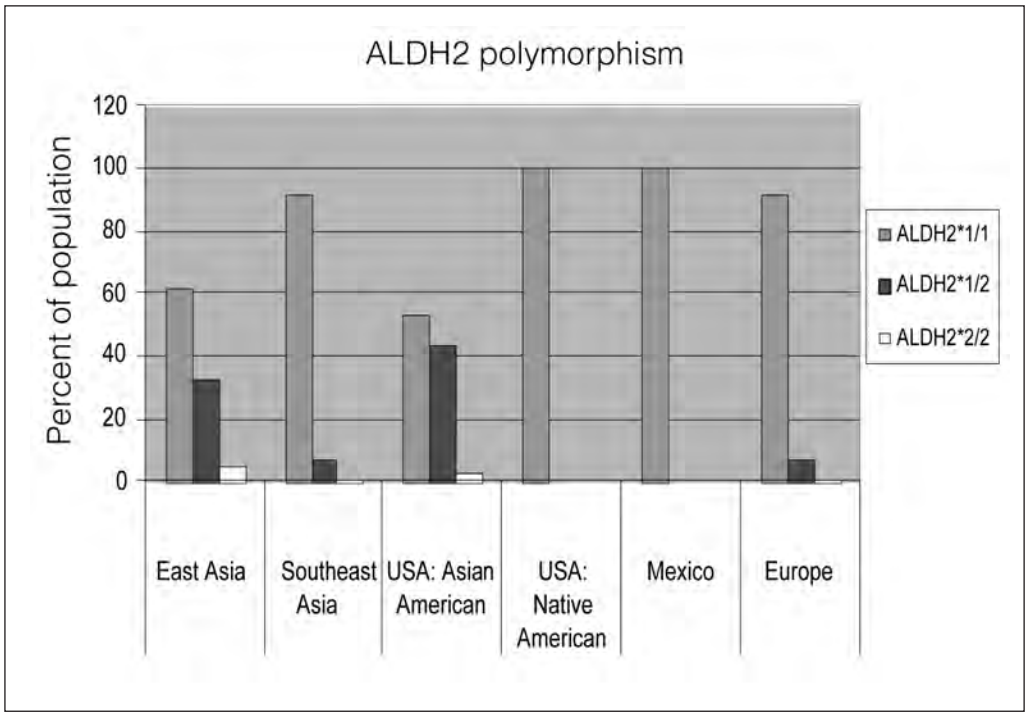

Fig. 4. Prevalence of ALDH2 polymorphism in several populations

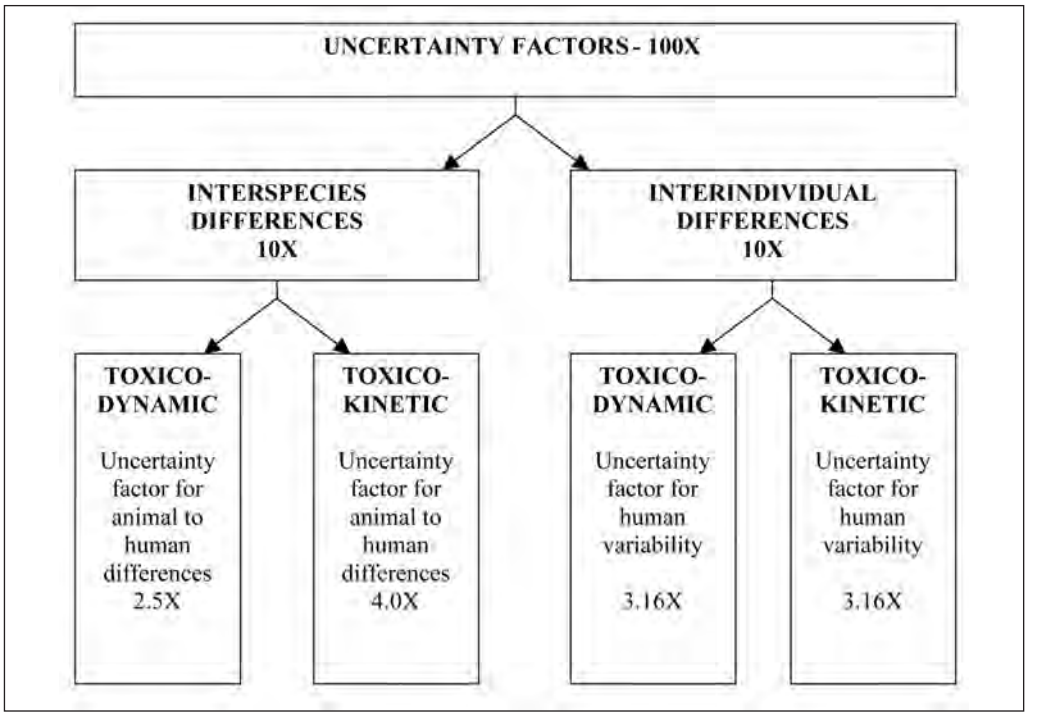

Fig. 5. Uncertainty factors for interspecies and interindividual variability

inevitably relies on a number of uncertainties, assumptions, and rationalizations.

Toxicological risk assessment of effects other than cancer has been traditionally based on the assumption that there is a threshold dose, such as a no-observed-adverse-effect level (NOAEL), below which an adverse effect will not occur. An uncertainty factor is used for the extrapolation of the NOAEL from an animal study to a human exposure value. This uncertainty factor is to account for: 1) the biological differences between human and the animal species, and 2) differences between individuals within the human population[83]. The overall uncertainty factor generally consists of 10-fold factors for each of the following: inter-species differences and inter-individual (human) variation (fig. 5). The inter-species extrapolation is then distinguished in kinetic and dynamic aspects that are subdivided 4- and 2.5-fold, respectively. Similarly, the 10-fold uncertainty factor for inter-individual (human-to-human) variation has been further subdivided into two factors of $10^{0.5}$ (3.16) to cover toxicokinetics and toxicodynamics, and this subdivision allows for the replacement of an uncertainty factor with a chemical-specific adjustment factor (CSAF) when compound-specific data are available. This inter-individual variability factor addresses uncertainties produced by several factors, such as age, gender, nutritional status, disease status, exposure to drugs, and genetic polymorphism.

Alternatively, human toxicokinetics can be predicted from physiologically-based pharmacokinetic (PB-PK) modelling, a method that is capable of using *in vivo* data from the most sensitive test species to predict the kinetics in humans[83]. Such predictive methodology has shifted the emphasis from external exposure toward predicting the dose of a compound at the molecular target site.

Identification of susceptibility genes provides an opportunity to replace the traditional generic default factor with a data-based factor for each agent. The variability of xenobiotic metabolism caused by genetic polymorphisms is expected to be captured by the 3.16-fold toxicokinetic uncertainty factor. If there is a 20-fold difference in susceptibility between major genotypes in population, is a 10X safety factor too big or too small?

Several examples in the literature show a more than ten-fold variation in metabolic capacity, depending on genetic polymorphism in bio-transforming enzymes. For example, the right dose of warfarin differs about ten-fold between patients having or lacking functional *CYP2C9*[84]. Warfarin is prescribed as a racemic mixture of (R)- and (S)- enantiomers. The (S)-warfarin, which is pharmacologically three to five times more potent than the (R)-enantiomer, is predominantly metabolized by the *CYP2C9* enzyme. This enzyme is polymorphic, and there are two common allelic variants – *CYP2C9*2* and *CYP2C9*3* – which encode enzymes that are about 12% and 5%, respectively, as efficient as the wild type of enzyme in (S)-warfarin hydroxylation. Therefore, the *CYP2C9*2* and *CYP2C9*3* variant alleles are associated with reduced warfarin dose requirements consequent to reduced warfarin clearance[84].

Individuals who are *ALDH2*2* homozygous and *ALDH2*1/2* heterozygous have 18-fold and 5-fold, respectively, higher average peak blood acetaldehyde concentrations than those who are *ALDH2*1/1/ homozygous*[85].

In another study, Mizoi *et al*[86] administered 0.4 g of ethanol per kg of body weight over 10 minutes to 68 healthy subjects. The acetaldehyde levels in the subjects with the *ALDH2*1/*2 heterozygote* (43%) increased to 23.4 μM, on average. Subjects homozygous for *ALDH2*2* (9%) showed very high levels of blood acetaldehyde, and the average value was 79.3 μM, whereas the average value of blood acetaldehyde was 4.1 μM for subjects homozygous (48%) to the wild type of variant. Therefore, individuals with the homozygous variant had approximately 20 times higher blood acetaldehyde levels than individuals with the homozygous wild type. In this case, the default toxicodynamic uncertainty factor (3.16 fold) is lower than the 20-fold difference due to the polymorphism. The default uncertainty factor is also lower than the 5.7-fold difference seen in individuals who are *ALDH2* heterozygous.

The data of Mizoi *et al*[86] are useful for generating a Monte Carlo population distribution for blood acetaldehyde levels in healthy adults after ethanol ingestion in populations other than Japanese, such as the United States population that has the significant presence of several races and ethnicities. The last US census conducted in 2000 showed that the people of Asian origin alone represented 3.6% of the population[87]. This relatively small contribution to the total US population did not alter significantly the mean distribution, the median, and the 95th and 99th percentile of blood acetaldehyde levels, compared to the same distribution in the population carrying the wild-type genotype *ALDH2*1/1* (Table 4). A significant polymorphism exists in a low-percentage ethnic subgroup, and it is a distinct, identifiable subgroup. In this subgroup, the variability in *ALDH2* functions is greater than the 3.2-fold uncertainty factor typically applied in non-cancer risk assessment. Nevertheless, the distribution of the Asian population is unequal among the different states (fig. 6). Its presence is higher in states such as Hawaii and California, representing 41.6% and 10.9% of the state population, respectively, according to the 2000 US Census. The contribution of this population among the genotypes is captured in the upper end of the distribution of serum blood acetaldehyde levels, in the 95th and 99th percentile in the Hawaii

Table 4 - Monte Carlo simulation of acetaldehyde blood concentration (μM) across populations

Population	Mean	Median	95th percentile	99th percentile	99th/Median
*ALDH2*1/1*	4.1	4.1	6.1	7	1.71
*ALDH2*1/2*	23.3	23.2	41.8	48.8	2.10
*ALDH2*2/2*	78.3	78	125.3	148.	6 1.91
Mixed distribution: all genotypes in Japanese population	16.9	5.6	63.6	107.1	19.13
US population					
1990 Census	4.2	4.1	6.3	7.5	1.83
2000 Census	4.3	4.1	6.3	7.5	1.83
2004 (estimates)	4.3	4.1	6.3	7.6	1.85
California					
1990 Census	4.6	4.1	6.4	22	5.37
2000 Census	4.6	4.1	6.4	23.4	5.71
2004 (estimates)	4.6	4.1	6.4	25	6.10
Hawaii					
1990 Census	6.5	4.2	22.6	68.1	16.21
2000 Census	6.1	4.2	17.8	57.1	13.60
2004 (estimates)	6.1	4.2	17.9	57.1	13.60
New Jersey					
1990 Census	4.3	4.1	6.3	7.5	1.83
2000 Census	4.4	4.1	6.3	7.8	1.90
2004 (estimates)	4.4	4.1	6.4	8.6	2.10
New York					
1990 Census	4.3	4.1	6.3	7.5	1.83
2000 Census	4.4	4.1	6.3	7.8	1.90
2004 (estimates)	4.4	4.1	6.3	8.1	1.98

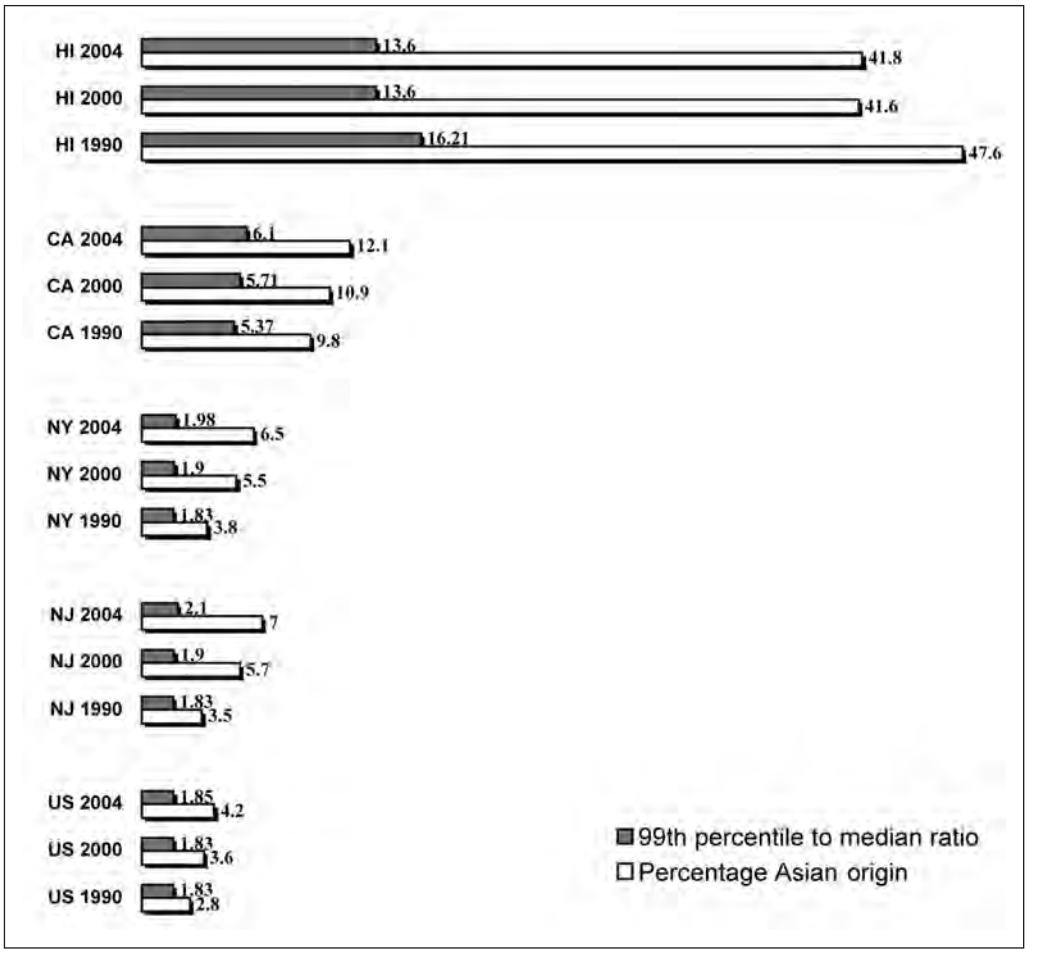

Fig. 6. Changes in Asian descendent percentage in the population and estimated toxicokinetics uncertainty factor over time
Hi=Hawaii
Ca=California
NY=New York
NJ=New Jersey
US=United States of America

population and the 99th percentile in the California population (Table 4). These percentiles are 4.24, 13.60, and 5.71-fold higher than their median ratios, and they are also higher than the 3.16-fold uncertainty factor captured by the toxicokinetics default factor.

Globalization, gene migration and health risk assessment

These findings have serious implications for public health in the economically more developed regions of the world, given the increase in migration. As a result of economic globalization and growing political instability, migration has become more pronounced than ever before. Approximately 175 million people, or 2.9% of the world's population, currently live temporarily or permanently outside their countries of origin[88]. This figure, which includes migrant workers, permanent immigrants, and refugees and asylum seekers, does not account for the growing irregular or undocumented movement that is coming to characterize migration in the global community. This world-wide migration and the other interconnected trans-border processes that constitute the heart of globalization will probably continue and become an even greater public health challenge.

Estimates of net migration between the major development groups show that since 1960 the more developed regions have been net gainers of emigrants from the less-developed regions (fig. 7). Net migration to the more developed regions increased steadily from 1960 to 2000. Between 1990 and 2000, the more developed regions were annually gaining 2.6 million migrants, with about half of that net flow directed to Northern America (1.3 million people annually). Between 2000 and 2010, estimates show the level of net migration to the more developed regions as a whole changing only slightly, but there is some increase in the net number of migrants received by Northern America (1.4 million annually). Over the rest of the projection period, net migration to the more developed regions is projected to remain at about 2.2 million per year, of which 1.3 million are directed to Northern America. Asia was the major source of migrants from 1990 to 2000 (1.4 million annually), followed by Latin America and the Caribbean (0.8 million annually) and then Africa (0.3 million annually). After 2000, it is expected that over half of all migrants leaving the less developed regions will be from Asia, with about 25% to

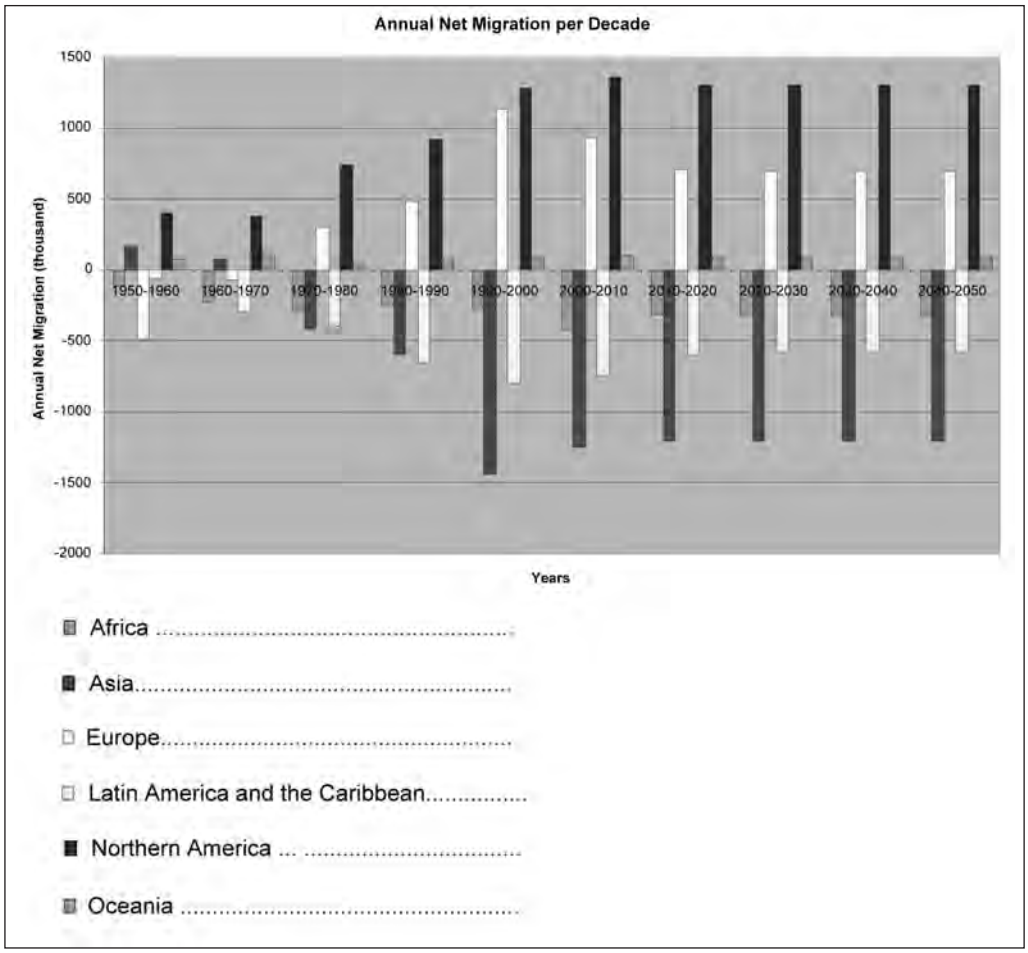

Fig. 7. Annual net migration over time by geographic area Data from United Nations

30% from Latin America and the Caribbean and the remainder from Africa.

The links between globalization and health are complex. Globalization is a multifaceted phenomenon that can affect health in myriad ways. Its consequences can be either direct – at the level of whole populations, individuals, and healthcare delivery systems – or indirect, through the economy and other factors, such as education, sanitation, and water supply[89]. Although increasing trade is certainly beneficial for economies, it also leads to a globalization of health risks. For example, the World Health Organization (WHO) estimates that the death toll from tobacco abuse alone will reach 10 million a year over the next two decades. Up to 70% of these deaths, caused by lung cancer, cardio-vascular diseases (CVDs), lung diseases, diabetes, and many other tobacco-related ailments, will occur in developing countries[90].

Alcohol consumption is another area in which the globalization of an industry has led to more health risks. In genetic terms, migration enables gene flow. This movement of genes from one population into another over time will cause the gene frequencies of different populations to converge. An obvious consequence for toxicological risk assessment will be the changes in the inter-individual variability factor, addressed not only by the genetic polymorphism, but also by other factors, such as age, gender, and nutritional disease status. In the last 15 years, the percentage of people of Asian origin in the United States has increased from 2.8 in 1990[91] to 3.6 in 2000 (fig. 6). It has been estimated that in 2004 this percentage increased to 4.2%[92]. From the example of the *ALDH2* genotype and Monte Carlo distribution of the blood acetaldehyde, the ratio between the 99th percentile and the median of the US population has barely changed over this period.

However, a different picture emerges at the state level. From 1990 to 2004, the Asian population in the states of New Jersey and New York doubled (fig. 6), whereas in California it increased from 9.8% in 1990 to 12.1%, according to a 2004 estimate. Consequently, there has been a steady increase of the ratio between the 99th percentile and the median of the population among the California, New Jersey, and New York State populations in the last 15 years (fig. 6 and Table 4). Although these estimated ratios for the New Jersey and New York populations are still lower than the default toxicokinetic uncertainty factor of 3.16-fold, should this migration trend be maintained, the ratios will probably be higher than the

default factor in less than a decade. These examples emphasize how migration may also influence the dynamic of risk assessment measurements.

Conclusion

In order to incorporate genetics in risk assessment, it is essential to: 1) understand the mechanisms and molecular bases involved in toxicological response; 2) identify polymorphic genes and determine the frequency of the polymorphisms in populations; 3) determine the functional activities of the product of the polymorphisms; and 4) incorporate kinetic or other functional data into PBPK risk models and interface the PBPK model with Monte Carlo analysis to incorporate functional variability caused by the polymorphism in the population and in the high-risk subgroup.

The cases we have presented in this paper illustrate that genotype-specific adjustment factors are essential in a formalized articulation of uncertainty and uncertainty analysis, in addition to chemical-specific adjustment factors. They also illustrate that population variability in genetic heterogeneity must be carefully scrutinized. "One size does not fit all" applies to both populations and inter-individual variations in vulnerability.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Agency for Toxic Substances and Disease Registry.

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Eur. J. Oncol., vol. 12, n. 3, pp. 171-173, 2007

Trattamento chirurgico dei GIST del duodeno

Surgical treatment of duodenal GISTs

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Riassunto

I GIST (gastrointestinal stromal tumours = tumori stromali gastrointestinali) sono neoplasie del tratto gastrointestinale e costituiscono un'entità nosologica relativamente nuova e in aumento negli ultimi 10 anni, rappresentando circa l'1-5% di tutte le neoplasie. All'analisi immunoistochimica, la positività per il CD117 è obbligatoria per porre diagnosi differenziale con tutti gli altri tumori di origine mesenchimale. La loro localizzazione è varia, interessando tutto il tratto gastroenterico con diverse percentuali; per quanto riguarda la localizzazione duodenale essa è dell'ordine del 3-5%. Vi sono diversi parametri per definire il grado di malignità anche se, in tutti i casi, la sola possibilità di cura è la completa resezione chirurgica. Anche se non necessitano di una estesa linfadenectomia perchè metastatizzano per via ematica, i GIST duodenali sono tumori che presentano notevoli difficoltà per quanto riguarda l'approccio terapeutico in relazione alla sede di insorgenza, al tipo di resezione chirurgica e all'elevata mortalità e complicanze post-operatorie. Eur. J. Oncol., 12 (3), 171-173, 2007

Parole chiave: **GIST, neoplasia duodenale, duodenocefalopancreasectomia (DCP)**

Summary

GISTs (gastrointestinal stromal tumours) are neoplasias of the gastrointestinal tract and represent a relatively new nosological entity, which has been increasing over the last 10 years, and now represents about 1-5% of all neoplasias. At immunohistochemical analysis, positivity for CD 117 is mandatory in order to make a differential diagnosis from all the other tumours of mesenchymal origin. Their location varies, interesting all the gastrointestinal tract with different percentages; duodenal location is in the order of 3-5%. There are various parameters to define the degree of malignancy, even if, in all cases, the only possibility of recovery is complete surgical resection. Even though extensive lymphadenectomy is not necessary, because they metastasize through the bloodstream, duodenal GISTs are tumours that present notable difficulties for the therapeutic approach, in relation to the site of onset, the type of surgical resection, and because of elevated mortality and post-operative complications. Eur. J. Oncol., 12 (3), 171-173, 2007

Key words: **GIST, duodenal neoplasias, duodenocephalopancreasectomy (DCP)**

Introduzione

I GIST (gastrointestinal stromal tumours = tumori stromali gastrointestinali) sono neoplasie di tipo mesenchimale che interessano il tratto gastrointestinale[1]. La loro incidenza, negli ultimi dieci anni, è progressivamente aumentata grazie all'avvento e al perfezionamento di nuove metodiche (immunoistochimica, microscopia elettroni-

Received/Pervenuto 29.4.2007 - Accepted/Accettato 6.6.2007
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ca), che hanno permesso di classificare come GIST alcune neoplasie precedentemente conosciute come leiomiosarcomi o tumori a origine nervosa[2]. Ad oggi i GIST rappresentano l'1-5% di tutte le neoplasie e si riscontrano più frequentemente sopra i 50 anni di età[2, 3].

La positività per il CD117 all'analisi immunoistochimica, per altro obbligatoria nella diagnosi differenziale con altri tumori stromali[4, 5], ne ha definito l'origine, facendo risalire tali neoplasie alle cellule di Cajal[6], cellule *pace-maker* del tratto gastrointestinale. Per tale motivo il loro interessamento è a carico della sottomucosa e della *muscularis propria*, spesso in continuità con sottosierosa e sierosa[6, 7].

La loro localizzazione è gastrica in oltre il 70% dei casi e nel 20-30% è a livello del piccolo intestino anche se, in piccole percentuali, tali neoplasie interessano il restante tratto gastrointestinale, l'omento e il retroperitoneo. Vi sono sostanziali differenze in termini di frequenza, morfologia e variabilità biologica in relazione al sito d'origine; i GIST a localizzazione gastrica, ad esempio, nonostante siano solitamente di dimensioni maggiori rispetto a quelli duodenali, sono generalmente a minor aggressività; quelli a localizzazione rettale hanno un alto tasso di recidiva locale e le lesioni con caratteristiche *GIST-like cells* (cellule fusiformi) a carico dell'esofago molto spesso sono negative al CD117 e pertanto esulano da questo gruppo di neoplasie[2, 8-12].

Discussione

I GIST del duodeno rappresentano localizzazioni rare di queste neoplasie: comprendono circa il 3-5% di tutti i GIST[13]. La maggior parte dei tumori si localizza nella 2ª e 3ª porzione duodenale. Per lo più sono singoli; possono anche essere multipli come nella triade di Carney, caratterizzata da GIST, condroma polmonare e paraganglioma extrasurrenalico. È nota un'associazione, più frequente che negli altri tipi di GIST, con la neurofibromatosi[14, 15].

Istologicamente essi sono costituiti da cellule epitelioidi, affusolate, che si organizzano a formare strutture simili ai paragangliomi e ai tumori carcinoidi[13, 16].

La maggior parte dei GIST duodenali è benigna, ma nel 30% dei casi essi danno metastasi[13], rare per via linfatica, per lo più per via ematica e più frequentemente al fegato. È difficile determinare la loro potenziale malignità e prognosi. È oggi comunemente accettata la divisione dei GIST in basso, intermedio, ed alto grado di rischio, riferendosi alla loro potenzialità di dare metastasi e recidive[16]. Il rischio è definito da una serie di parametri: la grandezza del tumore, la sede, l'invasione di organi e strutture adiacenti, l'invasione della mucosa, l'architettura cellulare, la conta mitotica, il pleiomorfismo nucleare, la necrosi, e la percentuale di cellule in proliferazione[17-20]. Una grandezza del tumore di >5 cm con un elevato indice mitotico (>50 divisione mitotiche per campo) rappresenta un GIST ad alto grado di metastatizzazione.

Il sanguinamento è la più comune presentazione clinica dei GIST duodenali[13]. Altri sintomi includono dolore, perdita di peso, comparsa di una massa addominale, vomito[13, 21]. La sola possibilità di cura è attualmente la completa resezione chirurgica[21]. Nella maggior parte dei casi si deve ricorrere ad interventi di duodenocefalopancreatectomia, senza la necessità di praticare estese linfoadenectomie, considerata la rara metastatizzazione per via linfatica. Altre possibilità chirurgiche sono rappresentate da resezioni duodenali parziali, laddove l'estensione della neoplasia consenta questo tipo di approccio conservativo.

L'obiettivo chirurgico deve essere quello di ottenere un intervento R0, con margini di resezione liberi da neoplasia. Infatti la sopravvivenza appare essere in parallelo con la completa radicalità chirurgica[17, 21].

La presenza di metastasi è il fattore principale che influenza la prognosi dei pazienti, con una sopravvivenza media di 14 mesi dopo la comparsa delle stesse[22]. Pazienti con metastasi epatiche isolate possono trarre beneficio da resezioni epatiche, portando in alcuni casi la sopravvivenza media a 3 anni[23]. Shima *et al*[22] dimostrano che la resezione di singole metastasi epatiche aumenta la sopravvivenza.

L'imatinib mesilato, un potente inibitore del gene c-kit, è stato utilizzato con successo nei pazienti con GIST avanzati o metastatici[24]; infatti questo composto ha dimostrato una buona attività antitumorale e una buona tolleranza da parte dei pazienti[25]. Il suo utilizzo in alcuni casi è stato esteso alla terapia neoadiuvante, adiuvante, e per prevenire metastasi dopo resezione[24].

Conclusioni

Pur essendo neoplasie rare, i GIST duodenali sono quelli che tra tutti i tipi di GIST presentano l'approccio chirurgico più difficile: infatti a causa della posizione anatomica che occupa il duodeno, per ottenere la totale radicalità oncologica bisogna ricorrere ad interventi altamente demolitivi, gravati da elevata mortalità e morbilità post operatoria.

La duodenocefalopancreatectomia (DCP), intervento chirurgico al quale vengono sottoposti la maggior parte dei pazienti affetti da questo tipo di neoplasia, come dimostra il grande numero di casi del Memorial Sloan-Kettering Cancer Center di New York, presenta una mor-

talità operatoria del 4%[24], percentuale alta rispetto ad altri tipi di interventi. Negli ultimi anni una serie di studi si sono concentrati sulla possibilità di ridurre la massa neoplastica per poter praticare, invece che una DCP, una resezione duodenale parziale. In casi selezionati, come dimostra lo studio di Kurihara *et al*[26] ciò si è reso possibile attraverso l'embolizzazione dell'arteria duodenale che ha ridotto le dimensioni della neoplasia consentendo una resezione parziale della seconda porzione duodenale sede della neoplasia.

Maggiore attenzione si è concentrata sull'utilizzo dell'imatinib mesilato come chemioterapico che, in terapia neoadiuvante, potrebbe ridurre le dimensioni del tumore e consentire una resezione duodenale parziale invece che interventi più demolitivi[24].

Inoltre, un ulteriore aumento della sopravvivenza potrebbe derivare, come accade per le patologie più rare, dalla gestione clinica e chirurgica di questi pazienti in centri selezionati con una casistica numerosa.

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Eur. J. Oncol., vol. 12, n. 3, pp. 175-181, 2007

Analisi di una casistica ospedaliera di neoplasie professionali

Analysis of a hospital case series of occupational cancer

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Riassunto

***Finalità*. Scopo del lavoro è quello di verificare la frequenza con la quale le neoplasie professionali sono diagnosticate nell'attuale pratica ospedaliera e di acquisire informazioni, utilizzabili in ambito preventivo, sulle loro caratteristiche eziologiche e anatomocliniche. *Pazienti e metodi*. Sono stati individuati i tumori segnalati dal nostro Istituto all'INAIL come professionali nel quinquennio 2001-2005. Dalla documentazione clinica dei singoli casi sono stati quindi ricavati i dati relativi a: sede d'insorgenza, tipo istologico, agenti causali, settori lavorativi e mansioni a rischio, durata dell'esposizione, periodo di latenza, abitudine al fumo, eventuali patologie concomitanti. *Risultati*. Nel periodo considerato, sono stati diagnosticati 16 tumori occupazionali in 15 soggetti maschi, in prevalenza fumatori o ex-fumatori, tutti in seguito deceduti. Tale numero è inferiore a quanto prevedibile in base ad alcune stime epidemiologiche. La casistica comprende 8 casi di carcinoma polmonare, 6 di mesotelioma pleurico maligno e 2 di urotelioma vescicale. L'amianto (tumori respiratori) è risultato l'agente eziologico più frequentemente implicato, seguito dagli idrocarburi policiclici aromatici (carcinomi polmonari e vescicali) e dalla silice cristallina (due carcinomi polmonari, di cui uno insorto in un minatore già affetto da silicosi). Edili (6 casi) e metalmeccanici (4 casi) sono stati i lavoratori più colpiti. I tumori sono insorti in seguito ad esposizioni reiterate negli anni e (tranne che in due soggetti) dopo lunghi**

Summary

***Aim*. The purpose of this study is to verify the frequency of diagnosis of occupational cancer in current hospital practice, and to acquire information, utilizable for prevention, on its aetiology, pathology and clinical aspects. *Patients and methods*. The study singles out those cases presented by our Institute to the Italian National Institute for Occupational Health Insurance (*Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro* = INAIL) as occupational cancers in the years 2001-2005. For each case, data were collected on the anatomical site of onset, histopathology, causative agents, occupational sector and hazardous job description, duration of exposure, latency period, smoking habit, and possible concomitant diseases. *Results*. In the period considered, 16 occupational neoplasms were diagnosed in 15 males, mostly smokers or ex-smokers, all subsequently deceased. Such number is less than expected on the basis of epidemiological estimates. The series includes 8 cases of lung carcinoma, 6 of malignant pleural mesothelioma, and 2 of bladder urothelioma. Asbestos (respiratory tumours) was the aetiologic factor most frequently involved, followed by polycyclic aromatic hydrocarbons (lung and bladder carcinomas), and crystalline silica (two lung carcinomas, one of which in a miner already affected with silicosis). Workers in the building (6 cases) and metal (4 cases) industries were the most frequently affected. The tumours were induced by long-lasting exposures and (with the**

Received/Pervenuto 6.12.2006 - Accepted/Accettato 12.4.2007
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periodi di latenza dall'allontanamento dal rischio. *Conclusioni*. Le neoplasie professionali sono probabilmente sottostimate nella pratica clinica. Un'accurata anamnesi lavorativa è fondamentale per la loro diagnosi eziologica. L'apparato respiratorio e la vescica urinaria continuano a essere bersagli preferenziali per l'oncogenesi occupazionale. L'edilizia e l'industria metalmeccanica sono le attività produttive a rischio maggiore, per le quali maggiormente si impongono migliori misure preventive. In particolare, sono necessari programmi di sorveglianza sanitaria per i soggetti con pregressa esposizione ad amianto. Eur. J. Oncol., 12 (3), 175-181, 2007

Parole chiave: **anamnesi lavorativa, amianto, carcinoma polmonare, mesotelioma, urotelioma**

exception of two cases) appeared many years after discontinuation of the jobs at risk. *Conclusions*. Occupational cancer is probably underestimated in clinical practice. The collection of each patient's in-depth occupational history is crucial for aetiological diagnosis. The respiratory system and the urinary bladder continue to be preferential targets for work-related carcinogenesis. Construction and metal working are the productive activities at highest risk and are those which, even more than other sectors, require improved preventive measures. In particular, health surveillance programmes are needed for people with previous asbestos exposure. Eur. J. Oncol., 12 (3), 175-181, 2007

Key words: **occupational history, asbestos, lung carcinoma, mesothelioma, urothelioma**

Introduzione

Si definiscono professionali le neoplasie nella cui eziopatogenesi abbiano svolto un ruolo causale o concausale agenti cancerogeni presenti nell'ambiente di lavoro[1]. A partire dalla storica descrizione di Pott, nel 1775, del carcinoma scrotale degli spazzacamini[2], numerose osservazioni cliniche ed epidemiologiche, accanto a numerosi studi sperimentali *in vivo* e *in vitro*, hanno portato all'identificazione di diverse centinaia di agenti (fisici, biologici e, soprattutto, chimici) in grado di aumentare il rischio oncogeno per l'uomo. Essi sono attualmente classificati secondo diversi criteri in apposite liste, periodicamente aggiornate, dalla IARC (*International Agency for Research on Cancer*) e da altri enti ed agenzie nazionali e internazionali[3]. Tra i più noti cancerogeni occupazionali rientrano le radiazioni ionizzanti (in grado di indurre leucemie, tumori cutanei e altre neoplasie solide), gli idrocarburi aromatici policiclici (IPA), quali benzopirene, dibenzantracene, metilcolantrene, contenuti nelle pece, nel catrame, nella fuliggine e nei prodotti di combustione in generale (cancerogeni per cute, apparato respiratorio e vescica urinaria), alcune amine aromatiche (benzidina, 2-naftilamina, 4-aminodifenile) in grado di indurre uroteliomi, i composti del cromo esavalente (tumori respiratori), il benzene (leucemie), il cloruro di vinile monomero (angiosarcoma epatico), e l'amianto (carcinoma polmonare e mesotelioma maligno)[1, 4].

Nel 1981, Doll e Peto[5] stimarono in circa il 4% (7% considerando solo i soggetti di sesso maschile) la proporzione di tutte le neoplasie maligne umane attribuibili ad esposizioni lavorative, che sarebbe possibile prevenire con opportuni interventi di contenimento del rischio. Percentuali ancora più alte derivano da stime epidemiologiche incentrate sugli organi (quali polmone e vescica urinaria) più comunemente sede di tumori professionali[6, 7].

Nella pratica clinica, tuttavia, la proporzione di neoplasie per le quali viene effettivamente riconosciuta un'eziologia professionale è molto inferiore[8, 9]; ancora più bassa è la percentuale di tumori riconosciuti come occupazionali in ambito medico-legale e assicurativo[10, 11]. Ciò in buona parte deriva dalla difficoltà di dimostrare il nesso di causa nei singoli casi. La patologia oncologica professionale è infatti costituita da tumori diffusi anche nella popolazione generale e non presenta caratteri anatomopatologici o clinici distintivi; inoltre, essa insorge dopo periodi di latenza di anni o decenni (rendendo difficile l'identificazione e la quantificazione delle esposizioni lavorative a rischio), colpendo siti esposti anche all'azione di cancerogeni ambientali o voluttuari (primo fra tutti il fumo di tabacco)[4, 10, 12]. In molti casi, tuttavia, la mancata formulazione della diagnosi eziologica dipende da un'inadeguata raccolta dell'anamnesi lavorativa[12].

In tale contesto, abbiamo analizzato la casistica di neoplasie professionali individuate nell'ultimo quinquennio presso l'Istituto Scientifico di Pavia della Fondazione Maugeri (al quale appartiene la nostra unità operativa di medicina del lavoro). Tale centro medico comprende reparti di degenza, *day-hospital* e ambulatori di oncologia, cure palliative, medicina interna, chirurgia generale e

pneumologia, con un afflusso annuo di circa 1.500 pazienti oncologici, provenienti in maggior parte dal Nord Italia, ossia da un'area geografica altamente industrializzata. Lo studio è stato intrapreso con il duplice scopo di verificare con quale frequenza i tumori professionali sono identificati nell'attuale pratica ospedaliera e di ricavare informazioni, utilizzabili in ambito preventivo, circa le loro caratteristiche eziologiche e anatomo-cliniche.

Pazienti e metodi

In base alle segnalazioni inoltrate all'Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro (INAIL) dalla Direzione Sanitaria dell'Istituto, sono state rintracciate le neoplasie diagnosticate come professionali nel periodo compreso tra il 1° gennaio 2001 e il 31 dicembre 2005. Deve essere precisato che tali diagnosi sono probabilistiche e in massima parte basate su quanto anamnesticamente riferito dai pazienti e dai loro familiari. In altre parole, secondo la prassi dell'Istituto, una malattia neoplastica è segnalata (all'INAIL e alle competenti autorità giudiziarie e ispettive) come "professionale" quando l'anamnesi lavorativa fornisce elementi tali da far ritenere che le riferite esposizioni occupazionali, per le loro intensità e durata, abbiano quanto meno svolto un importante ruolo concausale.

Le cartelle cliniche dei casi così identificati sono state recuperate nel Sistema Informativo Ospedaliero (SIO) del centro medico. Dalle cartelle sono stati quindi ricavati i dati clinici e anagrafici dei pazienti e le informazioni disponibili relative alla loro vita lavorativa. Le neoplasie costituenti la casistica sono state analizzate in base all'apparato interessato, al tipo istologico, alle mansioni lavorative con i relativi rischi, alla durata dell'esposizione agli agenti eziologici, al periodo di latenza tra la fine dell'esposizione al rischio professionale e l'insorgenza della malattia. In base all'abitudine al fumo, i pazienti sono stati classificati in fumatori, ex-fumatori e non fumatori. È stata infine verificata l'eventuale presenza di patologie non neoplastiche (in particolare: placche pleuriche, pneumoconiosi), dovute agli stessi agenti eziologici responsabili dei tumori.

Risultati

Nel quinquennio considerato sono stati segnalati all'INAIL come affetti da neoplasia professionale 15 pazienti (Tabella 1), tutti di sesso maschile, d'età compresa tra 41 e 75 anni (media: 65,6), dei quali uno (caso n. 14) portatore di due tumori (carcinoma polmonare e urotelioma). Oltre ad essere stati esposti ai cancerogeni occupazionali, molti soggetti erano fumatori o ex-fumatori. Tutti sono deceduti a causa della loro malattia neoplastica.

Delle 16 neoplasie che costituiscono la casistica, 14 interessano l'apparato respiratorio (6 casi di mesotelioma pleurico e 8 di carcinoma polmonare, così ripartiti: 2 adenocarcinomi, 2 carcinomi a cellule squamose, 1 microcitoma, 3 carcinomi non a piccole cellule, non meglio identificati dal punto di vista istologico). I restanti 2 tumori sono uroteliomi (carcinomi a cellule transizionali) della vescica urinaria.

Per quanto riguarda gli agenti causali, l'amianto è stato identificato come agente eziologico dei 6 casi di mesotelioma e di 5 neoplasie polmonari, in 2 delle quali (pazienti n. 8 e 10) è stato identificato un ruolo eziologico anche per gli IPA contenuti nei prodotti di combustione. L'esposizione professionale a IPA (in soggetti fumatori) è stata inoltre associata ai due tumori insorti a distanza di tre anni uno dall'altro nel paziente n. 14 e all'urotelioma del caso n. 15. I restanti due carcinomi polmonari (casi n. 11 e 12) sono stati attribuiti a polveri miste contenenti silice (SiO_2) libera cristallina. L'esposizione ai fattori di rischio era avvenuta principalmente in edilizia (6 casi) o nell'industria metalmeccanica (4 pazienti).

Tutti i pazienti erano stati esposti reiteratamente agli agenti cancerogeni in ambito lavorativo per periodi di tempo assai lunghi (Tabella 1): in due casi (n. 4 e 9) non è stato possibile ricostruire con esattezza la durata dell'esposizione (comunque pluriennale), nei rimanenti 13 questa varia da 10 a 46 anni. In due soggetti (casi n. 8 e 15) la neoplasia professionale è comparsa mentre l'esposizione era ancora in atto; negli altri casi in cui è stato possibile calcolare il periodo di latenza, questo è sempre risultato superiore al decennio con un massimo di 41 anni per il paziente n. 1.

Per quanto riguarda l'eventuale presenza di patologie associate, il soggetto n. 12 (minatore) era portatore di silicosi. Tre pazienti (n. 2, 4 e 10) erano affetti da bronchite cronica, uno (n. 8) da broncopmeumopatia cronica ostruttiva (BPCO). Negli altri non erano presenti malattie non neoplastiche correlabili, almeno in parte, agli stessi agenti causali delle patologie tumorali.

Discussione

Il primo dato che emerge dal presente studio è l'esiguo numero di tumori professionali (16 in 15 soggetti) diagnosticati nel quinquennio considerato. Poiché in tale periodo sono transitati presso l'Istituto circa 7.500 pazienti oncologici, ne deriva che una diagnosi di neoplasia occupazionale è stata posta solo nello 0,2% di essi, ossia in

Tabella 1 - Neoplasie professionali segnalate all'INAIL nel periodo 2001-2005 e loro caratteristiche

N.	Sesso	Età (anni)	Fumo	Patologia	Apparato	Settore produttivo	Mansione	Eziologia	Durata esposizione (anni)	Latenza[a] (anni)
1	M	74	sì	Mesotelioma pleurico	Respiratorio	Edilizia	Posatore cemento-amianto	Amianto	19	41
2	M	73	sì	Mesotelioma pleurico	Respiratorio	Edilizia	Marmista scultore	Amianto	46	14
3	M	73	no	Mesotelioma pleurico	Respiratorio	Metalmeccanica	Impiantista elettrico	Amianto	10	non nota[b]
4	M	44	no	Mesotelioma pleurico	Respiratorio	Industria chimica	Operaio, manutentore	Amianto	non nota[b]	non nota
5	M	68	sì	Mesotelioma pleurico	Respiratorio	Vari	Conduttore di caldaie	Amianto	22	non nota
6	M	72	ex	Mesotelioma pleurico	Respiratorio	Vari	Elettricista manutentore	Amianto	19	32
7	M	64	sì	Adenocarcinoma polmonare	Respiratorio	Edilizia	Muratore	Amianto	17	37
8	M	56	sì	Adenocarcinoma polmonare	Respiratorio	Edilizia	Impiantista, bitumatore	Amianto, IPA[c]	35	_[d]
9	M	71	non noto	Ca. squamocellulare polmonare	Respiratorio	Edilizia	Muratore	Amianto	non nota[b]	non nota[b]
10	M	63	ex	Ca. squamocellulare polmonare	Respiratorio	Petrolchimica	Operaio petrolchimico	Amianto, IPA[c]	29	non nota[b]
11	M	73	ex	Microcitoma polmonare	Respiratorio	Edilizia	Addetto a cottura laterizi	Polveri miste[e]	30	16
12	M	75	sì	Carcinoma polmonare n.a.s.[f]	Respiratorio	Industria estrattiva	Minatore	Polveri miste[e]	35	non nota[b]
13	M	68	ex	Carcinoma polmonare n.a.s.[f]	Respiratorio	Metalmeccanica	Impiantista elettrico	Amianto	35	non nota
14	M	69	sì	Carcinoma polmonare n.a.s.[f] Urotelioma vescicale	Respiratorio Urinario	Metalmeccanica	Addetto ai forni di cottura	IPA[c]	28	25 22
15	M	41	sì	Urotelioma vescicale	Urinario	Metalmeccanica	Verniciatore	IPA[c]	20	_[d]

[a] Definita come intervallo tra fine dell'esposizione all'agente cancerogeno e diagnosi di neoplasia
[b] Durata non specificata, ma comunque dell'ordine di anni
[c] Idrocarburi Policiclici Aromatici (presenti nei prodotti di combustione)
[d] Neoplasia diagnosticata mentre l'esposizione all'agente cancerogeno era ancora in atto
[e] Contenenti silice libera cristallina
[f] Non altrimenti specificato

una proporzione nettamente inferiore a quella prevedibile in base ad alcune stime epidemiologiche[5-7]. Rimarchevole è inoltre l'assenza nella casistica di soggetti di sesso femminile, tra cui pure notoriamente insorgono tumori professionali, anche se con incidenza inferiore rispetto agli uomini[5]. Tali riscontri appaiono di particolare rilievo se si considera che l'indagine è stata condotta presso un'istituzione (la Fondazione Maugeri) che ha tra le proprie finalità la tutela della salute dei lavoratori. Quanto osservato è verosimilmente dovuto a diversi fattori, non ultime le obiettive difficoltà, ricordate nell'introduzione, nella formulazione della diagnosi eziologica. Inoltre, presso il centro medico sono diagnosticati e trattati molti tumori (es. carcinomi mammari) per i quali non è nota in letteratura (e pertanto non dimostrabile nella pratica clinica) una chiara eziologia professionale. Infine, neoplasie che spesso hanno un'origine occupazionale, quali i carcinomi cutanei[1, 4], sono di regola convogliati presso ospedali provvisti di servizi specialistici (dermatologia) che mancano presso il nostro Istituto. Probabilmente per questo motivo, i tumori cutanei risultano assenti nella presente casistica.

Considerato quanto sopra, rimane il plausibile sospetto che una parte dei tumori professionali sia stata in realtà "perduta"[8], ossia sfuggita a un corretto inquadramento diagnostico circa l'eziologia, per carenza di un'anamnesi lavorativa sufficientemente approfondita. Si può in proposito ricordare che i pazienti neoplastici talora giungono al nostro Istituto con malattia in stadio avanzato e in condizioni generali alquanto compromesse, non risultando pertanto in grado di sostenere un'intervista accurata. Inoltre, anche soggetti in condizioni discrete, soprattutto se anziani, spesso non ricordano dettagli su attività professionali esercitate molti anni prima, o possono essere reticenti circa lavori svolti in nero o senza il rispetto delle misure preventive. A riprova di ciò, in alcuni dei casi qui presentati, l'esame della documentazione disponibile ha evidenziato alcune lacune circa la durata dell'esposizione ai cancerogeni occupazionali e/o il tempo intercorso tra fine dell'esposizione e comparsa della neoplasia.

Recentemente, Porru *et al*[12] hanno condotto una ricerca sistematica delle neoplasie polmonari professionali presso gli Spedali Civili di Brescia, durata sette anni: dopo adeguata sensibilizzazione, i medici dei vari reparti segnalavano, mediante una scheda informativa comprendente una sintetica anamnesi lavorativa, i nuovi casi di neoplasia polmonare primitiva al medico del lavoro, il quale effettuava una visita di consulenza laddove fosse ravvisabile un'eziologia professionale. Mentre nel periodo precedente allo studio venivano identificati solo pochi casi professionali (e ancor meno erano indennizzati dall'INAIL), nel periodo studiato sono stati attribuiti all'occupazione 182 tumori polmonari (il 26% dei casi sottoposti a consulenza), oltre un terzo dei quali riconosciuti dall'istituto assicuratore al momento della pubblicazione dei dati. Appare pertanto evidente come il medico del lavoro possa (e debba) coadiuvare gli specialisti di altre discipline nella ricerca sistematica delle neoplasie professionali, anche in considerazione delle importanti ricadute in ambito medico-legale e assicurativo.

Pur nella sua esiguità, la casistica qui descritta permette alcune osservazioni sugli attuali aspetti dell'oncogenesi occupazionale. Innanzi tutto spicca l'assenza di alcune neoplasie professionali "storiche", quali il carcinoma vescicale da amine aromatiche, le leucemie da benzene, l'angiosarcoma epatico da cloruro di vinile monomero. Tale dato concorda con quanto osservato in precedenza nel medesimo Istituto[9] e con ogni probabilità riflette i mutamenti dei cicli tecnologici e i miglioramenti in ambito preventivo e legislativo avvenuti negli ultimi decenni.

Sono stati invece riscontrati, in ordine decrescente di frequenza, carcinomi polmonari, mesoteliomi pleurici e uroteliomi vescicali (da IPA). L'apparato respiratorio e, in misura minore, la vescica urinaria si confermano pertanto bersagli preferenziali per l'oncogenesi occupazionale. Questo non sorprende, in quanto l'esposizione lavorativa a cancerogeni chimici avviene principalmente per via inalatoria, mentre, nel caso di esposizione a IPA, il lume vescicale è sede d'accumulo dei metaboliti attivi escreti con le urine[1, 4].

Tra i carcinomi polmonari sono comprese diverse forme istologiche, con un unico caso di microcitoma. Anche questo dato concorda con la letteratura, in quanto il tumore a piccole cellule è sensibilmente più raro rispetto agli altri istotipi, sia tra le neoplasie professionali sia tra quelle "spontanee"[12].

La casistica comprende sei casi di mesotelioma pleurico maligno causati dall'inalazione di fibre d'amianto, neoplasia assai aggressiva e, contrariamente al carcinoma polmonare, relativamente rara tra la popolazione generale[13]. Questa preoccupante osservazione concorda con recenti proiezioni epidemiologiche secondo le quali, nell'Europa occidentale, la mortalità per mesotelioma dovrebbe raddoppiare ogni anno fino attorno al 2018[14]. In tale ambito, l'Italia si colloca tra i Paesi con i tassi di mortalità più elevati tra i maschi e con una tendenza in maggior crescita tra le donne (assenti però nella presente indagine): tra il 2012 e il 2024 è atteso un picco di mortalità per mesotelioma pleurico di circa 800 casi per anno[15]. Questo fenomeno è facilmente spiegabile se si considera che, grazie alle sue proprietà fisico-chimiche e al relativo basso costo, l'amianto (o asbesto) è stato tra i materiali più largamente utilizzati in epoca contemporanea, per esempio in edilizia (manufatti in cemento-amianto, pan-

nelli antincendio, *etc*.), nelle industrie navale, aeronautica e ferroviaria (rivestimenti coibentanti e antincendio), automobilistica (freni e frizioni), spaziale (scudi antincendio), metallurgica e metalmeccanica (schermi, indumenti protettivi), alimentare (filtri per alimenti), delle materie plastiche (additivi, rinforzanti) e per la produzione di svariati altri manufatti d'uso comune (tute, isolanti elettrici, *etc*.)[16, 17]. Nel corso degli anni i livelli espositivi a fibre d'amianto negli ambienti di vita e di lavoro sono progressivamente diminuiti, in relazione ai progressi tecnici delle lavorazioni e dei limiti sempre più restrittivi imposti dalle legislazioni, in Italia e all'estero, in conseguenza delle conoscenze sempre più approfondite sui rischi derivanti dall'impiego di questo materiale. Nel nostro Paese, la legge 257/1992 definisce le norme applicative per la cessazione dell'impiego dell'asbesto. Tuttavia, l'esposizione negli ambienti di vita e di lavoro continua ad essere un problema di salute pubblica per almeno tre motivi[17, 18]: 1) i numerosi lavoratori esposti prima dell'entrata in vigore del decreto continuano a essere una popolazione a rischio; 2) una categoria di lavoratori tuttora esposti a rischio specifico da amianto è rappresentata dagli addetti ad operazioni di demolizione, smaltimento e bonifica; 3) persistono negli ambienti di vita e di lavoro manufatti in amianto che vanno incontro a processi di disgregazione con liberazione di fibre nell'aria. Per tali ragioni e in considerazione della lunga latenza clinica delle patologie asbesto-correlate[17, 19] (confermata anche nel presente studio), è indispensabile la sorveglianza sanitaria degli ex-esposti, sia per identificare e seguire la patologia derivante dal passato sia per valutare l'effetto delle basse esposizioni verificatesi negli ultimi anni[20, 21].

Oltre che per la pleura, l'amianto è sicuramente oncogeno per il peritoneo e per il polmone, sul quale può interagire con il fumo di tabacco o con altri cancerogeni professionali o ambientali[1, 4], come indicato anche dalla presente indagine dove l'amianto (in associazione con il fumo di tabacco e/o con altri prodotti di combustione presenti nell'ambiente di lavoro) è stato identificato come agente causale in oltre la metà dei carcinomi polmonari. Nella patogenesi del carcinoma, l'amianto sembra comportarsi da promotore (agendo in sinergia con i prodotti di combustione), mentre nell'induzione del mesotelioma esso agisce come cancerogeno completo: in questo processo sembrano svolgere un ruolo centrale radicali liberi dell'ossigeno provvisti di azione genotossica sulle cellule mesoteliali[22].

La patologia asbesto-correlata comprende anche diverse patologie respiratorie non neoplastiche: asbestosi, broncopatie ostruttive croniche, placche pleuriche e altre pleuropatie benigne[17, 20, 21]. I soggetti affetti da tali malattie rappresentano una popolazione ad alto rischio neoplastico. La tendenza a sviluppare mesoteliomi o carcinomi polmonari esiste comunque in tutti gli ex-esposti ad amianto, anche in assenza di placche pleuriche o di segni radiologici di asbestosi, come indicato sia da alcuni studi epidemiologici[23, 24] sia dalla presente indagine.

Oltre che all'amianto, nella casistica presentata è stato attruibuito un ruolo eziologico agli IPA e alla silice cristallina. Mentre gli IPA sono stati, da un punto di vista storico, tra i primi composti ad essere riconosciuti come cancerogeni[1, 4], la silice è stata classificata dalla IARC nel gruppo 1 ("cancerogeno accertato per l'uomo") solo nel 1997, soprattutto in considerazione di alcuni studi epidemiologici che evidenziano un eccesso di rischio per cancro polmonare in soggetti affetti da silicosi[25], come nel paziente n. 12 della presente casistica. Successive metaanalisi dei dati pubblicati hanno quindi indicato l'esistenza di un rischio neoplastico lievemente (ma significativamente) aumentato anche in assenza di pneumoconiosi[26, 27], come apparentemente si è realizzato in 19 soggetti compresi nella citata casitica di Porru *et al*[12] e nel caso n. 11 del nostro studio.

Il paziente n. 14 necessita un commento particolare in quanto trattasi di un fumatore che ha sviluppato due carcinomi (al polmone e alla vescica) tipicamente fumo-correlati. Questo caso è stato comunque segnalato all'INAIL come probabilmente professionale, in quanto si è ritenuto che l'esposizione per 28 anni ai prodotti di combustione derivanti dai forni abbia contribuito in modo concausale allo sviluppo delle due neoplasie.

L'analisi dei dati per attività produttive indica che gli edili e gli operai metalmeccanici sono i lavoratori maggiormente colpiti da malattie neoplastiche occupazionali, richiamando la necessità di migliori interventi preventivi in tali settori. Proprio in edilizia, per esempio, dove si può facilmente verificare l'esposizione ad amianto (demolizioni, bonifiche, ristrutturazioni), silice cristallina (refrattari, laterizi, sabbiature) ed IPA (bitume, pece, catrame), è particolarmente diffuso il fenomeno del lavoro sommerso e cottimista e sono meno rispettate le normative sulla sicurezza e sull'igiene del lavoro[28]. In metalmeccanica si possono realizzare esposizioni "atipiche" all'amianto (vecchie tubature, guarnizioni di macchinari, *etc*.), in un settore industriale dove questo materiale non era presente come materia prima e dove il rischio non è pertanto assicurato[11, 16]. Nella casistica esaminata, ad esempio, l'anamnesi lavorativa ha svelato l'esposizione ad amianto in due impiantisti elettrici (casi n. 3 e 13).

Infine, la presente indagine conferma che le neoplasie professionali insorgono in seguito a esposizioni assai protratte (anni o decenni) agli agenti cancerogeni, di regola dopo altrettanto lunghi periodi di latenza dalla cessazione della lavorazione a rischio. Se da un lato questo fenome-

no costituisce, come si è detto, un ostacolo per il corretto inquadramento eziologico dei casi che giungono all'osservazione clinica, dall'altro esso richiama l'opportunità di rinforzare le misure di prevenzione primaria (come previsto dalla legislazione vigente) e i programmi di sorveglianza sanitaria (anche post-esposizione) finalizzati alla diagnosi precoce.

Conclusioni

La presente indagine indica che le neoplasie professionali sono identificate solo raramente nell'attuale pratica ospedaliera e richiama l'importanza di un'accurata anamnesi occupazionale ai fini della loro diagnosi eziologica, alla quale conseguono importanti ricadute sul piano medico-legale e assicurativo. Le forme occupazionali che il clinico deve aspettarsi di osservare sono il carcinoma polmonare, il mesotelioma pleurico e l'urotelioma vescicale. L'amianto rappresenta l'agente causale più frequentemente implicato (nonostante il suo impiego sia vietato dal 1992), seguito dai prodotti di combustione e dalla silice cristallina. L'edilizia e l'industria metalmeccanica sono i settori produttivi più colpiti, per i quali maggiormente si impongono migliori misure preventive.

Ringraziamenti

Desideriamo ringraziare la Sig.ra Laura Villa (Direzione Sanitaria della Fondazione Maugeri) per la pronta e cortese collaborazione nel reperimento della documentazione clinica relativa al presente studio.

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Eur. J. Oncol., vol. 12, n. 3, pp. 183-187, 2007

An estimate of pleural mesothelioma incidence in Biancavilla, Sicily, Italy, 1998-2004

Stima dell'incidenza del mesotelioma pleurico a Biancavilla, Sicilia, 1998-2004

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Summary

***Aims*. The purpose of this study was to estimate the incidence of mesothelioma in Biancavilla, a town in Sicily, Italy contaminated by an asbestos-like fluoroedenitic fibre, in the period 1998-2004 and to ensure epidemiological monitoring of the area. *Materials and methods*. Pleural mesothelioma cases (ICD9 163) among residents of the municipality of Biancavilla diagnosed between 1/1/1998 and 31/12/2004, were identified using hospital admission data. Death certificates reporting mesothelioma among Biancavilla residents were gathered to make the survey exhaustive. The Ragusa Cancer Registry provided individual clinical records and pathology files. Sex-specific crude and standardized incidence rates were calculated for ascertained cases, and for total cases. The Italian population of 1991 was taken as the reference to calculate standardised rates with the direct method in order to compare findings with those of the National Mesothelioma Registry (*Registro Nazionale dei Mesoteliomi* = ReNaM). *Results*. Nineteen cases (9 men and 10 women) of mesothelioma occurred in the study period. For eight subjects clinical records and pathology files were found (ascertained cases): the crude incidence rate was $5.1x10^{-5}$ (males 6.6, females 3.7). After standardisation, the rate for overall cases was $5.4x10^{-5}$**

Riassunto

***Finalità*. Scopo del lavoro è quello di stimare l'incidenza del mesotelioma, negli anni 1998-2004, a Biancavilla, cittadina della Sicilia contaminata dalla fibra asbestiforme di fluoroedenite e assicurare il monitoraggio epidemiologico dell'area. *Materiali e metodi*. Con l'ausilio delle schede di dimissione ospedaliera (SDO), sono stati identificati i casi di mesotelioma pleurico (ICD9 163) nei residenti di Biancavilla diagnosticati tra il 1/1/1998 ed il 31/12/2004. Per rendere lo studio esaustivo, sono stati raccolti i certificati di morte con la diagnosi di mesotelioma tra i residenti di Biancavilla. Il Registro Tumori di Ragusa ha fornito su base individuale cartelle cliniche e dati di anatomia patologica. Per casi accertati e casi totali sono stati calcolati tassi di incidenza grezzi e standardizzati per sesso. Per i tassi standardizzati diretti è stata utilizzata la popolazione italiana del 1991 per confrontare i risultati con i dati del Registro Nazionale dei Mesoteliomi (ReNaM). *Risultati*. Nel periodo preso in esame, si sono verificati diciannove casi di mesotelioma (9 uomini, 10 donne). Per otto soggetti erano disponibili cartelle cliniche e dati di anatomia patologica (casi accertati): il tasso grezzo di incidenza è pari a $5,1x10^{-5}$ (6,6 uomini, 3,7 donne). Il tasso standardizzato era pari a $5,4x10^{-5}$ (7,4 uomini, 3,6 donne). Con i rimanenti 11**

Received/Pervenuto 20.4.2007 - Accepted/Accettato 24.5.2007
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(males 7.4, females 3.6). Including the remaining eleven cases with information to be completed (suspected cases), the crude and standardised rates were 12.1 (males 11.9, females 12.3) and 13.9 (males 13.5, females 14.4), respectively. Mesothelioma incidence in Biancavilla is comparable to that of Regions where the presence of important sources of asbestos exposure caused a significant increase in the occurrence of the disease. *Conclusions*. The elevated incidence rate of pleural mesothelioma in Biancavilla supports the notion of a high risk area. Experimental induction of mesothelioma in rats has confirmed the fibres' aetiological rôle. Land improvement and decontamination of the area were advised by the Italian National Health Institute. Further epidemiological surveillance is required in order to set priorities for environmental reclamation and to evaluate the efficacy of the public health interventions which have been adopted. Eur. J. Oncol., 12 (3), 183-187, 2007

Key words: **mesothelioma, incidence, fluoro-edenite, environmental exposure**

casi sospetti (raccolta informazioni in corso), i tassi erano 12,1 (grezzo) (uomini 11,9, donne 12,3) e 13,9 (standardizzato) (uomini 13,5, donne 14,4). L'incidenza del mesotelioma a Biancavilla era confrontabile a quella riscontrabile in Regioni italiane note per la presenza di importanti sorgenti di esposizione ad amianto. *Conclusioni*. L'elevato tasso di incidenza del mesotelioma pleurico a Biancavilla avvalora la nozione di area ad alto rischio. L'induzione sperimentale del mesotelioma nei ratti conferma il ruolo eziologico della fibra. Sono state suggerite da parte dell'Istituto Superiore di Sanità azioni per la bonifica ambientale. È opportuno proseguire la sorveglianza epidemiologica per contribuire alla definizione delle priorità della bonifica, e per valutare l'efficacia dell'azione di sanità pubblica intrapresa. Eur. J. Oncol., 12 (3), 183-187, 2007

Parole chiave: **mesotelioma, incidenza, fluoro-edenite, esposizione ambientale**

Introduction

A national geographic mortality study found a cluster of pleural malignant neoplasms (period 1988-1992) in Biancavilla, a town located on the south-western slope of the Etna volcano (Sicily, Italy)[1]. No asbestos-related industrial activities have ever been present in the area, but the Monte Calvario stone quarry, material from which was extensively used in the local building industry, was identified as a source of an asbestos–like fibre[2]. Over the period 1980-1997, 17 cases of pleural mesothelioma were identified (7 females and 10 males), and the relative hospital medical documentation was gathered and examined. After interviewing patients or their next of kin on prior fibre exposure, exposure categories were attributed following the Italian Registry of Mesothelioma (*Registro Nazionale dei Mesoteliomi* = ReNaM) guidelines. An annual incidence rate of around 7.0×10^{-4} was estimated. Successively the fibre was recognized as fluoro-edenite, a new fibrous mineral of the edenite-fluoroedenite series[3]. Fluoro-edenite is the third mineral fibre (with erionite and winchite), not classified as asbestos, with a demonstrable mesotheliomatogenous action in humans[4].

To reduce exposure to fluoro-edenite, the Italian National Health Institute (*Istituto Superiore di Sanità*) provided the Municipality of Biancavilla and the Sicily Region with public health recommendations: termination of quarrying activity, removal of sources of dust in the town centre of Biancavilla, and asphalting roads previously paved with local soil materials[5].

These recommendations were successfully adopted in 2001, when Biancavilla was included among the sites of national interest for environmental reclamation.

The framework within which this epidemiological study was being conducted was further characterised by the results of other scientific studies: Soffritti *et al* [6] demonstrated *in vivo* that fluoro-edenitic fibres can induce mesothelioma in rats, while Travaglione *et al* [7] showed *in vitro* cell changes similar to those induced by asbestos type crocidolite. Biggeri *et al* [8] found a significant association between chronic obstructive pulmonary disease (COPD) and pleural neoplasm mortality in a geographic mortality study encompassing the whole Etna volcano area; both diseases showed a high frequency in Biancavilla, especially in women. A higher Standardized Hospitalization Ratio (SHR) for acute respiratory diseases and cardiovascular diseases was identified in Biancavilla, as compared to data in other local municipalities, thus confirming the risks for mesothelioma and COPD[9]. Recently, fluoro-edenitic fibres were identified in the sputum of 6 out of 12 patients (farmers or housewives) living in Biancavilla and suffering from chronic

bronchitis, thus pointing to serious environmental exposure levels[10].

The aim of the present study is to estimate the incidence of mesothelioma in Biancavilla in the period 1998-2004, in order to ensure epidemiological monitoring of the area of interest.

Materials and methods

Sources of information were the hospital admission/discharge forms (Schede di Dimissione Ospedaliera - SDO), collected by the Department of Epidemiology - Sicily Region, and the Ragusa Cancer Registry covering eastern Sicily.

Cases of pleural mesothelioma (ICD9 163) among residents of the municipality of Biancavilla diagnosed between 1/1/1998-31/12/2004 were identified using hospital admission related data provided by the SDO record linkage system. Death certificates reporting mesothelioma among Biancavilla residents were gathered to make the survey exhaustive. To complete the medical histories of these cases, the Ragusa Cancer Registry provided individual clinical records and pathology files.

At the end of June 2006 cases were classified as either "ascertained" (with complete clinical records) or "suspected" (ongoing research for clinical records).

Sex-specific crude and standardized incidence rates were calculated separately for ascertained cases, and for total cases (ascertained and suspected). Crude incidence rates for ascertained and total cases were calculated dividing cases observed in the study period by the Biancavilla population in 1997, the latter estimated from the 1991 and 2001 Censuses[11].

The Italian population of 1991 was taken as the reference to calculate standardised rates with the direct method to compare our findings with those of the National Mesothelioma Registry (ReNaM). Confidence Intervals (CI) were set at 95% under the hypothesis that the observed cases follow a Poisson distribution. STATA software was used for the statistical analysis.

Results

Nineteen cases (9 men and 10 women) of mesothelioma occurred in the study period (1998-2004). For eight subjects, clinical records and pathology files, with information about histological, cytological and/or immunohistochemical features, were found (ascertained cases). Table 1 shows sex specific crude and standardised incidence rates for the ascertained cases: the crude incidence rate was $5.1x10^{-5}$ (males 6.6, females 3.7).

After standardisation, the rate for overall cases was $5.4x10^{-5}$ (males 7.4, females 3.6). When the remaining eleven cases with information to be completed (suspected cases) were included in the analysis, the rates were respectively 12.1 for crude rate (males 11.9, females 12.3) and 13.9 for standardised rate (males 13.5, females 14.4) (Table 2).

The results show elevated rates both for ascertained and total cases.

Discussion

In Biancavilla, environmental exposure to fluoroedenite, a previously unknown fibre, occurred, and a cluster of mesothelioma cases was first detected in the period 1988-1992. It is interesting to note that these

Table 1 - Ascertained cases of pleural mesothelioma in Biancavilla, 1998-2004

	Cases (N.)	Crude rate (x 100.000)	CI 95%	Standardised rate (x 100.000)	CI 95%
Men	5	6.6	2.1 - 15.4	7.4	0.9 - 13.8
Women	3	3.7	0.8 - 10.9	3.6	0.0 - 7.8
Total	8	5.1	2.2 - 10.1	5.4	1.6 - 9.2

Table 2 - Total cases of pleural mesothelioma in Biancavilla, 1998-2004

	Cases (N.)	Crude rate (x 100.000)	CI 95%	Standardised rate (x 100.000)	CI 95%
Men	9	11.9	5.4 - 22.5	13.5	4.6 - 22.3
Women	10	12.3	5.9 - 22.8	14.4	5.2 - 23.6
Total	19	12.1	7.3 - 18.9	13.9	7.5 - 20.3

patients had no previous exposure to asbestos, 7 out of 17 cases were women, and the age at diagnosis was less than sixty in most of the cases[2], features tipical of environmental exposure. Case detection was considered incomplete, and no accurate incidence estimate was possible.

The present study focussed on another observation period, with all the new cases included. Again, female gender was strongly involved (10 out of 19 cases). The "real" incidence rate for mesothelioma in Biancavilla, for the period under study, is likely to lie between the figures concerning ascertained and total cases.

The asbestos-like mesotheliomatogenous action of fluoro-edenitic fibres is clear, but there is as yet no evidence that the behaviour of fluoro-edenite is completely similar to that of asbestos fibres, when other cancers and non-neoplastic diseases are considered. No quantitative exposure data are available as yet.

The Italian Register, ReNaM, established guidelines for Regional Operating Centres (*Centri Operativi Regionali* = COR) to record mesothelioma cases in an appropriate manner, in order to estimate the incidence of the disease in Italy, collecting information on past exposure to asbestos, impact and spread of the disease among the population and identification of unexpected or unknown sources of contamination. The ReNaM classifies cases as "definite", "probable", "possible", and "non-mesothelioma", according to the level of diagnostic certainty achieved.

The ReNaM published incidence and survival data, as well as in-depth evaluations of asbestos exposure for incident cases for the periods 1993-1996[12], 1997[13], and 1998-2001[14]. An overall report on its activity was published in 2005[15].

We decided to compare our ascertained cases in Biancavilla with ReNaM definite cases, as well as our total cases with the ReNaM definite, probable and possible cases. We chose data from those Italian Regions, where high occupational asbestos exposure had occurred, elevated incidence rates of mesothelioma were found, and sufficiently long observation periods were available (Liguria, Piedmont, Veneto, Tuscany, Marche). Sicily was included as the Region where Biancavilla is located.

Table 3 - Definite cases of pleural mesothelioma according to the ReNaM level of diagnostic certainty achieved: incidence, crude and standardized rates, 2001

Region	Men		Women	
	Crude rate (x 100.000)	Standardized rate (x 100.000)	Crude rate (x 100.000)	Standardized rate (x 100.000)
Piedmont	3.44	2.72	1.83	1.49
Veneto	2.64	2.42	0.48	0.45
Liguria	9.53	6.57	1.92	1.36
Emilia Romagna	2.66	2.06	0.73	0.62
Tuscany	2.68	2.13	0.94	0.73
Marche	2.67	2.20	0.66	0.47
Apulia	1.02	1.05	0.19	0.19
Sicily	1.66	1.62	0.43	0.43

Modified from Marinaccio *et al*[14]

Table 4 - Definite, probable, possible cases of pleural mesothelioma according to the ReNaM level of diagnostic certainty achieved: incidence, crude and standardized rates, 2001

Region	Men		Women	
	Crude rate (x 100.000)	Standardized rate (x 100.000)	Crude rate (x 100.000)	Standardized rate (x 100.000)
Piedmont	5.06	3.95	2.70	2.13
Veneto	2.83	2.55	0.60	0.53
Liguria	15.98	10.40	3.60	2.31
Emilia Romagna	3.50	2.58	0.98	0.77
Tuscany	2.98	2.34	1.21	0.91
Marche	2.96	2.43	0.80	0.57
Apulia	1.02	1.05	0.19	0.19
Sicily	2.04	1.96	0.58	0.58

Modified from Marinaccio *et al*[14]

It is worth noting that mesothelioma incidence in Biancavilla (1998-2004), a town which has never had important asbestos-related industrial activities, is comparable to those of Regions where, for example, elevated mesothelioma incidence was attributed to the presence of important shipyards in both comparisons, i.e. ascertained (Table 3) and total cases (Table 4). Moreover, mesothelioma incidence rates in Biancavilla are about ten times higher than the regional incidence rates of Sicily.

Age at diagnosis less than 66 for 19 out of 33 total cases (period 1988-2004) and the number of women (16 out for 33) confirm the environmental origin of exposure. The higher SHR for acute respiratory diseases and cardiovascular diseases confirms the need for *ad hoc* studies to investigate the pattern of the biological effects of the fibre which are still to some extent unknown, while the high occurrence of COPD points to a possible fibrogenic action of fluoro-edenitic fibres that, again, should be the object of *ad hoc* studies.

Conclusions

The finding of a consistently elevated incidence rate of pleural mesothelioma in Biancavilla supports the notion of a high risk area suggested by previous observations. The experimental induction of mesothelioma in rats following intrapleural and intraperitoneal injection of fluoro-edenitic fibres confirms the fibres' aetiological rôle, ruling out possible hypotheses of bias and confounding. Land clearance and decontamination were advised, following the precautionary principle, by the Italian National Health Institute, soon after the mesothelioma cluster was detected and the fibre had been isolated, even though it had not yet been properly classified. Further epidemiological surveillance is required in order to set priorities for environmental reclamation and, allowing for latency time, to evaluate the efficacy of the public health interventions, which have been adapted.

Acknowledgement

This work was financially supported by the Italian National Institute of Health (Istituto Superiore di Sanità).

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Eur. J. Oncol., vol. 12, n. 3, pp. 189-195, 2007

Long latency periods in asbestos-related mesothelioma of the pleura

Lunghi periodi di latenza nel mesotelioma asbesto-correlato della pleura

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Summary

***Aim*. The duration of the latency periods (time intervals elapsing between first exposure to asbestos and diagnosis of mesothelioma) has been the object of diverging evaluations. The present study was carried out to determine the duration of the latency periods in a series of pleural mesotheliomas recently observed in the Trieste-Monfalcone area of north-eastern Italy.** ***Patients and methods*. One hundred and sixty-four cases of pleural mesothelioma, diagnosed in the Trieste-Monfalcone area in the period 2001-2006, were reviewed. Occupational data were obtained from the patients or from their relatives by means of personal interviews. Latency periods were calculated in 136 cases, for which precise chronological data were available (128 men and 8 women, aged between 43 and 89 years). The diagnosis was based on histological examination in all the cases but two, and was confirmed by necropsy in 76 cases. Lung asbestos bodies had been isolated in 15 cases.** ***Results*. A majority of patients (62.5%) had histories of working in shipbuilding. Latency periods ranged from 25 to 71 years (mean 48.8 years, median 49.0). Latency periods varied in the various occupational groups, being shorter among insulators. Lung asbestos bodies ranged from 900 to 230,000 bodies per gram of dried tissue. Long latency periods were observed even in people with high amounts of lung asbestos bodies.** ***Conclusions*. It is plausible that various factors, including the severity of**

Riassunto

***Finalità*. La durata del periodo di latenza (intervallo di tempo intercorrente tra la prima esposizione all'asbesto e la diagnosi di mesotelioma) è stata oggetto di valutazioni discordanti. Il presente studio è stato condotto allo scopo di determinare la durata del periodo di latenza in una serie di mesoteliomi pleurici osservati di recente nell'area di Trieste-Monfalcone dell'Italia nord-orientale.** ***Pazienti e metodi*. Sono stati esaminati 164 casi di mesotelioma pleurico, diagnosticati nell'area di Trieste-Monfalcone nel periodo 2001-2006. La storia professionale è stata raccolta dai pazienti o dai loro parenti attraverso interviste personali. Il periodo di latenza è stato calcolato in 136 casi per i quali erano disponibili dati cronologici precisi (128 uomini e 8 donne, di età compresa tra 43 ed 89 anni). La diagnosi era basata sull'esame istologico in tutti i casi tranne due e fu confermata dall'autopsia in 76 casi. I corpi dell'asbesto erano stati isolati dal polmone in 15 casi.** ***Risultati*. La maggioranza dei pazienti (62,5%) aveva una storia di attività nei cantieri navali. Il periodo di latenza variava da 25 a 71 anni (media 48,8 anni, mediana 49,0). La durata del periodo di latenza variava nei diversi gruppi professionali ed era più breve negli isolatori. I corpi dell'asbesto polmonari variavano tra 900 e 230.000 corpi per grammo di tessuto secco. Periodi di latenza lunghi furono osservati anche in casi con elevate quantità di corpi dell'asbesto polmonari.** ***Conclusioni*. È plausibile che la durata del periodo di**

Received/Pervenuto 10.5.2007 - Accepted/Accettato 31.5.2007
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exposure, may influence the length of the latency periods. The sequence of events occurring during the long latency period of asbestos-related mesothelioma remains mysterious. Recurrent inflammation-repair episodes in the pleura of people exposed to asbestos probably play a rôle in the pathogenesis of the tumour. Eur. J. Oncol., 12 (3), 189-195, 2007

Key words: **mesothelioma, pleura, asbestos, latency period, asbestos bodies**

latenza sia influenzata da svariati fattori, tra i quali la gravità dell'esposizione all'asbesto subita. La sequenza di eventi che si verifica durante la lunga latenza del mesotelioma da asbesto rimane enigmatica. Probabilmente gli episodi infiammatori-riparativi che si susseguono ripetutamente nella pleura dei soggetti esposti all'asbesto svolgono un ruolo nella patogenesi del tumore. Eur. J. Oncol., 12 (3), 189-195, 2007

Parole chiave: **mesotelioma, pleura, asbesto, periodo di latenza, corpi dell'asbesto**

Introduction

The relationship between exposure to asbestos and development of malignant mesothelioma has been explored extensively during the last five decades[1, 2]. Nevertheless, many questions about this subject remain unanswered. The tumour develops on pleural or peritoneal serosa, only decades after exposure to asbestos had started. The sequence of events occurring during this long period is unknown. In this context, it is relevant to investigate the features of the latency periods.

Latency periods are defined as the time intervals elapsing between first exposure to asbestos and diagnosis of the tumour (or alternatively between first exposure to asbestos and death). The length of the latency periods has variously been evaluated. Figures of 20-40 years were mostly reported. However, substantially longer periods have been observed in some series[3]. In the present study, latency periods were investigated in a series of pleural mesotheliomas, diagnosed in the Trieste-Monfalcone area during the last six years.

The Trieste-Monfalcone area is a narrow coastal district, located at the north-eastern border of Italy, with a total population of about 300,000 inhabitants. Shipbuilding is the principal industry in this district. Previous studies showed a high incidence of mesothelioma in the Trieste-Monfalcone area[3].

Patients and methods

One hundred and sixty-four cases of pleural mesothelioma, observed at the Hospitals of Trieste and Monfalcone in the period 2001-2006, were included in the study. The pathological diagnosis was based on histological examination in all cases but two, in which mesothelioma was diagnosed by cytological examination of pleural fluid. Detailed occupational histories were obtained from the patients themselves or from their relatives by means of personal interviews. In 91 cases, necropsy was carried out. In 21 cases, asbestos bodies were isolated from the lung after chemical digestion of pulmonary tissue, following the Smith-Naylor method[4]. Latency periods, defined as the time intervals elapsing between onset of the exposure to asbestos and diagnosis of mesothelioma, were calculated in 136 cases, for which sufficiently precise chronological data were available. Necropsy was performed in 76 of such cases, and lung asbestos bodies were isolated in 15 cases.

Results

The latency periods were calculated for 128 men and 8 women, aged between 43 and 89 years. The sex and age distribution of these people is reported in fig. 1.

In a large majority of cases, exposure had occurred in shipbuilding. Most patients had their first exposure before 1960 (fig. 2). The duration of the exposure, calculated in 117 cases, ranged between 6 months and 49 years (fig. 3). Latency periods ranged between 25 and 71 years (fig. 4), with a mean of 48.9 and a median of 49.0. The various occupational groups differed in the duration of the latency periods (Table 1), with shorter periods among insulators (4 cases, range 33-49, mean 38.5, median 36), dock workers (11 cases, range 32-46, mean 41.9, median 44), non-shipbuilding industries (16 cases, range 30-61, mean 41.9, median 41), and various occupations (10 cases, range 25-64, mean 46.9, median 47). Longer latency periods were observed among shipbuilding workers (85 cases, range 28-71, mean 51.4, median 53), seafarers (9 cases, range 42-66, mean 53.2, median 49),

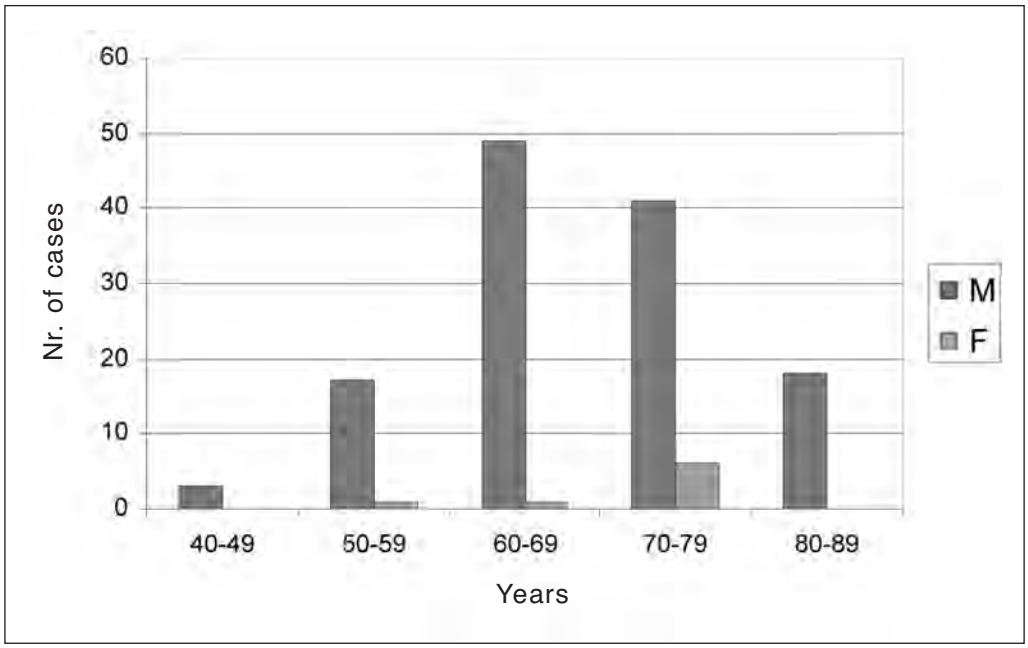

Fig. 1. Sex and age distribution in 136 cases of pleural mesothelioma, Trieste-Monfalcone, 2001-2006

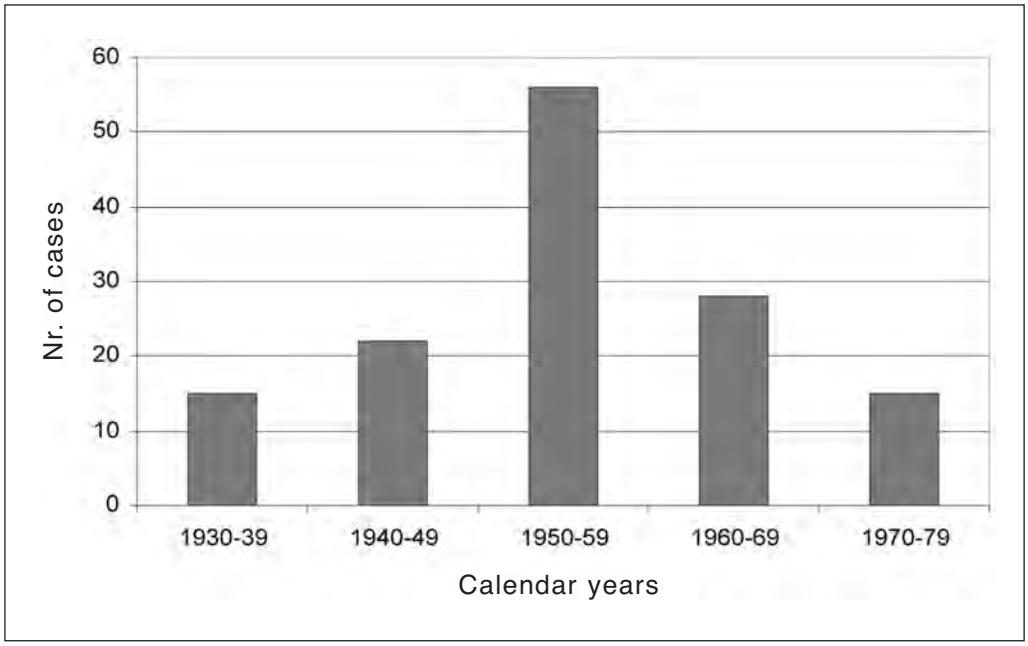

Fig. 2. First exposure to asbestos in 136 cases of pleural mesothelioma, Trieste-Monfalcone, 2001-2006

and patients with domestic exposure (one case, latency period of 50 years). Lung asbestos bodies ranged from 900 to 230,000 bodies per gram of dried tissue, with the highest values in some shipyard workers (Table 2). Very long latency periods were seen also in people with high amounts of lung asbestos bodies (Table 2).

Discussion

In the current series latency periods longer than 40 years were observed in 80.1% of cases, and latency periods longer than 60 years in 22% of cases. Such figures are substantially higher than those currently reported (20-40 years). These differences cannot be attributed to differences in methods. The onset of the occupation, source of exposure to asbestos, may generally be reconstructed with precision. Likewise, the calculation of the latency period is an extremely simple operation. The reason for the discrepancy is indicated by the fact that there are variations in the length of latency periods from one occupation to another. The consequence is that differences in the prevalences of the various occupations in mesothelioma series will reflect in different mean latency periods of the same series.

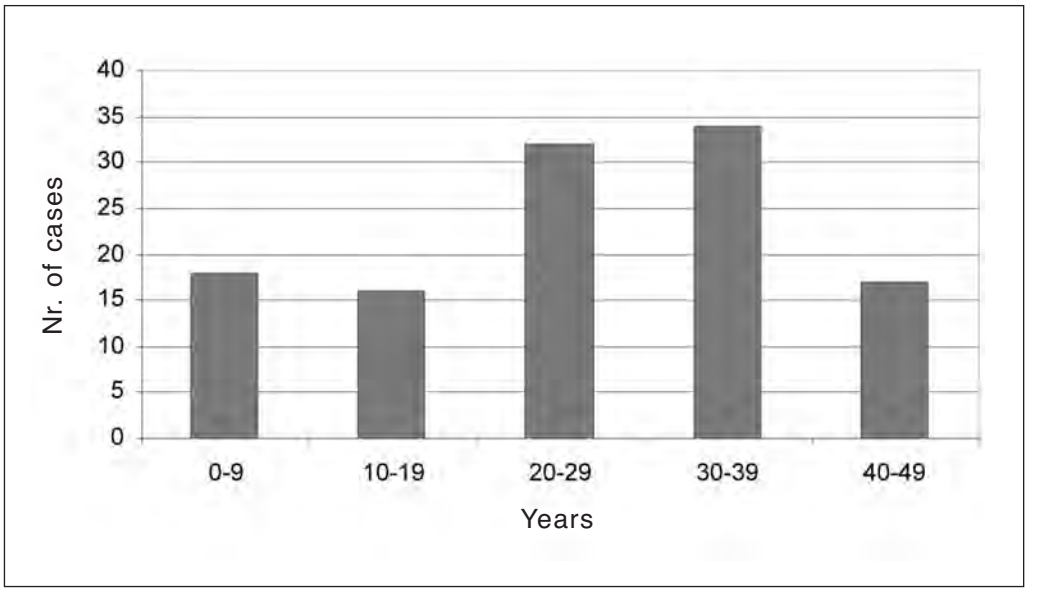

Fig. 3. Duration of exposure to asbestos in 117 cases of pleural mesothelioma, Trieste-Monfalcone, 2001-2006

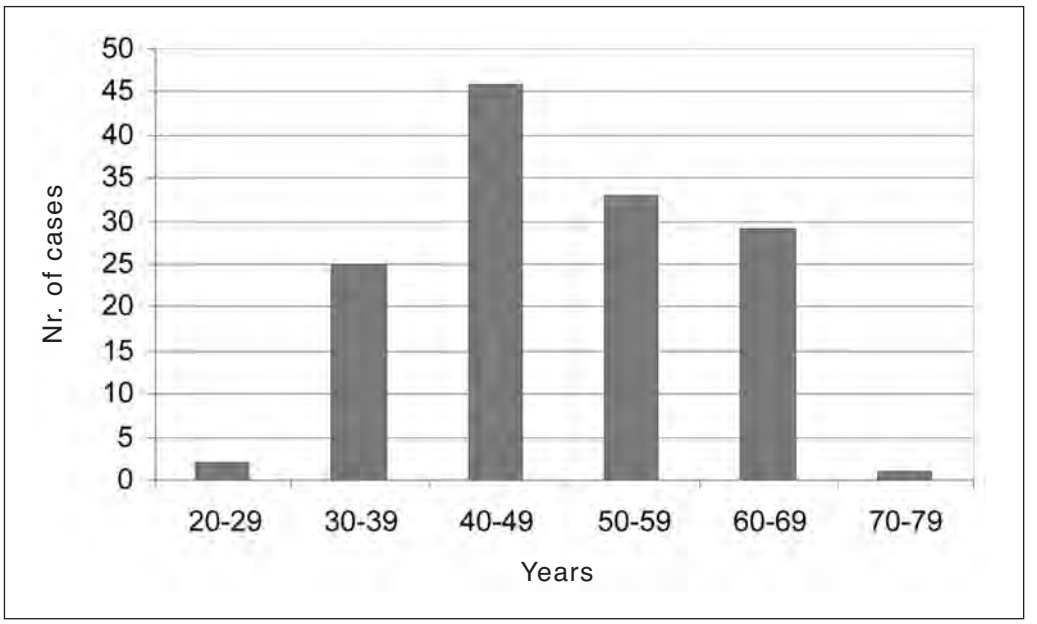

Fig. 4. Latency periods in 136 cases of pleural mesothelioma, Trieste-Monfalcone, 2001-2006

It has been observed[5] that the often-quoted latency periods of 20-40 years are derived from the studies of Selikoff *et al* on mesothelioma among insulators. Various data suggest that an inverse relationship exists between intensity of asbestos exposure and duration of the latency periods[3, 6-8]. The consequence is a wide range of latency periods from those relatively short, observed among insulators, the most heavily exposed group, to those markedly longer observed among the less heavily exposed seafarers[6, 7]. In a series of 400 pleural mesotheliomas examined in the Trieste-Monfalcone area[7], short latency periods were observed among insulators (5 cases, range 28-32 years, mean 29.6, median 29.0), and dock workers (14 cases, range 25-60, mean 36.2, median 31.5). Longer latency periods were seen among shipyard workers (276 cases, range 14-72, mean 49.1, median 51.5), among sailors (34 cases, range 35-75, mean 55.9, median 56.0), and among women with a history of domestic exposure (14 cases, range 27-62, mean 51.4, median 54.0).

In the present series a similar range was visible, even though the differences among the various occupational groups were not so pronounced as in the previous one[7].

Latency periods similar or superposable to those observed in the present cases were seen in other series. Notably, Hilliard *et al*[8], by studying 301 mesotheliomas of the pleura and peritoneum in workers from the Devonport Naval Dockyard, found a mean latency period of 48.5 years. Moreover, in the Devonport series the more heavily exposed trades showed latency periods shorter

Table 1 - Latency period by occupation in 136 cases of pleural mesothelioma, Trieste-Monfalcone, 2001-2006

Latency period (years)	Domestic exposure	Insulators	Seafarers	Various	Dock	Other industries	Shipbuilding	Total
20-29						1	1	2
30-39		3			3	7	12	25
40-49		1	5	6	8	5	21	46
50-59	1		1	3		2	26	33
60-69			3	1		1	24	29
70-79							1	1
Total	1	4	9	10	11	16	85	136

Table 2 - Lung asbestos body burdens in 15 cases of pleural mesothelioma, Trieste-Monfalcone, 2001-2006

Case Nr.	Nr. of bodies[a]	Occupational category	Latency period (years)	Period of exposure	Time intervals from end of exposure to death
1	900	Other industries	39	1964-1984?	19?
2	2,500	Other industries	42	1961-1984	19
3	2,500	Seafarers	49	1954-1982	22
4	3,600	Shipbuilding	59	1942-1946	55
5	6,000	Shipbuilding	61	1941-1977	25
6	7,500	Shipbuilding	66	1936-1976	28
7	14,000	Insulator	49	1954-1961	43
8	15,000	Shipbuilding	39	1964-1966	37
9	18,000	Insulator	37	1966-1981	25
10	26,000	Shipbuilding	47	1954-1994?	11?
11	80,000	Shipbuilding	60	1943-1992	12
12	103,000	Shipbuilding	60	1941-1978	24
13	164,000	Shipbuilding	31	1971-2001	1
14	169,000	Shipbuilding	48	1957-1985	21
15	230,000	Shipbuilding	43	1958-1972	30

[a] Lung asbestos body burdens per gram of dried tissue

than those less heavily exposed (42 years *versus* 49.5 years). In Italy, Mensi *et al*[9], by examining 11 pleural mesotheliomas in people exposed to asbestos in the Navy, observed a mean latency period of 56 years.

In other series mean latency periods were shorter. In a series of 710 mesotheliomas investigated in The Netherlands, the average latency period was 40.5 years[5]. In 100 pleural mesotheliomas examined in Japan, the mean latency period was 37.0[10]. In 2,544 mesotheliomas collected by the Italian Mesothelioma Registry, the mean latency period was 43.6 years[11].

As a whole, asbestos dose appears to be a major factor in determining the length of the latency periods. The intensity of asbestos exposure, however, does not seem to explain all the data. The long or very long latent periods seen in mesotheliomas of shipbuilding workers are in some ways unexpected. Studies conducted at necropsy showed that asbestos exposure occurring in shipyards was frequently heavy[12]. The very high amounts of asbestos bodies found in the lungs of some shipyard workers in the present series, also indicate an exposure severe in intensity. Consequently, the long latency periods among shipyard workers appear strange. Likewise, it is strange that in the present study even shipyard workers with very high amounts of lung asbestos bodies showed long latent periods.

In those cases, for which data on lung asbestos content were available, the time intervals elapsed between end of exposure to asbestos and death were frequently very long. Plausibly, a not negligible clearance occurred over these long periods. Therefore, the figures found at necropsy only partially reflect the intensity of exposure these people had had years previously.

Data on the latency periods have important implications for understanding the pathogenesis of asbestos-related mesothelioma. The figures of the latency periods indicate the time at which contact with asbestos fibres began. However, these figures do not indicate the time at

which the malignant process started. It is not plausible, for instance, that neoplastic transformation began 60-70 years before the first symptoms and signs of the tumour. One should rather distinguish between latent period and induction period, the latter being the time, obviously shorter, over which a multi-step malignant process occurs. This distinction, proposed both for malignant tumours in general as well as for mesothelioma[13], does not seem always to be clear in the literature. The distinction implies that a long period elapses, in which the presence of asbestos fibres in the tissues is well tolerated. Plausibly, in such period the surveillance mechanisms are efficacious in regulating cellular proliferations. On the other hand, malignant transformation is the exception rather than the rule, occurring only in a small proportion of persons severely exposed to asbestos[5]. In other words, a large majority of people show a resistance to the oncogenic effects of asbestos.

In order to explain the transition to malignancy, different co-factors co-operating with asbestos may be hypothesized[14]. A genetic susceptibility is suggested by the occurrence of several mesotheliomas in the same family[15-17]. However, such familial mesotheliomas do not differ in their natural history (including latency periods) from the sporadic cases, an argument speaking against the role of genetic factors. The co-existence of mesotheliomas and other malignancies[18,19] may be considered as a clue indicating a vulnerability to cancer. In particular, the association between mesotheliomas and lymphomas (especially extra-nodal lymphomas) suggests derangements in immune mechanisms[19].

Recurrent inflammation is also a factor to consider[20]. Mesothelioma mostly develops on pleura affected by pleural plaques[3,21]. Pleural plaque is the result of recurrent inflammatory-repair processes, occurring for decades: this sequence of episodes may favour malignant transformation. Such an hypothesis is supported by the fact that cases of peritoneal mesothelioma have been described in the course of familial Mediterranean fever[22-24], a condition characterized by recurrent episodes of fever and painful polyserositis[25], which mainly involves the peritoneum. Further findings suggesting a connection between long-term serosal inflammation and mesothelioma come from animal pathology. Pericardial mesotheliomas have been reported in dogs with idiopathic haemorrhagic pericardial effusion[26].

Contrary to what happens with many other malignancies, pre-malignant and early malignant phases cannot generally be detected in mesothelioma. Clinical manifestation of the tumour is frequently a bolt from the blue. In addition, sometimes a sudden event, like a thorax trauma, appears to mark the onset of the disease.

The knowledge of the events occurring during the long latency periods of mesothelioma remains fragmentary and hypothetic. A better understanding of this sequence could open up new prospects for prolonging latency periods, and preventing mesothelioma in asbestos-exposed people.

Acknowledgements

This study is supported by the Italian League against Cancer, Rome, Italy.

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Eur. J. Oncol., vol. 12, n. 3, pp. 197-203, 2007

La chemioterapia ipertermica intraoperatoria con cisplatino nel trattamento multimodale del mesotelioma pleurico: risultati preliminari

Intraoperative hyperthermic chemotherapy with cisplatin in the combined treatment of pleural mesothelioma: preliminary results

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Riassunto

***Introduzione*. Presentiamo il nostro protocollo terapeutico multimodale del mesotelioma pleurico maligno (MPM) aggiungendo all'intervento la chemioterapia ipertermica intraoperatoria con cisplatino (CII) a torace chiuso. Abbiamo valutato la fattibilità, gli effetti collaterali, la tossicità, le concentrazioni tessutali di cisplatino intratoraciche e l'efficacia terapeutica in termini di sopravvivenza e qualità di vita dei pazienti in stadio clinico I e II o III in assenza di coinvolgimento linfonodale. *Pazienti e metodi*. Gli Autori hanno trattato i pazienti secondo il seguente protocollo: toracoscopia/VATS, laparoscopia e/o mediastinoscopia - chirurgia con protocollo d'idratazione, eseguendo pneumonectomie extrapleuriche estese con resezione di pericardio e diaframma e ricostruzione con pericardio bovino (PPN) oppure pleurectomie e decorticazioni (PD), seguite da coagulazione diffusa e citoriduzione con argon. A torace chiuso si è eseguito il lavaggio con CII di cisplatino (150 - 200 mg/m^2) a 42,5°C per un'ora. Dopo l'intervento il paziente viene sottoposto a chemioterapia e radioterapia adiuvanti. Dal gennaio 1996 al dicembre 2006 sono stati operati 109 pazienti affetti da MPM epitelioide (75 PPN e 34 PD). Tra tutti questi, 41 pazienti sono stati trattati con l'aggiunta di CII (36 PPN e 5 PD) dal marzo 2003 al dicembre 2006. *Risultati*. Dal 1996 abbiamo riscontrato**

Summary

***Introduction*. We present our combined surgical treatment protocol for malignant pleural mesothelioma (MPM) with closed-chest intraoperative hyperthermic high dosage chemotherapy (IHC) with cisplatin. We evaluated feasibility, side effects, toxicity, platin concentrations in intrathoracic tissue, blood, plasma and urine. We describe the therapeutical efficacy, in terms of quality of life and survival rate, in patients with MPM in clinical stages I and II or III without node involvement. *Patients and methods*. The authors treated the patients according to the following protocol: thoracoscopy/VATS, laparoscopy and or mediastinoscopy - surgery and hydration protocol performing extended extrapleural pneumonectomy pericardiectomy and diaphragmectomy and reconstruction with bovine pericardium (EPP), or pleurectomy and decortication (PD) followed by argon beam cytoreduction. After chest closure, IHC was carried out with cisplatin 150 - 200 mg/m^2 at 42.5°C for an hour. After surgery, the patients undergo adjuvant chemo-radiotherapy. From January 1996 to December 2006 we surgically treated 109 patients with epithelial MPM (75 EPP, 34 PD). Of this group, 41 patients were treated with IHC (36 PPN and 5 PD) from March 2003 to December 2006. *Results*. From 1996 we reported a low surgical risk**

Received/Pervenuto 19.3.2007 - Accepted/Accettato 27.4.2007
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un basso rischio chirurgico, con mortalità globale del 1,8% (2,7% PPN, 0% PD): due decessi nelle PPN, di cui uno per polmonite "*ab ingestis*" ed uno per ipertensione polmonare, quest'ultimo nel gruppo con CII (1/36 pazienti). Le complicanze maggiori nel gruppo CII sono state osservate nel 7,3% dei casi (3/36 PPN, 0/5 PD), ma sono state tutte risolte: 1 polmonite + insufficienza respiratoria e fistola bronchiale dopo ventilazione meccanica, 1 erniazione gastrica ed 1 fistola esofagea. Alla stadiazione patologica i pazienti nel gruppo con CII sono risultati essere, secondo il sistema di classificazione IMIG 1995, 1 in stadio I, 8 in stadio II, 32 in stadio III. Dei 5 pazienti in stadio III sottoposti a PD, 2 sono deceduti a 25 e 12 mesi e 3 sono vivi a 3, 4 e 16 mesi dall'intervento. Dei pazienti sottoposti a PPN + CII, la sopravvivenza a 2 anni è del 38,5% (13 pazienti tutti in stadio III: 4 liberi da malattia, 1 vivo con malattia e 8 deceduti per malattia). Degli altri con *follow-up* inferiore a 2 anni: 3 sono deceduti a 15, 10 e 7 mesi; 1 è vivo con recidiva dopo 12 mesi; 19 pazienti sono vivi senza malattia con *follow-up* medio di 6,7 mesi. *Discussione e conclusione*. La CII permette di ottenere la neutralizzazione di foci tumorali microscopici residui e delle cellule esfoliate durante l'intervento. È una procedura sicura, senza effetti collaterali, non peggiora la morbilità nel perioperatorio e permette di ottenere concentrazioni tessutali di cisplatino intratoraciche molto elevate anche ad un centimetro di profondità. Eur. J. Oncol., 12 (3), 197-203, 2007

Parole chiave: **mesotelioma pleurico, chemioterapia, ipertermia, cisplatino, pleuropneumonectomia extrapleurica**

with global mortality of 1.8% (2.7% in EPP, 0% in PD): two deaths in the EPP group, of whom one from pneumonia due to an "*ab ingestis*" and one from pulmonary hypertension, the latter in the IHC group (1/36 patients). The most significant complications were observed in 7.3% (3/36 EPP, 0/5 PD) of the IHC group and were all resolved: 1 pneumonia + respiratory failure and following broncho-pleural fistula after mechanical ventilation, 1 pericardial patch insufficiency causing cardiac herniation, and 1 oesophageal fistula. The IMIG 1995 post-surgical staging of the IHC group was: 1 patient in stage I, 8 in stage II and 32 in stage III. Of the 5 patients in stage III who underwent PD, 2 died at 25 and 12 months and 3 are alive at 3, 4 and 16 months after the operation. The 2-year survival rate of the PPN + IHC group is 38.5% (13 patients all in stage III: 4 alive and free of disease, 1 alive with relapse, 8 have died due to their illness). Of the other patients, with a follow-up of less than 2 years, 3 died at 15, 10 and 7 months, 1 is alive with relapse after 12 months and 19 patients are alive without disease with a median follow-up of 6,7 months. *Discussion and conclusions*. IHC enables the neutralization of microscopic residual tumour sites and of cell spreading during surgery. It is a safe procedure, without side effects, which does not worsen postoperative morbidity, and allows very high tissue cisplatin concentrations to be reached in the intrathoracic wall up to a depth of 1 cm. Eur. J. Oncol., 12 (3), 197-203, 2007

Key words: **pleural mesothelioma, chemotherapy, hyperthermia, cisplatin, extrapleural pneumonectomy**

Introduzione

Si prevede per il mesotelioma pleurico maligno (MPM) un aumento dell'incidenza con un picco tra 15 anni. La storia naturale di questa malattia, se non curata, porta a decesso il paziente in 4-12 mesi. I pazienti che presentano un coinvolgimento linfonodale N2 presentano una prognosi peggiore con una bassa sopravvivenza. Il rapporto maschi/femmine è di 3/1. Di notevole importanza è lo *staging* di questi pazienti che deve essere il più accurato possibile.

Le indagini comunemente usate hanno vari limiti: l'Rx torace fornisce solo informazioni generiche; la TAC toraceaddome presenta ancora notevoli difficoltà nello *staging* clinico, la RNM dell'apice del torace e dell'addome superiore permettono una migliore definizione dell'eventuale infiltrazione extratoracica e del diaframma; la PET - TC risulta poco utile nella stadiazione dell'N nel MPM epitelioide per basso metabolismo[1, 2]; l'ecocardiogramma valuta il pericardio e la funzione ventricolare; e le prove di funzionalità respiratoria con la scintigrafia ventilo-perfusoria evidenziano o meno la permissività funzionale chirurgica.

Le indagini che permettono una corretta stadiazione ed eventuale indicazione chirurgica sono le seguenti:

- biopsie videotoracoassistite (toracoscopia - VATS) che permettono di definire con precisione l'istotipo, l'immunoistologia e la microscopia elettronica;
- la mediastinoscopia che ha sensibilità dell' 80%, specificità del 100% ed accuratezza del 93% nella definizione N mediastinica;

– la laparoscopia che valuta l'interessamento transmurale del diaframma.

Negli ultimi anni si è dimostrato che il trattamento multimodale (chemioterapia, chirurgia e radioterapia) del MPM[3-9] offre i risultati migliori in termini di qualità di vita e sopravvivenza per i pazienti allo stadio I e II secondo il sistema di classificazione proposto da Sugarbaker - Brigham Hospital/DFCI nel 1999[9] o stadi I, II, III in assenza di coinvolgimento linfonodale, secondo il sistema di classificazione IMIG del 1995.

Nel nostro studio si è aggiunto al protocollo terapeutico multimodale attualmente maggiormente utilizzato il lavaggio post-operatorio del cavo con chemioterapia ipertermica di cisplatino secondo il razionale di neutralizzazione di foci tumorali microscopici residui e di cellule esfoliate durante l'intervento. Appare anche interessante l'aumento della radiosensibilità tessutale determinata dalla penetrazione del cisplatino diretta nei tessuti. Sono state dosate le concentrazioni tessutali di cisplatino intratoraciche che si ottengono con tale metodica mediante biopsie e studi di farmacocinetica.

Pazienti e metodi

Presso la Divisione di Chirurgia Toracica degli Spedali Civili di Brescia gli Autori hanno ritenuto candidabili a questo studio i pazienti in stadio clinico I e II o III in assenza di coinvolgimento linfonodale.

Dal gennaio 1996 al dicembre 2006 sono stati operati 109 pazienti affetti da MPM: 75 eseguendo pneumonectomie extrapleuriche estese di pericardio e diaframma e ricostruzione del pericardio e del diaframma con pericardio bovino (PPN) e 34 eseguendo pleurectomie e decorticazioni (PD). Tra tutti questi, 41 pazienti, scelti con MPM epitelioide (38 maschi e 3 femmine con un *range* di età tra 36 e 75 anni, media 57,4), senza precedenti terapie, sono stati trattati con trattamento multimodale con l'aggiunta della chemioterapia ipertermica intraoperatoria ad alte dosi: 36 PPN e 5 PD dal 03/2003 al 12/2006.

I criteri di selezione comprendevano: assenza di precedenti o intercorrenti tumori, età tra 30 e 80 anni, Karnofsky *performance status* compreso tra 80 e 100. Adeguati esami ematici e normale funzionalità renale erano pre-requisiti per entrare nello studio con aggiunta di chemioipertermia. Prima di iniziare il protocollo, la valutazione del paziente prevedeva una completa visita generale, esami ematici, Rx torace, TAC torace-addome, RNM dell'apice del torace e/o dell'addome superiore, PET - TC[1], ecocardiogramma, prove di funzionalità respiratoria (sono stati esclusi dal nostro protocollo i pazienti con FEV_1 predetto post-operatorio <40% o DLCO <50%), scintigrafia ventilo-perfusoria, biopsie videotoracoassistite (toracoscopia - VATS), mediastinoscopia, laparoscopia. Tutti i pazienti sono stati valutati dal gruppo che si dedica al trattamento del mesotelioma composto da chirurgo toracico, pneumologo, oncologo medico, radioterapista e farmacologo.

I pazienti risultati idonei, dopo la firma del consenso informato, sono stati sottoposti al protocollo qui descritto.

La mediastinoscopia e/o laparoscopia + biopsie sono state eseguite, in un tempo chirurgico a se stante ed unico, quando sussistevano dubbi circa un interessamento linfonodale mediastinico o transmurale del diaframma con coinvolgimento peritoneale. Dopo acquisizione dell'esame istologico definitivo, in caso di idoneità, si sono sottoposti i pazienti al seguente protocollo d'idratazione da noi approntato in collaborazione con oncologi e nefrologi, prima di essere sottoposti all'intervento chirurgico toracico:

– 12 h prima della chirurgia:
  soluzione fisiologica 500 cc,
  5% soluzione glucosata 500 cc;
  1/6 mol bicarbonato 250 cc;
– durante la chirurgia:
  soluzione fisiologica 1000 cc + 20 mEq NaCl;
  soluzione fisiologica 1000 cc + 2 gr Mg solfato;
– durante chemioterapia ipertermica intraoperatoria:
  Na tiosolfato 12 gr/m^2 in 2 ore;
– dopo chirurgia:
  Na tiosolfato 15 gr/m^2 in 12 ore;
  soluzione fisiologica 1000 cc + 20 mEq NaCl x 2;
  soluzione fisiologica 1000 cc + 2 gr Mg solfato;
– nei 5 giorni seguenti:
  5% soluzione glucosata 500 cc x 2;
  soluzione fisiologica 500 cc;
  Mg solfato 1 gr.

Dal punto di vista chirurgico toracico sono state eseguite 36 PPN oppure, quando il paziente è risultato essere in stadio III secondo il Brigham Hospital/DFCI Boston-1999[9], per la presenza di un tumore non reso completamente resecabile per l'invasione massiva del mediastino o del diaframma e quindi con evidente impossibilità ad ottenere una radicalità chirurgica, si è proceduto, in 5 casi, a PD[10-12]. Successivamente è stata eseguita una coagulazione diffusa con argon del cavo toracico, posizionamento di 2 drenaggi (1 anteriore ed 1 posteriore) e 5 sonde di temperatura (1 ingresso liquido di lavaggio, 1 uscita liquido di lavaggio e 3 sonde intratoraciche). Una volta suturata definitivamente per strati la toracotomia, dopo protezione del moncone bronchiale con muscolo intercostale peduncolizzato, inizia il lavaggio del cavo toracico con macchina da perfusione extracorporea dedicata.

Il protocollo prevede l'introduzione di circa 3-4 litri di soluzione fisiologica nel cavo toracico fino a raggiungerne il riempimento. La temperatura del liquido di lavaggio viene gradualmente portata da 37° a 42,5°C in circa 30 minuti per evitare ipotensioni, e successivamente inizia il lavaggio con chemioterapia ipertermica intraoperatoria (CII). Si somministra cisplatino alla dose di 150-200 mg/m^2 diluito nella soluzione fisiologica presente nel cavo toracico a 42,5°C per un'ora.

Dopo conclusione della perfusione ipertermica si procede a svuotamento del cavo ed al successivo lavaggio dell'emitorace con soluzione antibiotica (vancomicina 3 gr) e pro-coagulante (acido tanexamico 2,5 gr) diluiti in 500 cc di soluzione fisiologia e successivo svuotamento dell'emitorace operato. Un drenaggio toracico viene rimosso in camera operatoria e si procede a toracoscopia con biopsie della parete toracica con carotaggio toracoscopico attraverso il tramite del drenaggio rimosso.

Dopo un mese circa dall'intervento chirurgico i pazienti sono valutati dal collega oncologo ed avviati a chemioterapia adiuvante comprendente gemcitabina e cisplatino per un ciclo (recentemente pemetrexed + cisplatino), seguito da radioterapia (45 Gy sull'emitorace d'interesse e mediastino) e infine da due ulteriori cicli di chemioterapia.

Tossicità del trattamento e *follow-up*

La tossicità acuta è stata valutata mediante controlli ematici giornalieri dei parametri emocromocitometrici della funzionalità renale ed epatica, Rx torace e visite cliniche accurate chirurgico-oncologiche. La tossicità tardiva così come le complicanze sono state valutate durante le visite di *follow-up* e terapeutiche a cui ogni paziente è stato avviato. I pazienti sono stati seguiti dopo la dimissione con un controllo a 15 giorni con nuovo Rx torace e successivamente visite cliniche chirurgiche mensili per i primi 3 mesi e successivamente ogni 3 mesi.

L'obiettività di assenza di tumore o ripresa di malattia alla fine del protocollo è stata studiata con nuova TAC torace-addome e visita clinica chirurgico-oncologica. Successivamente ogni 3 mesi sono stati eseguiti un Rx torace ed una visita di controllo.

Analisi statistiche

Il controllo locoregionale di assenza di malattia è definito come completa assenza di tumore nei siti del tumore primitivo e di tutto l'emitorace operato, in assenza di adenopatie. La data di ripresa della malattia è definita in base alla comparsa di sintomatologia clinica, o all'evidenza clinica o radiologica di ripresa del tumore escluse eventuali co-morbidità. I decessi vengono classificati in: per MPM, in assenza di ripresa di MPM per altre cause, con presenza di MPM ed altre cause. La sopravvivenza è stata misurata dall'intervento chirurgico. Lo studio è iniziato nel marzo 2003 ed è attualmente in corso, con analisi statistiche definitive previste per gennaio 2011, per ottenere risultati di *follow-up* a 5 anni su un numero cospicuo di pazienti.

Analisi della farmacocinetica del cisplatino

Il dosaggio di cisplatino nel liquido di lavaggio, per calcolare la dose totale di farmaco assorbita dal paziente, viene effettuato prima dell'inizio del lavaggio nella soluzione contenente fisiologica + cisplatino (10 ml di liquido) ed alla fine del lavaggio nella soluzione derivante dallo svuotamento del cavo residuo al lavaggio (10 ml) prima dell'inizio del lavaggio con soluzione antibiotica e pro-coagulante.

Dopo il lavaggio ipertermico vengono eseguite biopsie della parete toracica per via endoscopica con carotaggio di poco più di 1 cm di profondità nella parete toracica.

Successivamente all'intervento è stato eseguito il seguente protocollo di monitoraggio dei dosaggi di cisplatino nel sangue e nelle urine.

Sono stati prelevati 7 ml di sangue eparinato e 20 ml di urina alla fine, dopo 6 e 12 ore del lavaggio chemioterapico.

Si è eseguita una successiva analisi giornaliera di 20 ml di urina prodotta nelle 24 ore fino alla dimissione del paziente.

Si ottengono così i valori di platino totale nel sangue (BPt), platino totale nel plasma (UPt), platino nelle urine (PtU)[13].

Risultati

Dal marzo 2003 al dicembre 2006 sono stati reclutati nello studio 41 pazienti (38 maschi e 3 femmine) e sono stati trattati con l'aggiunta della CII: 36 PPN e 5 PD. L'età dei pazienti ha come *range* 36-75 anni, media 57,4.

Tutti i pazienti erano affetti da MPM di tipo epitelioide con associata una componente bifasica in due pazienti appartenenti 1 al gruppo delle PPN ed 1 al gruppo delle PD.

Tutti i pazienti hanno completato il protocollo di trattamento. La temperatura media osservata è stata di 41,6°C (*range* 40,9 - 43°C).

I dosaggi di cisplatino ematici, urinari e tessutali sono stati misurati in 16 pazienti.

Dal 1996 abbiamo riscontrato un basso rischio chirurgico con mortalità globale del 1,8% (2,7% mortalità PPN, 0% PD): due decessi nelle PPN di cui uno per polmonite "*ab ingestis*" da lacerazione tracheale post intubazione OT ed uno per ipertensione polmonare, quest'ultimo nel gruppo con CII (1/36 pazienti). Le complicanze maggiori nel gruppo CII sono state osservate nel 7,3% dei casi (3/36 PPN - 0/5 PD), ma sono state tutte risolte: 1 polmonite + insufficienza respiratoria e fistola bronchiale dopo ventilazione meccanica risolta con toraco-mio-omento-plastica, 1 distacco parziale del *patch* diaframmatico con conseguente ernia gastrica, risolta con re-intervento e nuova plastica, ed una fistola esofagea con empiema del cavo risolta con mio-omentoplastica, trasposizione intratoracica di muscolo grandorsale ed esclusione meccanica temporanea esofagea.

Sopravvivenza

Alla stadiazione patologica i pazienti nel gruppo con CII sono risultati essere secondo il sistema di classificazione IMIG 1995, 1 in stadio I, 8 in stadio II, 32 in stadio III. Dei 5 pazienti in stadio III sottoposti a PD, 2 sono deceduti a 25 e 12 mesi mentre 3 sono vivi a 3, 4 e 16 mesi dall'intervento.

Dei pazienti sottoposti a PPN + CII la sopravvivenza a 2 anni è del 38,5% (13 pazienti tutti in stadio III: 4 liberi da malattia, 1 vivo con malattia e 8 deceduti per malattia). Degli altri con *follow-up* inferiore a 2 anni: 3 sono deceduti a 15, 10 e 7 mesi; 1 è vivo con recidiva dopo 12 mesi; 19 pazienti sono vivi (compresi tutti i pazienti in stadio I e II) senza malattia con *follow-up* medio di 6,7 mesi.

Reazioni acute ed effetti collaterali

Non si è osservata tossicità renale significativa.

La creatinina pre-operatoria dei pazienti ha un *range* di 0,6 - 1,2 mg/dl (media 0,86 mg/dl), mentre quella massima riscontrata dall'intervento alla dimissione ha un *range* di 0,8 - 3,1 mg/dl (media di 1,28 mg/dl).

Dosaggi farmacocinetici

I dosaggi delle concentrazioni di cisplatino nelle soluzioni di lavaggio sono descritti nella Tabella 1, quelli ematici, plasmatici ed urinari nella Tabella 2, mentre quelli nei tessuti sono descritti nella Tabella 3.

Tabella 1 - Dosaggio di cisplatino nella soluzione di lavaggio chemioterapico

	Range
Pt soluzione lavaggio chemioterapico prima dell'inizio lavaggio μg/l	72 329 - 146 467
Pt soluzione lavaggio chemioterapico dopo lavaggio μg/l	21 939 - 105 728

Tabella 2 - Dosaggio di cisplatino nel sangue, plasma, urine dopo lavaggio chemioterapico

	6 ore dopo chirurgia	24/36 ore dopo chirurgia
Pt sangue μg/l	261-780	24h 111-384 36h 64-291
Pt plasma μg/l	443-1218	24h 209-524 36h 104-378
Pt urine μg/l	3 026-25 542	24h 2763-14848 36h 1471-7816

Tabella 3 - Dosaggio di cisplatino nelle biopsie intratoraciche dopo lavaggio chemioterapico

Tessuto intratoracico	Pt μg/g
Tessuto adiposo mediastinico	0,08
Parete toracica superficiale	101 - 1 986
Sotto 0,5 cm	20 - 368
Sotto 1 cm	2,4 - 250

Discussione

Il protocollo di trattamento multimodale con la CII appare fattibile con rischi chirurgici accettabili non influenzati dall'aggiunta di questa terapia[14].

La mortalità globale nel gruppo di studio del 2,4% con morbilità globale del 7,3% risulta correlabile alla precisa programmazione dell'intervento, alla selezione dei pazienti ed alla esperienza dello *staff* chirurgico.

Le complicanze osservate sono correlabili a quelle che si riscontrano in letteratura per gli interventi di pleuro-pneumo-pericardio-emidiaframmectomia in cui non viene impiegato il lavaggio ipertermico[15,16]. I dosaggi del cisplatino risultano confrontabili e sovrapponibili come cinetica di riduzione nel sangue, nel plasma e nelle urine (Tabella 2) e riproducibili in qualsiasi centro che applichi questo protocollo con l'impiego di CII con cisplatino.

Nel dosaggio urinario si possono identificare gruppi di pazienti in cui l'eliminazione del cisplatino a livello urinario presenta degli aumenti nella concentrazione urina-

ria a 12 ore dall'intervento pur avendo ps nella norma (8 pazienti); al contrario altri 3 pazienti mantengono la concentrazione stabile per 48 ore, 1 paziente presenta incrementi tra 12 e 48 ore, 1 paziente presenta un incremento in IV giornata post-operatoria mentre in tutti gli altri pazienti si ha una graduale diminuzione della concentrazione urinaria di Pt nel tempo, il tutto in assenza di episodi di insufficienza renale e con una cinetica che andrebbe meglio studiata con numeri più cospicui di pazienti.

L'assenza di tossicità sistemica, da ricercarsi soprattutto nella corretta idratazione prima della somministrazione del cisplatino, e l'assenza di complicanze cardiache maggiori, nonostante le concentrazioni di farmaco utilizzate, evidenziano la fattibilità di tale metodica.

Nel sangue i dosaggi di cisplatino risultano essere elevati ma, essendo il cisplatino chelato con tiosolfato, non è assorbito dai tessuti. Inoltre il cisplatino nel sangue e nelle urine decresce rapidamente dopo l'intervento, fino a quasi azzerarsi prima della dimissione in conseguenza delle diuresi indotte.

Di notevole rilevanza risultano essere gli assorbimenti tessutali di cisplatino che sono molto elevati. Non essendo liposolubile, il cisplatino non viene assorbito dal tessuto adiposo (valore medio nel tessuto adiposo mediastinico da noi riscontrato in questo studio: 0,08 μg/g), mentre in altri tessuti ha concentrazioni molto elevate anche ad un centimetro di profondità, soprattutto se paragonate ai dosaggi tessutali che si ottengono con una chemioterapia sistemica considerata terapeutica[13] (*range* 0,4-1,9 μg/g) risultando in media oltre 100 volte superiori nella superficie di contatto con il lavaggio ipertermico e anche 5 e 10 volte superiore in profondità nella parete.

A differenza del protocollo di lavaggio ipertermico del cavo eseguito da Sugarbaker il nostro protocollo prevede l'esecuzione dell'ipertermia a toracotomia chiusa, senza rischi per il personale[1].

Il razionale della CII consiste nella neutralizzazione di foci tumorali microscopici residui, di cellule esfoliate durante l'intervento e nell'aumento della radiosensibilità tessutale determinata dalla penetrazione del cisplatino: esso viene impiegato nel nostro protocollo, visto anche il provato elevato assorbimento tessutale, oltre alle altre note azioni dirette del cisplatino e dell'ipertermia a livello cellulare, presenti in letteratura.

In questo studio, volto soprattutto alla definizione della fattibilità del protocollo presentato, e degli effetti collaterali, e della morbilità e mortalità ad esso correlabili, i risultati in termini di sopravvivenza sono ancora preliminari, ma soddisfacenti per quanto riguarda la qualità di vita post-operatoria.

Nella nostra esperienza, nonostante una accurata stadiazione, abbiamo avuto un solo paziente in stadio patologico I e la sopravvivenza che riguarda gli 8 pazienti in stadio II ha un *follow-up* che attualmente non raggiunge i 2 anni; i numeri sono esigui per poter affermare che i risultati siano direttamente correlati con l'aggiunta della chemioipertermia oppure solo alla precocità della diagnosi o all'interessamento linfonodale. Tuttavia, la sopravvivenza preliminare a 2 anni dei pazienti in stadio III è incoraggiante con questo protocollo, rispetto a varie casistiche presenti in letteratura.

In conclusione la CII è una procedura sicura senza effetti collaterali rilevati. Non peggiora la morbilità nel perioperatorio ed è riproducibile. La numerosità dei pazienti non è elevata e pertanto si dovrà attendere un *follow-up* più lungo. Sono auspicabili per comprendere l'utilità di questo nuovo metodo di trattamento studi multicentrici, prospettici randomizzati.

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Eur. J. Oncol., vol. 12, n. 3, pp. 205-217, 2007

The first pilot study on characteristics and life style patterns of Kuwaiti breast cancer patients

Primo studio pilota sulle caratteristiche e modelli comportamentali delle pazienti con carcinoma mammario nel Kuwait

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Summary

***Background*. Non-genetic breast cancer risk factors have never been evaluated in Kuwait. Accordingly, we aimed at examining these factors as well as the immune profile of the patients. *Patients and methods*. Fifty stage I breast cancer patients and fifty age group-matched normal controls were assessed for the level of their peripheral blood lymphocyte subsets, and for risk factors associated with their demographic and reproductive characteristics, and with diet. *Results*. The percentages of $CD4^+$ T lymphocytes, $CD4^+$:$CD8^+$ ratio, and $CD19^+$ B lymphocytes were significantly higher in the patients as compared to controls, while the percentages of $CD8^+$ T lymphocytes and natural killer ($CD56^+$) cells were significantly reduced. Risk factors associated with the disease included higher body mass index (BMI), lack of regular exercise and physical activity in the past five years, early age at menarche, late age at first pregnancy, lack of previous information about breast cancer, hormonal therapy, and presence in Kuwait during the invasion/liberation. Other parameters included significantly more frequent consumption of**

Riassunto

***Finalità*. I fattori di rischio non genetici per il carcinoma mammario non sono mai stati valutati nel Kuwait. Di conseguenza abbiamo cercato di esaminare questi fattori ed il profilo immunologico delle pazienti. *Pazienti e metodi*. In 50 pazienti con carcinoma mammario in stadio I ed in 50 controlli normali appaiati per gruppi di età sono stati valutati i livelli delle sottoclassi di linfociti del sangue periferico, ed i fattori di rischio connessi con le loro caratteristiche demografiche e riproduttive e con la dieta. *Risultati*. Le percentuali di linfociti $CD4^+$ T, il rapporto $CD4^+$:$CD8^+$, ed i linfociti $CD19^+$ B erano significativamente più alti nei pazienti rispetto ai controlli, mentre le percentuali dei linfociti $CD8^+$ T e delle cellule natural killer ($CD56^+$) erano significativamente ridotte. I fattori di rischio associati con la malattia comprendevano un aumentato indice di massa corporea (body mass index = BMI), mancanza di regolare esercizio ed attività fisica negli ultimi 5 anni, menarca precoce, età avanzata alla prima gravidanza, mancanza di informazioni precedenti sul carcinoma mammario, terapia ormonale e presenza nel Kuwait durante l'invasione/liberazione. Altri**

Received/Pervenuto 12.3.2007 - Accepted/Accettato 3.5.2007
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carbohydrate, sweet, animal fat, and vegetable oil (margarine), and less frequent consumption of fresh vegetables and olive oil. *Conclusion*. This is the first study to highlight the environmental risk factors associated with breast cancer among Kuwaiti women. We recommend introducing a nation-wide campaign to further investigate these factors, and addressing them accordingly. Eur. J. Oncol., 12 (3), 205-217, 2007

parametri comprendevano un consumo significativamente più frequente di carboidrati, dolci, grassi animali ed olio vegetale (margarina), ed un consumo meno frequente di verdure fresche ed olio di oliva. *Conclusioni*. Questo è il primo studio che evidenzia i fattori di rischio ambientali associati con il carcinoma mammario tra le donne del Kuwait. Raccomandiamo di intraprendere una campagna su tutto il territorio nazionale del Kuwait per studiare questi fattori e per introdurre azioni di sanità pubblica per affrontarli. Eur. J. Oncol., 12 (3), 205-217, 2007

Key words: **breast cancer, demographic and reproductive characteristics, peripheral blood lymphocytes (PBLs), Mediterranean diet, Kuwait**

Parole chiave: **carcinoma mammario, caratteristiche demografiche e riproduttive, PBL, dieta mediterranea, Kuwait**

Introduction

Kuwait has one of the highest annual population growths in the world (4.5%)[1]. Breast cancer accounts for 30.3% of all cancer types in Kuwaiti females, and death occurs in approximately 43% of the patients[1].

Over eating of fast food and lack of exercise characterise the Kuwaiti society. One-third of adult Kuwaitis are obese, with a body mass index (BMI) exceeding 30 kg/m^2, and 4.7% of the females have severe obesity (BMI > 40 kg/m^2)[2]. Accordingly, obesity and associated diseases such as type-2 diabetes and coronary artery disease (CAD) have become major health problems in Kuwait[2-4].

Based on the recent Medline and PubMed searches that we performed, there were only nine studies conducted on Kuwaiti breast cancer patients, but none of these studies investigated the patients' characteristics and life style patterns[5-13]. Accordingly, we sought to investigate these parameters in line with those already published in other developed and developing countries. These parameters included information on demographic and reproductive risk factors, diet and physical activity, medical history, smoking, family history, breastfeeding habits, and presence in Kuwait during the invasion/liberation. Moreover, we determined the percentage of immunocyte subpopulations including CD3$^+$ (pan T cells), CD4$^+$ (helper T cells), CD8$^+$ (cytotoxic T cells), CD19$^+$ (B lymphocyte), CD56$^+$ (natural killer or NK cells), and CD3:CD4 ratio in the peripheral blood of the patients and their age group-matched normal controls in an attempt to examine their immune status.

Patients and methods

Ethics and criteria for exclusion

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. Both patients and controls gave informed consent after receiving a detailed description of the study.

Fifty stage I breast cancer patients seen at Mubarak Al-Kabeer Hospital at the time of diagnosis between 2004 and 2006 were included in this study. This hospital mainly serves two districts in Kuwait, Mubarak Al-Kabeer and Hawally (fig. 1). We excluded patients suffering from any sickness in the past three months before the study was conducted, such as common cold, 'flu, and any other infections, as well as those who had received blood transfusions or vaccinations, or who had undergone surgery during that period. We also excluded patients with a history of communicable diseases, chronic diseases, allergy, substance abuse, and those who were pregnant or lactating. None of the patients were exposed to chemo, radio, or adjuvant therapy before the inception of the study. Patients were matched by age group to 50 Kuwaiti volunteers (both visitors and staff of the hospital) who underwent the same exclusion criteria specified

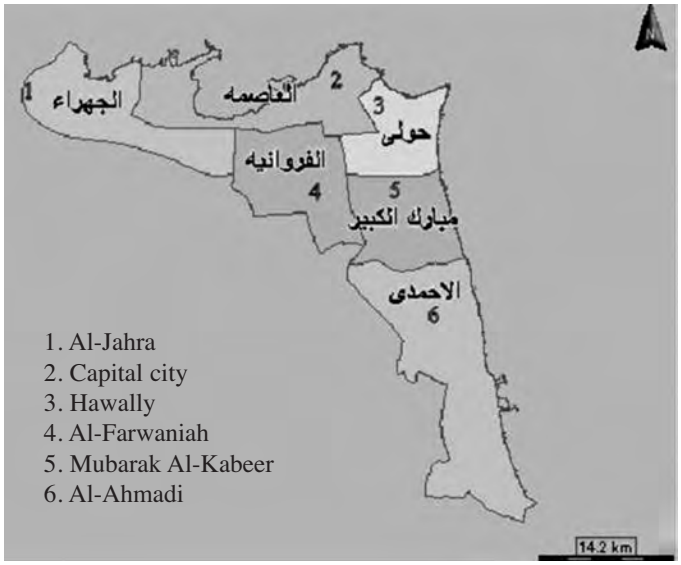

Fig. 1. A map of the State of Kuwait showing the six inhabited districts (modified after Kuwaitiah.net)

above, and who received the same information regarding the study as the breast cancer patients.

Determination of the immunophenotype status of the patients

Peripheral venous blood (10 ml) was collected from patients and controls in EDTA tubes between 08:00 and 10:00 a.m. using standard phlebotomy procedures. The blood sample was processed for flow cytometry analysis within two hours from the venipuncture time[14].

The monoclonal antibodies (Cyto-Stat/Coulter Clone, Beckman-Coulter Electronics Ltd., FL, USA) used to stain the different subsets of T and B lymphocytes included the double-labelled antibodies T4-RD1/T8-FITC against $CD4^+$ and $CD8^+$ T cells, B4-FITC against $CD19^+$ (pan B cell) lymphocytes, and CD3 FITC/$CD56^+$ RDI against $CD3^+$ mature T lymphocytes and natural killer (NK) cells. We used appropriate isotype controls for the above antibodies as recommended by the supplier. Counting of the white blood cells (WBCs) was done using a Coulter cell counter, and optimal staining was achieved with WBC counts in the range of $3x10^6$ to $10x10^6$ cells ml^{-1}. Those exceeding $10x10^6$ cells ml^{-1} were diluted to achieve counts in the above range, while those below $3x10^6$ cells ml^{-1} were centrifuged and re-suspended for the same purpose.

Eight 12x75 mm silicon tubes were used for each sample. Monoclonal antibodies (10 µl each) against the lymphocyte subsets were added to five tubes, while isotype control monoclonal antibodies (10 µl each) were added to the remaining tubes. This was followed by adding 100 µl of the venous blood to each tube and vortexing gently. Processing of the samples was performed after 10-12 minutes of incubation at 20-25^{0}C.

WBCs were prepared using Coulter Immunoprep Leukocyte System, a gentle no-wash erythrocyte lysing system that preserves both the morphology and cell surface of the leukocytes. Following the staining procedure, the test tubes were placed into a Coulter Q-prep Immunology workstation, and reagents such as formic acid (600 µl per sample), sodium (carbonate, chloride, and sulfate; 265 µl per sample), and paraformaldehyde (100 µl per sample) were added to the tubes. The tubes were allowed to stand at room temperature for half an hour before being transferred to the flow cytometer.

The EPICS-Profile II (Coulter Electronics, USA) flow cytometer was used. The fluorescence intensity of the cells was measured with a 488 nm air-cooled argon laser. Calibration of the instrument was done daily using various quality control reagents. Data acquisition was triggered by cell size (forward *versus* 90 light scatter). Filtration of the green fluorescence was done through a 530/30-band pass absorption filter. Dead cells and debris were excluded from the analysis by the conventional scatter gating method. Data was expressed in an exponential fluorescence histogram form. The threshold of positivity for the green fluorescence intensity was arbitrarily set based on the negative control sample.

Patients' and controls' characteristics and life style patterns

A structured questionnaire was used which included questions adopted from previous studies[15-17]. It included questions regarding diet and physical activity, medical history, smoking, family history, demographic and reproductive risk factors, breastfeeding habits, and presence in Kuwait during the invasion/liberation. BMI was determined at the time the questionnaire was delivered. It was calculated based on the height and weight of the participant. Questions that seemed ambiguous to the participants were clearly defined by the person doing the interview. For instance, regular exercise and physical activity were explained as simple exercises such as walking or doing aerobic exercises or as heavy exercises such as swimming or jogging being performed for ≥ 1/2 hr each time three times a week for the past five years. A stressful lifestyle was explained as being exposed to stressful events more than once a week for the past five years. The socioeconomic status was determined based on the annual income in Kuwaiti Dinar (Kuwaiti currency) in reference to the cost of living as calculated by the Kuwaiti government. When asked about any family

history of breast or other type of cancer, the degree of kinship was clearly defined. First-degree kinship included mother, sister, or daughter, while other degrees of kinship included grandmother, granddaughter, aunt, or niece. The question regarding the participant's previous information about breast cancer was explained as being information acquired through seminars, books, magazines, newspapers, television, radio, internet, friends, or relatives. A demonstration of a proper self-examination of the breast was performed in front of the participant before she was asked whether she had carried out more or less similar examinations over the past five years. The reason for asking the participants about whether they were present in Kuwait during the invasion/liberation (during the summer of the year 1990) is that we were interested in finding out whether the environmental pollution caused at that time by setting on fire 737 oil fields, some of which remained on fire for almost nine months, had influenced the subsequent rise in breast cancer incidence among Kuwaiti women. Such environmental pollution has been considered as being one of the worst environmental disasters in the 21st Century. The questionnaire also included questions regarding how frequently the participant consumed carbohydrates, sweets, fresh fruits, proteins, fresh vegetables, animal fat, vegetable oil (margarine), and olive oil during a typical week. Telephone calls to the participants were made to double check the answers a week after the interview was conducted.

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 chi-square (χ^2) test was used to assess the association between two qualitative variables. The student t-test was used to assess the difference between two independent normal variables. The Mann-Whitney U test was used to compare two independent non-normal variables. Unadjusted Odds Ratios (OR) and their 95% Confidence Intervals (CI) were calculated for different risk factors for breast cancer separately. Multiple logistic regression analysis was used to estimate the independent risk of these factors for breast cancer after controlling confounding among them. The adjusted ORs and their 95% CI for associated factors were computed from the coefficients of the logistic regression model.

Results

Immunophenotype status of patients and controls

The percentage of peripheral blood $CD4^+$ T helper lymphocytes, $CD8^+$ cytotoxic T lymphocytes, $CD4^+$:$CD8^+$ ratio, $CD19^+$ B lymphocytes, and $CD56^+$ NK cells differed between the breast cancer patients and their age group-matched normal controls (Table 1). The former had significantly ($p<0.001$; Mann-Whitney U test) higher percentage (median 47.2 [range 34.8-59.9]) of $CD4^+$ T helper lymphocytes as compared to the latter (median 40.2 [range 38.0-46.2]). The same trend was observed in relation to the $CD4^+$:$CD8^+$ ratio where the median was 1.75 (range 0.89, 5.52) in the patients, as compared to 1.26 (range 1.14, 1.49) in the controls ($p<0.001$). The percentage $CD19^+$ B lymphocytes was also significantly ($p=0.02$) higher in the patients (median 15.7 [range 9.4-24.7]) as compared to controls (median 13.9 [range 11.2-16.1]).

On the other hand, both the percentages of $CD8^+$ cytotoxic T lymphocytes and $CD56^+$ NK cells were significantly ($p<0.001$) lower in the patients (median 26.8 [range 10.2-42.7] and median 8.4 [range 2.3-15.9], respectively) as compared to controls (median 32.1 [range 30.8-34.6] and median 11.1 [range 9.4-15.2] respectively).

Table 1 - Subpopulations of immunocytes in the peripheral blood of the breast cancer patients and the age group-matched normal controls

Subpopulations of immunocytes	Breast cancer patients (N.=50)		Normal controls (N.=50)[a]		p value[b]
	Median	range	Median	range	
$CD3^+$	74.7	67.2-82.8	74.7	71.1-77.4	0.80
$CD4^+$	47.2	34.8-59.9	40.2	38.0-46.2	<0.001
$CD8^+$	26.8	10.2-42.7	32.1	30.8-34.6	<0.001
$CD4^+$:$CD8^+$ ratio	1.75	0.89-5.52	1.26	1.14-1.49	<0.001
$CD19^+$	15.7	9.4-24.7	13.9	11.2-16.1	0.02
$CD56^+$	8.4	2.3-15.9	11.1	9.4-15.2	<0.001

[a] Population normal percentage range ($CD3^+$=67-76; $CD4^+$=38-46; $CD8^+$=31-40; $CD4^+$: $CD8^+$ ratio=1-1.5; $CD19^+$=11-16; $CD56^+$=10-19)
[b] The p values were generated using the Mann-Whitney U test: $p<0.05$ was considered significant

No significant difference was found between the patients and controls in relation to the percentage of $CD3^+$ pan T lymphocytes (median 74.7 [range 67.2-82.8] *versus* median 74.7, range 71.1-77.4; p = 0.80).

Patients' and controls' characteristics and life style patterns

Demographic, lifestyle, and obstetric parameters

Our results showed that only 7 out of 24 parameters that we tested were significantly associated with breast cancer risk (Table 2). The percentage of breast cancer patients who performed regular exercise and physical activity was 14% as compared to 76% in the control group (OR=19.45; 95%CI=6.95-54.45; p<0.001; χ^2 test). Patients whose BMI fall in the 26-30 and >30 categories had an OR of 5.00 (95%CI 6.50-72.57, p<0.001) and 3.42 (95%CI=3.27-78.28, p<0.001), respectively. Seventy-four percent of the patients reported their age at menarche being ≤13 yrs (OR=2.42; 95%CI=1.04-5.63; p=0.04), and 39.1% and 17.4% reported their age at first pregnancy being between 25-29 yrs and ≥30 yrs, respectively (OR= 5.14; 95%CI=1.62-16.35; p=0.006; OR=7.62; 95%CI= 1.57-37.05; p=0.01, respectively). Lack of previous information about breast cancer was reported by 82% of the

Table 2 - General characteristics of the breast cancer patients and the age group-matched normal controls

Characteristics	Patients (N.=50)		Controls (N.=50)		OR[a]	95%CI[b]	p value[c]
	N.	%	N.	%			
Age (years)							
<40	17	34	19	38	1[Rd]		
40-59	24	48	21	42	0.97	0.13-7.30	0.98
>59	9	18	10	20	1.07	0.13-8.67	0.95
Mean ± SD[e]	47.2±11.8		49.9±12.6				0.90
Marital status							
Single	4	8	3	8	1.36	0.29-6.43	0.70
Married/divorced	46	92	47	92	1[R]		
Number of children							
None	4	8	3	6	2.22	0.40-12.39	0.36
1-3	9	18	15	30	1[R]		
≥4	37	74	32	64	1.93	0.74-4.99	0.18
Educational level							
Primary	4	8	3	6	0.79	0.17-3.75	0.77
Secondary	41	82	39	78	0.49	0.07-3.04	0.43
Tertiary	5	10	8	16	1[R]		
Working status							
Not working	4	8	3	6	1[R]		
Housewife	41	82	39	78	0.79	0.17-3.75	0.77
Housewife and employed	5	10	8	16	0.49	0.07-3.04	0.43
Socioeconomic status							
Poor	5	10	4	8	1.16	0.28-4.80	0.84
Average	27	54	25	50	1[R]		
Rich	18	36	21	42	0.79	0.35-1.82	0.59
Regular exercise and physical activity							
No	43	86	12	24	19.45	6.95-54.45	<0.001
Yes	7	14	38	76	1[R]		
Smoking status							
Non-smoker	48	96	44	88	1[R]		
Current/ex-smoker	2	4	6	12	0.31	0.06-1.59	0.16
Living a stressful lifestyle							
No	39	78	36	72	1[R]		
Yes	11	22	14	28	0.73	0.29-1.80	0.49

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Table 2 - continued

Characteristics	Patients (N.=50)		Controls (N.=50)		OR[a]	95%CI[b]	p value[c]
	N.	%	N.	%			
BMI[f]							
19-25.9	4	8	32	64	1[R]		
26-30	38	76	14	28	5.00	6.50-72.57	<0.001
>30	8	16	4	8	3.42	3.27-78.28	<0.001
Menopause							
No	24	48	19	38	1[R]		
Yes	26	52	31	62	0.66	0.30-1.47	0.31
Age at menarche (years)							
≤13	37	74	27	54	2.42	1.04-5.63	0.04
>13	13	26	23	46	1[R]		
Age at first pregnancy (years)							
<20	7	15.2	20	42.6	1[R]		
20-24	13	28.3	14	29.8	2.65	0.84-8.34	0.09
25-29	18	39.1	10	21.3	5.14	1.62-16.35	0.006
≥30	8	17.4	3	6.4	7.62	1.57-37.05	0.01
History of breastfeeding (years)							
Never	4	8	3	6	1[R]		
<1	20	40	21	42	0.71	0.14-3.60	0.68
1- 2	19	38	13	26	1.10	0.21-5.74	0.91
>2	7	14	13	26	0.40	0.07-2.34	0.31
History of being breastfed							
No	21	42	24	48	0.78	0.36-1.73	0.55
Yes	29	58	26	52	1[R]		
Family history of breast cancer							
No	45	90	48	96	1[R]		
Yes	5	10	2	4	2.67	0.49-14.44	0.26
Degree of kinship							
First degree[g]	2	4	1	2	3.20	0.32-31.90	0.32
Other[h]	3	6	1	2	1[R]		
Family history of other types of cancer							
No	30	60	34	68	1[R]		
Yes	20	40	16	32	1.42	0.62-3.22	0.40
Degree of kinship							
First degree[g]	7	14	10	20	2.45	0.83-7.27	0.10
Other[h]	13	26	6	12	1[R]		
Previous information about breast cancer[i]							
No	41	82	28	56	3.58	1.44-8.91	0.006
Yes	9	18	22	44	1[R]		
Previous self-examination of the breast							
No	45	90	43	86	1.47	0.43-4.97	0.54
Yes	5	10	7	14	1[R]		

(footnotes next page)

(to be continued on the next page)

Table 2 - continued

Characteristics	Patients (N.=50)		Controls (N.=50)		OR[a]	95%CI[b]	p value[c]
	N.	%	N.	%			
Previous mammography							
No	47	94	44	88	2.14	0.50-9.07	0.30
Yes	3	6	6	12	1[R]		
Hormonal therapy including contraceptive pills							
No	16	32	31	62	1[R]		
Yes	34	68	19	38	3.47	1.52-7.90	0.003
Presence in Kuwait during the invasion/liberation							
No	21	42	35	70	1[R]		
Yes	29	58	15	30	3.22	1.41-7.36	0.005

[a] OR = odds ratio
[b] CI = confidence interval
[c] The p values were generated using the chi square (χ^2) test except for the comparison of mean ages where the student t test was used ($p<0.05$ was considered significant)
[d] R = Reference category
[e] SD = standard deviation
[f] BMI = body mass index
[g] First degree kinship i.e. mother, sister, or daughter
[h] Other i.e. grandmother, granddaughter, aunt, or niece
[i] Knowledge acquired through seminars, books, magazines, newspapers, television, radio, internet, friends, or relatives

patients (OR=3.58; 95%CI=1.44-8.91; p=0.006). Lastly, hormonal therapy including contraceptive pills, as well as presence in Kuwait during the invasion/liberation were also significantly associated with breast cancer risk. Sixty-eight percent of the breast cancer patients were on hormones (OR=3.47; 95%CI=1.52-7.90; p=0.003), and 58% were present in Kuwait during the invasion/ liberation (OR=3.22; 95%CI=1.41-7.36; p=0.005).

The above seven parameters were reduced to only four when we attempted to determine which of them remained independently associated with breast cancer risk using multiple logistic regression analysis. These four parameters were lack of regular exercise and physical activity (OR=43.2; 95%CI=5.0-374.5; p=0.001), BMI in patients with a BMI between 26-30 (OR=63.7; 95%CI=5.9-682.4; p=0.001), early age at menarche (OR=12.0; 95%CI= 1.62-89.3; p=0.01), and lack of previous information about breast cancer (OR=13.4; 95%CI=1.96-91.2; p= 0.008).

Diet and nutrition parameters

The questionnaire (Table 3) included questions regarding how frequently the participants consumed carbohydrates including rice, bread, pasta, and cereals, sweets including Arabic sweets, cake, and chocolate, fresh fruits, proteins including red meat, poultry, fish, seafood, and beans, raw or cooked fresh vegetables, animal fat, vegetable oil (margarine), and olive oil during a typical week. A significant association with breast cancer risk was found in the patients consuming carbohydrates four to seven times a week (OR=5.21; 95%CI= 1.79-15.19; p=0.003; χ^2 test), and sweets (OR=5.23; 95%CI=1.87-14.59; p=0.002). A similar trend was observed regarding the intake of vegetable oil (margarine) (OR=14.12; 95%CI=1.65-120.89; p=0.02), and even in the patients who consumed vegetable oil (margarine) two to three times a week (OR=10.87; 95%CI=1.29-91.67; p=0.03). Patients who consumed animal fat four to seven times a week had an OR of 3.29, a 95%CI of 0.97-11.11, and a p value of 0.06. This shows a trend towards a significant association between high intake of animal fat and breast cancer risk in our patients; such significance could probably be more clearly expressed with a larger sample size. In addition to the above foodstuffs, less frequent consumption of fresh vegetables (once a week) was found to be significantly associated with breast cancer risk (OR=4.16; 95%CI= 1.38-12.49; p=0.01), and so was the less frequent consumption of olive oil (OR=4.17; 95%CI=1.16-14.91; p=0.03).

Investigations into which of the above five foodstuffs persisted as an independent risk factor associated with

Table 3 - Nutritional information about patients and matching controls

Diet	Cases (N.=50)		Controls (N.=50)		OR[a]	95%CI[b]	p value[c]
	N.	%	N.	%			
Carbohydrates (rice, bread, pasta, cereals)							
once a week	12	24	20	40	1[Rd]		
2-3 times a week	13	26	22	44	0.98	0.37-2.65	0.98
4-7 times a week	25	50	8	16	5.21	1.79-15.19	0.003
Sweets (Arabic sweets, cake, chocolate)							
once a week	11	22	25	50	1[R]		
2-3 times a week	16	32	15	30	2.42	0.89-6.59	0.08
4-7 times a week	23	46	10	20	5.23	1.87-14.59	0.002
Fresh fruits							
once a week	2	4	3	6	0.72	0.11-4.67	0.73
2-3 times a week	23	46	20	40	1.24	0.55-2.79	0.60
4-7 times a week	25	50	27	54	1[R]		
Proteins (red meat, poultry, fish and sea food, beans)							
once a week	2	4	3	6	1[R]		
2-3 times a week	22	44	32	64	0.98	0.15-6.40	0.99
4-7 times a week	26	52	15	30	2.7	0.40-18.00	0.31
Fresh vegetables (raw or cooked)							
once a week	22	44	10	20	4.16	1.38-12.49	0.01
2-3 times a week	19	38	23	46	1.56	0.57-4.29	0.39
4-7 times a week	9	18	17	34	1[R]		
Animal fat							
once a week	7	14	10	20	1[R]		
2-3 times a week	20	40	30	60	0.95	0.31-2.92	0.93
4-7 times a week	23	46	10	20	3.29	0.97-11.11	0.06
Vegetable oil (margarine)							
once a week	1	2	10	20	1[R]		
2-3 times a week	25	50	23	46	10.87	1.29-91.67	0.03
4-7 times a week	24	48	17	34	14.12	1.65-120.89	0.02
Olive oil							
once a week	25	50	12	24	4.17	1.16-14.91	0.03
2-3 times a week	20	40	28	56	1.43	0.42-4.83	0.57
4-7 times a week	5	10	10	20	1[R]		

[a] OR = odds ratio
[b] CI = confidence interval
[c] The p values were generated using the chi square (χ^2) test ($p<0.05$ was considered significant)
[d] R = Reference category

breast cancer following multiple logistic regression analysis revealed that such an association was narrowed down to only two foodstuffs, namely sweets and vegetable oil (margarine). The consumption of sweets four to seven times a week remained significantly associated with breast cancer risk (OR=4.48; 95%CI=1.09-18.37; p=0.04), and so was the consumption of vegetable oil (margarine) (OR=15.14; 95%CI=1.44-159.01; p=0.02). Moreover, such a significant association was also seen in patients who consumed vegetable oil (margarine) even two to three times a week (OR=21.14; 95%CI=2.00-223.14; p=0.01).

Discussion

Our results showed, and for the first time in Kuwait that there are some significant environmental factors

associated with breast cancer risk among Kuwaiti women. Although some p values were significant, we acknowledge the fact that there were some limitations to our study. These include a small sample size, and, accordingly, any conclusions drawn from this study may not be generalised. It is worth mentioning here that due to issues directly related to the Kuwaiti culture, it is difficult to conduct a survey or to follow up on that survey. Another limitation is that, even if a survey could be conducted, some women are educated in such a way that talking about issues related to one's own body, such as menarche and menstrual cycle, usage of oral contraceptives, self-examination of the breast, and about habits like smoking could be humiliating and not accepted by their upbringing. Still another limitation is the possibility of underestimation or overestimation of facts in response to some questions included in the questionnaire. Nevertheless, this anticipated recall bias limitation is sometimes an inherent component of case-control studies.

This study demonstrated that Kuwaiti women with stage I breast cancer and residing in two major districts in Kuwait, namely Mubarak Al-Kabeer and Hawally, for which Mubarak Al-Kabeer hospital is the major referral centre, share some common characteristics, some of which were found to be significantly associated with breast cancer risk. The immunophenotype of the patients showed that they had higher percentages of T helper and B lymphocytes in their peripheral blood as well as a higher T helper/ T cytotoxic ratio. They also had lower percentages of T cytotoxic and NK cells. The characteristics that were significantly associated with the disease included high BMI, lack of regular exercise and physical activity, early age at menarche, late age at first pregnancy, lack of previous information about breast cancer, hormonal therapy including contraceptive pills, presence in Kuwait during the invasion/liberation, more frequent consumption of carbohydrate, sweets, and vegetable oil (margarine), and less frequent consumption of fresh vegetables and olive oil. The patients also had a high intake of animal fat, but this did not reach significance in relation to breast cancer risk, probably due to a small sample size.

T helper lymphocytes are required for the generation and maintenance of effective anti-tumour immunity which is mediated by the cytotoxic T lymphocytes. The literature has reported various results in relation to the level of peripheral blood lymphocyte (PBL) subsets in patients having breast or other types of cancer as compared to normal controls[18-24]. In a study comparing PBLs from patients with breast cancer with those from normal controls, Schroder *et al*[18] reported no significant difference in the level of $CD19^+$ B lymphocytes or in the $CD4^+$:$CD8^+$ ratio. However, the level of $CD3^+$ T cells was decreased in the former group. Similar to Schroder *et al*, Whitford *et al*[19], reported no significant difference in the level of $CD19^+$ B lymphocytes in the peripheral blood of breast cancer patients as compared to normal controls. The authors reported the same trend in relation to the level of $CD3^+$ T lymphocytes, unlike the trend reported by Schroder *et al*, where the level of $CD3^+$ T lymphocytes was decreased. Analysis of the peripheral blood of patients with ovarian cancer revealed that they had a significantly lower $CD4^+$:$CD8^+$ ratio, and a higher percentage of $CD56^+$ NK cells than normal controls[18]. On the other hand, the $CD4^+$:$CD8^+$ ratio was found to be significantly increased in another study conducted on patients with ovarian cancer, and so were the levels of $CD56^+$ NK cells and $CD19^+$ B lymphocytes[20]. Such ratio was also found to increase in patients with gastric cancer[21]. This, however, contradicts what was reported by Lee *et al*[22], where the percentage of $CD4^+$ T lymphocytes and the $CD4^+$:$CD8^+$ ratio were found to decrease without a significant change in the level of $CD8^+$ T lymphocytes in the peripheral blood of patients with gastric cancer. In a study conducted on patients with bladder cancer, the peripheral blood levels of $CD3^+$ T lymphocytes, $CD4^+$ T lymphocytes, and the $CD4^+$:$CD8^+$ ratio were significantly lower than those of healthy men. However, the level of peripheral blood $CD8^+$ T lymphocytes was significantly elevated in the cancer patients[23]. Patients with bladder cancer were also investigated in a study conducted by Kaver *et al*[24]. The authors reported a decrease in the level of peripheral blood $CD4^+$ T lymphocytes and in the $CD4^+$:$CD8^+$ ratio in the patients with infiltrating bladder cancer, as compared to age-matched patients with benign urological disease, who served as controls. While the patients with superficial bladder carcinoma or with renal cell carcinoma did not show any difference in relation to the various immunocyte subsets as compared to the control group in the same study, those with prostate cancer displayed a higher $CD4^+$:$CD8^+$ ratio, resulting from a significant decrease in the level of $CD8^+$ T lymphocytes in their peripheral blood[24].

In some of the above reported studies as well as in our current study, one possible explanation for the lower levels of $CD8^+$ T lymphocytes and $CD56^+$ NK cells in the peripheral blood of the cancer patients as compared to normal controls is that such cell-mediated arm of the immune system becomes suppressed by the tumour itself due to the release of various immunosuppressive factors by the tumour cells[25]. Another possible explanation is that the inflammatory condition, associated with increased oxidative stress during tumour development, could in turn lead to tumour-induced immuno-suppression[26]. As far as the simultaneous increase in the levels of $CD4^+$ T

lymphocytes and $CD19^+$ B lymphocytes is concerned, this could be explained by the phenomenon whereby proliferation and activation of B lymphocytes require T helper cells. As the number of T helper cells increase in response to tumour development, there could be a subsequent increases in the level of B lymphocytes[27]. One should also not exclude an important rôle for nutrition in possibly altering the immune function in the 50 breast cancer patients that we studied. Earlier studies provided some evidence on the effect of nutrition on the immune system, and more recent studies showed that nutritional supplements, given to severely ill patients and to those with generalized malignancy were capable of restoring their immunodeficiency status and prolonging their survival [28-30]. Dietary omega-3 polyunsaturated fatty acids were found to exert an anti-tumour effect via alteration of prostaglandin E2 synthesis resulting in immunomodulation, prevention of inflammation, and direct inhibition of tumour cell proliferation[31]. In a study conducted on rats, Robinson *et al* [32] found that feeding long-chain n-3 fatty acids in a low polyunsaturated/saturated fatty acid diet increased the NK cell cytotoxicity as well as the proportion of activated $CD25^+$, $CD8^+$, and $CD28^+$ T cells in their blood. The group also found that feeding the rats with a diet containing 5% fish oil increased the proportion of peripheral blood $CD4^+$ and $CD8^+$ T lymphocytes[33]. Since cytokines have been used as markers of inflammation, monitoring these glycoproteins, which are secreted by activated lymphocytes in response to diet, could provide more evidence on the effect of diet on the immune system[34]. This has been demonstrated in studies where specific nutrients as well as nutritional status were found to influence the production and concentration of these cytokines[35, 36]. Another area of immunology where nutrition was shown to have an influence is gene expression. It was demonstrated that nutrients have an impact on early signals for gene expression in immunocytes, and on the message and proteins of genes including cytokine activation markers[33, 37]. Immunocytes could also be affected by glucose and glutamine, which constitute the critical energy source for these cells[38, 39]. The uptake of glutamine by immunocytes has been proposed as an early marker of lymphocyte activation[38, 39]. More powerful evidence on the effect of nutrition on cells of the immune system is probably demonstrated by the membrane composition of these cells. Earlier studies where the n-6 and n-3 content of the immune cell lipids were changed showed that such changes influenced the degree of proliferation of lymphocytes, their cell-to-cell adhesion, activity of their membrane-bound enzymes, fluidity of their plasma membranes, the amount of cytokine they produced, and the degree of expression of activation epitopes on their surface[40, 41]. More recent studies showed that the proportion of total n-6 and n-3 polyunsaturated fatty acids in the phospholipid component of the plasma membrane of peripheral blood mononuclear cells positively correlated with the degree of phagocytosis by neutrophils and monocytes, the degree of proliferation of lymphocytes, the level of neutrophil oxidative burst, and the degree of production of interferon gamma[42]. In our study, the low consumption of olive oil and the high consumption of vegetable oil (margarine) by the breast cancer patients were significantly associated with breast cancer risk. This could explain the abnormally low levels of cytotoxic T lymphocytes and NK cells in the peripheral blood of these patients, based on the results of the studies described above. In fact, the low consumption of olive oil and the high consumption of vegetable oil (margarine) by the breast cancer patients in our study persisted as two factors significantly associated with breast cancer risk, even after the multiple logistic regression analysis was performed.

Our results on the demographic and reproductive characteristics of the patients confirm what has been published so far regarding the existence of an association between breast cancer and some risk factors such as high BMI, lack of regular exercise and physical activity, menarche before age 11, late age at first pregnancy, and hormone use[43, 44]. Interestingly, two other demographic factors were found to be associated with the disease in our patients, namely lack of previous information about the disease and presence in Kuwait during the invasion/liberation in 1990.

Lack of previous information about the disease could usually result from one or more of the following reasons: low educational achievement, low socioeconomic status, working status, living distant from where information about the disease could be sought, or lack of proper health promotion programmes in the community[45, 46]. In our study, we could not detect a significant association between socioeconomic status, educational level, or working status and the disease. This could probably be attributed to the small sample size. Being geographically isolated could be ruled out as a reason for the lack of previous information about the disease, since the inhabited area in Kuwait is relatively small, and the concept of rural *versus* urban geographical location does not exist. The only remaining possible reason is that proper health promotion programmes in the community in Kuwait are still lacking. Having investigated this matter, we found out that a breast cancer society had recently been established in Kuwait. It is being headed by Professor Fayzah Al-Khourafi, who happened to be an ex-President of Kuwait University.

Concerning the presence in Kuwait during the invasion/liberation, we mentioned earlier that we were interested to explore whether the environmental pollution caused at that time by setting on fire 737 oil fields, some of which remained on fire for almost nine months, had influenced the subsequent rise in breast cancer incidence among Kuwaiti women. The smoke that is produced by burning crude oil often contains various heavy metals including cadmium. The latter has recently been shown to mimic the action of oestrogen in even very low doses in rats[47]. Whether cadmium contributed to the aetiology of breast cancer in our patients remains to be seen.

Lastly, our study showed that diet seems to play an important rôle in predisposing to breast cancer in Kuwaiti females. The patients seem to have a high intake of animal fat, carbohydrate, sweets, and vegetable oil (margarine), while their intake of olive oil and fresh vegetables is less frequent. This probably explains the obesity problem and the related high incidence of type-2 diabetes and CAD from which the Kuwaiti society is currently suffering[2-4]. The main protein intake in our patients was from imported red meat and grain-fed chicken, rather than from fish, seafood, or beans (data not shown). Since the diet seen in our breast cancer patients seems to favour the development of cancer, one possible approach to decrease the incidence of breast cancer in Kuwait is to introduce the Mediterranean diet to the society. This is based on the fact that several studies have documented a significant decrease in the incidence of cancer, including breast cancer, in individuals following such a diet[48, 49]. In a recently published cohort study, Lagiou *et al*[48] studied Swedish women aged 40 years or older at enrolment in relation to a possible effect of the Mediterranean diet on cancer incidence. The authors focussed on this age group since they argued, as we have shown in our current study, that breast cancer is often associated with environmental factors (including diet), rather than genetic aetiology in women aged 40 years and above. This was recently supported by the review published by Loman[50]. Following a 12-year follow-up, Lagiou *et al* demonstrated that the Mediterranean diet was significantly associated with a substantial reduction in the incidence of cancer in the Swedish women. Thus, this confirms the beneficial effect of the Mediterranean diet on diseases like cancer, where some nutritional components could play a significant protective rôle even in individuals who are in their forties. The Mediterranean diet entails a more frequent consumption of olive oil as the main source of dietary lipids, fresh fruits as the typical daily deserts, limiting sweet intake to feast days, minimising consumption of red meat while relying more on fish and seafood (rich in antioxidants like omega-3, vitamin E, selenium and zinc) as a source of protein, daily consumption of fresh vegetables, low to moderate consumption of dairy products (mainly cheese and yoghurt), and limiting egg intake to an average of four eggs per week[51]. The Mediterranean diet is considered as the diet of Crete, which is the biggest island and the most southern one in Greece, except for the little island of Gavdos. Such a diet is similar to the Neolithic diet which existed thousands of years ago, when people used to rely more on fish, seafood, and olive oil (rich in *n*-3 fatty acids), and on fresh vegetables and fruits, fresh legumes, wild plants like purslane, and nuts (rich in folate, glutathione, α-linolenic acid, and vitamins C and E), and to rely less on red meat, the source of which was mainly from animals gazing in the wild prairies rather than being grain-fed[49]. Earlier reports have shown that the population of Crete had the lowest rates of cancer and cardiovascular diseases according to the Seven Countries Study[52]. The same trend was observed in Crete twenty-five years later[53]. More recently, the Lyon Heart Study clearly showed that the Cretan diet resulted in anticancer effects, including clinical manifestation of the disease in the French population[54, 55]. It has been demonstrated that the protection against cancer through the Cretan diet is strongly related to the ratio of *n*-6 to *n*-3 fatty acids. Such a ratio has been found to be between 1 and 2 in the Cretan diet as compared to 15 in the UK and Northern Europe, 16.74 in the US, and up to 50 in urban India[49]. Cowing and Saker[56] reported that *n*-3 fatty acids could be potentially used as an adjuvant therapy to prevent recurrence and metastases of mammary tumours. Moreover, such tumours could become more sensitive to several cytotoxic drugs by the *n*-3 fatty acids[57]. In a recent study conducted on human MDA-MB-231 breast cancer cells, *n*-3 fatty acids were shown to be capable of significantly decreasing the proliferation of these cells, as well as inducing apoptosis[58]. Singapore Chinese women consuming high levels of *n*-3 fatty acids had a 26% reduction in breast cancer risk, while those who consumed less of these fatty acids had a relative risk ranging between 1.87 and 2.45[59]. Finally, a high level of *n*-3 fatty acids in the adipose tissue of the breast was found to be significantly associated with lower breast cancer risk in a recent case-control study conducted by Maillard *et al*[60].

According to our knowledge, our study is the first of its kind to be conducted in Kuwait. It described the characteristics and life style patterns of the Kuwaiti breast cancer patients. Our study had a small sample size due to the cultural difficulties that we described earlier, but we hope that it will form a platform on which future breast cancer research in Kuwait will be based.

Acknowledgements

A special thank you goes to our patients and the controls who kindly volunteered to participate in this study. The authors would also like to thank Mrs. Smitha Sivanandan for assisting in transferring the blood samples from the hospital to the lab, and for preparing the samples for flow cytometry analysis. This work was supported by Kuwait University, Research Grant No. [MA 01/07].

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