EUROPEAN JOURNAL OF ONCOLOGY

GIORNALE EUROPEO DI ONCOLOGIA

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

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

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

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

Issue 1 **Bernardino Ramazzini - Biografia e Bibliografia**
Pericle Di Pietro - 20 €

Issue 2 **The Precautionary Principle Implications for Research and Prevention in Environmental and Occupation Health**
P. Grandjean, M. Soffritti, F. Minardi, J.V. Brazier (Editors) - 25 €

Issue 3 **Ban on asbestos in Europe**
Messa al bando dell'amianto in Europa
C. Bianchi, M. Soffritti, F. Minardi, J.V. Brazier (Editors) - 18 €

European Journal of Oncology

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

title (titolo)

European Journal of Oncology
Giornale Europeo di Oncologia

notes (note)

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

Subscription rates (prezzi abbonamenti)

	Individual (Privati)			Institution (Istituzioni)	
	Italy	Europe	out of Europe Air Mail	Italy and Europe	out of Europe Air Mail
Annual subscription (Abb. annuo)	**52,00** euro	65,00 euro	76,00 euro	78,00 euro	88,00 euro
Back Numbers (Arretrati)					
Single Issue (Numero singolo)	**19,00** euro	24,00	28,00 euro	28,00 euro	32,00 euro
Complete Year (Annata compl.)	**62,00** euro	78,00	91,00 euro	84,00 euro	106,00 euro
Special Issues (Num. speciali)	Issue 1 ☐ Issue 2 ☐ Issue 3 ☐			number of copies	

I WOULD LIKE TO

☐ SUBSCRIBE TO THE "EUROPEAN JOURNAL OF ONCOLOGY" YEAR .....

☐ RECEIVE THE FOLLOWING ISSUES OF "RAMAZZINI LIBRARY"
ISSUE 1 ☐ ISSUE 2 ☐ ISSUE 3 ☐

PLEASE SEND THE ISSUES TO:

MR/DR/PROF.

ADDRESS N

TOWN

POSTAL CODE COUNTRY

E-MAIL

PHONE/FAX

☐ (IF ITALIAN) CODICE FISCALE E PARTITA IVA (MANDATORY):

PAYMENT MAY BE MADE:

☐ BY CHECK N° .....

☐ BY BANK TRANSFER intested to
Mattioli 1885 spa Via Coduro 1/B, 43036 Fidenza (PR),
Bank: Cassa di Risparmio di Parma e Piacenza, Ag. 3 di Fidenza; cod. IBAN: IT 39 S 06230 65732 000094186751
cod. BIC: CRPPIT2P487

☐ BY CREDIT CARD: ☐ VISA ☐ MASTERCARD

CARD NUMBER ☐☐☐☐ ☐☐☐☐ ☐☐☐☐ ☐☐☐☐

EXPIRY DATE ☐☐ ☐☐ ☐☐

CARDHOLDER'S NAME (IN CAPITAL LETTERS):.....

SIGNATURE .....

Mattioli 1885 Casa Editrice
via Coduro 1/b
43036 Fidenza (Pr)

Mattioli 1885 Casa Editrice - spa - via Coduro 1/b 43036 Fidenza (Pr) tel 0039/0524/84547 fax 0039/0524/84751 e-mail subscribe@mattioli1885.com - www.mattioli1885.com

EUROPEAN JOURNAL OF ONCOLOGY
GIORNALE EUROPEO DI ONCOLOGIA

CONTENTS/INDICE

VOLUME 10 - N. 2 - 2005

Eur. J. Oncol., vol. 10, n. 2, pp. 89-106, 2005

Position paper/Commentario

Melatonin levels and exposure to electromagnetic fields: biologic background and epidemiological implications

Livelli di melatonina ed esposizione a campi elettromagnetici: conoscenze biologiche e implicazioni epidemiologiche

Marta Benedetti*, Pietro Comba*, Maurizio Nordio**

* Department of Environment and Primary Prevention, National Institute of Health, Rome, Italy

** Department of Medical Physiopathology, University "La Sapienza", Rome, Italy

Summary

Exposure to extremely low frequency (ELF) magnetic and electric fields (10-100 Hz), including the 50-60 Hz fields generated by power lines, has been associated to a number of adverse health effects including cancer, reproductive problems, cardiovascular diseases, neuropsychological and psychiatric disorders, neurodegenerative diseases, and alteration of the immunologic and haematologic parameters. According to what has been described as the "melatonin hypothesis", the mechanism by which exposure to ELF magnetic fields may cause adverse biological effects is by reducing or suppressing the normal nocturnal rise in melatonin. Melatonin, a methoxyindole, is synthesized and secreted principally by the pineal gland. Under normal environmental conditions, pineal melatonin synthesis exhibits a pronounced circadian rhythm: its concentrations are lower during the day and reach maximal levels at night. Once synthesized in the pineal, melatonin is immediately released into the circulation and rapidly metabolised (half-life = 20-30 min), chiefly in the liver, and then excreted in the urine. In humans the main metabolite is 6-sulphatoxymelatonin: its urinary concentration closely parallels the plasma melatonin profile. In numerous animal studies, exposure to ELF magnetic fields has been shown to alter melatonin synthesis and secretion. On the contrary, in humans, the data on the influence of ELF magnetic fields on melatonin synthesis and secretion are contradictory. A reduction of nocturnal melatonin plasma or urinary 6-sulphatoxymelatonin concentrations has been

Riassunto

L'esposizione ai campi magnetici ed elettrici a frequenza estremamente bassa (ELF) (10-100 Hz), inclusi i campi a 50-60 Hz generati dalle linee elettriche, è stata associata alla comparsa di numerosi effetti avversi quali tumori, problemi della sfera riproduttiva, malattie cardiovascolari, disordini neuropsicologici e psichiatrici, malattie neurodegenerative ed alterazioni dei parametri immunologici ed ematologici. Secondo quella che è stata definita l'"ipotesi della melatonina", il meccanismo attraverso il quale l'esposizione ai campi magnetici ELF potrebbe indurre effetti avversi, consiste nella riduzione o nella soppressione dell'incremento dei livelli notturni della melatonina. La melatonina, un metossindolo, è principalmente sintetizzata e secreta dalla ghiandola pineale e, in condizioni ambientali normali, la sintesi e la liberazione di melatonina da parte della pineale avviene seguendo una ben evidente ritmicità circadiana. Tale ritmo è caratterizzato da concentrazioni plasmatiche molto basse durante il giorno e, al contrario, elevate durante le ore notturne. Dopo essere stata sintetizzata nella pineale, la melatonina è immediatamente rilasciata nel torrente circolatorio e rapidamente metabolizzata (emivita = 20-30 min), per la maggior parte nel fegato, e quindi escreta nelle urine. Nell'uomo il principale metabolita è la 6-sulfossimelatonina, la cui concentrazione urinaria è correlata significativamente con i livelli plasmatici di melatonina. Numerosi studi sperimentali condotti su specie animali hanno evidenziato che l'esposizione ai campi ELF è in grado di alterare la sintesi e la se-

Received/Pervenuto 1.3.2005 - Accepted/Accettato 24.3.2005

Address/Indirizzo: Dr. Marta Benedetti, Department of Environment and Primary Prevention, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italia - E-mail: marta.benedetti@iss.it

reported in some occupational and residential studies. However, these changes were not observed in the majority of the laboratory-based exposure studies. According to the International Agency for Research on Cancer (IARC), a small reduction of melatonin concentration has been observed both in occupational and residential settings, but it is difficult to distinguish between the effects of ELF magnetic fields and those of other environmental factors. Subsequent to this evaluation, one study corroborated the hypothesis of an aetiological rôle of magnetic fields in the complex determination of melatonin levels, while another did not detract from the credibility of the hypothesis, since its non-positive results could not be adequately evaluated given the heterogeneous composition of the study subjects. On the basis of these considerations, we have recently developed guidelines for assessing the health status of subjects resident close to sources of ELF magnetic fields. Measurement of 6-sulphatoxymelatonin in daytime (from 08:00 to 20:00) and nighttime (from 20:00 to 08:00) urine samples is part of this protocol, together with assessment of the immune function, of the heart rate variability and with a health check with special reference on neuropsychological and psychiatric aspects. As many factors (pathological states, use of drugs, alcohol consumption, light at night, jet-leg, etc.) can influence the normal physiological production of melatonin, we have also made available an *ad hoc* questionnaire that may be submitted by a trained physician the day before the 24 hour urine sample collection. Subjects affected by certain pathological conditions, or subjects assuming particular drugs are not be included in the study protocol. Ratio nighttime *versus* daytime urinary 6-sulphatoxymelatonin concentrations are examined and commented, also taking into account the outcomes of the clinical and laboratory examinations. The abovementioned procedure is currently being tested in populations exposed to relatively high levels of 50 Hz magnetic fields. Eur. J. Oncol., 10 (2), 89-106, 2005

Key words: **melatonin, cancer, epidemiology, electromagnetic fields, humans**

crezione della melatonina. Al contrario, nell'uomo, i dati riguardanti l'influenza dei campi ELF sulla sintesi e rilascio di melatonina sono alquanto contraddittori. Infatti, se, da un lato, sono state evidenziate modificazioni dei livelli plasmatici notturni di melatonina o della concentrazione urinaria di 6-sulfossimelatonina in alcuni studi occupazionali e residenziali, in altri casi, studi sperimentali condotti in laboratorio non hanno potuto documentare alcuna modificazione dei livelli di melatonina. Secondo la International Agency for Research on Cancer (IARC), una modesta riduzione di melatonina è stata osservata in situazioni di esposizione occupazionale e residenziale, ma appare alquanto difficile distinguere tra gli effetti dovuti ai campi ELF e quelli dovuti ad altri fattori ambientali. In seguito a tali osservazioni, uno studio ha avvalorato l'ipotesi di un ruolo eziologico dei campi magnetici nel meccanismo che regola la produzione e secrezione di melatonina, mentre un altro, anche se con esiti negativi, non ha diminuito la credibilità dell'ipotesi in quanto l'eterogeneità dei soggetti studiati ha reso impossibile una valutazione dei risultati ottenuti. Sulla base di queste considerazioni, abbiamo recentemente messo a punto delle linee guida per la determinazione dello stato di salute di soggetti residenti nelle vicinanze di fonti di emissione di campi ELF. Fa parte di questo protocollo anche la determinazione della concentrazione urinaria diurna (08.00-20.00) e notturna (20.00-08.00) di 6-sulfossimelatonina, oltre al monitoraggio di alcune variabili immunologiche, alla valutazione della variabilità del ciclo cardiaco e ad un controllo generale dello stato di salute, con particolare riferimento agli aspetti riguardanti possibili disordini neuropsicologici o psichiatrici. Poiché molti fattori (patologie, uso di farmaci, assunzione di alcolici, luce notturna, *jet-lag*, etc.) possono influenzare la normale sintesi della melatonina, abbiamo anche previsto un questionario *ad hoc*, sottoposto da un medico esperto il giorno prima della raccolta delle urine delle 24 ore. Soggetti affetti da alcune patologie o che fanno uso di determinati farmaci non possono essere inclusi nello studio. Una volta raccolti i dati dai pazienti in esame, verrà esaminato e commentato il rapporto tra la concentrazione urinaria notturna di 6-sulfossimelatonina e la concentrazione diurna, tenendo anche conto della valutazione clinica e dei parametri delle indagini di laboratorio. Attualmente questo protocollo viene testato in un'ampia popolazione di soggetti esposti a livelli relativamente alti di campi magnetici a 50 Hz. Eur. J. Oncol., 10 (2), 89-106, 2005

Parole chiave: **melatonina, tumori, epidemiologia, campi elettromagnetici, uomo**

Introduction

The purpose of the present paper is to review the recent biological research on melatonin and discuss its relevance to the design of epidemiological studies on the variation of melatonin levels associated with electromagnetic fields exposure. In addition, we present some guidelines on the design of a correct study concerning the biological effects of extremely low frequency (ELF) fields, with particular attention to the determination of a melatonin metabolite in human urines. The underlying point that is regarded as crucial is that the use of indicators of pineal melatonin secretion in population studies may be appropriate inasmuch as there is full awareness of the complex pattern of regulation and modulation of melatonin secretion in individuals.

On the basis of these considerations, recent advances in biological studies concerning the rôle of melatonin on living organisms will be summarized. In addition, the epidemiological studies focussed on the supposed relationship between melatonin and electromagnetic fields will also be reviewed, and finally, some indications on how to design future studies will be presented.

The pineal gland and melatonin

Definition

Known initially as "the site of the soul" or "the third eye" for its supposed connections with the highest mental activities, the pineal gland (or epiphysis) has captured increasing attention due to the fact that tadpole gland extracts demonstrated a potent skin-lightening effect by inhibiting the dispersion of melanin in epidermal melanocytes. After some years, its main metabolic product, the indoleamine melatonin (N-acetyl-5-methoxytryptamine) was finally isolated from bovine pineal tissue[1]. Since then, this simple, lipophilic and therefore ubiquitous molecule has been studied in a progressively increasing number of research fields, ranging from its effects upon the reproductive system of mammals, to its ability in the maintenance of biological rhythms, and, more recently, to its potent antioxidant properties[2-4].

Originally regarded as a hormone restricted to vertebrates, melatonin has now been detected in several phylogenetically distinct organisms, as well as insects and even a unicell, the dinoflagellate *Gonyaulax polyedra*[5]. Data collected during the last decade have revealed numerous facts which make the classical definition, of melatonin exclusively as a hormone, seem too narrow. According to the literature, many features of melatonin distinguish it from the classical hormones; one of these is the fact that melatonin is synthesized by a number of extrapineal organs which are regarded as non-endocrine. Moreover, pineal melatonin is not exclusively released into the systemic circulation and, in contrast with many hormones, there is no identified storage machinery for melatonin in the pineal gland. Since melatonin is considered a highly conserved molecule that is virtually present in all evolutionary life forms, from the simplest bacteria to human beings, the initial primary function of melatonin may not have been that of a hormone. Its hormonal properties were probably acquired during the evolutionary stage of multicellular organization. Additional proofs of the not-only-hormonal nature of melatonin derive from its non-receptor-mediated actions. In fact, it is now well accepted that melatonin has a noteworthy activity as radical scavenger and a broad-spectrum antioxidant, with its activity being independent of its receptor-mediated actions. In addition to the above, the most obvious features which distinguish melatonin from classical hormones are its alternative sources (i.e. vegetables, fruits, seeds, rice, wheat and medical herbs). Finally, from a nutritional point of view, melatonin may also be considered as a vitamin, because the definition of a vitamin is that of an ingested micronutrient important for life processes, not or insufficiently formed in the animal[6].

Regulation of the pineal function

Day/night and seasonal changes in the environment dominate the lives of plants and animals, including humans. This is due to the Earth's rotation, which causes significant changes in sunlight conditions. For the timing of biological rhythms, it has been crucial that these variations in the physical environment were not random but could be "predicted". Therefore, many facets of physiology are adapted to anticipate these changes. In vertebrates, the endocrine system plays a pivotal rôle in directing temporal changes in physiology, through the presence of a well characterized circadian and seasonal rhythmicity in blood hormone levels. From this point of view, the presence of an endogenous circadian pace-maker (or clock) has a fundamental rôle. Daily input of light or other stimuli continually reset this clock and synchronize it with the environment. For that reason, light may today be defined as "a visually and chronobiologically effective radiant energy for human beings"[7].

Briefly, external light is received by the retina, which contains specific photosensitive cells that can respond to light due to the presence of a previously unsuspected opsin photopigment that seems to mediate circadian photo-entrainment[8]. On the other hand, the well-known clas-

sical photoreceptor cells, rods and cones, seem not to be involved in light perception that modulates pineal melatonin production. The photic information is transduced into a neural signal which is projected, via the axons of the retinal ganglion cells, through the optic nerves (the retinohypothalamic tract), to a specific nuclear complex in the anterior hypothalamus of the brain, referred to as the suprachiasmatic nuclei (SCN), known as the biological clock, or internal pacemaker. The intrinsic neurons of the SCN are inherently rhythmic, thus alternating periods of quiescence and firing slightly longer than the 24 hours light/dark cycle[9]. Indispensable for a self-sustained rhythmic generation in the SCN are the so called "clock genes" and their proteins, which are biologically active as transcription factors and activate positive and negative loops in order to allow the perpetuation of the circadian cycle in the SCN[10]. Given that the intrinsic neurons do not have a period of 24 hours, one function of the prevailing light/dark period is to synchronize the biological clock to 24 hours via the neural pathways described above. Output signals, generated in the SCN during darkness at night, run into a complex neural pathway which includes axons of the SCN that project to the paraventricular nuclei of the hypothalamus, whose fibres descend to the upper thoracic cord where they terminate on preganglionic sympathetic cell bodies. The axons of these neurons exit from the spinal cord and synapse on parikarya of postganglionic sympathetic cells in the superior cervical ganglia. Ultimately, the axons of these neurons innervate the pineal gland where they control the production of melatonin[7]. On the other hand, light at night prevents SCN from signalling the pineal gland to activate the molecular machinery to produce melatonin.

Light exposure has two basic functions on the melatonin synthesis cycle: acute light exposure at night (even of very short duration) causes the decrease of melatonin production, while alternating periods of light and darkness, as it is well known, serve to synchronize the melatonin rhythm to 24 hours. A recent paper demonstrated that the amount of light that is needed to suppress melatonin night plasma levels is less than what was suspected in earlier works. This difference appears to be dependent upon the previous light history of the subjects. In fact, it seems that background light intensities used in many studies may have desensitized the circadian photoreceptive system and thereby elevated the apparent threshold of melatonin suppression[11].

Melatonin production, release and excretion

Melatonin is synthesized primarily in the pineal gland from the essential aminoacid tryptophan as a precursor that is hydroxylated to 5-hydroxytryptophan and then decarboxylated to serotonin. The latter is N-acetylated by the rate-limiting enzyme in melatonin production arylalkylamine-N-acetyltransferase (AA-NAT) to N-acetylserotonin and finally converted to melatonin by the enzyme hydroxyindole-O-methyltransferase[12]. The synthesis of melatonin is initiated by the release of norepinephrine (NE) into the synaptic clefts between the sympathetic nerve endings and the pinealocytes. NE is released during the dark phase of the light/dark cycle and activates adenylate cyclase, which induces cyclic adenosine monophosphate (cAMP) production. This, in turn, activates AA-NAT, the key enzyme in melatonin synthesis, as well as its transcription and translation[13]. Rhythmic activation of AA-NAT is centered around the transcriptional regulation of the AA-NAT gene, involving two antagonistic transcription factors of the cAMP signalling pathway, CREB (cAMP response element binding protein) as activator of gene expression, and ICER (inducible cAMP early repressor) as an inhibitor[14].

Once synthesized in the pineal gland, melatonin is immediately released into the circulation and possibly directly into the cerebrospinal fluid of the third ventricle[15]. Due to the cyclic activation and inhibition of melatonin production by the pinealocyte, plasma levels of the indole follow a well-known high-amplitude circadian rhythm, synchronized with the external environment, with highest values at night. The amount of melatonin produced in the pineal is genetically determined. Among individuals of the same age the nocturnal rises in blood melatonin concentrations vary considerably. Thus, while some individuals exhibit what is considered to be a robust night peak, in others the amplitude of the peak may be greatly attenuated[16]. In addition, given that the amplitude of the melatonin circadian rhythm is highly consistent from night to night in subjects of any age, clearly some people over the course of their lifetime produce much more melatonin than others. The significance of these marked differences in the total amount of melatonin generated by the pineal gland remains unknown[17]. Moreover, nocturnal peak concentrations of the pineal indole vary considerably according to age. Infants younger than three months of age secrete very little melatonin. It increases and becomes circadian in older infants, while the peak nocturnal concentrations are highest (about 325 pg/ml) at the age of one to three years, after which they decline gradually. In normal young adults, the average daytime melatonin levels are about 5 pg/ml, whilst during the night they range from about 40 to approximately 150 pg/ml[18]. Moreover, in ageing animals and humans melatonin nighttime levels progressively decrease to about half of the values seen in young people. However, in 70-90-year-old individuals,

only 53% exhibited a decrease in melatonin levels, while 33% and 14% demonstrated no variations and increased concentrations, respectively[19]. A recent paper, in which melatonin saliva concentrations in young and old subjects were evaluated, confirmed the reduction in old and oldest individuals, also demonstrating that the decrease begins during the fourth decade[20].

The physiological age-dependent decrease in melatonin blood levels at nighttime, together with its multiple biologic effects, has led several investigators to suggest that melatonin may have a rôle in ageing and age-related diseases. Studies in rats and mice have shown that diminished melatonin secretion may be associated with an acceleration of the ageing process. In addition, as will be better specified later in this review, melatonin may provide protection against ageing through the attenuation of the effects of cell damage induced by free radicals or through immunoenhancement. However, it cannot be excluded that age-related reduction in nighttime melatonin secretion could well be a consequence of the ageing process, rather than its cause. In addition, no data supporting an antiageing effect of melatonin in humans exist to date, despite the worldwide use of the drug[18]. The mechanism causing decreased melatonin levels during ageing in humans is not yet understood. In fact, the pineal gland shows no obvious signs of degeneration[21]. On the other hand, studies indicate that the increasing number of pineal gland calcifications with age seems to be able to determine alterations in melatonin synthesis[22].

In totally blind people the rhythm of melatonin production is maintained, even though it may be desynchronized with respect to the external environment. In fact, in the absence of information regarding the prevailing light/dark cycle as a synchronizer, the melatonin circadian rhythm free-runs with a period different from 24 hours and therefore, due to the difference between the duration of the day/night rhythm (24 hours) and the cycling of the SCN neurons (about 24.5 hours), becomes out-of-phase with respect to the external light/dark cycle[23, 24].

Since melatonin is not stored in the conventional sense, its release follows production and, once into the blood stream, only around 30% of melatonin escapes binding to plasma albumin. The active compound has a reported half-life of 20 minutes, and is rapidly metabolized, chiefly in the liver, by microsomal hydroxylation to 6-hydroxymelatonin and, after conjugation with sulphuric or glucuronic acid, is excreted in the urine. The urinary excretion of 6-sulphatoxymelatonin (aMT6-s) (the main metabolite of melatonin) closely parallels serum melatonin concentratons[25, 26] and therefore the measurement of the melatonin metabolite in urines by radioimmunoassay is now widely used in clinical studies.

Extrapineal melatonin

In higher vertebrates melatonin was initially thought to be synthesized exclusively in the pineal gland. Subsequent studies have shown that it is also synthesized in numerous other organs or tissues, besides the pineal. These include the lens, gut, liver, reproductive organs, bone marrow cells, lymphoid cells, several brain regions and possibly many other tissues as well[27-30]. The concentrations of melatonin detected in the bone marrow cells, gut and liver are several orders of magnitude higher than levels reported in the blood[28, 31, 32]. However, even if melatonin is synthesised in other extrapineal organs, the pineal remains the organ that mainly contributes to the levels of melatonin present in the blood. It has been hypothesized that the melatonin produced in extrapineal organs could contribute to the numerous effects ascribed to this indole and, in particular, its presence may be related to its antioxidant and free radical scavenging actions (see following paragraph on the physiological rôle of melatonin).

Whereas in the pineal gland and in the visual system (retina and Harderian gland) the melatonin secretion rhythm complies with the light-darkness rhythm, in the other organs and tissues located outside the above its secretion probably does not depend on the degree of illumination[33].

It remains unknown whether melatonin synthesised by extrapineal organs is the source of baseline melatonin levels in the blood[6]. Pinealectomy reduces nighttime blood levels of melatonin, but it does not affect daytime blood levels nor does it affect the presence of melatonin in the gastrointestinal tract. According to Bubenik[28] most of the daytime level of melatonin in the blood is probably derived from synthesis in the gastrointestinal tract, in which melatonin concentrations exceed blood melatonin levels by 10-100 times. However, the mechanisms of synthesis and the mode of secretion of gastrointestinal melatonin have not jet been fully elucidated. Unlike pineal melatonin production, which is regulated by photoperiodicity, circadian secretion of melatonin produced by the gastrointestinal tract appears to be regulated by food intake. The gastrointestinal tract seems to be responsible for the increase of circulating melatonin levels observed after tryptophan administration, food intake and, paradoxically, long-term food deprivation[34].

Melatonin receptors

The physiological effects of melatonin are mediated, at least in part, by the activation of specific receptors which are classified into three related, but distinct, high affinity subtypes (MT_1, MT_2, MT_3), according to their kinetic and

pharmacological properties. Two of the melatonin receptor subtypes have seven transmembrane domains and belong to the G-protein-coupled receptor superfamily (MT_1 and MT_2); while the third, which was recently affinity-purified from Syrian hamster kidney, is a protein that displays a binding profile similar to that of MT_2 receptor and belongs to quinone reductase enzyme family (MT_3)[35]. Each of the G-protein-coupled melatonin receptor subtypes can couple to multiple signal transduction cascades, whereas the signal transduction cascades mediating MT_3 responses are still unclear. The cloning of genes encoding the melatonin receptors (from the simplest organisms to humans) confirmed that this family of receptors has all the major structural characteristics of the G-protein-coupled receptors, although several highly conserved sequence motifs found in all other previously cloned G-protein receptors were not present in the melatonin receptors[36].

The discovery of selective melatonin receptor ligands and the creation of mice with targeted disruption of melatonin receptor genes have been valuable tools to investigate the localization and functional rôles of the receptors in native systems. As far as the MT_1 receptor is concerned, it has been possible to determine that it has a vast tissue distribution and it can couple to a wide variety of G-proteins, thus explaining its diversity of response within the body. As shown in numerous studies, the MT_1 receptor has been shown to produce inhibitory responses on the cAMP signal transduction cascade, resulting in decreases in protein kinase A (PKA) activity and cAMP response element binding protein (CREB) phosphorilation[37]. Besides the cAMP-dependent cascade, MT_1 receptors can couple to a stimulation of phospholipase C-dependent (PLC-dependent) signal transduction cascades, directly or indirectly via a specific G-protein subunit ($G\beta\gamma$), they can activate protein kinase C (PKC)[38], and can also modulate the formation of arachidonic acid, and stimulate a number of kinases[39]. Therefore, it has been possible to determine that, for example, the activation of the MT_1 melatonin receptor inhibits the neuronal firing rate in the SCN, prolactin secretion from the *pars tuberalis* and induces vasoconstriction. In fact, MT_1 receptors are expressed in the SCN and cardiac vessels where they are involved in modulating circadian rhythms and constricting cardiac vessels[40, 41]. In summary, as data continue to accumulate, it seems that only a few tissues are devoided of melatonin membrane receptors[17].

The rôle of MT_2 receptors in mammalian physiology, as well as their signalling properties, is now becoming clearer. To date, what is known is that MT_2 receptors, even though they are not present as functional receptors in all mammals, are involved in retinal physiology, in modulating circadian rhythms in the SCN, in dilating cardiac vessels, and in the inflammatory response at the level of microcirculation[41]. Therefore, not surprisingly, activation of the MT_2 melatonin receptor phase shifts circadian rhythms generated within the SCN, inhibits dopamine release in the retina, induces vasodilation, enhances splenocyte proliferation and inhibits leukocyte rolling in the microvasculature. In a similar way to that of MT_1, MT_2 receptors are able to determine an inhibition of cAMP formation and a stimulation of phosphoinositide hydrolysis[42]. On the other hand, unlike the MT_1 receptors, MT_2 are more restricted in their localization, which includes the SCN of the hypothalamus, the cerebellum, the retina, the kidney, the ovary, cardiac vessels and various cancerous cell lines[43].

Recently, a protein that demonstrated a binding profile similar to that of the MT_2 receptor was named MT_3. Data showed that this protein shares 95% homology to the human quinine reductase 2, which is an enzyme involved in detoxification[44]. This protein and its associated activity, as revealed through radioligand binding and enzymatic assays, shows that it is expressed in the liver, kidney, brain, heart, brown adipose tissue, skeletal musculature, lung, intestine, testis and spleen of different mammalian species[45]. Very recent data showed that the MT_3 protein may be involved in the regulation of intraocular pressure in rabbits and in inflammatory responses in the microvasculature, inducing a reduction of intraocular pressure and inhibiting leukotriene B_4-induced leukocyte adhesion[46, 47].

Melatonin may also act at intracellular sites, through binding to cytosolic calmodulin, which affects calcium signalling by interacting with target enzymes such as adenylate cyclase and phosphodiesterase, as well as with structural proteins[48]. In addition, melatonin has also been identified as a ligand for two orphan receptors (a and b) in the family of nuclear retinoid Z receptors[49]. Interestingly, due to the lipophylic nature of melatonin, other studies[50], in accordance with the results obtained in our laboratory, demonstrated the accumulation of melatonin in the nuclei of the cells of a large number of organs (Nordio, unpublished results).

Taken together, the findings that melatonin can regulate a great number of nuclear cellular factors involved in signal transduction may account, at least in part, for the complexity of melatonin's rôle in modulating an increasing variety of physiological processes.

Melatonin in non vertebrates

Melatonin synthesis is not restricted to vertebrates and to the animal kingdom. This indolamine is present in many other organisms that lack a pineal gland, including invertebrates, fungi, algae, plants and unicellular organisms[51, 52]. Recently, it was reported that in some inverte-

brates and plants melatonin levels cycle with a nocturnal peak (maximum) in some way analogous to that of the vertebrates[53, 54].

In edible plants melatonin has been identified in a variety of vegetables, fruits, seeds and medicinal herbs[55]. Particularly high levels of melatonin have been detected in many species of edible plants with melatonin concentrations being several orders of magnitude higher than those in the blood of vertebrates, especially the two medicinal herbs St. John's wort (*Hypericum perforatum*) and feverfew (*Chrysanthemum parthenium*)[56].

In mammals, dietary melatonin is readily bioavailable[6]. Foodstuffs containing melatonin seem to enhance circulating melatonin levels in the blood[56]. Furthermore, melatonin extracted from plants is capable of binding to melatonin binding sites in the brain of mammals. These findings suggest that the consumption of plant material with a high content of melatonin could alter blood melatonin levels and therefore influence physiological process in mammals, including humans[51].

Effects of light on melatonin

An interesting definition of light could be "visually and chronobiologically effective radiant energy for human beings"[57]. This because in man and in many other species, both support of vision and regulation of biological rhythms are mediated via electromagnetic radiation in the range of 10^{14}-10^{15} Hz. In fact, the presence of external light of appropriate wavelength and intensity enables vision and actions, whilst the absence of external light impedes actions and leads to physiological rest which is reinforced by internal biological clocks. Therefore, the exclusive use of the signal "light" alone (i.e. its presence or absence) which allows vision and activity, on the one hand, and disallows vision and enforces rest and sleep, on the other, constitutes a very efficient binary environmental switch[7]. Rhythmic alternation of external light and darkness (the light/dark cycle) provides information that is conveyed within the organism through the well known endogenous circadian system. It is important to underline that light exposure is not able to generate biological rhythms, but it is able to alter their timing. In this sense, the hypothalamic SCN has been identified as a circadian oscillator in mammals and is a critical component of the circadian system. The functional utility of the SCN circadian oscillator is a result of its susceptibility to entrainment by the environmental light/dark cycle, a process that does not impose rhythmicity, but provides stable and appropriate phasing of the innate oscillatory programme, with respect to local time[58]. The development and maintenance of biological rhythms should be regarded as an adaptive mechanism by which many species, including man, prepare their physiology for the challenges of an environment that changes regularly through light.

As already described, rhythmic light/dark perception at the retinal level evocates a series of alternating signals which, ultimately, control melatonin synthesis by the pineal gland, with highest plasma concentrations during nighttime. Thus, in mammals one circadian message from the clock to the body is based on the nocturnally elevated production of melatonin, the immediate release of which reflects the duration of the dark period and mirrors the seasonal changes in the length of day and night. However, light of sufficient intensity and suitable spectral quality suppresses melatonin production; the most effective wavelengths being in the range of 460-470 nm[3]. In addition, the magnitude of melatonin suppression demonstrates marked individual differences and seems to be dependent by recent photic history[11].

Physiological rôle of melatonin

In recent years, there have been numerous advances in the knowledge of the physiology and biochemistry of the pineal gland and its main hormonal product, melatonin. It is now evident that melatonin, in mammals, besides playing an important rôle in the circadian organization of biological rhythms[3], is also related to a variety of other functions including, among others: 1) the enhancement of the immune response; 2) the control of tumour promotion and growth; 3) a protective effect on the cardiovascular system; 4) marked anti-inflammatory and analgesic effects. These actions of melatonin are considered to be receptor-mediate[59-61].

Recent studies have uncovered other non-receptor-mediated actions of melatonin such as its free radical scavenging and antioxidant action. There is now clear evidence that melatonin is a highly effective free radical scavenger and general antioxidant, at both physiological and pharmacological concentrations. Melatonin directly neutralizes a number of free radicals and reactive oxygen and nitrogen species. Melatonin has also been shown to have indirect antioxidative actions such as the stimulation of enzymes involved in metabolizing reactive oxigen intermediates, thereby further increasing its capability to protect against free radicals. It is possible that melatonin's ability to augment the activities of antioxidative enzymes may be in part also receptor-mediated. The melatonin radical scavenging action is particularly important as free radical damages have been linked to a wide variety of diseases[62-64].

Currently, there is an increasing number of experimental data suggesting melatonin's pro-apoptotic and anti-

apoptotic action. Various human diseases are characterized by alteration of the apoptotic processes. Inappropriate apoptosis has been suggested to be involved in pathological neuronal death in various neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis and spinal muscolar atrophy[65]. Many reports support the idea that melatonin may act as a neuroprotective agent preventing neurons from apoptosis. On the contrary, many tumours and cancer cell lines show resistance to the triggering of apoptosis; melatonin, in this case, seems to have a rôle in increasing apoptotic cell death. The mechanism by which melatonin controls cell death is not entirely known[66]. Recently, mitochondria, which are implicated in the intrinsic pathway of apoptosis, have been identified as a target for melatonin actions[67].

Recently, it has been suggested that melatonin may also affect bone metabolism, both in a direct and a non-direct way[68]. Several experiments have documented that the pineal indole induces an increase in the proliferation of human osteoblasts, and an increase in proteins that are incorporated into the bone matrix, like procollagen type I c-peptide. On the other hand, melatonin probably impairs osteoclast activity in bone through its free radical scavenging and antioxidant properties .

The evaluation of the wide spectrum of melatonin effects upon the biological rhythms needs to consider the interaction between the pineal hormone and the thyroid gland, which has been, for a long time, a subject of intensive research. The abundant evidence to-date, which relates mostly to the inhibitory effect of melatonin on thyroid growth and function and, to a lesser extent, to the stimulatory effects of thyroid hormones on the pineal gland, seems widely accepted, even though general agreement is still lacking[69, 70]. It is known that melatonin administration may inhibit (and pinealectomy stimulates) thyroid growth, while melatonin supplementation to pinealectomized rats reverses this condition[71]. Melatonin injections lead to a reduction in blood thyroid-stimulating hormone (TSH) and thyroid hormone concentrations in experimental animals, in a dose-dependent fashion. In fact, high doses suppressed thyroid gland response to TSH, but this effect was not observed at low doses[72]. Moreover, recent data demonstrate that the TSH-inhibiting effect of melatonin is prevented by zinc, which is known to be important in normal thyroid physiology and is absorbed from the digestive system through the intervention of melatonin[69].

In addition to their direct relationship, melatonin and the thyroid have been shown to be related also at other levels. In particular, it has been possible to identify that the type 2 iodothyronine deiodinase gene, which is important for thyroid hormones synthesis, is involved in the regulation of seasonal reproduction[73]. Moreover, the hypothalamic-pituitary-thyroid axis and melatonin possibly interact in the control of body temperature in humans. In fact, core body temperature is characterized by circadian variations with lower levels during nighttime, when melatonin concentrations are the highest. On the other hand, it is known that thyroid hormones influence thermogenesis. On these bases, data obtained concerning the changes in TSH and FT4 levels indicate that the response of anterior pituitary to hypothalamic thyroid-releasing hormone (TRH) and of thyroid to hypophyseal TSH may be influenced by the pineal hormone which, therefore, may modulate the hypothalamic-pituitary-thyroid axis function and influence the circadian rhythm of body temperature[74].

Variation of melatonin secretory patterns

There is a quite extensive literature reporting modifications in melatonin levels in numerous human physiological and pathological conditions[75, 76], as well as modifications due to several other external factors (e.g. use of drugs or other substances, shift-work, environmental light at night, etc.)

In pathological states in which an impairment of melatonin levels has been detected, it is still unclear whether melatonin alterations have an aetiological rôle or are secondary to the disorders[77]. Furthermore, since melatonin production is influenced by many endogenous and exogenous factors, it could be difficult to interpret the results obtained as only dependent on the pathological state itself.

Melatonin and psychiatric and neurological disorders

Desynchronization of circadian melatonin patterns in humans has been found in many psychiatric disorders like mood disorders, anxiety disorders, psychotic disorders, eating disorders, suicidal behaviour and autism. Data about melatonin secretion in psychiatric disorders are controversial and discrepancies exist among the different studies, so that different authors have reported for the same disorder a normal, an increased or a decreased level of melatonin, and/or a normal, a phase-delay or a phase-advanced melatonin peak[77]. Several factors could explain these discrepancies: different patients' characteristics such as the duration of illness, subtypes of the disorders, being drug-free or under psychopharmaceutical treatments, and duration of drug wash-out[78]. Other methodological factors that could have influenced the results are the small number of patients in the study group,

not strictly matched controls, different age of the subjects, inclusion and exclusion criteria not thoroughly controlled, study conducted without consideration of the period of the year[79].

A decrease of plasma melatonin levels and/or an alteration in its rhythms have been found in patients affected by several neurodegenerative diseases[80, 81]. In subjects with Alzheimer's disease a decrease in blood melatonin levels has been found in cerebrospinal fluid and in pineal gland extracts[82]. Moreover, in patients with dementia of the degenerative type, the impairment of melatonin secretion seems to be related to the severity of mental impairment[83]. In recent years, numerous investigations have evidenced that oxidative stress[84], apoptotic loss of post mitotic cells and aberrant mitogenic changes[85] have important rôles in the pathogenesis of Alzheimer's disease and probably of other neurodegenerative diseases. As melatonin exerts both a protective action at the central nervous system level[86] and an anti-apoptotic action[66], a decrease in melatonin concentrations may be involved in the pathogenesis of Alzheimer's disease as well as of other neurodegenerative processes.

Some authors suggest a possible association between sleep disorders and a decrease in melatonin output, especially in aged people[87]. It has been suggested that melatonin improves sleep function and, according to some clinical studies, administration of melatonin results in an increase of sleep quality and a decrease of awakening in patients with sleep disorders[88]. Altered melatonin rhythm is also evident in other circadian phase disorders, such as the shift-work syndrome, in which a decreased level of melatonin has been observed during night work[89].

It has also been proposed that melatonin deficiency may significantly contribute to cluster headache. A number of studies have shown reduced melatonin levels during the cluster periods, suggesting that the disorder is associated with a periodic dysfunction of hypothalamic structures[90]. Some authors have demonstreted that melatonin administration could alleviate cluster attacks and significantly reduce headache frequency[91]. Besides cluster headache, reduced melatonin levels seem to be involved in the pathogenesis of other painful illnesses, such as migraine[92] and chronic neurogenic or idiopathic pain syndromes[93]. Low melatonin levels found in patients with chronic pain syndromes were related by Almay *et al*[93] to the increase in depressive symptomatology. As low concentrations of melatonin were also found by some authors in patients with depressive disorders, they suggest that the chronic pain syndrome may be a variant of depressive disease.

Controversial data exist concerning the rôle of melatonin in the pathogenesis of some types of epilepsy. Bazil *et al*[94] have found that patients with intractable epilepsy have low baseline melatonin levels, while Schapel *et al*[95] found an altered circadian pattern with an increased melatonin production in untreated patients with active epilepsy.

Melatonin and cancer

Numerous *in vivo* and *in vitro* experimental studies have documented that melatonin has oncostatic and anticarcinogenetic effects[96] and preliminary clinical studies seem to confirm its antitumoural properties in selected human malignancies[97]. The biological mechanisms by which melatonin exerts its antiproliferative and oncostatic properties on some types of neoplastic cells seem to be due to its ability: 1) to suppress cancer cell proliferation by increasing cell-to-cell interactions: in cancer cells, defective cell adhesion and/or malfunctioning gap-junction contacts are present; 2) to increase the degradation of calmodulin which plays an important rôle in the proliferation of normal and cancer cells; 3) to act as an indirect antioxidant and a free radical scavenger: tumour cells at an advanced stage of carcinogenesis are characterized by a persistent oxidative stress which is insufficient to cause cell death, because of the reduced sensitivity to oxidative stress of tumour cells; 4) to act on the immune system by activating the cytokine system which demonstrates growth-inhibitory properties over a wide range of tumour cells; 5) to suppress the uptake and metabolism of tumour fatty acids: fatty acids serve as specific tumour growth signalling molecules and their high concentrations in neoplastic cells seem to increase tumour growth[98]. Moreover, recent data suggest that melatonin may control tumour growth by inducing apoptosis[66] and, at least in part, by acting as a natural antiangiogenic molecule[99].

In addition to its potential direct antitumour activity, melatonin, given orally at night, has shown the ability to modulate the effects of cancer chemotherapy, by reducing the toxic side effects (asthenia, thrombocytopenia, lymphocytopenia, stomatitis, cardiotoxicity and neurotoxicity) and by slowing down the tumour progression[100].

Some evidence exists that the rôle of the pineal gland in malignancy may not be confined to melatonin, since potent antineoplastic fractions of yet unknown pineal gland chemicals have been detected which are capable of inhibiting *in vitro* cell lines resistant to melatonin. Moreover, some data indicate that the presence of a malignant tumour may alter the morphology of the host pineal gland[101].

Alterations in melatonin concentrations in the blood, as well as in the excretion of its main metabolite sulphatoxymelatonin, have been demonstrated in patients suffering from different types of both endocrine-dependent (mammary, endometrial, prostate cancer) and non endocrine-depen-

dent cancers (lung, gastric, colorectal cancer)[102-105]. In the majority of studies melatonin tends to be depressed or to show a desynchronization of its secretory pattern.

Bartsch and Bartsch[106], in their clinical studies, have found that a reduction of circulating melatonin is most pronounced in patients with advanced localized primary tumours, with a clear negative correlation between melatonin levels and tumour size. The phenomenon of melatonin reduction seems to be a transient finding since patients with secondary tumours, either local recurrences or distant metastases, did not show reduced circulating melatonin levels. Surgical ablation of the primary tumour does not lead however to normalization of the melatonin levels. Since the reduction of the melatonin levels is not simply affected by operation, they concluded that the changes in melatonin level are not the immediate effect of the tumour presence, but may be due to a systemic alteration in response to tumour growth.

Melatonin and cardiovascular diseases

The clinical importance of circadian biological rhythms has been strengthened by a number of studies showing a circadian distribution of cardiovascular events like myocardial infarction, stroke, complex arrhythmia, or sudden cardiac death. Their incidence shows a maximum during the early morning hours, after awakening from sleep. In addition, a number of pathophysiological mechanisms has been identified to coincide with this peak, including blood pressure and heart surges, decreased endothelial dilatory capacity of coronary arteries, enhanced sympathetic activity, decreased cardiac electrical stability, and increased platelet aggregation, therefore indicating in early morning hours a high risk window for the incidence of cardiovascular events. With this in mind, the hormone melatonin is presently under evaluation, to study its ability to maintain the synchronization of circadian rhythms with the external environment. In fact, recent data demonstrate that repeated melatonin administration to hypertensive patients results in a significant reduction of nocturnal blood pressure[107], through a direct action on the cardiovascular system[108]. In fact, hypertensive patients and patients with coronary artery disease seem to have an impaired nocturnal melatonin secretion[109, 110]. Finally, the recent identification of both types of melatonin receptors expression in human arteries and left ventricle strengthens the rôle of the pineal hormone in the management of a series of cardiovascular diseases from the circadian perspective.

Melatonin and other diseases

A sparse literature exists reporting an increase in the nocturnal melatonin secretion and/or a variation in its rhythm in patients suffering from several diseases including hypothyroidism[111], hyperparathyroidism[112], Cushing's disease[113], in female patients with polycystic ovary[114], primary[115], secondary[116] and hypothalamic amenorrhea[117], in male patients with hypogonadotropic hypogonadism[118, 119], and patients with rheumatoid arthritis[120] and nocturnal asthma[121]. A marked alteration of plasma melatonin rhythm is also found in cirrhotic patients, who present elevated melatonin levels during daytime hours, in addition to a delay in the timing of the onset of melatonin nocturnal increase[122, 123].

Conversely, low melatonin levels are reported by some authors in patients affected by chronic renal failure[124], fibromyalgia[125], duodenal ulcer[126], acute intermittent porphyria with epileptic seizures[127], sudden infant death syndrome[128] and scoliosis[129].

Controversial data exist reporting lowered or augmented melatonin levels in individuals with chronic fatigue syndrome[130, 131].

Variation of melatonin levels due to other factors

It has been shown that several groups of drugs may affect melatonin production in humans. The use of β-blockers[132], calcium antagonists[133], some benzodiazepines[134, 135] and clonidine significantly reduces or suppresses nocturnal melatonin secretion or alters its circadian phase. Also, melatonin levels are reduced in individuals using non steroidal anti-inflammatory drugs[136]. Other substances that seem to have a depressant effect on melatonin production are caffeine[137] and alcohol[138].

Conversely, melatonin secretion is increased by the administration of a certain number of antidepressants[139, 140], psoralens[141], opiates[142] and probably interferon[143].

Besides pathological states and the use of chemicals, other factors seem to influence the endogenous production of melatonin. Age has been considered as a factor that can influence melatonin production[13, 144], but a general agreement is still lacking. Although numerous reports have shown that circulating melatonin[20] declines with age, some recent studies by Fourtillan *et al*[145] and Zeiter *et al*[146] do not support this hypothesis. Zeiter *et al* explain the difference between their results and those of other investigators with the use of very strict selection in the study subjects, that were healthy and drug-free. Kennaway *et al*[147] have found, as other investigators, a reduction of melatonin production in elderly people, but the change occurs very

early in life, around 20-30 years of age.

An inverse relationship between body weight and melatonin was observed by Davis *et al*[148]. Also, a reduction of melatonin levels has been seen in subjects with a low intake of tryptophan with the diet[149]. Furthermore, intense physical training seems to reduce melatonin levels[150].

As seen above, light of sufficient intensity, duration and appropriate wavelength reduces or suppresses melatonin production. The profile of melatonin secretion is also influenced by changing daylength, so that seasonal changes in night length (scotoperiod) induce parallel changes in the duration of melatonin secretion, which is longer in winter and shorter in summer[151].

Posture changing, from a lying to a standing position, results in an increase in plasma melatonin concentrations during the rising phase of melatonin. These changes seem to be explained through the differences in haemoconcentration and haemodilution associated with posture changes[152].

Karkela *et al*[153] have found that both spinal and general anaesthesia in conjunction with surgery may alter the melatonin circadian rhythm by delaying the onset of nocturnal melatonin secretion.

Melatonin and electromagnetic fields

Electromagnetic fields: definition and ascertained health effects

A detailed presentation on electric, magnetic and electromagnetic fields can be found in the report of the International Commission on Non-Ionizing Radiation Protection (ICNIRP)[154]. In brief, 50 or 60 Hz electric or magnetic fields generated by electrical power lines above a certain level of intensity have the potential for inducing electrical current in the body, thus stimulating cell activity. Radiofrequency electromagnetic fields generated by TV and radio transmitters above a certain intensity of field may cause an increase in the temperature of the tissues of exposed subjects. In both cases (ELF and radiofrequencies) there is a great interest in the study of possible long-term health effects occurring at lower exposure levels than those required for the above-mentioned, ascertained, direct health effects. Even if no firm conclusion has so far been reached for long-term health effects, the available data on ELF magnetic fields have been evaluated by the International Agency for Research on Cancer (IARC) as indicative of a possible carcinogenic risk[155]. No such evaluation by IARC is so far available for radiofrequencies. Recent reviews by the British National Radiological Protection Board (NRPB)[156], the Canadian Government[157] and the ICNIRP Standing Committee on Epidemiology have consistently stated that the available evidence is inadequate to confirm or refute the existence of long-term health effects. For the above-mentioned reasons, the following sections of this paper will concentrate on ELF magnetic fields.

The "melatonin hypothesis"

One of the most salient features of the change in human habits during the past 100 years has been the increased generation and use of electric power. Modern technologies using electric power are clearly of significant benefit to society, but it is essential to examine whether these changes in the electromagnetic environment can affect human health. In recent decades interest has increased in the possibility that exposure to ELF electromagnetic fields (10-100 Hz), including the 50-60 Hz fields generated by the power lines, may cause biological effects in the human population, increasing the risk of numerous health problems such as cancer (particularly childhood leukaemia)[158], reproductive problems[159], heart diseases[160], neuropsychological and psychiatric disorders[161], and neurodegenerative diseases[162]. Moreover, some investigators have proposed that ELF fields may affect haematologic and immunologic parameters[161, 163].

On the basis of experimental studies, that showed reductions in melatonin concentrations in animals exposed to ELF fields, and of epidemiological studies in humans, that suggested an association between certain cancers and ELF fields exposure, Stevens[164] proposed what has been described as the "melatonin hypothesis". According to this hypothesis, the exposure to ELF fields may alter the normal function of the pineal gland, suppressing or reducing the nocturnal increase in melatonin synthesis and release[165]. The "melatonin hypothesis", proposed firstly by Stevens to interpret the increased risk of breast cancer associated with the exposure to ELF fields, was then extended to other health outcomes[166]. Currently, the "melatonin hypothesis" represents one of the most studied and plausible mechanisms by which electromagnetic fields could lead to several outcomes.

Epidemiological studies on melatonin levels and exposure to electromagnetic fields

Occupational exposure

Pfluger and Minder[167] compared a group of 66 Swiss railway engineers mainly exposed to 16.7 Hz magnetic fields (average exposure level close to 20 μT) with a

group of 42 train attendants and station managers (average exposure close to 1 μT). Urinary concentration of aMT6-s was measured in the morning and evening during leisure time, after one day and after one week of work, and during the last day following the leisure period. During working days, evening aMT6-s was lowered by a factor of 0.81 compared to leisure days among engine drivers but not among controls, while the evening concentrations recovered during leisure time.

Burch *et al* [168, 169] studied the effects of 60 Hz magnetic field and light on aMT6-s levels in a group of 142 electric utility workers (85 exposed electricians, operators and linemen and 57 non-exposed maintenance and administration workers). Urinary concentration of aMT6-s was measured at the beginning of the week (base line) and after three consecutive workdays. Both field intensity and temporal stability of the field were measured. Men in the highest quartile of the temporal stability parameter had lower aMT6-s concentrations on the 2nd and 3rd day, compared to the men in the lowest quartile. The subjects who were in the highest quartile of temporal stability both at home and at work had mean aMT6-s/creatinine concentrations 39% lower than those in the lowest quartile. Light exposure modified the magnetic field effect: in subjects with workplace light exposure below the median, temporally stable magnetic field exposures were associated with decreased aMT6-s/creatinine excretion, but no association was observed in workers with light exposure above the median. The authors hypothesize that elevated light exposure may suppress post work aMT6-s/creatinine levels so that further decreases associated with magnetic field exposure would not be detectable.

Burch *et al* [170] further investigated the issue contrasting electric utility workers working 2 hours or more per day in substations with 3-phase environments, with those working less than 2 hours or working only in 1-phase environments. In workers with more than 2 hours of work in 3-phase environments, a trend of decreasing nocturnal aMT6-s/creatinine excretion with increasing magnetic field exposure was observed.

Juutilainen *et al* [171] studied urinary aMT6-s excretion in female workers exposed to magnetic fields in the garment industry (22 subjects exposed to more than 1 μT, 10 subjects exposed to less than 1 μT, 8 subjects possibly exposed and 20 non exposed controls).

The average aMT6-s excretion on Friday was lower than on Monday among garment workers but not among controls; no dose-response relation was detected.

Residential exposures

Davis *et al* [148] measured urinary aMT6-s levels in the bedrooms of 203 women who had been selected as controls in a case-control study on breast cancer in two different seasons of the year. Higher mean night time bedroom magnetic field levels were associated with lower aMT6-s levels in night urine, especially in seasons with longest day light, and in women of older age, higher body mass index, current alcohol consumption, and consumption of β-blockers, calcium channel blockers and psychotropic drugs.

Levallois *et al* [172] studied urinary excretion of aMT6-s in a sample of 221 women living near a 735 kV line and in 195 women living away from power lines. Geometric means of magnetic field exposure at night were 0.29 μT and 0.08 μT respectively. The decrease in aMT6-s concentration with age and body mass index was significantly more pronounced for those living close to the lines than for the controls.

Assumption of β-blockers, calcium channel blockers, anti-anxiety and nonsteroidal anti-inflammatory drugs resulted in an aMT6-s decrease.

Youngstedt *et al* [173] studied aMT6-s urinary excretion in a group of 242 older adults including 194 participants in the Women's Health Initiative study, some of whom had been treated for breast cancer and 49 subjects with problems of insomnia or depression. No significant association between nocturnal magnetic field and aMT6-s excretion was detected (all subjects except five had exposure levels lower than 0.5 μT).

Evidence from experimental studies in humans

In the light of the complex patterns underlying melatonin levels in humans that are reflected in the abovementioned epidemiologic studies, it is not surprising that laboratory studies on volunteers, with their unavoidable oversimplification of real life, have so far generated conflicting results.

Wilson *et al* [165] measured overnight excretion of aMT6-s in 42 volunteers who used for 8 weeks normal electric blankets or continuous polymer wire (CPW) modified blankets, whose magnetic fields were about 50% stronger than those of normal blankets.

A higher proportion of CPW blanket users showed a decrease in aMT6-s excretion during the study period.

Karasek *et al* [174] showed a significant reduction of nocturnal melatonin rise among 12 patients with low-back pain treated for three weeks with daily exposure to 40 Hz magnetic fields of 2.9 μT.

Åkerstedt *et al* [175] exposed 18 subjects to 1 μT for one night and compared sleep quality and melatonin level with those of a night without exposure. Total sleep time and efficacy of sleep were reduced by exposure, while

circulating melatonin levels were not affected.

Graham *et al*[176] exposed 30 subjects for four nights to magnetic fields of 28.3 μT. At the end of the exposure cycle, average night aMT6-s excretion was not affected, but the stability of individual measurements was reduced.

Graham *et al*[177] exposed 37 women to 60 Hz magnetic fields of 28.3 μT for one night and observed no reduction of blood levels of melatonin; exposure to light, on the contrary, significantly affected night melatonin production.

Crasson *et al*[178] exposed 21 subjects to 50 Hz magnetic fields of 100 μT for three sessions of half an hour each. There was no significant reduction of aMT6-s night excretion after exposure, but a tendency for a smaller increase of night aMT6-s excretion was observed among men with low excretion patterns.

Hong *et al*[179] exposed 9 subjects for 11 weeks to 50 Hz magnetic fields generated by electric blankets (median field: 9 μT at waist). Urinary excretion rate of melatonin did not significantly differ between exposure and non exposure periods.

Evaluation of epidemiologic studies

According to IARC, a small reduction of melatonin concentration has been observed both in occupational and residential settings, but it is difficult to distinguish between the effects of magnetic fields and those of other environmental factors. Subsequent to this evaluation, one study corroborated the hypothesis of an aetiologic rôle of magnetic fields in the complex determination of melatonin levels, while another did not detract from the credibility of the hypothesis, since its non-positive results could not be adequately evaluated given the heterogeneous composition of the study subjects.

The lack of a robust demonstration of a causal rôle of magnetic fields in the reduction of melatonin levels may reflect inappropriate study designs, and point to the need that future studies take into account more direct markers of circadian clock status.

Recommendations for future studies

In the light of the aforementioned discussion, it seems reasonable to provide some indications for future studies.

A multidisciplinary Italian group studying the neurobehavioural effects of electromagnetic fields has recently published a report with guidelines for assessing the health status of subjects resident close to sources of ELF magnetic fields[180].

Measurement of aMT6-s in day and night urine samples is part of this protocol, together with the assessment of immune function and of heart rate variability, together with a health check with special reference to neurological and psychiatric aspects, through the submission of an *ad hoc* questionnaire.

With reference to the aMT6-s determinations, some restrictions to the inclusion of subjects have to be recommended. Subjects affected by some defined conditions, or subjects who are assuming particular drugs should not be included in the study protocol. The exclusion criteria are the following: 1) subjects affected by malignant neoplasias, kidney failure and diabetes; 2) alcohol abusers; 3) subjects assuming β-blockers, calcium antagonists, benzodiazepines and antidepressants. Once these requirements are fulfilled, subjects may be included in the study, and their melatonin levels can be examined and evaluated, while taking into account the outcomes of the other clinical and laboratory examinations.

The aforementioned approach provides the rationale for an ongoing cross-sectional investigation into the health status of subjects living in the neighbourhood of power lines; the information on melatonin can thus be compared between groups characterized by different current and past exposure levels.

The suggested outcome is the ratio night *versus* day urinary levels of aMT6-s. Given that multiple urinary determinations of melatonin metabolite concentrations parallel melatonin immission into the blood circulation, it is important to underline that our approach does not intend to obtain a circadian rhythm of the melatonin metabolite in urines. However, in the attempt to build up a versatile, simple and "easy-to-use" tool for future studies on the biological effects of ELF fields, we decided to limit aMT6-s determinations to day and night samples. These measurements will give information about the melatonin rhythm whose presence (or absence) is so important for the maintenance of a stable circadian rhythmicity. In addition, results may be expressed as difference between night and day values, percentage, or night/day ratio. This last approach seems to give better results since the higher the ratio, the higher the difference between night and day melatonin content is seen to be. In other words, a ratio below the number of 3, which has been arbitrarily taken as cut-off point, indicates that the melatonin rhythm is almost absent. On the other hand, a ratio higher than 3 suggests that the melatonin rhythm is conserved, therefore being somehow insensitive to ELF.

Concluding remarks

Subsequently to the allocation of ELF magnetic fields to the category of "possibile carcinogens" by IARC, sev-

eral independent research lines have shown increased risks of neurodegenerative diseases and adverse reproductive outcomes in populations exposed to relatively elevated field levels.

A moderate decrease of nocturnal blood melatonin levels has been reported in a number of individuals with fairly high occupational or environmental ELF field exposures.

Detailed protocols for monitoring urinary excretion of aMT6-s in individuals and populations of interest are now available, and this issue should be adequately addressed in all newly designed epidemiological studies.

Biologically-based epidemiological studies may thus contribute to a proper evaluation of the plausibility of the association between ELF field exposure and various health outcomes, enabling, among others, the validation of man *versus* animal models.

References

1. Lerner AB, Case JD, Takahashi Y, *et al*. Isolation of melatonin, the pineal factor that lightens melanocytes. J Am Chem Soc 1958; 80: 2587.
2. Reiter RJ. Melatonin: its sources, its message, and the interpretation of the message. In Reiter RJ, Pang SF. Advances in pineal research. London: Libbey, 1989, 165-73.
3. Arendt J. Importance and relevance of melatonin to human biological rhythms. J Neuroendocrinol 2003; 15: 427-31.
4. Reiter RJ, Tan DX, Gitto E, *et al*. Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage. Pol J Pharmacol 2004; 56: 159-70.
5. Poeggeler B, Harderland R. Detection and quantification of melatonin in a dinoflagellate, *Gnyaulax polyedra*. Solutions to the problem of methoxyindole destruction in non-vertebrate material. J Pineal Res 1994; 17: 1-10.
6. Tan DX, Manchester LC, Hardeland R, *et al*. Melatonin: a hormone, a tissue factor, an autocoid, a paracoid, and an antioxidant vitamin. J Pineal Res 2003; 34 (1): 75-8.
7. Erren TC, Reiter RJ, Piekarski C. Light, timing of biological rhythms, and chronodisruption in man. Naturwissenschaften 2003; 90: 485-94.
8. Charman WN. Age, lens transmittance, and the possible effects of light on melatonin suppression. Ophtal Physiol 2003; 23: 181-7.
9. Gillette MU, Tischkau SA. Suprachiasmatic nucleus: the brain's circadian clock. Rec Progr Harm Res 1999; 54: 33-58.
10. Karolczak M, Burbach GJ, Sties G, *et al*. Clock gene mRNA and protein rhythms in the pineal gland of mice. Eur J Neurosci 2004; 19: 3382-8.
11. Smith KA, Schoen MW, Czeisler CA. Adaptation of human pineal melatonin suppression by recent photic history. J Clin Endocrinol Metab 2004; 89: 3610-4.
12. Klein DC. Circadian rhythms in the pineal gland. In Krieger DT (ed.). Endocrine rhythms New York: Raven Press, 1979, 203-23.
13. Touitou Y. Human aging and melatonin. Clinical relevance. Exp Gerontol 2001; 36: 1083-100.
14. Stehle JH, von Gall C, Korf HW. Melatonin: a clock-output, a clock-input. J Neuroendocrinol 2003; 15: 383-9.
15. Tricoire H, Locatelli A, Chemineau P, *et al*. Melatonin enters the cerebrospinal fluid through the pineal recess. Endocrinology 2002; 143 (1): 84-90.
16. Bergiannaki JD, Soldatos CR, Paparrigopoulos TJ, *et al*. Low and high melatonin excretors among healthy individuals. J Pineal Res 1995; 18 (3): 159-64.
17. Reiter RJ. Melatonin: clinical relevance. Best Pract Res Clin Endocrinol Metab 2003; 17: 273-85.
18. Brzezinski A. Mechanisms of disease: melatonin in humans. N Engl J Med 1997; 336 (3): 186-95.
19. Touitou Y, Fevre M, Lagoguey M. Age and mental health related circadian rhythm of plasma levels of melatonin, prolactin, luteinizing hormone, and follicle stimulating hormone in man. J Endocrinol 1981; 91: 467-75.
20. Zhou J, Liu R, van Heerikhuize J, *et al*. Alterations in the circadian rhythm of salivary melatonin begin during middle-age. J Pineal Res 2003; 34: 11-6.
21. Tapp E, Huxley M. The histological appearance of the human pineal gland from puberty to old age. J Pathol 1971; 108: 137-44.
22. Kunz D, Schmitz S, Mahlberg R, *et al*. A new concept for melatonin deficit: on pineal calcification and melatonin excretion. Neuropsychopharmacology 1999; 21: 765-72.
23. Reinberg AE, Touitou Y. Synchronisaton et dyschronisme des rythmes circadiens humains. Pathol Biol 1996; 44: 487-95.
24. Skene DJ, Lockley SW, Arendt J. Use of melatonin in the treatment of phase shift and sleep disorders. Adv Exp Med Biol 1999; 467: 79-84.
25. Lynch HJ, Wurtman RJ, Moskowitz MA, *et al*. Daily rhythm in human urinary melatonin. Science 1975; 187: 169-71.
26. Graham C, Cook MR, Kavet R, *et al*. Prediction of nocturnal plasma melatonin from morning urinary measures. J Pineal Res 1998; 24 (4): 230-8.
27. Carrillo-Vico A, Calvo JR, Abreu P, *et al*. Evidence of melatonin synthesis by human lymphocytes and its physiological significance: possible role as intracrine, autocrine, and/or paracrine substance. FASEB J 2004; 18 (3): 537-9.
28. Bubenik GA. Gastrointestinal melatonin: localization, function, and clinical relevance. Dig Dis Sci 2002; 47: 2336-48.
29. Stefulj J, Hortner M, Hosh M, *et al*. Gene expression of the key enzymes of melatonin synthesis in extrapineal tissues of the rat. J Pineal Res 2001; 30 (4): 243-7.
30. Kvetnoy IM. Extrapineal melatonin: location and role within diffuse neuroendocrine system. Histochem J 1999; 31 (1): 1-12.
31. Messner M, Huether G, Lorf T, *et al*. Presence of melatonin in the human hepatobiliary-gastrointestinal tract. Life Sci 2001; 69 (5): 543-51.
32. Tan DX, Manchester LC, Reiter RJ, *et al*. Identification of highly elevated levels of melatonin in bone marrow: its origin and significance. Biochim Biophys Acta 1999; 1472 (1-2): 206-14.
33. Kvetnoy IM. Extrapineal melatonin in pathology: new perspectives for diagnosis, prognosis and treatment of illness. Neuroendocrinol Lett 2002; 23 suppl 1: 92-6.
34. Bubenik GA, Ball RO, Pang SF. The effect of food deprivation on brain and gastrointestinal tissue levels of tryptophan, serotonin, 5-hydroxyindoleacetic acid, and melatonin. J Pineal Res 1992; 12 (1): 7-16.
35. Witt-Enderby PA, Bennett J, Jarzynka MJ, *et al*. Melatonin receptors and their regulation: biochemical and structural mechanisms. Life Sci 2003; 72 (20): 2183-98.

36. Barrett P, Conway S, Morgan PJ. Digging deep-structure-function relationships in the melatonin receptor family. J Pineal Res 2003; 35: 221-30.
37. Witt-Enderby PA, Masana MI, Dubocovich ML. Physiological exposure to melatonin supersensitizes the cyclic adenosine 3',5'-monophosphate-dependent signal transduction cascade in Chinese hamster ovary cells expressing the human mt1 melatonin receptor. Endocrinology 1998; 139 (7): 3064-71.
38. Witt-Enderby PA, Jarzynka MJ, Melan MA. Microtubules modulate melatonin receptor function. Neurosci Abstracts 2001; 27: 142.
39. Godson C, Reppert SM. The Mel 1a melatonin receptor is coupled to parallel signal transduction pathways. Endocrinology 1997; 138: 397-404.
40. Doolen S, Krause DN, Dubocovich ML, *et al*. Melatonin mediates two distinct responses in vascular smooth muscle. Eur J Pharmacol 1998; 345 (1): 67-9.
41. Dubocovich ML, Yun K, Al-Ghoul WM, *et al*. Selective M2 receptor antagonist block melatonin-mediated phase advances of circadian rhythms. FASEB J 1998; 12: 1211-20.
42. MacKenzie RS, Melan MA, Passey DK, *et al*. Dual coupling of MT(1) and MT(2) melatonin receptors to cyclic AMP and phosphoinositide signal transduction cascades and their regulation following melatonin exposure. Biochem Pharmacol 2002; 63: 587-95.
43. Von Gall C, Stehle JH, Weaver DR. Mammalian melatonin receptors: molecular biology and signal transduction. Cell Tissue Res 2002; 309: 151-62.
44. Nosjean O, Ferro M, Coge F, *et al*. Identification of the melatonin-binding site MT3 as the quinine reductase 2. J Biol Chem 2000; 275: 311-7.
45. Nosjean O, Nicolas JP, Klupsch F, *et al*. Comparative pharmacological studies of melatonin receptors: MT1, MT2 and MT3/QR2. Tissue distribution of MT3/QR2. Biochem Pharmacol 2001; 61: 1369-79.
46. Pintor J, Martin L, Pelaez T, *et al*. Involvement of melatonin MT(3) receptors in the regulation of intraocular pressure in rabbits. Eur J Pharmacol 2001; 416: 251-4.
47. Lotufo CM, Lopes C, Dubocovich M, *et al*. Melatonin and N-acetylserotonin inhibit leukocyte rolling and adhesion to rat microcirculation. Eur J Pharmacol 2001; 430: 351-7.
48. Benitez-King G, Anton-Tav F. Calmodulin mediates melatonin cytoskeletal effects. Experientia 1993; 49: 635-41.
49. Becker-Andre M, Wiesenberg I, Schacren-Wiemers N. Pineal gland hormone melatonin binds and activates an orphan of the nuclear receptor superfamily. J Biol Chem 1994; 269: 28531-4.
50. Menendez-Pelaez A, Poeggeler B, Reiter RJ, *et al*. Nuclear localization of melatonin in different mammalian tissues: immunocytochemical and radioimmunoassay evidence. J Cell Biochem 1993; 53 (4): 373-82.
51. Reiter RJ, Tan DX, Burkhardt S, *et al*. Melatonin in plants. Nutrition Rev 2001; 59 (9): 286-90.
52. Vivien-Roels B, Pevet P. Melatonin: presence and formation in invertebrates. Experientia 1993; 49(8): 642-7.
53. Hardeland R, Poeggeler B. Non-vertebrate melatonin. J Pineal Res 2003; 34 (4): 233-41.
54. Van Tassel DL, O'Neill SD. Putative regulatory molecules in plants: evaluating melatonin. J Pineal Res 2001; 31 (1): 1-7.
55. Chen G, Huo Y, Tan DX, *et al*. Melatonin in Chinese medicinal herbs. Life Sci 2003; 73 (1): 19-26.
56. Hattori A, Migitaka H, Iigo M, *et al*. Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. Biochem Mol Biol Int 1995; 35 (3): 627-34.
57. Stevens RG, Rea MS. Light in the built environment: potential role of circadian disruption in endocrine disruption and breast cancer. Cancer Causes Control 2001; 12 (3): 279-87.
58. Pickard GE, Rea MA. Serotonergic innervation of the hypothalamic suprachiasmatic nucleus and photic regulation of circadian rhythms. Biol Cell 1997; 89 (8): 513-23.
59. Macchi MM, Bruce JN. Human pineal physiology and functional significance of melatonin. Front Neuroendocrinol 2004; 25 (3-4): 177-95.
60. Cuzzocrea S, Reiter RJ. Pharmacological actions of melatonin in acute and chronic inflammation. Curr Topics Med Chem 2002; 2 (2): 153-65.
61. Barrett T, Kent S, Voudouris N. Does melatonin modulate beta-endorphin, corticosterone, and pain threshold? Life Sci 2000; 66 (6): 467-76.
62. Reiter RJ, Tan DX, Qi W, *et al*. Pharmacology and physiology of melatonin in the reduction of oxidative stress *in vivo*. Biol Signals Recept 2000; 9 (3-4): 160-71.
63. Reiter RJ, Tan DX. Melatonin: reducing intracellular hostilities. Endocrinologist 2004; 14 (4): 222-8.
64. Rodriguez C, Mayo JC, Sainz RM, *et al*. Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res 2004; 36 (1): 1-9.
65. Deigner H-P, Haberkorn U, Kinscherf R. Apoptosis modulators in the therapy of neurodegenerative diseases. Expert Opinion Invest Drugs 2000; 9 (4): 747-64.
66. Sainz RM, Mayo JC, Rodriguez C, *et al*. Melatonin and cell death: differential actions on apoptosis in normal and cancer cells. Cell Mol Life Sci 2003; 60 (7): 1407-26.
67. Leon J, Acun~a-Castroviejo D, Escames G, *et al*. Melatonin mitigates mitochondrial malfunction. J Pineal Res 2005; 38 (1): 1-9.
68. Cardinali DP, Ladizesky MG, Boggio V, *et al*. Melatonin effects on bone: experimental facts and clinical perspectives. J Pineal Res 2003; 34 (2): 81-7.
69. Baltaci AK, Mogulkoc R, Kul A, *et al*. Opposite effects of zinc and melatonin on thyroid hormones in rats. Toxicology 2004; 195: 69-75.
70. Lewinski A. The problem of goitre with particular consideration of goitre resulting from iodine deficiency. II. Management of non-toxic nodular goitre and of thyroid nodules. Neuroendocrinol Lett 2002; 23: 356-64.
71. Wajs E, Lewinski A. Inhibitory influence of late afternoon melatonin injections and the counter-inhibitory action of melatonin containing pellets on thyroid growth process in male Wistar rats: comparison with effects of other indole substances. J Pineal Res 1992; 13: 158-66.
72. Wright ML, Pikula A, Babski AM, *et al*. Effect of melatonin on the response of the thyroid to thyrotropin stimulation *in vitro*. Gen Comp Endocrinol 1997; 108: 298-305.
73. Watanabe M, Yasuo S, Watanabe T, *et al*. Photoperiodic regulation of type 2 deiodinase gene in Djungarian hamster: possible homologies between avian and mammalian photoperiodic regulation of reproduction. Endocrinology 2004; 145 (4): 1546-9.
74. Mazzoccoli G, Giuliani A, Carughi S, *et al*. The hypothalamic-pituitary-thyroid axis and melatonin in humans: possible inter-

actions in the control of body temperature. Neuroendocrinol Lett 2004; 25: 368-72.
75. Claustrat B, Brun J, Chazot G. The basic physiology and pathophysiology of melatonin. Sleep Med Rev 2005; 9 (1): 11-24.
76. Reiter RJ, Tan DX. What constitutes a physiological concentration of melatonin? J Pineal Res 2003; 34 (1): 79-80.
77. Pacchierotti C, Iapichino S, Bossini L, *et al*. Melatonin in psychiatric disorders: a review on the melatonin involvement in psychiatry. Front Neuroendocrinol 2001; 22 (1): 18-32.
78. Miller HL, Ekstrom RD, Mason GA, *et al*. Noradrenergic function and clinical outcome in antidepressant pharmacotherapy. Neuropsychopharmacology 2001; 24 (6): 617-23.
79. Szymanska A, Rabe-Jablonska J, Karasek M. Diurnal profile of melatonin concentrations in patients with major depression: relationship to the clinical manifestation and antidepressant treatment. Neuroendocrinol Lett 2001; 22 (3): 192-8.
80. Ohashi Y, Okamoto N, Uchida K, *et al*. Daily rhythm of serum melatonin levels and effect of light exposure in patients with dementia of the Alzheimer's type. Biol Psych 1999; 45 (12): 1646-52.
81. Sandyk R. Pineal melatonin and sensory symptoms in Parkinson disease. Ital J Neurol Sci 1989; 10 (4): 399-403.
82. Maurizi CP. Loss of intraventricular fluid melatonin can explain the neuropathology of Alzheimer's disease. Med Hypoth 1997; 49 (2): 153-8.
83. Skene DJ, Swaab DF. Melatonin rhythmicity: effect of age and Alzheimer's disease. Exp Gerontology 2003; 38 (1-2): 199-206.
84. Zhu X, Raina AK, Lee HG, *et al*. Oxidative stress signalling in Alzheimer's disease. Brain Res 2004; 1000 (1-2): 32-9.
85. Zhu X, Raina AK, Perry G, *et al*. Alzheimer's disease: the two-hit hypothesis. Lancet Neurol 2004; 3 (4): 219-26.
86. Reiter RJ, Acun~a-Castroviejo D, Tan DX, *et al*. Free radical-mediated molecular damage: mechanisms for the protective actions of melatonin in the central nervous system. Ann NY Acad Sci 2001; 939: 200-15.
87. Leger D, Laudon M, Zisapel N. Nocturnal 6-sulfatoxymelatonin excretion in insomnia and its relation to the response to melatonin replacement therapy. Am J Med 2004; 116 (2): 91-5.
88. Brusco LI, Fainstein I, Marquez M, *et al*. Effect of melatonin in selected populations of sleep-disturbed patients. Biol Signals Rec 1999; 8 (1-2): 126-31.
89. Yamauchi H, Iwamoto M, Harada N. Effects of night nork on urinary excretion rates of 6-sulfatoxymelatonin and norepinephrine in hospital nurses. Int Med J 2003; 10 (3): 185-90.
90. Leone M, Lucini V, D'Amico D, *et al*. Abnormal 24-hour urinary excretory pattern of 6-sulphatoxymelatonin in both phases of cluster headache. Cephalalgia 1998; 18 (10): 664-7.
91. Peres MFP, Rozen TD. Melatonin in the preventive treatment of chronic cluster headache. Cephalalgia 2001; 21 (10): 993-5.
92. Toglia JU. Melatonin: a significant contributor to the pathogenesis of migraine. Med Hypoth 2001; 57 (4): 432-4.
93. Almay BG, von Knorring L, Wetterberg L. Melatonin in serum and urine in patients with idiopathic pain syndromes. Psych Res 1987; 22 (3): 179-91.
94. Bazil CW, Short D, Crispin D, *et al*. Patients with intractable epilepsy have low melatonin, which increases following seizures. Neurology 2000; 55 (11): 1746-8.
95. Schapel GJ, Beran RG, Kennaway DL, *et al*. Melatonin response in active epilepsy. Epilepsia 1995; 36 (1): 75-8.
96. Pawlikowski M, Winczyk K, Karasek M. Oncostatic action of melatonin: facts and question marks. Neuroendocrinol Lett 2002; 23 suppl 1: 24-9.
97. Bartsch C, Bartsch H, Karasek M. Melatonin in clinical oncology. Neuroendocrinol Lett 2002; 23 suppl 1: 30-8.
98. Blask DE, Sauer LA, Dauchy RT. Melatonin as a chronobiotic/anticancer agent: cellular, biochemical, and molecular mechanisms of action and their implications for circadian-based cancer therapy. Curr Topics Med Chem 2002; 2 (2): 113-32.
99. Lissoni P, Rovelli F, Malugani F, *et al*. Anti-angiogenic activity of melatonin in advanced cancer patients. Neuroendocrinol Lett 2001; 22 (1): 45-7.
100. Lissoni P, Chilelli M, Villa S, *et al*. Five years survival in metastatic non-small cell lung cancer patients treated with chemotherapy alone or chemotherapy and melatonin: a randomized trial. J Pineal Res 2003; 35 (1): 12-5.
101. Karasek M, Pawlikowski M. Pineal gland, melatonin and cancer. Neuroendocrinol Lett 1999; 20 (3-4): 139-44.
102. Grin W, Grunberger W. A significant correlation between melatonin deficiency and endometrial cancer. Gynecol Obstetr Invest 1998; 45 (1): 62-5.
103. Bartsch C, Bartsch H, Karenovics A, *et al*. Nocturnal urinary 6-sulphatoxymelatonin excretion is decreased in primary breast cancer patients compared to age-matched controls and shows negative correlation with tumor-size. J Pineal Res 1997; 23 (2): 53-8.
104. Karasek M, Dec W, Kowalski AJ, *et al*. Serum melatonin circadian profile in women with adenocarcinoma of uterine corpus. Int J Thymol 1996; 4 suppl 1: 80-3.
105. Mazzoccoli G, Carughi S, De Cata A, *et al*. Neuroendocrine alterations in lung cancer patients. Neuroendocrinol Lett 2003; 24 (1-2): 77-82.
106. Bartsch C, Bartsch H. Melatonin in cancer patients and in tumor-bearing animals. Adv Exp Med Biol 1999; 467: 247-64.
107. Scheer FA, Van Montfrans GA, van Someren EJ, *et al*. Daily nighttime melatonin reduces blood pressure in male patients with essential hypertension. Hypertension 2004; 43 (2): 192-7.
108. Sletten T, Burgess H, Savic N, *et al*. The effects of bright light and nighttime melatonin administration on cardiac activity. J Hum Ergol (Tokyo) 2001; 30 (1-2): 273-8.
109. Jonas M, Garfinkel D, Zisapel N, *et al*. Impaired nocturnal melatonin secretion in non-dipper hypertensive patients. Blood Press 2003; 12 (1): 19-24.
110. Yaprak M, Altun A, Vardar A, *et al*. Decreased nocturnal synthesis of melatonin in patients with coronary artery disease. Int J Cardiol 2003; 89 (1): 103-7.
111. Rojdmark S, Berg A, Rossner S, *et al*. Nocturnal melatonin secretion in thyroid disease and in obesity. Clin Endocrinol 1991; 35 (1): 61-5.
112. Wikner J, Wetterberg L, Rojdmark S. Does hypercalcaemia or calcium antagonism affect human melatonin secretion or renal excretion? Eur J Clin Invest 1997; 27 (5): 374-9.
113. Terzolo M, Piovesan A, Ali A, *et al*. Circadian profile of serum melatonin in patients with Cushing's syndrome or acromegaly. J Endocrinol Invest 1995; 18 (1): 17-24.
114. Luboshitzky R, Qupti G, Ishay A, *et al*. Increased urinary 6-

sulfatoxymelatonin excretion in women with non-classical steroid 21-hydroxylase deficiency. Neuroendocrinol Lett 2001; 22 (5): 332-6.
115. Walker AB, English J, Arendt J, *et al*. Hypogonadotrophic hypogonadism and primary amenorrhoea associated with increased melatonin secretion from a cystic pineal lesion. Clin Endocrinol 1996; 45 (3): 353-6.
116. Gruber DM, Schneeberger C, Laml T, *et al*. 6-sulfatoxymelatonin in women with secondary amenorrhea. Wiener Klinische Wochenschrift 1997; 109 (18): 750-2.
117. Kadva A, Djahanbakhch O, Monson J, *et al*. Elevated nocturnal melatonin is a consequence of gonadotropin-releasing hormone deficiency in women with hypothalamic amenorrhea. J Clin Endocrinol Metab 1998; 83 (10): 3653-62.
118. Kumanov P, Tomova A, Nordio M. Altered melatonin secretion in hypogonadisms: clinical evidence. Int J Androl 2005, in press.
119. Luboshitzky R, Wagner O, Lavi S, *et al*. Abnormal melatonin secretion in hypogonadal men: the effect of testosterone treatment. Clin Endocrinol 1997; 47 (4): 463-9.
120. Maestroni GJ, Cardinali DP, Esquifino AI, *et al*. Does melatonin play a disease-promoting role in rheumatoid arthritis? J Neuroimmunol 2005; 158 (1-2): 106-11.
121. Sutherland ER, Ellison MC, Kraft M, *et al*. Elevated serum melatonin is associated with the nocturnal worsening of asthma. J Allergy Clin Immunol 2003; 112 (3): 513-7.
122. Ardizzi A, Grugni G, Saglietti G, *et al*. Circadian rhythm of melatonin (aMT) in liver cirrhosis. Minerva Med 1998; 89 (1-2): 1-4.
123. Steindl DJ, Ferenci P, Marktl W. Impaired hepatic catabolism of melatonin in cirrhosis. Ann Int Med 1997; 127 (4): 494.
124. Karasek M, Szuflet A, Chrzanowski W, *et al*. Circadian serum melatonin profiles in patients suffering from chronic renal failure. Neuroendocrinol Lett 2002; 23 suppl 1: 97-102.
125. Citera G, Arias MA, Maldonado-Cocco A, *et al*. The effect of melatonin in patients with fibromyalgia: a pilot study. Clin Rheumatol 2000; 19 (1): 9-13.
126. Malinovskaya N, Komarov FI, Rapoport SI, *et al*. Melatonin production in patients with duodenal ulcer. Neuroendocrinol Lett 2001; 22 (2): 109-17.
127. Bylesjo I, Forsgren L, Wetterberg L. Melatonin and epileptic seizures in patients with acute intermittent porphyria. Epileptic Disorders 2000; 2 (4): 203-8.
128. Maurizi CP. Could exogenous melatonin prevent sudden infant death syndrome? Med Hypoth 1997; 49 (5): 425-7.
129. Moreau A, Wang DS, Forget S, *et al*. Melatonin signaling dysfunction in adolescent idiopathic scoliosis. Spine 2004; 29 (16): 1772-81.
130. Knook L, Kavelaars A, Sinnema G, *et al*. High nocturnal melatonin in adolescents with chronic fatigue syndrome. J Clin Endocrinol Metab 2000; 85 (10): 3690-2.
131. Korszun A, Sackett-Lundeen L, Papadopoulos E, *et al*. Melatonin levels in women with fibromyalgia and chronic fatigue syndrome. J Rheumatol 1999; 26 (12): 2675-80.
132. Stoschitzky K, Sakotnik A, Lercher P, *et al*. Influence of beta-blockers on melatonin release. Eur J Clin Pharmacol 1999; 55 (2): 111-5.
133. Lusardi P, Piazza E, Fogari R. Cardiovascular effects of melatonin in hypertensive patients well controlled by nifedipine: a 24-hour study. Br J Clin Pharmacol 2000; 49 (5): 423-7.
134. Norman TR, Piccolo J, Voudouris N, *et al*. The effect of single oral doses of zopiclone on nocturnal melatonin secretion in healthy male volunteers. Progr Neuro-Psychopharmacol Biol Psychiatr 2001; 25 (4): 825-33.
135. Hajak G, Rodenbeck A, Bandelow B, *et al*. Nocturnal plasma melatonin levels after flunitrazepam administration in healthy subjects. Eur Neuropsychopharmacol 1996; 6 (2): 149-53.
136. Murphy PJ, Myers BL, Badia P. Nonsteroidal anti-inflammatory drugs alter body temperature and suppress melatonin in humans. Physiol Behav 1996; 59 (1): 133-9.
137. Shilo L, Sabbah H, Hadari R, *et al*. The effects of coffee consumption on sleep and melatonin secretion. Sleep Med 2002; 3 (3): 271-3.
138. Stevens RG, Davis S, Mirick DK, *et al*. Alcohol consumption and urinary concentration of 6-sulfatoxymelatonin in healthy women. Epidemiology 2000; 11 (6): 660-5.
139. Skene DJ, Bojkowski CJ, Arendt J. Comparison of the effects of acute fluvoxamine and desipramine administration on melatonin and cortisol production in humans. Br J Clin Pharmacol 1994; 37 (2): 181-6.
140. Hajak G, Rodenbeck A, Adler L, *et al*. Nocturnal melatonin secretion and sleep after doxepin administration in chronic primary insomnia. Pharmacopsychiatry 1996; 29 (5): 187-92.
141. Garde E, Micic S, Knudsen K, *et al*. 8-methoxypsoralen increases daytime plasma melatonin levels in humans through inhibition of metabolism. Photochem Photobiol 1994; 60 (5): 475-80.
142. Lissoni P, Esposti D, Esposti G, *et al*. A clinical study on the relationship between the pineal gland and the opioid system. J Neural Transmission 1986; 65 (1): 63-73.
143. Uchimura Y, Uchimura N, Kumashiro R, *et al*. Changes in melatonin concentrations during IFN therapy. J Hepatol 1999; 31 (6): 1131.
144. Skene DJ, Swaab DF. Melatonin rhythmicity: effect of age and Alzheimer's disease. Exp Gerontol 2003; 38 (1-2): 199-206.
145. Fourtillan JB, Brisson AM, Fourtillan M, *et al*. Melatonin secretion occurs at a constant rate in both young and older men and women. Am J Physiol Endocrinol Metab 2001; 280 (1): E11-E22.
146. Zeiter JM, Daniels JE, Duffy JF, *et al*. Do plasma melatonin concentrations decline with age? Am J Med 1999; 107 (5): 432-6.
147. Kennaway DJ, Lushington K, Dawson D, *et al*. Urinary 6-sulfatoxymelatonin excretion and aging: new results and a critical review of the literature. J Pineal Res 1999; 27 (4): 210-20.
148. Davis S, Kaune WT, Mirick DK, *et al*. Residential magnetic fields, light-at-night, and nocturnal urinary 6-sulfatoxymelatonin concentration in women. Am J Epidemiol 2001; 154 (7): 591-600.
149. Zimmerman RC, McDougle CJ, Schumacher M, *et al*. Urinary 6-hydroxymelatonin sulfate as a measure of melatonin secretion during acute tryptophan depletion. Psychoneuroendocrinology 1993; 18 (8): 567-78.
150. Buxton OM, L'Hermite-Baleriaux M, Hirschfeld U, *et al*. Acute and delayed effects of exercise on human melatonin secretion. J Biol Rhythms 1997; 12 (6): 568-74.
151. Luboshitzky R, Yanai D, Shen-Orr Z, *et al*. Daily and season-

al variations in the concentration of melatonin in the human pineal gland. Brain Res Bull 1998; 47 (3): 271-6.
152. Nathan PJ, Jeyaseelan AS, Burrows GD, *et al*. Modulation of plasma melatonin concentrations by changes in posture. J Pineal Res 1998; 24 (4): 219-23.
153. Karkela J, Vakkuri O, Kaukinen S, *et al*. The influence of anaesthesia and surgery on the circadian rhythm of melatonin. Acta Anaesthesiol Scand 2002; 46 (1): 30-6.
154. International Commission on Non-Ionizing Radiation Protection. Guideline for limiting exposure to time-varyng electric, magnetic, and electromagnetic fields (up to 300 GHz). Health Physics 1998; 74: 494-509.
155. International Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risks to humans. Vol. 80. Non-ionizing radiation. Part 1. Static and extremely low-frequency (ELF) electric and magnetic fields. Lyon: IARC, 2002.
156. National Radiological Protection Board. ELF electromagnetic fields and risk of cancer. Report of an advisory group on ionizing radiation. NRPB Documents 2001; 12: 38-60.
157. Krewski D, Byus CV, Lotz WG, *et al*. Potential health risks of radiofrequency fields from wireless telecommunication devices. J Toxicol Environ Health 2001 part B; 4: 1-143.
158. Lagiou P, Tamimi R, Lagiou A, *et al*. Is epidemiology implicating extremely low frequency electric and magnetic fields in childhood leukemia? Environ Health Prev Med 2002; 7 (2): 33-9.
159. Lee GM, Neutra RR, Hristova L, *et al*. A nested case-control study of residential and personal magnetic field measures and miscarriages. Epidemiology 2002; 13 (1): 21-31.
160. Sait ML, Wood AW, Sadafi HA. A study of heart rate and heart rate variability in human subjects exposed to occupational levels of 50 Hz circularly polarised magnetic fields. Med Engin Phys 1999; 21 (5): 361-9.
161. Bonhomme-Faivre L, Marion S, Bezie Y, *et al*. Study of human neurovegetative and hematologic effects of environmental fields produced by transformers. Arch Environ Health 1998; 53: 87-92.
162. Hakansson N, Gustavsson P, Johansen C, *et al*. Neurodegenerative diseases in welders and other workers exposed to high levels of magnetic fields. Epidemiology 2003; 14 (4): 420-6.
163. Boscolo P, Bergamaschi A, Di Sciascio MB, *et al*. Effects of low frequency electromagnetic fields on expression of lymphocyte subsets and production of cytokines of men and women employed in a museum. Sci Total Environ 2001; 270 (1-3): 13-20.
164. Stevens RG. Electric power use and breast cancer: a hypothesis. Am J Epidemiol 1987; 125 (4): 556-61.
165. Wilson BW, Wright CW, Morris JE, *et al*. Evidence for an effect of ELF electromagnetic fields on human pineal gland function. J Pineal Res 1990: 9 (4): 259-69.
166. Wilson BW, Stevens RG, Anderson LE. Neuroendocrine mediated effects of electromagnetic-field exposure: possible role of the pineal gland. Life Sci 1989; 45 (15): 1319-32.
167. Pfluger DH, Minder CE. Effects of exposure to 16.7 Hz magnetic fields on urinary 6-hydroxymelatonin sulfate excretion of Swiss railway workers. J Pineal Res 1996; 21 (2): 91-100.
168. Burch JB, Reif JS, Yost MG, *et al*. Reduced excretion of a melatonin metabolite in workers exposed to 60 Hz magnetic fields. Am J Epidemiol 1999; 150 (1): 27-36.
169. Burch JB, Reif JS, Yost MG, *et al*. Nocturnal excretion of a urinary melatonin metabolite among electric utility workers. Scand J Work Environ Health 1998; 24 (3): 183-9.
170. Burch JB, Reif JS, Noonan CW, *et al*. Melatonin metabolite levels in workers exposed to 60-Hz magnetic fields: work in substations and with 3-phase conductors. J Occup Environ Med 2000; 42 (2): 136-42.
171. Juutilainen J, Stevens RG, Anderson LE, *et al*. Nocturnal 6-hydroxymelatonin sulfate excretion in female workers exposed to magnetic fields. J Pineal Res 2000; 28 (2): 97-104.
172. Levallois P, Dumont M, Touitou Y, *et al*. Effects of electric and magnetic fields from high-power lines on female urinary excretion of 6-sulfatoxymelatonin. Am J Epidemiol 2001; 154 (7): 601-9.
173. Youngstedt SD, Kripke DF, Elliott JA, *et al*. No association of 6-sulfatoxymelatonin with in-bed 60-Hz magnetic field exposure or illumination level among older adults. Environ Res 2002; 89 (3): 201-9.
174. Karasek M, Woldanska-Okonska M, Czernicki J, *et al*. Chronic exposure to 2.9 mT, 40 Hz magnetic fields reduces melatonin concentrations in humans. J Pineal Res 1998; 25 (4): 240-4.
175. Åkerstedt T, Arnetz B, Ficca G, *et al*. A 50-Hz electromagnetic field impairs sleep. J Sleep Res 1999; 8 (1): 77-81.
176. Graham C, Cook MR, Sastre A, *et al*. Multi-night exposure to 60 Hz magnetic fields: effects on melatonin and its enzymatic metabolite. J Pineal Res 2000; 28 (1): 1-8.
177. Graham C, Cook MR, Gerkovich MM, *et al*. Examination of the melatonin hypothesis in women exposed at night to EMF or bright light. Environ Health Perspect 2001; 109 (5): 501-7.
178. Crasson M, Beckers V, Pequeux C, *et al*. Daytime 50 Hz magnetic field exposure and plasma melatonin and urinary 6-sulfatoxy melatonin concentration profiles in humans. J Pineal Res 2001; 31 (3): 234-41.
179. Hong SC, Kurokawa Y, Kabuto M, *et al*. Chronic exposure to ELF magnetic fields during night sleep with electric sheet: effects on diurnal melatonin rhythms in men. Bioelectromagnetics 2001; 22 (2): 138-43.
180. Vanacore N, Benedetti M, Conte D, *et al*. Approccio multidisciplinare allo studio degli effetti neurocomportamentali associati all'esposizione al campo magnetico a 50 Hz. Roma: Istituto Superiore di Sanità, 2004 (Rapporti ISTISAN 04/019).

Eur. J. Oncol., vol. 10, n. 2, pp. 107-116, 2005

Aspartame induces lymphomas and leukaemias in rats[a]

L'aspartame induce linfomi e leucemie nei ratti

Morando Soffritti, Fiorella Belpoggi, Davide Degli Esposti, Luca Lambertini
Cancer Research Centre, European Ramazzini Foundation of Oncology and Environmental Sciences, Bologna, Italy

Summary

Aspartame, a widely used artificial sweetener, was administered with feed to male and female Sprague-Dawley rats (100-150/sex/group), 8 weeks-old at the start of the experiment, at concentrations of 100,000; 50,000; 10,000; 2,000; 400; 80 and 0 ppm. Treatment lasted until spontaneous death of the animals. In this report we present the first results showing that aspartame, in our experimental conditions, causes a statistically significant, dose-related increase in lymphomas and leukaemias in females. No statistically significant increase in malignant brain tumours was observed among animals from the treated groups as compared to controls. Eur. J. Oncol., 10 (2), 107-116, 2005

Key words: **aspartame, artificial sweetener, carcinogenesis, rats, lymphoma, leukaemia**

Riassunto

L'aspartame, un dolcificante artificiale largamente diffuso, è stato somministrato con il mangime a ratti Sprague-Dawley, maschi e femmine (100-150/sesso/gruppo), di 8 settimane di età all'inizio dell'esperimento, a concentrazioni di 100.000; 50.000; 10.000; 2.000; 400; 80 e 0 ppm. Il trattamento è durato fino alla morte spontanea degli animali. In questo articolo vengono presentati i primi risultati che dimostrano come l'aspartame, nelle nostre condizioni sperimentali, causa un incremento statisticamente significativo, dose-correlato, di linfomi e leucemie nelle femmine. Nei gruppi trattati rispetto al controllo non è stato osservato nessun aumento statisticamente significativo dei tumori maligni del cervello. Eur. J. Oncol., 10 (2), 107-116, 2005

Parole chiave: **aspartame, dolcificante artificiale, cancerogenesi, ratti, linfoma, leucemia**

Introduction

Aspartame (APM) is a widely used artificial sweetener consumed by hundreds of millions of people around the world[1, 2]. It is found in more than 6,000 products, including soft drinks, chewing gum, candy, yoghurt, table-top sweeteners and some pharmaceuticals such as vitamins and sugar-free cough drops[2].

Dietary surveys, performed among APM consumers, have shown that the average APM daily intake in the general population ranged from 2 to 3 mg/kg b.w. and was even more in children and pregnant women[1]. The Accept-

Received/Pervenuto 15.3.2005 - Accepted/Accettato 11.4.2005
Address/Indirizzo: Dr. Morando Soffritti, Centro di Ricerca sul Cancro, Fondazione Europea di Oncologia e Scienze Ambientali "B. Ramazzini", Castello di Bentivoglio, 40010 Bentivoglio (BO), Italia - Tel. 0039/051/6640460 - Fax 0039/051/6640223 - E-mail: crcfr@ramazzini.it

[a]Research supported by European Ramazzini Foundation of Oncology and Environmental Sciences, Bologna, Italy

able Daily Intake (ADI) both in the US and in Europe is 50 and 40 mg/kg b.w., respectively[1].

In rodents and humans, APM is metabolised in the gastrointestinal tract into three constituents: aspartic acid, phenylalanine and methanol[3].

Three long-term feeding carcinogenicity bioassays on APM were performed on rats, and one on mice, during the 1970s. Overall, the carcinogenicity studies were considered negative[4], but it must be noted that these studies did not comply with the basic requirements which must nowadays be met when testing the carcinogenicity potential of a chemical or physical agent. Because of these limitations, we decided to perform a mega-experiment following the currently accepted Good Laboratory Practices.

In the present paper we are reporting our first results on the incidence of haemolymphoreticular malignancies (lymphomas and leukaemias) and malignant brain tumours.

Materials and methods

The APM used was produced by Nutrasweet and supplied by Giusto Faravelli S.p.A., Milan, Italy. As an active ingredient, its purity was more than 98%. To simulate an assumed daily intake by humans of 5,000; 2,500; 500; 100; 20; 4; or 0 mg/kg b.w., APM was added to the standard Corticella diet, used for 30 years at the laboratory of the Cancer Research Centre (CRC) of the European Ramazzini Foundation (ERF), at concentrations of 100,000; 50,000; 10,000; 2,000; 400; 80; or 0 ppm. APM-treated feed was administered *ad libitum* to Sprague-Dawley rats (100-150/sex/group), 8 weeks old at the start of the experiment, and the treatment lasted until spontaneous death. Control animals received the same feed without APM. The plan of the experiment is shown in Table 1.

Male (M) and female (F) rats from the colony of the CRC were used. This colony of rats has been employed

Table 1 - Long-term carcinogenicity bioassay on aspartame administered with feed supplied *ad libitum* to male (M) and female (F) Sprague-Dawley rats from 8 weeks of age until spontaneous death. Plan of the experiment

Groups No.	Animals			Treatment			
	Age at start (weeks)	Sex	No.	Dose			Duration
				ppm	mg/kg b.w. [a]	Human ADI equivalent [b]	
I	8	M	100				
		F	100	100,000	5,000	100X	Life span
		M+F	200				
II	8	M	100				
		F	100	50,000	2,500	50X	Life span
		M+F	200				
III	8	M	100				
		F	100	10,000	500	10X	Life span
		M+F	200				
IV	8	M	150				
		F	150	2,000	100	2X	Life span
		M+F	300				
V	8	M	150				
		F	150	400	20	0.4X	Life span
		M+F	300				
VI	8	M	150				
		F	150	80	4	0.08X	Life span
		M+F	300				
VII	8	M	150				
		F	150	0	-	-	Life span
		M+F	300				

[a] The daily assumption in mg/kg b.w. was calculated considering the average weight of a rat for the duration of the experiment as 400 g, and the average consumption of feed as 20 g per day, both for males and females

[b] Considering the Acceptable Daily Intake (ADI) of 50 mg/kg b.w. for humans

for various experiments in the CRC laboratory for nearly 30 years. Data are available on the tumour incidence among untreated Sprague-Dawley rats. These animals were monitored for feed, water consumption, and body weight, for their life span and, at death, underwent complete necropsy and histopathological evaluation (historical controls).

The experiment was conducted according to the Italian law regulating use of animals for scientific purposes [5].

After weaning, at 4-5 weeks of age, the experimental animals were identified by ear punch, randomised in order to have no more than one male and one female from each litter in the same group, and housed in groups of 5 in makrolon cages (41x25x15 cm), with stainless-steel wire tops and a shallow layer of white wood shavings as bedding. All the animals were kept in rooms assigned only to this experiment, at 23 ± 2°C and 50-60% relative humidity.

Mean daily drinking water and feed consumption were measured per cage, and body weight individually, once a week for the first 13 weeks, and then every two weeks until 110 weeks of age. Body weight continued to be measured every 8 weeks until the end of the experiment. Status and behaviour of the animals were examined 3 times daily, and they were clinically examined for gross changes every 2 weeks. All animals were kept under observation until spontaneous death.

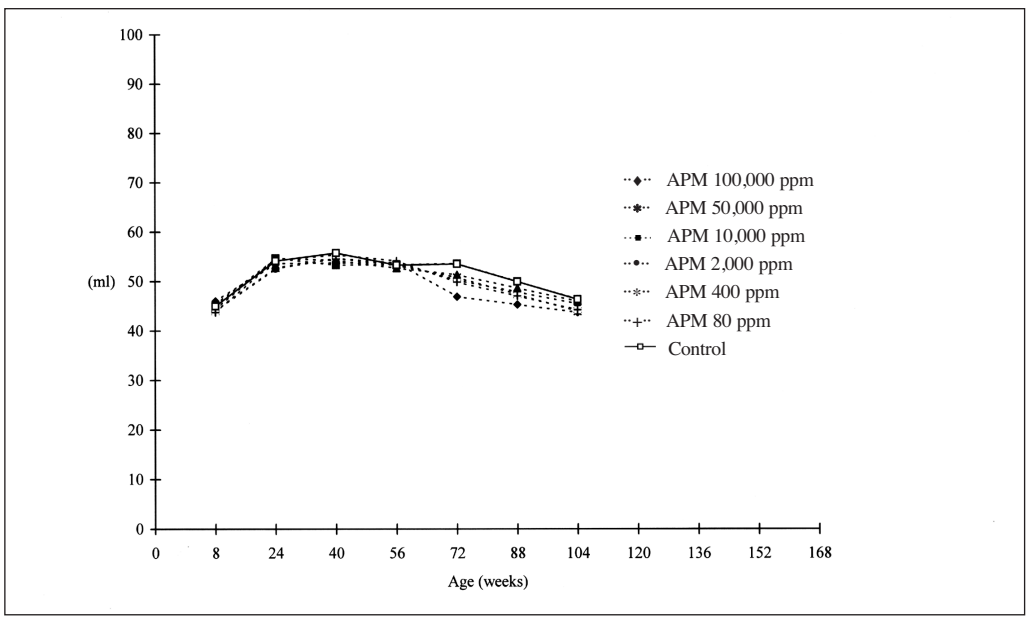

Fig. 1. Mean daily water consumption in male Sprague-Dawley rats

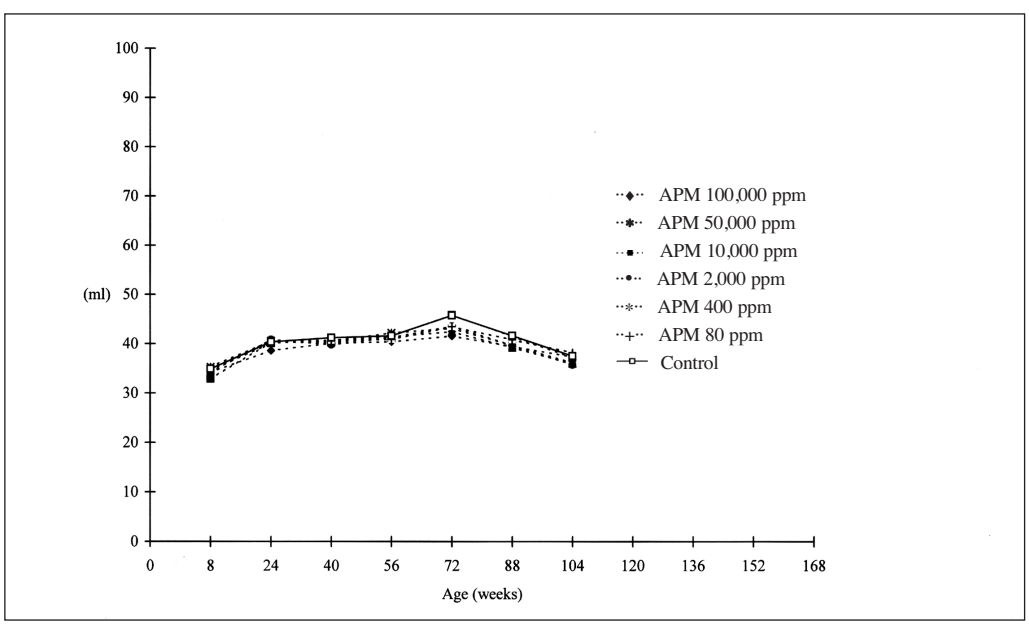

Fig. 2. Mean daily water consumption in female Sprague-Dawley rats

The biophase of the experiment terminated after 151 weeks, with the death of the last animal at the age of 159 weeks.

Upon death, the animals underwent complete necropsy.

Histopathology was routinely performed on the following organs and tissues of all animals from each group: skin and subcutaneous tissue, mammary gland, the brain (3 sagittal sections), pituitary gland, Zymbal glands, salivary glands, Harderian glands, cranium (five sections, with oral and nasal cavities and external and internal ear ducts), tongue, thyroid, parathyroid, pharynx, larynx, thymus and mediastinal lymph nodes, trachea, lung and mainstem bronchi, heart, diaphragm, liver, spleen, pancreas, kidneys, adrenal glands, oesophagus, stomach (fore and glandular), intestine (four levels), urinary bladder, prostate, gonads, interscapular brown fat pad, subcutaneous and mesenteric lymph nodes and other organs or tissues with pathological lesions.

All organs and tissues were preserved in 70% ethyl alcohol, except for bones which were fixed in 10% formalin and then decalcified with 10% formaldehyde and 20% formic acid in water solution. The normal specimens were trimmed, following the Standard Operating Procedures at the CRC laboratory: i.e. parenchymal organs were dissected through the hilus to expose the widest surface, and hollow organs were sectioned across the greatest diameter.

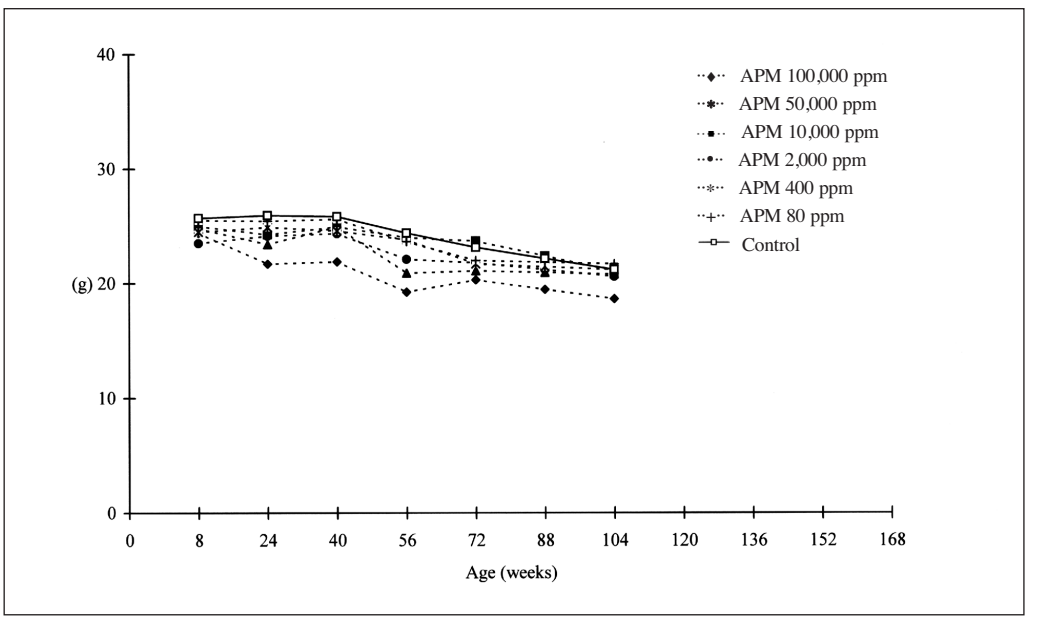

Fig. 3. Mean daily feed consumption in male Sprague-Dawley rats

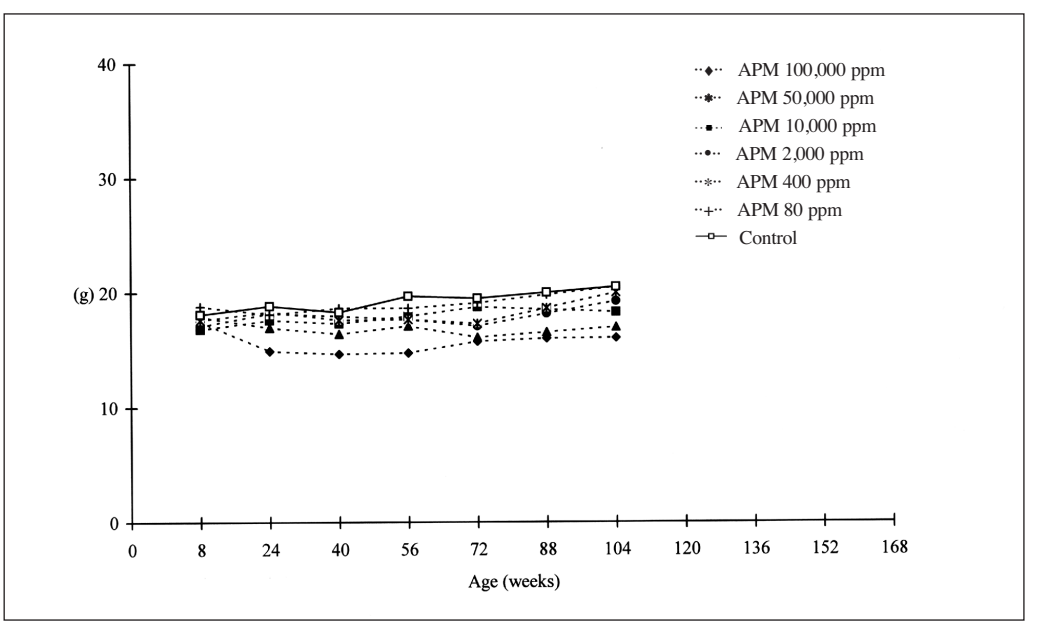

Fig. 4. Mean daily feed consumption in female Sprague-Dawley rats

Any pathological tissue was trimmed through the largest surface, including normal adjacent tissue. Trimmed specimens were processed as paraffin blocks, and 3-5 micron sections of every specimen were obtained. Sections were routinely stained with haematoxylin-eosin.

Statistical analyses were performed using the poly-k test (k = 3). This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account [6-8].

Results

During the experiment no differences were observed among the various groups in mean daily water consumption (figs. 1 and 2). A dose-related difference in feed consumption was observed between the various treated groups and the control group in both males and females (figs. 3 and 4). No differences in mean body weight were observed among treated and control groups in either males or females (figs. 5 and 6). No substantial difference in survival was observed among treated and control groups, males or females (figs. 7 and 8).

Yellowing of the coat was observed in animals exposed to APM, mainly at the highest concentrations. This change was previously observed in our laboratory in rats exposed to formaldehyde administered with drinking water[9].

The occurrence of lymphomas and leukaemias among male and female rats in treated and control groups is

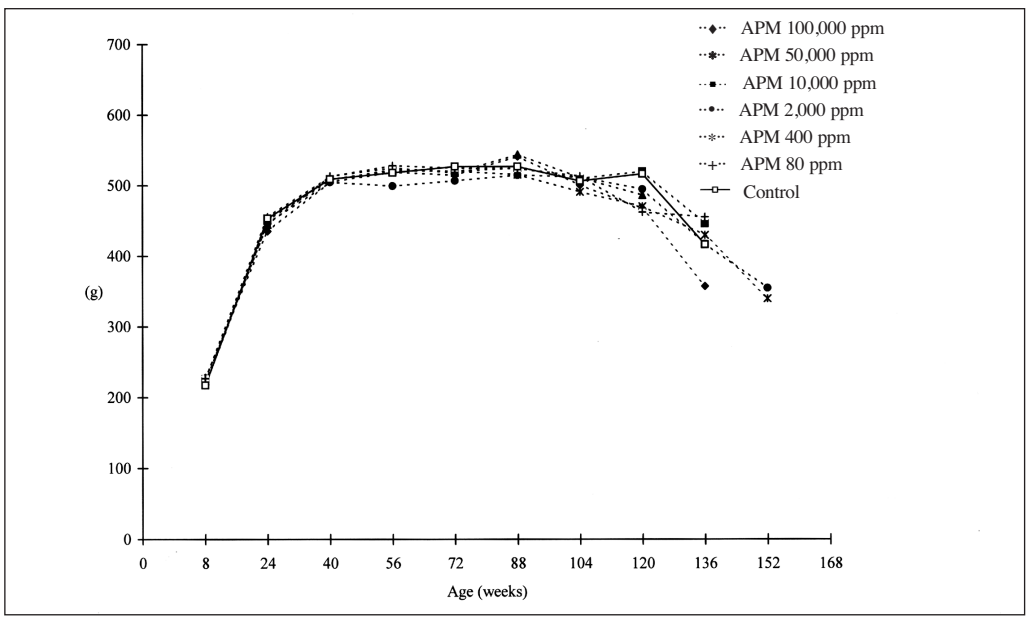

Fig. 5. Mean body weights in male Sprague-Dawley rats

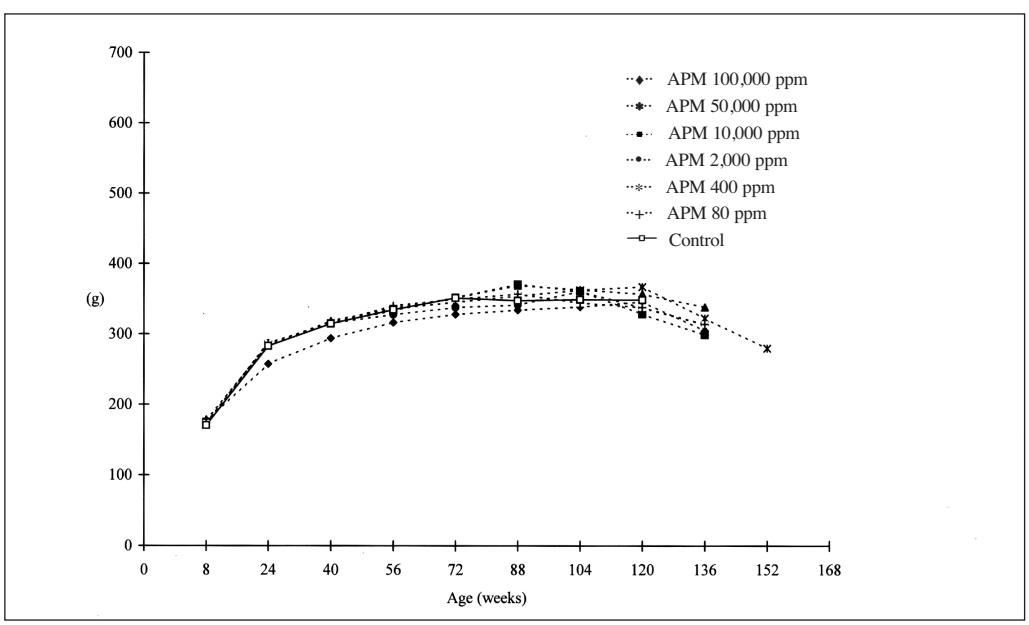

Fig. 6. Mean body weights in female Sprague-Dawley rats

shown in Table 2. The data indicate that APM causes a statistically significant increase in the incidence of lymphomas and leukaemias in females, at concentrations of 100,000 ($p \leq 0.01$); 50,000 ($p \leq 0.01$); 10,000 ($p \leq 0.05$); 2,000 ($p \leq 0.05$) and 400 ($p \leq 0.01$) ppm as compared to untreated controls. This increase is dose-related ($p \leq 0.05$).

Although not statistically significant, an increase was also observed in females treated with 80 ppm and in males treated with the highest dose.

The haemolymphoreticular neoplasias observed in the experiment include: lymphoblastic lymphoma and leukaemia, lymphocytic lymphoma, lymphoimmunoblastic lymphoma, histiocytic sarcoma and monocytic leukaemia, myeloid leukaemia. The most frequent type of neoplasia was the lymphoimmunoblastic lymphoma (figs. 9 and 10).

Lymphomas and leukaemias are considered together, since both solid and circulating phases are present in many lymphoid neoplasms, and distinction between them is artificial[10].

The occurrence of brain malignancies is shown in Table 3. Sparse malignant brain tumours were observed among males and females in the treated groups and none in the controls.

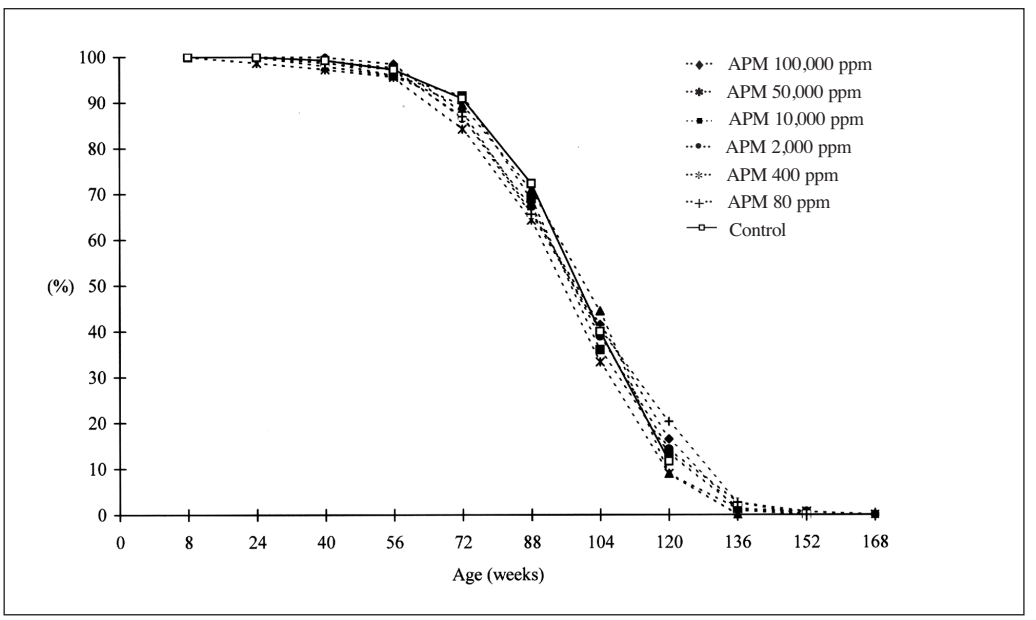

Fig. 7. Survival in male Sprague-Dawley rats

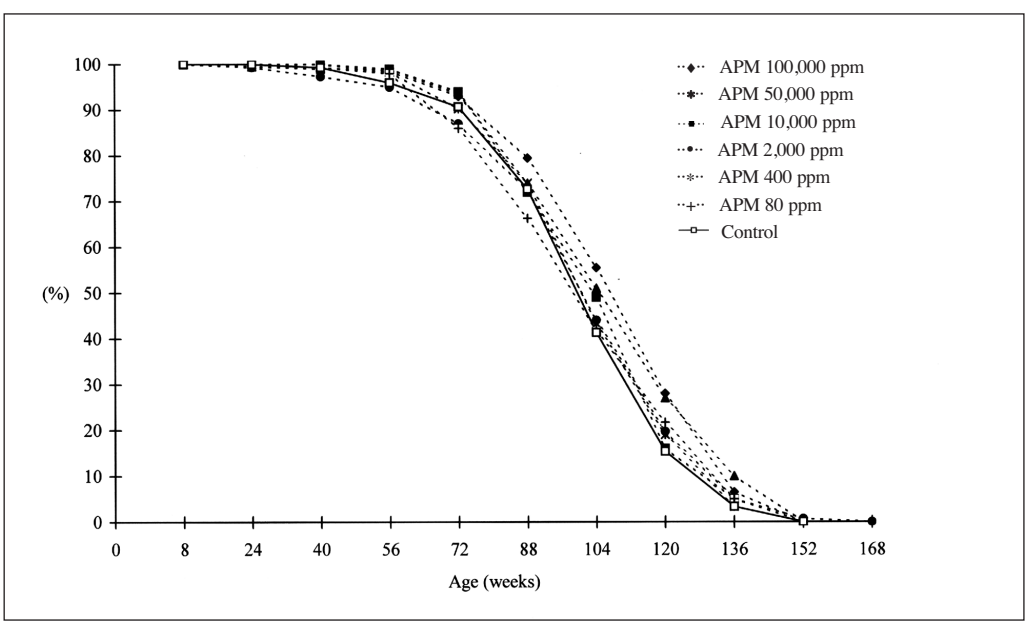

Fig. 8. Survival in female Sprague-Dawley rats

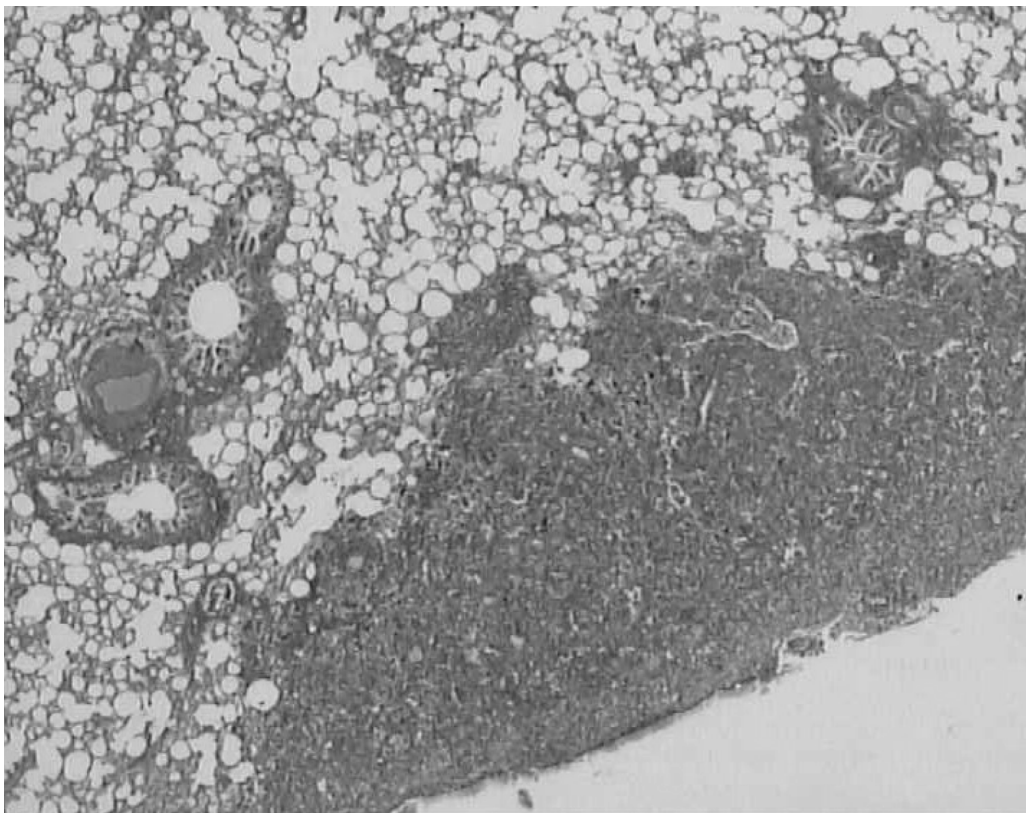

Fig. 9. Lymphoimmunoblastic lymphoma in a female rat administered 80 ppm aspartame in feed (lung). HE X 25

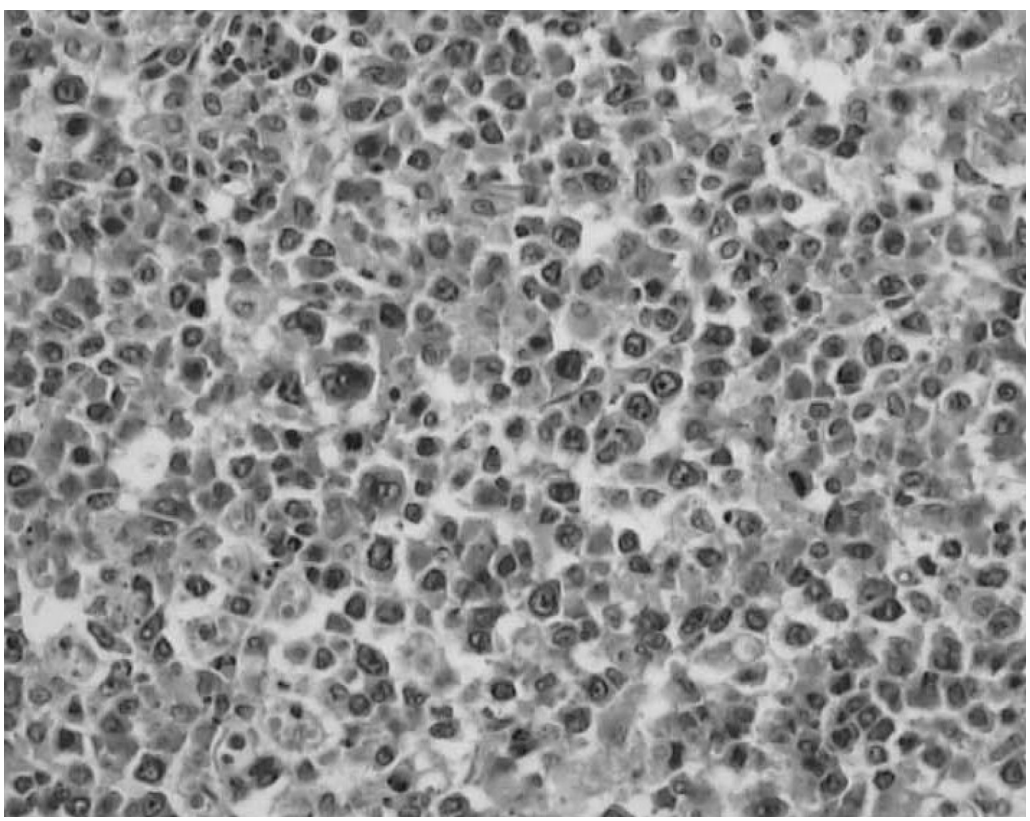

Fig. 10. A detail of the lymphoimmunoblastic lymphoma shown in fig. 9. HE X 400

Table 2 - Long-term carcinogenicity bioassay on aspartame administered with feed supplied *ad libitum* to male (M) and female (F) Sprague-Dawley rats from 8 weeks of age until spontaneous death. Incidence of lymphomas and leukaemias

Group No.	Animals			Treatment				Animals with lymphomas and leukaemias	
	Age at start (weeks)	Sex	No.	Dose			Duration	No.	%
				ppm	mg/kg b.w.[a]	Human ADI equivalent [b]			
I	8	M	100					29	29.0
		F	100	100,000	5,000	100X	Life span	25	25.0**
		M+F	200					54	27.0
II	8	M	100					20	20.0
		F	100	50,000	2,500	50X	Life span	25	25.0**
		M+F	200					45	22.5
III	8	M	100					15	15.0
		F	100	10,000	500	10X	Life span	19	19.0*
		M+F	200					34	17.0
IV	8	M	150					33	22.0
		F	150	2,000	100	2X	Life span	28	18.7*
		M+F	300					61	20.3
V	8	M	150					25	16.7
		F	150	400	20	0.4X	Life span	30	20.0**
		M+F	300					55	18.3
VI	8	M	150					23	15.3
		F	150	80	4	0.08X	Life span	22	14.7
		M+F	300					45	15.0
VII	8	M	150					31	20.7
		F	150	0	-	-	Life span	13	8.7
		M+F	300					44	14.7

[a] Considering the life-span average weight of a rat (male and female) as 400 g and the average consumption of food as 20 g per day
[b] Considering the Acceptable Daily Intake (ADI) of 50 mg/kg b.w. for humans
* Statistically significant p≤ 0.05; ** Statistically significant p≤ 0.01 using poly-k test (k = 3)

In our historical controls over the last 20 years, when we consider groups of 100 or more animals per sex (1934 males and 1945 females), the overall incidence of lymphomas and leukaemias in males is 20.7% (8.0-30.9) and in females 12.4% (7.0-18.4). The overall incidence of malignant brain tumours is 1.7% (0-5.0) in males and 0.7% (0-3.0) in females respectively.

Conclusions

In our experimental conditions, it has been demonstrated, for the first time, that APM causes a statistically significant, dose-related increase in lymphomas and leukaemias in females at dose levels very near those to which humans can be exposed. Moreover, it can hardly be overlooked that, at the lowest exposure of 80 ppm, there was a 69% increase in lymphomas and leukaemias compared to controls, even though this was not statistically significant. When compared to the concurrent control group, an increase in the incidence of these neoplasias was also observed in males exposed to the highest dose; even though not statistically significant, this observation confirms and extends the result in females.

The significance of the increase in haemolymphoreticular neoplasias is further reinforced by the following considerations, based on the results of experiments performed in the CRC laboratory.

These experiments demonstrate that the increase in lymphomas and leukaemias, observed in the APM study, could be related to methanol, a metabolite of APM, which is metabolised to formaldehyde and then to formic acid, both in humans and rats[3]. In fact we have shown that: 1) methanol administered in drinking water increased the in-

Table 3 - Long-term carcinogenicity bioassay on aspartame administered with feed supplied *ad libitum* to male (M) and female (F) Sprague-Dawley rats from 8 weeks of age until spontaneous death. Incidence of malignant brain tumours

Group No.	Animals			Treatment				Animals with malignant brain tumours[a]	
	Age at start (weeks)	Sex	No.	Dose			Duration	No.	%
				ppm	mg/kg b.w.[b]	Human ADI equivalent [c]			
I	8	M	100					1	1.0
		F	100	100,000	5,000	100X	Life span	1	1.0
		M+F	200					2	1.0
II	8	M	100					2	2.0
		F	100	50,000	2,500	50X	Life span	1	1.0
		M+F	200					3	1.5
III	8	M	100					0	-
		F	100	10,000	500	10X	Life span	1	1.0
		M+F	200					1	0.5
IV	8	M	150					2	1.3
		F	150	2,000	100	2X	Life span	1	0.7
		M+F	300					3	1.0
V	8	M	150					0	-
		F	150	400	20	0.4X	Life span	0	-
		M+F	300					0	-
VI	8	M	150					2	1.3
		F	150	80	4	0.08X	Life span	1	0.7
		M+F	300					3	1.0
VII	8	M	150					0	-
		F	150	0	-	-	Life span	0	-
		M+F	300					0	-

[a] The malignancies observed were: 10 malignant gliomas or mixed gliomas, 1 medulloblastoma, and 1 malignant meningioma
[b] Considering the life-span average weight of a rat (male and female) as 400 g and the average consumption of food as 20 g per day
[c] Considering the Acceptable Daily Intake (ADI) of 50 mg/kg b.w. for humans

cidence of lymphomas and leukaemias in female rats[11]; 2) the same effect was induced in females treated with the gasoline oxygenated additive methyl-*tert*-butyl-ether (MTBE), which is also metabolised to methanol[12]; and finally 3) an increase in the incidence of lymphomas and leukaemias was also observed in females treated with formaldehyde[9, 13].

These results further highlight the important rôle that formaldehyde has on the induction of haematological malignancies in rodents. Moreover, in a recent reevaluation of the carcinogenicity of formaldehyde by the International Agency for Research on Cancer (IARC), strong, although not considered sufficient, evidence of an association with leukaemias in humans was found[14].

Since the results of carcinogenicity bioassays in rodents, mainly rats and mice, have been shown to be a consistent predictor of human cancer risk[15-17], the first results of our study call for urgent re-examination of permissible exposure levels of APM in both food and beverages, especially to protect children.

References

1. Butchko HH, Stargel WW, Comer CP, *et al*. Preclinical safety evaluation of aspartame. Regul Toxicol Pharmacol 2002; 35: S7-S16.
2. Aspartame Information Center. Available on http://www.aspartame.org, 2004.
3. Ranney RE, Opperman JA, Maldoon E, *et al*. Comparative metabolism of aspartame in experimental animals and humans. Toxicol Environ Health 1976; 2: 441-51.
4. Food and Drug Administration. Aspartame: Commissioner's Final Decision; 1981 Fed Regist 46, 38285-308.
5. Repubblica Italiana. Decreto Legislativo 116. Attuazione della direttiva n. 86/609/CEE in materia di protezione degli animali

utilizzati a fini sperimentali o ad altri fini scientifici. Supplemento ordinario alla Gazzetta Ufficiale 1992; 40: 5-25.
6. Bailer AJ, Portier CJ. Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. Biometrics 1988; 44**:** 417-31.
7. Portier CJ, Bailer AJ. Testing for increased carcinogenicity using a survival-adjusted quantal response test. Fundam Appl Toxicol 1989; 12: 731-7.
8. Piergorsh WW, Bailer AJ. Statistics for environmental biology and toxicology. London: Chapman, 1997.
9. Soffritti M, Belpoggi F, Lambertini L, *et al*. Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. In Mehlman MA, Bingham E, Landrigan PJ, *et al*. Carcinogenesis bioassays and protecting public health. Commemorating the lifework of Cesare Maltoni and colleagues. Ann NY Acad Sci 2002; 982: 87-105.
10. Harris NL, Jaffe ES, Vardiman JW, *et al*. WHO classification of tumours of haematopoietic and lymphoid tissues. Introduction. In Jaffe ES, Harris NL, Stein H, *et al*. Tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press, 2001, 12-3.
11. Soffritti M, Belpoggi F, Cevolani D, *et al*. Results of long-term experimental studies on the carcinogenicity of methyl alcohol and ethyl alcohol in rats. In Mehlman MA, Bingham E, Landrigan PJ, *et al*. Carcinogenesis bioassays and protecting public health. Commemorating the lifework of Cesare Maltoni and colleagues. Ann NY Acad Sci 2002; 982: 46-69.
12. Belpoggi F, Soffritti M, Maltoni C. Methyl-tertiary-butyl ether (MTBE), a gasoline additive, causes testicular and lymphohaematopoietic cancers in rats. Toxicol Ind Health 1995; 11: 119-49.
13. Soffritti M, Maltoni C, Maffei F, *et al*. Formaldehyde: an experimental multipotent carcinogen. Toxicol Ind Health 1989; 5**:** 699-730.
14. International Agency for Research on Cancer. Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxy-2-Propanol. Vol. 88 (in press). Available on http://www.iarc.fr.
15. Huff J. Long-term chemical carcinogenesis bioassays predict human cancer hazards. Issues, controversies, and uncertainties. In Bailer JA, Maltoni C, Bailar III JC, *et al*. Uncertainty in the risk assessment of environmental and occupational hazards. Ann NY Acad Sci 1999; 895: 56-79.
16. Tomatis L, Aitio A, Wilbourn J, *et al*. Human carcinogens so far identified. Jpn J Cancer Res 1989; 80: 795-807.
17. Rall DP. Can laboratory animal carcinogenicity studies predict cancer in exposed children? Environ Health Perspect 1995; 103 suppl 6: 173-5.

Eur. J. Oncol., vol. 10, n. 2, pp. 117-122, 2005

Genetic polymorphism of toxicant-metabolizing enzymes and prognosis of Chinese workers with chronic benzene poisoning [a]

Polimorfismo genetico degli enzimi che metabolizzano le sostanze tossiche e prognosi dei lavoratori cinesi con avvelenamento cronico da benzene

Jun-Xiang Wan*, Zhong-Bin Zhang*, Ji-Ru Guan**, Duo-Zhi Cao***, Rong Ye **, Xi-Peng Jin*, Zhao-Lin Xia*
* Department of Occupational Health and Toxicology, School of Public Health, Fudan University, Shanghai, China
** Hangzhou Centers for Disease Control and Prevention, Hangzhou, Zhejiang Province, China
*** Institute of Occupational Health attached to Maanshan Steel & Iron Group, Maanshan, Anhui Province, China

Summary

There are many factors related to the prognosis of workers with chronic benzene poisoning (CBP), whose count of white blood cells (WBC) was still below 4x10^9/l even after their benzene exposure had ceased some years previously. In order to explore these factors, 120 workers who had been exposed to CBP were divided into two groups depending on the WBC count. The mean age at diagnosis of CBP, benzene-exposure duration, body mass index (BMI) and the proportion of genotypes of cytochrome P450 2E1 *(CYP2E1)*, glutathione-S-transferase mu-1 *(GSTM1)*, glutathione-S-transferase theta-1 *(GSTT1)*, myeloperoxidase *(MPO)*, and NAD(P)H-quinone oxidoreductase 1 *(NQO1)* were compared between the WBC<4x 10^9/l group and the WBC≥4x10^9/l group. Using the method of logistic regression, a risk model was set up to predict the prognosis of the CBP workers. The results indicated that the BMI of workers with WBC<4x10^9/l was lower than that of workers with ≥4x10^9/l (21.40 ± 2.76 *vs* 23.09 ± 3.36, p = 0.01). The logistic regression model suggested that there was a 4.5-fold increased risk among workers carrying the *GSTT1 null* genotype (95% CI: 1.13-17.54) compared with that of workers with the *GSTT1 non-null* genotype. In conclusion, our results suggest that benzene-

Riassunto

Ci sono molti fattori correlati alla prognosi di lavoratori colpiti da avvelenamento cronico da benzene (CBP), la cui conta leucocitaria (WBC) fosse ancora inferiore a 4x10^9/l nonostante l'esposizione a benzene fosse cessata da diversi anni. Per indagare su questi fattori, 120 lavoratori colpiti da CBP sono stati suddivisi in due gruppi in base alla conta leucocitaria. L'età media alla diagnosi di CBP, la durata dell'esposizione a benzene, l'indice di massa corporea (BMI) e la distribuzione dei genotipi del citocromo P450 2E1 (*CYP2E1*), la glutatione-S-transferasi mu-1 (*GSTM1*), la glutatione-S-transferasi teta-1 (*GSTT1*), la mieloperossidasi (*MPO*), e la NAD(P)H-chinone ossidoreduttasi 1 (*NQO1*) sono stati confrontati tra il gruppo con una conta inferiore a 4x10^9/l e il gruppo con una conta almeno pari a 4x10^9/l. Con il metodo di regressione logistica, è stato costruito un modello di rischio per predire la prognosi dei lavoratori affetti da CBP. I risultati hanno mostrato che il BMI di lavoratori con una conta inferiore a 4x10^9/l era minore di quello dei lavoratori con una conta almeno pari a 4x10^9/l (21,40 ± 2,76 *vs* 23.09 ± 3.36, p = 0.01). Il modello di regressione logistica ha suggerito che ci sia un rischio 4,5 volte superiore tra i lavoratori portatori del genotipo *null* del gene *GSTT1* (95% CI: 1.13 - 17.54)

Received/Pervenuto 4.3.2005 - Accepted/Accettato 7.4.2005
Address/Indirizzo: Prof. Zhao-Lin Xia, School of Public Health, Medical Center of Fudan University 138, Yi Xue Yuan Road, Shanghai, PR 200032 China - Tel. 0086/21/54237050 - Fax 0086/21/64178160 - E-mail: zlxia@shmu.edu.cn - E-mail: xzl580428@yahoo.com

[a] This study was supported by the National Natural Science Foundation of China (30271113) and 973 Program (2002CB512902)

exposure duration, BMI and *GSTT1* genotype may be important factors that affect the prognosis of the CBP workers. Eur. J. Oncol., 10 (2), 117-122, 2005

***Key words*: chronic benzene poisoning, prognosis, genetic polymorphism, life style**

confrontato con lavoratori portatori di un genotipo *non-null* per il gene *GSTT1*. In conclusione, si suggerisce che la durata dell'esposizione a benzene, il BMI e il genotipo del gene *GSTT1* possano essere fattori importanti sulla prognosi dei lavoratori affetti da CBP. Eur. J. Oncol., 10 (2), 117-122, 2005

***Parole chiave:* avvelenamento cronico da benzene, prognosi, polimorfismo genetico, stile di vita**

Introduction

Exposure to benzene produces haematotoxicities, including pancytopenia, aplastic anaemia, myelodysplasia, and acute myeloid leukaemia[1-4]. Humans are exposed to benzene via occupational exposure or environmentally, via contact with cigarette smoke, gasoline emissions, or products of incomplete combustion. Substantive research has been performed that elucidates the mechanism of benzene poisoning (BP)[5-7], however, to date there have been few studies on the recovery status of the benzene poisoned workers after their disengagement from benzene exposure for a long time[8,9]. Recently, we found that the white blood cells (WBC) count of some workers was still below $4x10^9$/l even after their benzene exposure at work had ceased years previously.

Using the method of logistic regression, we analyzed the relationship between the prognosis and the age at diagnosis of CBP, benzene-exposure duration, body mass index (BMI) and the proportion of genotypes of cytochrome P450 2E1 (*CYP2E1*), glutathione-S-transferase mu-1 (*GSTM1*), and glutathione-S-transferase theta-1 (*GSTT1*), myeloperoxidase (*MPO*), and NAD(P)H-quinone oxidoreductase 1 (*NQO1*), in subjects in the WBC<$4x10^9$/l group and the WBC≥$4x10^9$/l group.

Patients and methods

Patients

One hundred and twenty BP workers were recruited from Maanshan and Hangzhou, China, including 46 males and 74 females. BP was diagnosed from 1980 to 1998 by the local authorized Occupational Disease Diagnostic Team, and patients were registered in the hospitals of prevention and treatment for occupational diseases, which cooperated with our study group. The diagnostic criteria for occupational BP, according to the Chinese Ministry of Health, include: 1) a total WBC count of <4,000/μl or a WBC count between 4,000 and 4,500/μl and a platelet count of < 80,000/μl, with repeated confirmation of this count after a few months in a peripheral blood examination; 2) the individual with documented benzene exposure has been employed for at least 6 months in the factory; and 3) exclusion of other causes of abnormal blood counts such as chloromycetin use and ionizing radiation. The medical records of patients were independently reviewed, especially those WBC counts of > 3,500/μl, to confirm the BP diagnosis. There were in all 44 workers whose WBC counts were below $4x10^9$/l.

WBC count

Routine blood examination and assay of alanine transaminase (ALT) were performed at the Institute of Occupational Health attached to the Maanshan Steel & Iron Group, and the Hangzhou Hospital for Occupational Diseases. The method used in ALT determination was ELISA linked-continuously ultraviolet monitoring assay.

ALT is an important serum marker of hepatic damage, so in our study it was used to evaluate the hepatic damage in BP workers.

Benzene-exposure duration and age at diagnosis of BP

Benzene-exposure duration is defined as the actual time period during which workers were engaged in benzene-exposed work, and the age at diagnosis of BP denotes the age at which the workers were shown to have BP (the date of diagnosis minus their date of birth).

Calculation of BMI

BMI is defined as the body weight (kg) divided by the square of the body height (m^2).

Determination of genetic polymorphisms of CYP2E1, NQO1, MPO, GSTM1 and GSTT1

The single nucleotide polymorphisms (SNPs) in the promoters and the complete coding regions of *CYP2E1*,

NQO1 and *MPO* were determined by PCR sequencing and DHPLC (denaturing high performance liquid chromatography)[10, 11]. The genotypes of *GSTT1* and *GSTM1* were determined by PCR[12].

Statistical analysis

A database was established and the data were analyzed with SPSS 10.0. The age at diagnosis, the benzene-exposure duration and the BMI in the WBC<4x10^9/l group and the WBC≥4x10^9/l group were analyzed with *t-test*. The *chi-square test* was adopted to compare the differences in life style (smoking and alcohol consumption) and the proportion of genotypes of *CYP2E1*, *GSTM1*, *GSTT1*, *MPO*, and *NQO1* in the two groups. Multiple factor analysis was implemented with the method of logistic regression.

Results

Distribution of gender, age at diagnosis and years of benzene exposure

The distributions of gender, age at diagnosis, years of benzene exposure and ALT in subjects are shown in Table 1.

Comparison of age at diagnosis, benzene exposure duration and BMI

There was no significant difference in age at diagnosis and benzene exposure duration between the two groups, but the BMI of the WBC<4x10^9/l group was lower than that of the WBC≥4x10^9/l group (p=0.01) (Table 2).

Relationship between life style, polymorphisms in toxicant metabolizing genes and WBC recovery level of BP workers

The proportion of *null* genotype of *GSTT1* in the WBC<4x10^9/l group was higher than that of the WBC≥4x10^9/l group (64.29% *vs* 46.67%), but, although suggestive, did not reach statistical significance (p=0.07), as was the case with other factors between the two groups (Table 3).

Logistic analysis of WBC recovery level of BP workers

Logistic analysis of WBC recovery level of BP workers was implemented with WBC level (WBC≥4x10^9/l = 0; WBC<4x10^9/l = 1) as a dependent variable and the gender, age at diagnosis, benzene exposure duration, BMI, and polymorphisms of benzene toxicant-metabolizing genes as covariables (logistic methods of Forward Wald)[13]. The results suggested that benzene exposure duration, BMI and genetic polymorphisms of *GSTT1* may affect the WBC recovery of BP workers (Table 4).

Discussion

Previous studies have shown that the toxicity of benzene derives from its metabolites. Once absorbed, benzene is metabolized by *CYP2E1* to yield phenol, hydroquinone (HQ), catechol (CAT), and 1,2,4-benzenetriol[14]. These metabolites accumulate in the bone marrow[15],

Table 1 - Demographics of subjects with WBC<4x10^9/l and WBC≥4x10^9/l

Variables	WBC <4x10^9/l		WBC ≥4x10^9/l	
	N.	%	N.	%
Gender				
Male	14	31.82	32	42.11
Female	30	68.18	44	57.89
Age at diagnosis				
<30	7	15.91	18	23.68
~35	14	31.82	20	26.32
~40	13	29.54	17	22.37
~45	7	15.91	11	14.47
>45	3	6.82	10	13.16
Years of benzene exposure				
<5	7	15.91	13	17.11
~10	12	27.27	23	30.26
~15	11	25.00	14	18.42
~20	5	11.36	15	19.74
>20	9	20.46	11	14.47
ALT				
≤40	44	100.00	76	100.00
>40	0	0.00	0	0.00

Table 2 - Comparison of age at diagnosis, benzene exposure duration and BMI in WBC<4x10^9/l group and WBC≥4x10^9/l group

Variables	No of workers	WBC<4x10^9/l	WBC≥4x10^9/l	t	p
Age at diagnosis (years)	120	35.59±6.98	36.39±7.95	0.557	0.578
Duration of benzene exposure (years)	120	13.85±8.63	12.64±7.09	-0.830	0.408
BMI (kg/m^2)	99	21.40±2.76	23.09±3.36	2.636	0.010

Table 3 - Effects of gender, lifestyle, and genetic polymorphisms of toxicant-metabolizing enzymes on WBC recovery level of BP workers

Variables	WBC<4x10^9/l (%)	WBC≥4x10^9/l (%)	OR (95% CI)
Gender			
Male	14 (31.82)	32 (42.11)	0.64 (0.27~1.50)
Female	30 (68.12)	44 (57.89)	
Lifestyle			
Smoking			
Yes	5 (11.90)	9 (12.33)	0.96 (0.26~3.47)
No	37 (88.10)	64 (87.67)	
Alcohol consumption			
Yes	4 (9.30)	10 (13.51)	0.66 (0.16~2.50)
No	39 (90.70)	64 (86.49)	
Genetic factors			
NQO1			
c.559C>T			
T/T	10 (28.57)	19 (26.03)	1.14 (0.42~3.05)
C/T and C/C	25 (71.43)	54 (73.97)	
CYP2E1			
96bp insertion			
Ins_{96}-/-	26 (63.41)	46 (63.01)	1.02 (0.43~2.43)
Ins_{96}-/+ and +/+	15 (36.59)	27 (36.99)	
c.-1293G>C			
G/G	24 (54.55)	46 (60.53)	0.78 (0.35~1.77)
G/C and C/C	20 (45.45)	30 (39.47)	
c.1263C>T			
C/C	26 (74.29)	43 (74.14)	1.01 (0.35~2.93)
C/T	9 (25.71)	15 (25.86)	
MPO			
c.-463G>A			
G/G	37 (86.05)	60 (81.08)	1.44 (0.46~4.64)
G/A	6 (13.95)	14 (18.92)	
IVS8 +19G>A			
G/G	7 (19.44)	16 (21.92)	0.86 (0.28~2.55)
G/A	29 (80.56)	57 (78.08)	
GSTM1			
Null	21 (51.22)	34 (50.70)	1.02 (0.44~2.39)
Non-null	20 (48.78)	33 (49.25)	
GSTT1			
Null	27 (64.29)	35 (46.67)	2.06 (0.88~4.83)
Non-null	15 (35.71)	40 (53.33)	

where they undergo autoxidation or activation by peroxidases to yield the corresponding quinones[16-18], which are believed to be among the ultimate toxic metabolites of benzene[19]. In theory, when BP workers cease to be exposed to benzene, the metabolites of benzene in their bodies will decrease gradually, so that the effects of these metabolites will diminish over time. If there is no significant damage in function of DNA, the cellular functions will also recover gradually and the WBC count will return to the normal levels.

However, in the study on the association of genetic polymorphisms of toxicant-metabolizing enzymes with susceptibility to benzene poisoning, we found that not all the BP workers' WBC quantities would exceed 4x10^9/l even after their disengagement from benzene exposure for years. There were 36.7% BP workers whose WBC count in peripheral blood was still below 4x10^9/l in this study. The study of susceptibility to BP indicates that there are joint effects between life style, such as smoking and alcohol consumption, and polymorphisms in toxicant-metabolizing genes such as *NQO1 c.559C >T, CYP2E1 c.-1293G >C*, which lead to the supposition that

Table 4 - Logistic analysis of WBC recovery level of workers with chronic benzene poisoning

Variables	β	Wald	p	OR (95%CI)
Duration of benzene exposure[a]	0.0907	4.6626	0.0308	1.0949 (1.0084-1.1888)
GSTT1[b]	1.4941	4.5640	0.0327	4.4553 (1.1313-17.5459)
BMI[c]	-0.3245	5.4900	0.0191	0.7229 (0.5511-0.9483)
Constant	4.4734	2.6245	0.1052	

[a] Benzene exposure duration (years) as a continuous variable (19,20,21……)
[b] *GSTT1* null genotype *vs* non-null genotype
[c] BMI as a continuous variable kg/m^2 (16.81,16.82,16.83……)

both the life style and the polymorphisms in toxicant-metabolizing genes may affect the prognosis of the BP workers.

The results suggested that there was an extremely significant difference for BMI between the WBC<4x10^9/l and the WBC≥4x10^9/l groups (p = 0.01); the results of logistic regression also indicated that there would be a 0.2771-fold decrease in the risk of CBP for individuals exposed to benzene with a 0.01 kg/m^2 increase in BMI, which may be related to the nutritional conditions of the BP workers. However, it has been reported that the mean blood pressure, blood sugar and triglyceride levels in Chinese people whose BMI exceeds 22.6 are higher than those in Chinese people whose BMI is 22.6. So the recovery of the BP workers needs appropriate instead of excessive nutrition. In the logistic regression model, the risk of BP for individuals with the *null GSTT1* gene was 4.4553 times higher than that for individuals with the *non-null GSTT1* gene (95% CI: 1.1313-17.5459). Moreover, individuals with the *null GSTT1* gene and the *NQO1* c.559C >T at the same time were susceptible to BP[12]. As a result, individuals with the *null GSTT1* gene should avoid being engaged in work which exposes them to benzene.

Benzene is furthermore a confirmed carcinogen, and previous studies have indicated that excess risk of leukaemia is associated with cumulative benzene exposures[20-23]. In a retrospective cohort study conducted in China, some cases of leukaemia were also found in workers with a history of CBP before the leukaemia developed[22]. Thus, these factors involved in the prognosis of CBP may also contribute to the development of leukaemia.

However, this study was not specially designed for exploring the various factors in the prognosis of BP workers, but merely as an initial exploration for factors affecting prognosis of BP workers according to the present data. There are no data about factors that may affect the prognosis of BP workers, such as therapy schemes and nutrition in this study, and more attention should be given to this in subsequent research projects.

References

1. Aksoy M, Dincol K, Akgun T, *et al*. Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene. Br J Ind Med 1972; 29: 56-64.
2. Yin SN, Li GL, Tain FD, *et al*. Leukemia in benzene workers, a retrospective cohort study. Br J Ind Med 1987; 44: 124-8.
3. Linet MS, Yin SN, Travis LB, *et al*. Clinical features of hematopoietic malignancies and related disorders among benzene-exposed workers in China. Environ Health Perspect 1996; 104: 1353-64.
4. Yardley-Jones A, Anderson D, Parke DV. The toxicity of benzene and its metabolism and molecular pathology in human risk assessment. Br J Ind Med 1991; 48: 437-44.
5. Yoon BI, Hirabayashi Y, Kawasaki Y, *et al*. Mechanism of action of benzene toxicity: cell cycle suppression in hemopoietic progenitor cells (CFU-GM). Exp Hematol 2001; 29: 278-85.
6. Hiraku Y, Kawanishi S. Oxidative DNA damage and apoptosis induced by benzene metabolites. Cancer Res 1996; 56: 5172-8.
7. Tuo J, Wolff SP, Loft S, *et al*. Formation of nitrated and hydroxylated aromatic compounds from benzene and peroxynitrite, a possible mechanism of benzene genotoxicity. Free Radic Res 1998; 28: 369-75.
8. Guberan E, Kocher P. Long-term prognosis of chronic benzene poisoning checking of a population ten years after exposure. Schweiz Med Wochenschr 1971; 101: 1789-90.
9. Drogichina EA, Zorina LA, Gribova IA. Clinical picture and prognosis of nervous system changes in chronic benzene poisoning. Gig Tr Prof Zabol 1971; 15: 18-21.
10. Liu W, Smith DI, Rechtzigel KJ, *et al*. Denaturing high performance liquid chromatography (DHPLC) used in the detection of germline and somatic mutations. Nucleic Acids Res 1998; 26: 1396-400.
11. Kuklin A, Munson K, Gjerde D, *et al*. Detection of single-nucleotide polymorphisms with the WAVE DNA fragment analysis system. Genet Test 1997-98; 1: 201-6.
12. Wan J, Shi J, Hui L, *et al*. Association of genetic polymorphisms in CYP2E1, MPO, NQO1, GSTM1, and GSTT1 genes with benzene poisoning. Environ Health Perspect 2002; 110: 1213-8.
13. Fang JQ. Medical statistics and computerized experiments (2nd ed). Shanghai: Scientific and Technical Publishers, 2001, 484-91.
14. Seaton MJ, Schlosser PM, Bond JA, *et al*. Benzene metabolism by human liver microsomes in relation to cytochrome P450 2E1 activity. Carcinogenesis 1994; 15: 1799-806.

15. Rickert DE, Baker TS, Bus JS, *et al*. Benzene disposition in the rat after exposure by inhalation. Toxicol Appl Pharmacol 1979; 49: 417-23.
16. Greenlee WF, Sun JD, Bus JS. A proposed mechanism of benzene toxicity: formation of reactive intermediates from polyphenol metabolites. Toxicol Appl Pharmacol 1981; 59: 187-95.
17. Thomas DJ, Sadler A, Subrahmanyam VV, *et al*. Bone marrow stromal cell bioactivation and detoxification of the benzene metabolite hydroquinone: comparison of macrophages and fibroblastoid cells. Mol Pharmacol 1990*;* 37: 255-62.
18. Levay G, Ross D, Bodell WJ. Peroxidase activation of hydroquinone results in the formation of DNA adducts in HL-60 cells, mouse bone marrow macrophages and human bone marrow. Carcinogenesis 1993; 14: 2329-34.
19. Irons RD. Quinones as toxic metabolites of benzene. J Toxicol Environ Health 1985; 16: 673-8.
20. Glass DC, Gray CN, Jolley DJ, *et al*. Leukemia risk associated with low-level benzene exposure. Epidemiology 2003; 14(5): 569-77.
21. Rinsky RA, Smith AB, Hornung R, *et al*. Benzene and leukemia. An epidemiologic risk assessment. N Engl J Med 1987; 316 (17): 1044-50.
22. Yin SN, Li GL, Tain FD, *et al*. Leukaemia in benzene workers: a retrospective cohort study. Br J Ind Med 1987; 44 (2):124-8.
23. Hayes RB, Songnian Y, Dosemeci M, *et al*. Benzene and lymphohematopoietic malignancies in humans. Am J Ind Med 2001; 40 (2): 117-26.

Eur. J. Oncol., vol. 10, n. 2, pp. 123-127, 2005

Il trattamento dei GIST

The treatment of GIST

Luca Bortolasi*, Rosario Barbagiovanni*, Alireza Hasheminia*, Ivano Dal Dosso*, Piero Brosolo**, Luigi Scrinzi***, Giuseppe Bogina****, Francesco Orcalli*

* U.O. Chirurgia Generale, Ospedale di San Bonifacio, Verona, Italia
** U.O. Gastroenterologia, Ospedale di San Bonifacio, Verona, Italia
*** Servizio di Oncologia, Ospedale di San Bonifacio, Verona, Italia
**** Servizio di Anatomia Patologica, Ospedale Don Calabria, Negrar, Verona, Italia

Riassunto

I tumori stromali del tratto gastrointestinale (GIST) sono rare neoplasie mesenchimali a comportamento incerto, la cui sede più frequente è quella gastrica, dove presentano un minor rischio di aggressività rispetto a quella intestinale. I GIST derivano dalle cellule interstiziali di Cajal e come queste sono caratterizzati dalla positività immunoistochimica alla proteina CD 117. Il rischio di comportamento aggressivo è indicato in basso, medio o alto, in base alle dimensioni e all'indice mitotico. Dall'ottobre 2002 al dicembre 2004, presso il Dipartimento di Chirurgia dell'Ospedale di San Bonifacio (Verona), 11 pazienti sono risultati affetti da GIST: 4 pazienti sono in *follow-up* endoscopico per lesioni inferiori a 2 cm, 6 pazienti sono stati sottoposti ad intervento chirurgico (*2 wedge resection* gastriche, 1 gastroresezione e 3 resezioni digiunali, con resezione epatica in un paziente dopo terapia con imatinib) e un paziente è in terapia neoadiuvante con imatinib. La chirurgia rimane il trattamento di scelta, con accesso videolaparoscopico nelle lesioni gastriche < 5-6 cm. In presenza invece di malattia non resecabile o di metastasi è indicato trattamento medico con imatinib, riservando l'eventuale approccio chirurgico a quei casi divenuti resecabili. Eur. J. Oncol., 10 (2), 123-127, 2005

Parole chiave: **GIST, chirurgia, endoscopia, imatinib**

Summary

Gastrointestinal stromal tumours (GIST) are rare mesenchymal neoplasms, the biological behaviour of which is uncertain. The most frequent localization is the stomach, where they show a lesser risk of malignancy than those localized in the intestine. They originate from the interstitial cells of Cajal and, like these, give CD 117 positive immunostaining. The risk of aggressive behaviour is indicated as low, moderate or high, depending on the size of the lesion and the mitotic index. From October 2002 to December 2004 at the Surgical Department of the San Bonifacio Hospital in Verona, 11 patients were diagnosed with GIST. Four patients are on endoscopic follow-up for lesions of less than 2 cm, 6 patients underwent surgical resection (2 gastric wedge resections, 1 gastric resection, and 3 jejunal resections, plus a hepatic resection in one patient after imatinib therapy) and one patient is on neoadjuvant treatment with imatinib. Surgery represents the treatment of choice of GIST, with laparoscopic access in the case of gastric lesions of less than 5-6 cm. However, in the case of inoperable tumours or metastases, the approach must be medical therapy with imatinib, reserving surgical therapy to the cases that have been downstaged. Eur. J. Oncol., 10 (2), 123-127, 2005

Key words: **GIST, surgery, endoscopy, imatinib**

Received/Pervenuto 10.3.2005 - Accepted/Accettato 8.4.2005
Address/Indirizzo: Dr. Luca Bortolasi, U.O. Chirurgia Generale, Ospedale di San Bonifacio, Viale Trieste 21, 37047 Verona, Italia

Introduzione

I tumori stromali del tratto gastrointestinale (GIST) sono neoplasie mesenchimali a comportamento incerto, che originano dalla parete muscolare dei visceri cavi, e possono interessare tutto il tratto digestivo dall'esofago al retto e, seppur raramente, anche l'omento e il retroperitoneo[1].

La maggior parte dei GIST, circa il 70%, ha localizzazione gastrica, mentre il 20-30% viene diagnosticato nel piccolo intestino[1], dove presenta un maggiore rischio di comportamento maligno. La principale innovazione nel campo diagnostico è stata la scoperta di una proteina specifica rilevabile all'immunoistochimica, la CD 117, che è espressione della mutazione del gene KIT; questa positività è espressa solo nei GIST ed in quelle che sembrano essere le cellule da cui deriva questo tumore, ossia le cellule interstiziali di Cajal[1]. I criteri di distinzione dei GIST benigni dai maligni sono stati analizzati per anni e, tra i vari parametri, sono risultati predittivi di una migliore sopravvivenza il basso indice mitotico e il minor diametro. Tuttavia, in considerazione del fatto che anche piccole lesioni o lesioni con un basso indice mitotico possono metastatizzare, sembra più prudente indicare il rischio di un eventuale comportamento aggressivo, basso, medio o alto, piuttosto che definire la lesione benigna o maligna[2].

Dal punto di vista terapeutico, la più importante novità è stata l'introduzione delle metodiche chirurgiche mininvasive e, in campo medico, l'avvento dell'imatinib, un farmaco specifico che inibisce selettivamente l'attività dell'enzima tirosin-chinasi[3].

Pazienti e metodi

Dall'ottobre 2002 al dicembre 2004, presso il Dipartimento di Chirurgia Generale dell'Ospedale di San Bonifacio di Verona, 11 pazienti sono stati riscontrati affetti da GIST. Quattro pazienti sono in *follow-up* endoscopico, sei pazienti sono stati sottoposti ad intervento chirurgico e un paziente è in trattamento neoadiuvante con imatinib (Tabella 1).

I quattro pazienti (pazienti 7, 8, 9 e 10) in *follow-up* endoscopico presentano lesioni gastriche con caratteristiche ecoendoscopiche di GIST ed un diametro inferiore a 2 cm.

Nel gruppo dei pazienti sottoposti ad intervento chirurgico, in tre casi il tumore interessava lo stomaco mentre negli altri tre il digiuno. Il sintomo principale è stato l'emorragia digestiva, mentre tra le indagini diagnostiche notevole importanza ha dimostrato l'ecoendoscopia nel definire specificamente le lesioni gastriche (Tabella 2).

Due pazienti (pazienti 1, 2) con lesioni gastriche di 6 cm sono stati sottoposti a *wedge resection* gastrica videolaparoscopica (VLS), mentre una paziente (paziente 3) con lesione di 20 cm a gastroresezione laparotomica; l'esame istologico ha confermato trattarsi di GIST in tutti i casi con positività al CD 117 e mitosi < 10/50 CFI; i pazienti risultano viventi ed esenti da malattia rispettivamente a 16, 15 e 5 mesi (Tabella 3).

I tre pazienti con GIST digiunale, multifocale in un caso, sono stati sottoposti a resezione intestinale; un paziente (paziente 4), dopo 7 mesi dalla resezione del tumore primitivo, che presentava 25-30 mitosi/50 CFI, ha sviluppato 3 metastasi epatiche al lobo sinistro, ed è stato sottoposto a terapia con imatinib 400 mg/die per 19 mesi; pur essendo la PET risultata negativa, la TAC addominale di controllo dimostrava ancora le lesioni ipodense, per cui è stato sottoposto a settoriectomia laterale sinistra e resezione del IVb. L'esame ha confermato trattarsi di metastasi da GIST con scarsa componente cellulare attiva (<5%); il paziente è attualmente vivente a 26 mesi. Il paziente 5 presentava una perforazione da GIST multifocale digiunale con metastasi polmonari. E' stato sottoposto a resezione digiunale: l'esame istologico ha confermato trattarsi di GIST per le caratteristiche morfologiche, pur in assenza di positività al CD 117 (mitosi 80-100/50 CFI).

Tabella 1 - Sede, dimensioni e provvedimento terapeutico

Paziente	Età	Sede	Diametro (cm)	Terapia
1	45	Stomaco (corpo)	6	Chirurgia
2	77	Stomaco (fondo)	6	Chirurgia
3	74	Stomaco (corpo)	20	Chirurgia
4	65	Digiuno	5	Chirurgia
5	57	Digiuno (multifocale)	5	Chirurgia
6	71	Digiuno	6,5	Chirurgia
7	72	Stomaco (antro)	1,2	*Follow-up* endoscopico
8	73	Stomaco (antro)	2	*Follow-up* endoscopico
9	77	Stomaco (fondo)	1,6	*Follow-up* endoscopico
10	72	Stomaco (antro)	1,2	*Follow-up* endoscopico
11	70	Duodeno + metastasi epatica	10	Imatinib

Tabella 2 - Sintomi, diagnostica e trattamento chirurgico

Paziente	Diagnosi	Sede	EGDS	Ecoendoscopia	TAC addome	Trattamento
1	Emorragia digestiva	Stomaco (corpo)	Positiva	Positiva	Positiva	*Wedge resection* gastrica VLS
2	Emorragia digestiva	Stomaco (fondo)	Positiva	Positiva	Positiva	*Wedge resection* gastrica VLS
3	Presenza di massa	Stomaco (corpo)	Positiva	No	Positiva	Resezione gastrica
4	Emorragia digestiva	Digiuno	Positiva	No	Positiva	Resezione digiunale + imatinib + resezione epatica
5	Addome acuto	Digiuno (multifocale)	No	No	No	Resezione digiunale + chemioterapia
6	Occasionale	Digiuno	Negativa	No	Positiva	Resezione digiunale

Tabella 3 - Caratteristiche immunoistochimiche e *follow-up* dei pazienti operati

Paziente	mitosi/50 CFI	Ki67	CD117	CD34	ProtS-100	Vimentina	*Follow-up*
1	3	1%	+	+	-	+	Vivente a 16 mesi
2	6-7	<5%	++	++	-	-	Vivente a 15 mesi
3	6	<10%	+	+	-	n.v.	Vivente a 5 mesi
4	25-30	>10%	+	+	-	+	Vivente a 26 mesi
5	80-100	40%	-	-	-	++	Deceduto dopo 10 mesi
6	3	2%	+	-	n.v.	+++	Vivente a 4 mesi

L'evoluzione della malattia è stata rapidamente progressiva senza alcuna risposta alla chemioterapia di I e II linea e il paziente è deceduto dopo 10 mesi. In un caso (paziente 6) il riscontro è stato occasionale e la diagnosi confermata con digiunoscopia; è stata eseguita una resezione digiunale; l'esame istologico ha confermato la positività al CD 117 con 3 mitosi/50 CFI, ed il paziente è vivente a 4 mesi (Tabella 3).

L'ultimo paziente della nostra serie (paziente 11) si è presentato con emorragia digestiva; l'esofagogastrodigiunoscopia (EGDS) ha dimostrato una lesione ulcerata del duodeno, la cui biopsia ha posto diagnosi di GIST, confermato alla TAC, con un diametro di 10 cm e lesione metastatica al fegato. Il paziente è stato posto in trattamento con imatinib 400 mg/die che continua da 14 mesi, e alla TAC di controllo la lesione primitiva è ridotta in diametro del 60% mentre la metastasi epatica risulta stabile.

Risultati

I GIST sono tumori a comportamento incerto e per questo motivo devono essere distinti da quelle lesioni che sono chiaramente benigne o maligne, quali leiomiomi, schwannomi o leiomiosarcomi. L'ecoendoscopia ha permesso di identificare i GIST mostrando l'origine dalla muscolatura propria, l'aspetto lobulato e la diversa ecogenicità rispetto ai leiomiomi e schwannomi[4]. La conferma della diagnosi è stata resa possibile, solo negli ultimi anni, grazie all'identificazione immunoistochimica del CD 117, che è una proteina specifica dei GIST. La diagnosi di GIST dovrebbe essere posta solo in presenza di una positività al CD 117, tuttavia le caratteristiche morfologiche, ossia la presenza di cellule fusate ed epitelioidi, la storia clinica e l'esperienza dell'anatomo-patologo possono portare alla diagnosi di GIST pur in assenza di positività al CD 117[1]. Questa definizione ha aperto nuovi orientamenti secondo i quali, in caso di CD 117 negativo, bisogna approfondire lo studio a livello di biologia molecolare per dimostrare eventuali alterazioni del gene KIT[5]. La dimostrazione della proteina CD 117 è fondamentale anche a scopi terapeutici, poiché l'imatinib, l'inibitore selettivo dell'enzima tirosin-chinasi, deve essere somministrato solo quando il CD 117 è positivo[1].

La chirurgia rappresenta il trattamento di scelta dei GIST: l'obiettivo è la resezione completa con margine macroscopicamente indenne ed evitando la rottura del tumore. La dimostrazione che i GIST non danno metastasi

linfonodali, associata al fatto che la sede gastrica comporta un minore rischio di aggressività, ha consentito l'approccio VLS delle lesioni gastriche con un diametro inferiore a 5-6 cm col vantaggio di poter eseguire la resezione sotto controllo gastroscopico. Nella via VLS le lesioni della parete anteriore del corpo, grande curvatura e fondo possono essere resecate mediante approccio diretto con endo-suturatrice o dissettore ad ultrasuoni e successiva applicazione di suturatrice, mentre le lesioni della parete posteriore del corpo possono essere aggredite per via transgastrotomica o intragastrica con particolari *port* auto-espandibili[6]. Il limite della VLS è la sede della neoplasia: ossia, lesioni prossime alla giunzione gastroesofagea o al piloro comportano un rischio maggiore di stenosi.

Buoni risultati sono stati ottenuti in campo medico con l'avvento dell'imatinib soprattutto nel trattamento delle metastasi epatiche da GIST che, assieme alle peritoneali, sono le più frequenti[7], e delle lesioni non resecabili. Ciò è dimostrato anche in due dei nostri pazienti sottoposti a terapia con imatinib: nel primo si è ottenuta la riduzione volumetrica e funzionale delle metastasi epatiche e il paziente è stato sottoposto ad una resezione epatica radicale, mentre nell'altro paziente si è avuta una buona risposta con riduzione di circa il 60% della massa primitiva. Un ruolo importante, nel valutare la risposta terapeutica al trattamento, è svolto dalla TAC-PET, che fornisce informazioni sia sulle dimensioni e la densità del tumore che sull'attività metabolica[8].

Discussione

La nostra, seppur limitata, casistica è stata ottenuta negli ultimi 2 anni e pertanto l'indagine immunoistochimica per la positività del CD 117 è stata eseguita in tutti i pazienti. Ciò ha sicuramente permesso di selezionare i GIST da lesioni il cui comportamento è certo, benigno o maligno che esso sia. La diagnosi preoperatoria di GIST, ottenuta con biopsia endoscopica, ha determinato la scelta del trattamento chirurgico essendo il comportamento di questi tumori incerto. Se questa indicazione non è cambiata dall'era pre-imatinib[9] ai nostri giorni, ciò che invece ha sicuramente modificato il trattamento del GIST avanzato e metastatico è stata l'introduzione dell'imatinib, che ora è considerato il trattamento di prima scelta in questi casi non resecabili[3]. Tali casi possono, comunque, essere successivamente sottoposti a valutazione chirurgica per eventuale resezione. Quest'approccio aggressivo è supportato dal fatto che sono stati riportati casi di resistenza all'imatinib insorti dopo trattamento[10], i *follow-up* sono di breve durata ed infine il costo della terapia non è trascurabile. È quindi indicato un *follow-up*, con TAC-PET, del paziente in trattamento con imatinib per porre eventuale indicazione chirurgica qualora siano presenti le condizioni di resecabilità.

L'altro aspetto che merita considerazione è l'approccio VLS delle lesioni primitive dello stomaco, soprattutto se associato al *rendez-vous* endoscopico che permette una più precisa localizzazione della lesione e resezione con un margine di tessuto sano[6, 11, 12]. Nelle lesioni a sede intestinale, a nostro avviso, è indicato un intervento laparotomico, sia perché i GIST intestinali presentano un maggior rischio di aggressività, che per la possibilità di lesioni multifocali con il conseguente rischio di un intervento non macroscopicamente radicale.

Conclusioni

La chirurgia rappresenta il trattamento di scelta dei GIST, tuttavia, in presenza di lesioni non resecabili o con metastasi, una valida opzione è rappresentata dalla terapia con imatinib che, determinando una regressione della malattia, può permettere un successivo approccio chirurgico. La terapia adiuvante con imatinib è indicata in caso di lesioni ad elevato rischio di aggressività, grossi tumori con coinvolgimento di altri organi pur in resezioni R0 ed in caso di rottura del tumore durante la resezione.

Ringraziamenti

Si ringrazia per la collaborazione il Professor Giuseppe Zamboni.

Bibliografia

1. Berman J, O'Leary TJ. Gastrointestinal stromal tumor workshop. Hum Pathol 2001; 32: 578-82.
2. Fletcher CDM, Berman JJ, Corless C, *et al*. Diagnosis of gastrointestinal stromal tumors: a consensus approach. Hum Pathol 2002; 33: 459-65.
3. DeMatteo R, Heinrich MC, El-Rifai WM, *et al*. Clinical management of gastrointestinal stromal tumors: before and after STI-571. Hum Pathol 2002; 33: 466-77.
4. Okai T, Minamoto T, Ohtsubo K, *et al*. Endosomographic evaluation of c-kit positive gastrointestinal tumors. Abdom Imaging 2003; 28: 301-7.
5. Blay JY, Bonvalot S, Casali P, *et al*. Consensus meeting for the management of gastrointestinal stromal tumors. Report of the GIST Consensus Conference, 20-21 March 2004, under the auspices of ESMO. Ann Oncol 2005; 16: 566-78.
6. Avital S, Brasesco O, Szomstein S, *et al*. Technical considerations in laparoscopic resection of gastric neoplasms. Surg Endosc 2003; 17: 763-5.

7. Reddy MP, Reddy P, Lilien DL. F-18 FDG PET imaging in gastrointestinal stromal tumors. Clin Nucl Med 2003; 28: 677-9.
8. Goerres GW, Stupp R, Barghouth G, *et al*. The value of PET, CT and in-line PET/CT in patients with gastrointestinal stromal tumours: long-term outcome of treatment with imatinib mesylate. Eur J Nucl Med Mol Imaging 2005; 32: 153-62.
9. DeMatteo RP, Lewis JJ, Leung D, *et al*. Two hundred gastrointestinal stromal tumors: recurrence patterns and prognostic factors for survival. Ann Surg 2000; 231: 51-8.
10. Debiec-Rychter M, Cools J, Dumez H, *et al*. Mechanism of resistence to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. Gastroenterology 2005; 128: 270-9.
11. Yahchouchy-Chouillard E, Etienne JC, Fagniez PL, *et al*. A new "no touch" technique for the laparoscopic treatment of gastric stromal tumors. Surg Endosc 2002; 16: 962-4.
12. Ludwig K, Wilhelm L, Scharlan U, *et al*. Laparoscopic-endoscopic rendez-vous resection of gastric tumors. Surg Endosc 2002; 16: 1561-5.

Eur. J. Oncol., vol. 10, n. 2, pp. 129-135, 2005

Port site metastasis: è una complicanza specifica della chirurgia laparoscopica del carcinoma colorettale?

Port site metastasis: is this a specific side effect of laparoscopic surgery of colorectal cancer?

Francesca Natale, Massimiliano Marinaccio, Costanzo Natale
Dipartimento di Chirurgia, Azienda Ospedaliera - Universitaria, Ospedali Riuniti, Foggia, Italia

Riassunto

Finalità. **Viene presentata una revisione della letteratura sulla incidenza e sulle cause di insorgenza di metastasi sulle porte di entrata dei *trocars* nella chirurgia laparoscopica del carcinoma colorettale. I dati più recenti ridimensionano il problema equiparando l'incidenza di *port site metastasis* alla recidiva di malattia in sede di ferita in chirurgia *open*. Vengono riportati i principi di prevenzione della complicanza. *Pazienti e metodi.* Viene esaminata una serie omogenea di 92 pazienti operati in tre anni per carcinoma colorettale con procedura laparoscopica. Sono state eseguite 33 colectomie destre, 28 colectomie sinistre e 31 resezioni del retto. I principi oncologici seguiti sono i medesimi della chirurgia *open*. Accorgimenti di prevenzione adottati sono stati: incisioni adeguate per ridurre il trauma della parete, adeguato controllo dell'emostasi, *open laparoscopy*, modesti traumatismi con gli strumenti, scarsa o nessuna manipolazione del tumore, uso di contenitori per il preparato chirurgico, protezione con adatto dispositivo della minilaparotomia di servizio, insufflazione lenta e progressiva e desufflazione da un solo *trocar*. *Risultati.* Nel periodo di osservazione nessuna metastasi parietale è stata diagnosticata con la clinica e con la ecografia. *Conclusioni.* Le recidive di parete possono attualmente essere considerate il risultato di una sfortunata curva di apprendimento. Eur. J. Oncol., 10 (2), 129-135, 2005**

***Parole chiave: port site metastasis*, carcinoma colorettale, chirurgia laparoscopica**

Summary

Aim. **A review of the literature on the incidence and causes of the onset of trocar port metastases in the laparoscopic surgery of colorectal cancer is presented. The most recent data reappraise the problem, equating the incidence of port site metastases to that of disease recurrence at the site of open surgery. The principles of the prevention of complications are set forth. *Patients and methods*. An homogeneous series of 92 patients operated for colorectal cancer by laparoscopic surgery over three years is examined: 33 right colectomies, 28 left colectomies and 31 resections of the rectum were performed. The oncological principles adopted are the same as those of open surgery. The preventive techniques adopted were adequate incisions to reduce abdominal wall trauma, adequate control of haemostasis, open laparoscopy, limited traumatisms due to the use of instruments, limited or no handling of the tumour, use of bags for the surgical specimen, protection with a suitable device of the service minilaparotomy, slow and progressive insufflation, and desufflation through a single trocar. *Results.* In the period of observation no parietal metastases have been diagnosed by clinical examination or echography. *Conclusions.* Abdomen wall recurrences may today be considered the result of an unfortunate, early stage of the learning curve. Eur. J. Oncol., 10 (2), 129-135, 2005**

Key words: **port site metastasis, colorectal cancer, laparoscopic surgery**

Received/Pervenuto 28.2.2005 - Accepted/Accettato 30.3.2005
Address/Indirizzo: Dr. Francesca Natale, Via M. Natola 11, 71100 Foggia, Italia - E-mail: costanzo.natale@virgilio.it

Introduzione

L'accesso videolaparoscopico (VLS) (perché solo di accesso si tratta e non di una diversa chirurgia) nel trattamento delle neoplasie maligne addominali, risulta ancora fonte di numerose controversie.

Ciò nonostante, sono da anni riconosciute l'affidabilità e la riproducibilità della procedura, che ripete i medesimi principi di correttezza oncologica dell'accesso *open*. La procedura laparoscopica, che pure ha gli stessi tempi di ospedalizzazione e gli stessi costi di quella *open*, offre il vantaggio del minor dolore, di una più precoce ripresa della peristalsi, di una buona cosmesi e di una più rapida riabilitazione.

Uno dei più importanti motivi di controversia è rappresentato dalla comparsa di metastasi sui siti di introduzione dei *trocars*.

Il primo caso di questo fenomeno clinico è stato segnalato da Drouard *et al*[1] e si riferisce alla comparsa di una metastasi nel sito di introduzione di un *trocar* dopo colecistectomia eseguita per calcolosi con un carcinoma misconosciuto.

Altre segnalazioni sono seguite, anche relative ad ampie casistiche in cui erano rappresentate quasi tutte le neoplasie digestive ed in particolare colorettali[2-5].

Queste segnalazioni hanno frenato notevolmente l'entusiasmo iniziale dell'approccio laparoscopico, tanto che attualmente solo il 5-6% delle neoplasie addominali viene trattato con metodica videolaparoscopica[6, 7].

I dati pubblicati, tuttavia, dimostrano che verosimilmente l'approccio laparoscopico non è responsabile del fenomeno, e che molta importanza deve essere attribuita alla tecnica chirurgica adottata ed allo stadio della malattia.

D'altra parte, con l'aumento dell'esperienza e con una più accurata selezione dei pazienti, l'incidenza di tale complicanza ha toccato, più di recente, valori che non si discostano da quelli segnalati per la chirurgia *open*[8, 9].

Incidenza

Una stima affidabile dell'incidenza di *port site metastases* diventa difficile, perché le statistiche sono eterogenee per tipologia di neoplasia e per criteri di selezione. Un importante valore di riferimento ci deriva dagli studi di Hughes *et al* del 1983[10] e di Reilly *et al* del 1996[8] sull'incidenza degli impianti neoplastici sulla ferita laparotomica ed in particolar modo in corrispondenza dell'ombelico nei pazienti operati con tecnica *open* per neoplasie colorettali. Essa è risultata variabile dallo 0,6% all'1,5% su un totale di 3000 casi esaminati. Questa incidenza non è molto dissimile da quella denunciata nelle più recenti casistiche videolaparoscopiche.

Dall'inizio degli anni '90 l'incidenza di localizzazioni neoplastiche nelle sedi di introduzione dei *trocars* si è progressivamente ridotta, parallelamente alla migliore esperienza acquisita dagli autori[11].

In una interessante rassegna del 1995, Wexner e Cohen[12] riportano le esperienze di O'Rourke, di Nduka, di Gionnone e di Ngoi, registrando un'incidenza di *port site metastasis* variabile fra l'1,5% e il 21%.

Sulla scorta delle segnalazioni acquisite, Wexner e Cohen[12] ritengono il fenomeno particolarmente interessante per quattro motivi:

1) non tutte le metastasi si verificano nella sede di estrazione del preparato chirurgico;
2) il numero dei casi riportati rappresenta solo la punta dell'*iceberg*;
3) il fenomeno non è esclusivo delle lesioni avanzate;
4) il problema investe non solo i tumori dell'apparato digerente, ma anche altre neoplasie e soprattutto i tumori ovarici[13].

Viene naturale chiedersi se la recidiva parietale rappresenta una complicanza anche della chirurgia *open*. Molti chirurghi esperti e con larghe casistiche sanno che questa evenienza esiste e che esprime una progressione della malattia ed una prognosi infausta. Le segnalazioni in letteratura sono scarse[8,10], verosimilmente perché il fenomeno viene considerato una fatale evenienza legata allo stadio e alla biologia del tumore e non certo alla modalità dell'intervento.

Tuttavia, nelle casistiche videolaparoscopiche più recenti e più significative, l'incidenza delle recidive sulle porte dei *trocars* si è sensibilmente ridotta rispetto a quella denunciata da Wexner e Cohen nel 1995[12].

Lo studio controllato di Schiedek *et al*[11] riferisce una percentuale dello 0,2% su 399 casi operati.

Già in precedenza i dati del Clinical Outcome of Surgical Therapy Study Group (1,08%)[14] e quelli del registro dell'American Society of Colorectal Surgeons e della Society of American Gastrointestinal Endoscopic Surgeons (1,1%)[15] avevano confermato una diminuzione dell'incidenza delle *port site metastases* che tende a sovrapporsi a quella della chirurgia tradizionale.

La revisione più recente della letteratura (2003-2004) ha dimostrato che l'incidenza delle *port site metastases* è comparabile a quella delle metastasi parietali dopo chirurgia *open*[16-18].

La complicanza compare fra i 3 ed i 26 mesi, con una maggiore incidenza entro i 12 mesi, si presenta con uguale frequenza a livello dei siti di estrazione del pezzo e in corrispondenza dei *trocars*, è chiaramente prevalente nel trattamento delle neoplasie in stadio III, spesso associate

a carcinosi peritoneale (espressione di per sé di una prognosi infausta per elevata aggressività biologica).

Le metastasi parenchimali non sembrano rappresentare un fattore favorente l'insorgenza di metastasi sulla sede dei *trocars*. L'associazione della chemioterapia adiuvante sembra ridurre l'incidenza della complicanza.

Valutazione delle cause

Nella patogenesi delle metastasi nelle sedi di introduzione dei *trocars* concorrono diversi fattori, tutti però riconducibili all'impianto per contatto diretto di cellule neoplastiche. E' quindi necessaria l'esistenza di cellule neoplastiche libere, di un terreno di crescita e di una depressione immunitaria del paziente.

Le cause maggiormente invocate sono lo pneumoperitoneo, il gas utilizzato, il trauma dei tessuti dovuto alla introduzione dei *trocars*, la manipolazione viscerale (e quindi del tumore) con gli strumenti, la frequente reintroduzione dei *trocars*.

Una condizione frequentemente riportata in letteratura è quella relativa all'estrazione di un tumore maligno attraverso una minilaparotomia di servizio senza protezione alcuna. Le porte dei *trocars* possono venir contaminate da cellule neoplastiche che aderiscono ai *trocars*. Cellule neoplastiche sarebbero state infatti isolate dal liquido di lavaggio degli strumenti laparoscopici.

L'incidenza di citologia peritoneale positiva è risultata alquanto infrequente e circoscritta a neoplasie in stadi molto avanzati. Nello studio di Lucha *et al* [19] cellule maligne nel liquido peritoneale furono rinvenute in un solo caso di cancro del retto in stadio IV con carcinosi peritoneale.

Una certa importanza sulla contaminazione delle porte sembra essere legata anche al materiale dei *trocars*. Un interessante e complesso studio sperimentale di Brundell *et al* [20] ha dimostrato che l'adesione delle cellule neoplastiche è maggiore sui *trocars* metallici che su quelli di plastica, e che la differenza è statisticamente significativa anche in ordine alla capacità di contaminare le ferite.

Lo pneumoperitoneo gioca anch'esso un ruolo non indifferente nella patogenesi della complicanza in oggetto. Esso provoca un aumento della pressione endoaddominale e riduce il flusso ematico della parete addominale. Questo meccanismo si associerebbe ad un aumento della capacità di crescita delle cellule tumorali, come è stato recentemente dimostrato in ricerche sperimentali sui ratti[21].

Meno importanti sarebbero la turbolenza delle correnti di anidride carbonica[22] e le modificazioni che questa induce sull'equilibrio acido-base. La minore pericolosità dell'elio rispetto all'anidride carbonica non è stata dimostrata.

In un altro studio sperimentale è stato invece dimostrato che l'aumento di aggressività delle cellule neoplastiche indotto dallo pneumoperitoneo (con CO_2 o con He) viene abolito dagli inibitori delle metalloproteasi della matrice[23].

Maggiore importanza riveste il trauma tissutale grazie ai fattori di crescita che si sviluppano nei tessuti contusi ed ai coaguli che rappresentano un vero ostacolo ai meccanismi di difesa.

Accorgimenti di tecnica

Da sempre la chirurgia è stata un continuo divenire alla ricerca di soluzioni tecniche che potessero ridurre le complicanze. In modo particolare sono stati adoperati sempre più sofisticati accorgimenti per evitare o limitare prima l'inquinamento batterico e poi quello neoplastico delle ferite addominali.

I primi periodi della chirurgia laparoscopica avanzata sono stati invece caratterizzati soprattutto dalla necessità di verificare la fattibilità di alcuni interventi attraverso una laboriosa curva di apprendimento. Ciò ha comportato il trascurare alcuni accorgimenti che in chirurgia *open* erano ormai standardizzati.

Con adeguate incisioni è possibile introdurre i *trocars* senza traumatizzare i tessuti, evitando sanguinamenti e curando la buona tenuta. L'uso di materiali monouso rappresenta una garanzia per la riduzione della contaminazione cellulare. È assolutamente da evitare il movimento di entrata e di uscita dei *trocars*; una volta sistemati, questi devono essere tenuti sempre nella stessa posizione.

I movimenti degli strumenti in cavità addominale devono essere misurati in modo da evitare traumi ai visceri, soprattutto in prossimità del tumore. Questo deve essere manipolato il meno possibile e comunque non prima che siano stati completati i tempi preliminari. Le interruzioni intestinali devono essere eseguite con suturatrici meccaniche *endo-gastrointestinal anastomosis* (endo-GIA). Il preparato chirurgico deve essere preferibilmente introdotto, appena libero, in una sacca di dimensioni adeguate. La minilaparotomia deve essere di lunghezza sufficiente in base al volume del preparato chirurgico che deve essere estratto, e deve essere comunque protetta con un anello di diametro adeguato. Al termine dell'intervento, la minilaparotomia deve essere irrigata con acqua bidistillata (che ha azione citolitica) e così pure le porte dei *trocars*. Ricerche sperimentali hanno dimostrato che l'irrigazione con povidone, una miscela di tourolidina ed eparina, o cloruro di sodio, non apportano beneficio anche se non risultano dannose[24].

Uno studio sperimentale sugli effetti antiproliferativi dell'aspirina e dell'indometacina ha evidenziato che, a differenza dei promettenti risultati di uno studio *in vitro*,

non vi erano *in vivo* benefici clinico-terapeutici nella prevenzione delle metastasi peritoneali e nelle sedi dei *trocars*[25].

Una sperimentazione *in vivo* sui maiali ha consentito di ottenere soddisfacenti risultati nella riduzione dell'incidenza delle metastasi nelle sedi di introduzione dei *trocars,* mediante un aumento della pressione di insufflazione, riduzione degli episodi di desufflazione e di perdite di gas, e frequenti lavaggi endo-addominali[26].

Pazienti e metodi

Sono stati eseguiti in videolaparoscopia, dal 2002 al 2004, 92 (78 nella struttura complessa ospedaliera e 14 in quella universitaria) interventi di resezione ad intento curativo di neoplasie colorettali (Tabella 1).

L'età dei pazienti era compresa fra i 35 anni ed i 92 anni, con età media di 71,4 anni per il colon destro, 68,6 anni per il colon sinistro e 70,7 anni per il retto (Tabella 2).

L'incidenza del sesso è indicata nella Tabella 3, che mostra una prevalenza del sesso maschile di circa 2:1.

Lo stadio indicato secondo la classificazione di Dukes vede una prevalenza di pazienti in stadio Dukes B piuttosto uniforme fra le varie localizzazioni (Tabella 4).

Il volume della neoplasia era T3 nella maggioranza dei pazienti (Tabella 5).

Le metastasi linfonodali risultavano localizzate alla prima stazione nel 91% dei pazienti N+; l'incidenza degli N- era di oltre il 70% (Tabella 6).

Il *grading* risultava in prevalenza ad alta e media differenziazione (Tabella 7).

Nelle neoplasie del colon destro è stata eseguita una colectomia destra ed in quelle localizzate al colon sinistro una colectomia sinistra, secondo i criteri oncologici seguiti nella chirurgia *open* (Tabella 8).

Tabella 1 - Casistica neoplasie colorettali operate in VLS

Sede	N.	%
Colon dx	33	35,9
Colon sn	28	30,4
Retto	31	33,7
Totale	92	100,0

Tabella 2 - Casistica neoplasie colorettali operate in VLS: età

Sede	Età media	*Range*
Colon dx	71,4	53-92
Colon sn	68,6	49-86
Retto	70,7	35-85

Tabella 3 - Neoplasie colorettali operate in VLS: sesso

Sede	Maschi	Femmine	Totale
Colon dx	20	13	33
Colon sn	18	10	28
Retto	22	9	31
Totale	60	32	92

Tabella 4 - Neoplasie colorettali operate in VLS: stadio

Stadio	Colon dx N. (%)	Colon sn N. (%)	Retto N. (%)	Totale N. (%)
I Dukes A	5 (15,1)	5 (17,8)	2 (6,4)	12 (13,0)
II Dukes B	22 (66,7)	15 (53,6)	22 (70,1)	59 (64,2)
III Dukes C	6 (18,2)	8 (28,6)	7 (22,5)	21 (22,8)
IV Dukes D	-	-	-	-

Tabella 5 - Neoplasie colorettali operate in VLS: volume del tumore

Valore di T	Colon dx	Colon sn	Retto	Totale
T 1	5	6	2	13
T 2	1	3	8	12
T 3	26	17	17	60
T 4	1	2	4	7

Tabella 6 - Neoplasie colorettali operate in VLS: metastasi linfonodali

Valore di N	Colon dx	Colon sn	Retto	Totale
N-	26	20	24	70
N+	7	8	7	22
Totale	33	28	31	92

Tabella 7 - Neoplasie colorettali operate in VLS: grading

Grading	Colon dx	Colon sn	Retto	Totale
G 1	12	11	13	36
G 2	16	16	15	47
G 3	5	1	3	9

In un caso in cui esisteva un polipo degenerato del sigma, alla colectomia destra è stata associata una resezione segmentaria del sigma. In un altro paziente in cui vi erano localizzazioni neoplastiche multiple nel settore destro ed in quello sinistro, è stata eseguita una colectomia totale con ileo-rettostomia.

Nelle neoplasie del retto è stata eseguita una resezione anteriore in 29 casi ed una amputazione addomino-peri-

Tabella 8 - Interventi in VLS per neoplasie del colon

Intervento	N.	%
Emicolectomia dx	33[a]	54,1
Emicolectomia sn	28[b]	45,9
Totale	61	100,0

[a] In un caso è stata associata una resezione segmentaria del sigma
[b] In un caso è stata eseguita una colectomia totale con ileo-retto anastomosi per neoplasie multiple

neale in 2 casi. In tre pazienti con neoplasia a 4 cm dall'orifizio anale la ricostruzione è stata realizzata mediante anastomosi colo-anale (Tabella 9).

Le amputazioni addomino-perineali sono state eseguite nei casi di infiltrazione neoplastica del canale anale.

Le resezioni anteriori e le amputazioni addomino-perineali sono state realizzate sempre con exeresi totale del mesoretto e conservazione delle strutture nervose.

La laparoscopia è stata eseguita sempre in anestesia generale. Il primo accesso laparoscopico viene realizzato con microincisione (*open laparoscopy*) subito al di sopra dell'ombelico. La pressione di CO_2 endoaddominale viene tenuta fra 10 e 12 mm Hg. Al *trocar* da 12 mm, inserito in *open laparoscopy* e che viene utilizzato per l'ottica, si aggiungono altri 2 *trocars* (uno da 12 mm ed uno da 5 mm) all'ipocondrio e alla fossa iliaca di sinistra per il trattamento dei tumori del colon destro, e di destra per il trattamento dei tumori del colon sinistro e del retto.

Un ulteriore *trocar* da 5 mm può essere necessario all'epigastrio per l'emicolectomia destra e al fianco sinistro e/o in sede sovrapubica per l'emicolectomia sinistra e per la resezione del retto.

Un'esplorazione della cavità addominale (per escludere una condizione di carcinosi peritoneale) e del fegato viene eseguita prima di iniziare i tempi della resezione. Quando vi è il sospetto di una lesione ripetitiva epatica, viene effettuato un esame ecolaparoscopico, eventualmente con studio in color-doppler.

La desufflazione dello pneumoperitoneo viene effettuata attraverso un solo *trocar* e quindi le ferite dei *trocars* vengono irrigate con acqua bidistillata. Un drenaggio secondo Penrose viene collocato in prossimità delle anastomosi.

Una trasversostomia di protezione viene eseguita nelle resezioni anteriori con anastomosi sottomesorettale.

Tre interventi sono stati convertiti per il volume della neoplasia: uno per un tumore del sigma e due per un tumore del retto.

Tabella 9 - Interventi in VLS per neoplasie del retto

Intervento	N.	%
Resezione anteriore	29	93,6
- proctectomia subtotale	20	
- proctectomia totale	9[a]	
Amputazione addomino-perineale	2	6,4
Totale	31	100,0

[a] In tre casi con anastomosi colo-anale

Risultati

I pazienti operati in laparoscopia hanno meno dolore ed un addome più trattabile nei primi due giorni postoperatori, poi il decorso è sovrapponibile a quello dei pazienti operati con tecnica tradizionale. La canalizzazione è talvolta più precoce. Il tempo di degenza postoperatoria e la ripresa dell'alimentazione sono sovrapponibili a quelli della procedura *open*.

Fra le complicanze abbiamo lamentato una discreta incidenza di suppurazione della ferita della minilaparotomia di servizio, specie per l'emicolectomia destra. In due pazienti si è verificata un'occlusione intestinale precoce: una dopo emicolectomia destra per stenosi cicatriziale perianastomotica ed una dopo resezione anteriore per angolazione di un'ansa digiunale. Entrambi i pazienti sono stati rioperati con tecnica *open*. Il paziente operato di colectomia totale con ileo-retto anastomosi termino-terminale eseguita con suturatrice meccanica circolare ha presentato un sanguinamento discreto e persistente dell'anastomosi per 3-4 giorni, trattato con cure mediche. Una paziente operata di emicolectomia destra ha presentato precocemente (in prima giornata) i segni clinici ed ematochimici di una pancreatite acuta necrotico-emorragica. In dodicesima giornata, per il sovrapporsi di segni di sepsi, la paziente è stata rioperata per eseguire la necrosectomia, la toilette addominale e la collocazione di drenaggi peritoneali. La paziente è deceduta dopo due giorni dal reintervento (Tabella 10).

Nel *follow-up* a distanza i siti dei *trocars* e delle minilaparotomie di servizio sono stati oggetto di esame al momento del controllo ambulatoriale del paziente, mensile e bimestrale. L'esame del paziente è stato clinico e strumentale.

Tabella 10 - Complicanze

Tipo	N.	%
Infezione ferita	8	8,7
Occlusione intestinale precoce	2	2,2
Emorragia anastomosi ileo-rettale	1	1,1
Pancreatite acuta necrotico-emorragica	1[a]	1,1

[a] Paziente deceduta

Al momento nessun paziente ha presentato segni clinici ed ecografici di recidiva neoplastica sulla sede delle incisioni.

Considerazioni

I dati della nostra esperienza sull'accesso laparoscopico per la patologia neoplastica dell'addome sono circoscritti al trattamento laparoscopico del carcinoma colorettale. Si tratta di un'esperienza molto concentrata nel tempo (tre anni) per cui è necessario un periodo più lungo per poter trarre conclusioni positive sulla mancanza, nella nostra casistica, di metastasi nelle sedi di introduzione dei *trocars* o di estrazione del preparato chirurgico.

Tuttavia se si considera che il periodo di maggiore incidenza del fenomeno si verifica fra 3 e 12 mesi, con delle punte, riportate in letteratura, di 26 mesi, i nostri dati diventano significativi.

Nella nostra esperienza sono stati seguiti precisi criteri di esclusione riguardanti il tumore e il paziente. Sono infatti stati esclusi dalla procedura laparoscopica i pazienti con tumori voluminosi (T4 allo stadio preoperatorio), con carcinosi peritoneale, con metastasi epatiche. I tre pazienti convertiti avevano infatti una neoplasia T4 (sottostadiata prima dell'intervento).

Per quanto concerne il paziente, la vera controindicazione all'accesso laparoscopico è costituita dallo scompenso cardiaco congestizio. L'insufficienza ventilatoria e l'età non hanno costituito controindicazione alla procedura laparoscopica rispetto a quella *open*.

Come nella chirurgia *open*, meticolosi accorgimenti sono stati messi in atto durante la procedura laparoscopica fin dai primi casi (durante la curva di apprendimento): incisioni adeguate per evitare traumatismi dei tessuti, emostasi accurata, insufflazione lenta e progressiva, limitazione dei traumatismi, scarsa o nessuna manipolazione del tumore, uso di sacche per racchiudere il preparato chirurgico quando le dimensioni di questo lo hanno consentito, sezione dei visceri con endo-GIA, protezione della minilaparotomia di servizio con anello di giuste dimensioni, desufflazione da un solo *trocar*.

I vantaggi della laparoscopia nel trattamento della litiasi biliare e della patologia del giunto cardioesofageo hanno favorito l'estensione della procedura alle condizioni di patologa neoplastica. Ciò non è universalmente accettato soprattutto in considerazione delle iniziali segnalazioni di metastasi precoci sulle porte dei *trocars*.

Conclusioni

Una revisione della letteratura basata su studi randomizzati, su ricerche sperimentali *in vitro* e sugli animali, e su studi comparativi fra resezioni laparoscopiche e *open*, ha dimostrato che i primi rapporti allarmanti sull'alta incidenza di *port site metastases* si sono progressivamente ridimensionati fino ad incidenze omologhe a quelle delle recidive sulle ferite degli interventi laparotomici.

In questa prospettiva il ruolo della laparoscopia nel trattamento delle lesioni addominali maligne è destinato ad espandersi in futuro[27].

Nella esperienza di Patankar *et al*[28] i risultati a 10 anni delle resezioni laparoscopiche per carcinoma colorettale eseguite in un centro dedicato non sono diversi da quelli della chirurgia *open*.

Sulla base dell'esperienza di 613 pazienti operati con accesso laparoscopico, Meyer *et al*[29] ritengono che è giustificato aspettarsi un'espansione di questa procedura.

La chirurgia laparoscopica del carcinoma colorettale certamente presenta numerosi vantaggi e non si associa ad una maggiore incidenza di morbilità e di mortalità. Principi oncologici standardizzati e risultati a lungo termine sono paragonabili a quelli della chirurgia *open*[30]. Le recidive di parete possono attualmente essere considerate il risultato di una sfortunata curva di apprendimento[31].

Bibliografia

1. Drouard F, Delamare J, Capron JP. Cutaneous seeding of gallbladder cancer after laparoscopic cholecystectomy. N Engl J Med 1991; 325: 1316-8.
2. Champault G, Lauroy J, Rizk N, *et al*. Colonisation néoplastique des trajets de trocarts: faut-il cesser d'opérer les cancers digestifs par laparoscopie? Presse Med 1994; 23 (28): 1313.
3. Bouvier S. Metastases sur les trajets de trocarts après traitment coeliochirurgical des tumeurs digestives. J Coelio Chir 1994; 10: 13-22.
4. Montorsi M, Fumagalli U, Rosati R, *et al*. Early parietal recurrence of adenocarcinoma of the colon after laparoscopic colectomy. Br J Surg 1995; 82: 1036-7.
5. Fusco MA, Paluzzi MW. Abdominal wall recurrence after laparoscopic assisted colectomy for adenocarcinoma of the colon. Dis Col Rect 1993; 36: 858-61.
6. Desrousseaux B. Place de la vidéo-chirurgie dans le traitement des cancers digestifs abdominaux. Ann Chir 1994; 48: 10886-8.
7. Jaquet P, Elias D, Sugarbarker P. L'implantation tumorale dans les sites de cicatrisation aprés chirurgie des cancers digestifs. J Chir (Paris) 1996; 133: 175-82.
8. Reilly WT, Nelson H, O'Connel M. Wound recurrence following conventional treatment of colorectal cancer. Dis Colon Rectum 1996; 29: 200-7.
9. Huscher C, Silecchia G, Croce E, *et al*. Laparoscopic colorectal resection. A multicenter Italian study. Surg Endosc 1996; 10: 875-9.
10. Hughes ES, McDermott FT, Polglase AL, *et al*. Tumor recurrence in the abdominal wall scar tissue after large-bowel cancer surgery. Dis Colon Rectum 1983; 26: 571-2.

11. Schiedeck THK, Scwandner O, Baca I. Laparoscopic surgery fort the cure of colorectal cancer. Results of a German five center study. Dis Colon Rectum 2000; 43: 1-8.
12. Wexner SD, Cohen SM. Port site metastases after laparoscopic colorectal surgery for cure of malignancy. Br J Surg 1995; 82: 295-8.
13. Nagarsheth NP, Rahaman J, Cohen CJ, *et al*. The incidence of port-site metastases in gynecologic cancers. JSLS 2004; 8: 133-9.
14. Fleshman JW, Nelson H, Peters WR. Clinical outcomes of surgical therapy (COST) study group. Early results of laparoscopic surgery for colorectal cancer: retrospective analysis of 372 patients treated by clinical COST study group. Dis Colon Rectum 1996; 39: 53-8.
15. Vukasin P, Ortega A, Greene F. Wound recurrence following laparoscopic colon cancer resection. Results of the American Society of Colon and Rectal Surgeons laparoscopic registry. Dis Colon Rectum 1996; 39: 20-3.
16. Itano O, Watanabe T, Jinno H, *et al*. Port site metastasis of sigmoid colon cancer after a laparoscopic sigmoidectomy: report of a case. Surg Today 2003; 33: 379-82.
17. Kuhry E, Jeekel J, Bonjer HJ. Effect of laparoscopy on the immune system. Semin Laparosc Surg 2004; 11: 37-44.
18. Curet MJ. Port site metastases. Am J Surg 2004; 187: 705-12.
19. Lucha PA Jr, Ignacio R, Rowley D, *et al*. The incidence of positive peritoneal cytology in colon cancer: a prospective randomized blinded trial. Am Surg 2002; 68: 1018-21.
20. Brundell S, Tucker K, Chatterton B, *et al*. Experimental study of the material composition of laparoscopic ports on tumour cell adherence. Br J Surg 2002; 89 (7): 928-32.
21. Lundberg O, Kristoffersson A. Pneumoperitoneum impairs blood flow and augments tumor growth in the abdominal wall. Surg Endosc 2004; 18: 293-6.
22. Zayyan KS, Christie-Brown JS, Van Noorden S, *et al*. Rapid flow carbon dioxide laparoscopy disperses cancer cells into the peritoneal cavity but not the port sites in a new rat model. Surg Endosc 2003; 17: 273-7.
23. Ridgway PF, Smith A, Ziprin P. Pneumoperitoneum augmented tumor invasiveness is abolished by matrix metalloproteinase blockade. Surg Endosc 2002; 16: 533-6.
24. Wittich P, Mearadji A, Marquet RL. Irrigation of port sites: prevention of port site metastases? J Laparoendosc Adv Surg Tech A 2004; 14: 125-9.
25. Kinugasa S, Smith E, Drew PA, *et al*. Aspirin and indomethacin for the prevention of experimental port-site metastases. Surg Endosc 2004; 18: 834-8.
26. Brundell SM, Tucker K, Texler M, *et al*. Variables in the spread of tumor cells to trocars and port sites during operative laparoscopy. Surg Endosc 2002; 16: 1413-9.
27. Are C, Talamini MA. Laparoscopy and malignancy. J Laparoendosc Adv Surg Tech A 2005; 15: 38-47.
28. Patankar SK, Larach SW, Ferrara A, *et al*. Prospective comparison of laparoscopie vs. open resections for colorectal adenocarcinoma over a ten-year period. Dis Colon Rectum 2003; 46: 601-11.
29. Meyer C, Kanor MA, Rohr S, *et al*. Critical analysis of the laparoscopic approach in colorectal surgery based on the personal experience of 613 interventions. Bull Acad Natl Med 2003; 187: 507-19.
30. Barlehner E, Benhidjeb T, Anders S, *et al*. Laparoscopic surgery for colon and rectal cancer. Surg Technol Int 2004; 13: 93-9.
31. Zmora O, Gervaz P, Wexner SD. Trocar site recurrence in laparoscopic surgery for colorectal cancer. Surg Endosc 2001; *15*: 788-93

Eur. J. Oncol., vol. 10, n. 2, pp. 137-141, 2005

Trattamento del tumore non a piccole cellule (NSCLC) del polmone in stadio avanzato con l'associazione cisplatino e vinorelbina: risultati di uno studio multicentrico[a]

Cisplatin and vinorelbine in the treatment of advanced non small cell lung cancer (NSCLC): results of a multicentre study

Virginio Filipazzi*, Maria Teresa Cattaneo*, Paola Candido**, Daniela D'Adda ***, Luciano Isa ****, Gianfranco Pavia**, Benedetto Rho*****, Maria Grazia Corradini******, Nicoletta Tosca*, Luigi Isabella*, Gabriella Esani*, Sabrina Ferrario*, Anna Gambaro*, Giuseppe Colosini*******, Eugenia Damiani*, Walter Legnani**, Valentina Gambi*, Elisabetta Schiavello*, Cinzia Fasola*, Elena Piazza*

* Divisione di Oncologia, Ospedale "Luigi Sacco", Milano, Italia
** Divisione di Oncologia, Ospedale Rho (MI), Italia
*** Divisione di Pneumologia, Ospedale "Luigi Sacco", Milano, Italia
**** Divisione di Oncologia, Ospedale Gorgonzola (MI), Italia
***** Divisione di Pneumologia, Ospedale di Garbagnate (MI), Italia
****** Divisione di Oncologia, Ospedale Bollate, Milano, Italia
******* Divisione di Medicina, Ospedale Manerbio (BS), Italia

Riassunto

Finalità. **La chemioterapia rimane l'unica risorsa disponibile nel tumore non a piccole cellule (NSCLC) del polmone diagnosticato in fase avanzata. Diversi studi e metanalisi hanno infatti confermato il vantaggio in termini di sopravvivenza e gestione dei sintomi rispetto alla sola terapia di supporto.** ***Pazienti e metodi.*** **Gli autori riferiscono la loro esperienza con l'associazione chemioterapica cisplatino 80 mg/m² giorno 1 + vinorelbina 25 mg/m² giorni 1-8 per 3 cicli ogni 21 giorni su 138 pazienti affetti da NSCLC in fase avanzata.** ***Risultati.*** **Il tasso di risposte è stato del 33%, la sopravvivenza mediana registrata è stata di 10 mesi, mentre la sopravvivenza complessiva ad un anno è del 31% ed a 2 anni del 10%. Il trattamento è stato nel complesso ben tollerato e solo il 4% di pazienti lo ha interrotto per effetti collaterali.** ***Conclusioni.*** **Attualmente tale associazione si conferma una valida opzione e si inserisce tra quelle standard disponibili per il trattamento di questo ampio gruppo di pazienti. Eur. J. Oncol., 10 (2), 137-141, 2005**

Parole chiave: **chemioterapia, cisplatino, vinorelbina**

Summary

Background. **Polychemotherapy is the only treatment for non small cell lung cancer (NSCLC) diagnosed at a late stage. Many studies and metanalayses have confirmed the real impact on survival and on quality of life compared with supportive care.** ***Patients and methods.*** **The authors present their experience with association chemotherapy of cisplatin 80 mg/m² day 1 + vinorelbine 25 mg/m² days 1-8 for 3 cycles every 21 days, in 138 patients with advanced NSCLC.** ***Results.*** **After chemotherapy the response rate was 33% with a median survival time of 10 months, while the overall survival was 31% at one year and 10% at two years. The treatment was generally well tolerated and only 4% of patients had to interrupt it because of the side effects.** ***Conclusions.*** **The efficacy of this association regimen has been confirmed as treatment for advanced stage NSCLC, and it could be included among those considered gold standard treatment for this large group of patients. Eur. J. Oncol., 10 (2), 137-141, 2005**

Key words: **chemotherapy, cisplatin, vinorelbine**

Received/Pervenuto 22.2.2005 - Accepted/Accettato 29.3.2005
Address/Indirizzo: Dr. Virginio Filipazzi, U.O. Oncologia Medica, Ospedale "L. Sacco", Via GB Grassi 74, 20147 Milano, Italia
Tel. 0039/02/39042492 - E-mail: doc.gino@libero.it

[a] Studio del Gruppo Cooperativo Nord Milano

Introduzione

Il cancro del polmone è la neoplasia più frequente al mondo e detiene ormai da anni il primato come principale causa di morte per tumore. Gli istotipi squamoso, adenocarcinoma e carcinoma a grandi cellule, raggruppati nei "non microcitomi" (NSCLC), rappresentano circa l'80% di tutti i tumori polmonari e la maggior parte dei soggetti affetti da tale patologia non è operabile al momento della diagnosi. La prognosi in questi casi è infausta a breve termine e solo il 10% dei pazienti raggiunge l'anno di sopravvivenza con le sole terapie di supporto.

La chemioterapia rappresenta l'unica possibilità per un trattamento sistemico quando la malattia si presenta in forma metastatica. Una recente metanalisi ha confermato il vantaggio della chemioterapia rispetto alle sole terapie di supporto, sia in termini di sopravvivenza che di controllo dei sintomi[1].

Lo schema considerato standard prevede l'associazione di un derivato del platino con un farmaco di terza generazione (vinorelbina o gemcitabina o un taxano o un inibitore delle topoisomerasi).

Gli autori riferiscono la loro esperienza collaborativa con l'associazione cisplatino e vinorelbina (P-V) nel NSCLC in stadio metastatico.

Pazienti e metodi

Dal 1.1.1999 al 31.12.2002 abbiamo seguito 138 pazienti consecutivi affetti da NSCLC in IV stadio escluse le metastasi cerebrali, con malattia misurabile, età inferiore a 72 anni, *performance status* (PS) 0-2 secondo la scala dell'Eastern Cooperative Oncology Group (ECOG), assenza di malattie concomitanti scompensate, normalità della riserva midollare e delle funzionalità epatica e renale e nessun trattamento chemioterapico precedente. La diagnosi cito-istologica era indispensabile, mentre la stadiazione completa prevedeva broncoscopia, TAC torace, addome superiore ed encefalo, e scintigrafia ossea.

Lo schema terapeutico, dopo acquisizione del consenso informato, comprendeva: cisplatino, 80 mg/m^2 giorno 1, seguito da vinorelbina, 25 mg/m^2 giorni 1 e 8, ripetuti per 3 cicli ogni 21 giorni. Come profilassi antiemetica abbiamo utilizzato ondansetron 8 mg iv + desametasone 16 mg iv il giorno 1, e ondansetron 8 mg + metoclopramide 20 mg per os nei 3 giorni successivi alla somministrazione del platino. Lo schema di idratazione prevedeva l'infusione di 2000 ml di soluzione salina seguita da diuresi forzata con mannitolo 250 cc e furosemide 20 mg.

Una riduzione della dose del 50% del cisplatino o il rinvio di una settimana della terapia era previsto in caso di tossicità ematologica di grado 3 o superiore, o in caso di insufficienza renale (*clearance* della creatinina < 50 ml/min) conclamata e persistente, mentre la riduzione della dose della vinorelbina veniva eseguita in caso di insufficienza epatica (incremento delle transaminasi 3 volte il valore di partenza).

La valutazione della risposta veniva eseguita dopo 3 cicli di chemioterapia con una TAC *total body* secondo i parametri WHO. In caso di risposta obiettiva, il trattamento continuava fino a 6 cicli. La radioterapia palliativa veniva permessa come terapia sintomatica delle metastasi ossee, associata a pamidronato 90 mg iv ogni 28 giorni, o di quelle linfonodali. Il tempo alla progressione, la sopravvivenza mediana e globale sono state calcolate dalla data della diagnosi.

La tossicità è stata raccolta secondo i parametri del National Cancer Institute degli USA.

La qualità di vita è stata valutata con PS (ECOG), scala analogico visiva per il dolore e la valutazione dei sintomi. I pazienti venivano poi rivalutati clinicamente ogni mese e radiologicamente ogni 3 mesi durante il *follow-up*.

Risultati

Abbiamo trattato complessivamente 138 pazienti, di cui 130 valutabili per la risposta, mentre 2 soggetti sono deceduti per sepsi e tromboembolia polmonare, e 6 non hanno completato il trattamento per tossicità. Nella Tabella 1 sono elencate le caratteristiche dei pazienti: notiamo che oltre il 50% dei soggetti presentava più di una sede metastatica ed il 30% dei pazienti un PS già compromesso (PS = 2).

Le risposte evidenziate sono state: 40 (30%) risposte parziali (RP) , 41 (31%) stabilizzazioni (SD) e 49 (37%)

Tabella 1 - Caratteristiche dei pazienti

Numero		138
Valutabili		130
Sesso	(M/F)	116/22
Età media (*range*)		61 (33-72)
PS	0	51
	1	37
	2	42
Stadio IV	(escluse le metastasi cerebrali)	130
	Più sedi metastatiche	68
Istologia		
	Adenocarcinoma	62
	Carcinoma squamocellulare	68
	Carcinoma a grandi cellule	8

progressioni (PD). Tra gli 81 soggetti che avevano evidenziato un vantaggio clinico (RP+SD), 56 pazienti hanno continuato la terapia fino al 6° ciclo.

Alla rivalutazione dopo 6 cicli si rilevava un ulteriore incremento di 4 RP (3%) mentre gli altri pazienti confermavano il risultato ottenuto in precedenza. Nel complesso 22 pazienti venivano poi sottoposti a radioterapia palliativa toracica o ossea. Il tempo medio alla progressione (mTTP) per i *responders* è stato di 4 mesi (range 1-11), la sopravvivenza mediana (MST) di 10 mesi per tutto il gruppo e di 13 mesi per i *responders*, mentre le sopravvivenze a 1 e 2 anni sono state del 31% e 10% rispettivamente.

La Tabella 2 riassume i risultati e la fig. 1 evidenzia la curva di sopravvivenza.

Complessivamente sono stati somministrati 537 cicli di chemioterapia, con una media di 4 cicli a paziente (range 1-6). Il trattamento è stato nel complesso ben tollerato: solo 43 cicli, pari all'8%, sono stati rinviati per neutropenia, mentre un altro 17% è stato supportato da fattori di crescita per i granulociti neutrofili. In circa il 14% dei cicli è comparsa anemia che ha richiesto l'utilizzo di eritropoietina, mentre non sono state effettuate riduzioni di dose, anche se 6 soggetti (4%) hanno sospeso il trattamento per gli effetti collaterali della chemioterapia. Le tossicità sono riassunte nella Tabella 3. La qualità di vita è stata conservata ed un decremento del PS si è registrato solo nei pazienti in progressione di malattia, mentre nel 61% (RP+SD) si è assistito ad un miglioramento dei sintomi quali tosse, dolore e dispnea durante il trattamento.

Tabella 2 - Risultati dopo chemioterapia

	3 cicli		6 cicli
RP[a]	40 (30%)	→	44 (33%)
SD[b]	41 (31%)	→	12 (9%)
PD[c]	49 (39%)		
Totale	130		56
mTTP[d]	4 m (*range* 1-11)	Sopravvivenza a 1 anno: 31%	
MST[e]	10 m (*range* 2-36)	Sopravvivenza a 2 anni: 10%	

[a] Risposte parziali
[b] Stabilizzazioni
[c] Progressioni
[d] Tempo medio alla progressione
[e] Sopravvivenza mediana

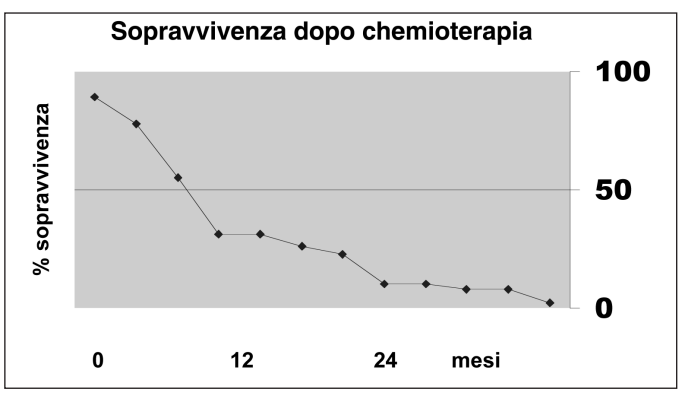

Fig. 1. Curva di sopravvivenza

Tabella 3 - Tossicità

Effetto collaterale	Grado I-II		Grado III-IV	
	N. pazienti	%	N. pazienti	%
Anemia	12	9,4	9	6,5
Neutropenia	65	50,0	33	25,0
Parestesie	4	3,0	2	1,8
Affaticamento	14	10,5	11	8,1
Emesi	16	12,0	3	2,1
Tossicità venosa	21	16,0	13	10,0
Stipsi	65	50,0	12	9,4
Altre	10	7,0	3	2,1

Discussione e Conclusioni

Sempre più frequentemente siamo chiamati a rispondere alle richieste di pazienti e loro famigliari riguardanti la possibilità di trattamento in casi di neoplasie polmonari metastatiche. Se fino agli anni '90 molti trattamenti venivano decisi più per l'insistenza degli interessati che per reali evidenze cliniche, oggi è possibile mostrare con certezza quali, anche se piccoli, reali vantaggi possa offrire un trattamento chemioterapico in questi soggetti.

Infatti la metanalisi del British Medical Journal[2] ha evidenziato un vantaggio significativo di sopravviveza a 6 mesi dell'ordine del 10%, che si stempera poi nel tempo. Lo schema terapeutico da privilegiare è quello contente platino ed un farmaco di terza generazione.

La nostra scelta sull'associazione platino-vinorelbina (P-V) deriva da esperienze precedenti nelle quali avevamo ottenuto discreti risultati in termini di efficacia e buona tollerabilità[3].

La riproducibilità dei risultati ottenibili con tale combinazione, inoltre, è stata dimostrata su oltre 250.000 pazienti trattati globalmente nel corso dell'ultimo decennio. Nella Tabella 4 sono rappresentati solo alcuni tra gli studi più rappresentativi in Italia e all'estero, con l'associazione P-V secondo un protocollo molto simile al nostro ed i cui risultati sono del tutto sovrapponibili sia in termini di tassi di risposte obiettive (RR) che di MST. Dobbiamo però sottolineare che nella nostra casistica tutti i

Tabella 4 - Confronto con risultati di studi analoghi

Autore (paese, anno)	Schema	N. pazienti	RR[a] (%)	MST[b] (mesi)
De Pierre *et al*[4] (Francia, 1994)	VNB[c] 30 mg/m²/sett DDP[d] 80 mg/m² g. 1 ogni 3 sett	116	43	7,7
Gil Deza *et al*[5] (Argentina, 1996)	VNB 30 mg/m²/sett DDP 100 mg/m² g. 1 ogni 4 sett	83	42	9,4
Colucci *et al*[6] (Italia, 1997)	VNB 25 mg/m² gg. 1-8 DDP 100 mg/m² g. 1 ogni 3 sett	50	47	9
Martoni *et al*[7] (Italia, 1998)	VNB 25 mg/m² gg. 1-8 DDP 60 mg/m² g. 1 ogni 3 sett	103	27	9,6
Wozniack *et al*[8] (USA, 1998)	VNB 25 mg/m²/sett DDP 100 mg/m² g. 1 ogni 4 sett	206	26	8
Tan *et al*[9] (internazionale, 2001)	VNB 30 mg/m²/sett DDP 80 mg/m² g. 1 ogni 3 sett	133	35	10
Kakolyris *et al*[10] (Grecia, 2002)	VNB 30 mg/m² gg. 1-8 DDP 80 mg/m² g. 1 ogni 3 sett	162	38	11
Martoni *et al*[11] (Italia, 2005)	VNB 25 mg/m² gg. 1-8 DDP 75 mg/m² g. 1 ogni 3 sett	137	32	11
Gruppo Cooperativa Nord Milano (Filipazzi *et al*, 2005)	VNB 25 mg/m² gg. 1- 8 DDP 80 mg/m² gg. 1 ogni 3 sett	138	33	10

[a] Tassi di risposta obiettiva
[b] Mediana di sopravvivenza
[c] Vinorelbina
[d] Dicloro di amminoplatino

pazienti sono in IV stadio, mentre gli studi segnalati contengono, in percentuali variabili, anche pazienti in stadio IIIb.

Gli studi di confronto con altri schemi contenenti cisplatino (Cis-Gem e Cis-Tax)[10, 12] hanno confermato la stessa efficacia in termini di risultati, mentre a livello di farmacoeconomia i costi sono risultati più contenuti per P-V[9-11]. Anche nei confronti di triplette più impegnative (ifosfamide - platino - vinorelbina / mitomicina - vindesina - platino) l'associazione P-V ha dimostrato pari efficacia con ridotta tossicità[9].

Tutti gli studi di confronto attualmente disponibili hanno confermato come la combinazione P-V si possa considerare uno *standard* terapeutico di facile applicazione, con risultati ripetibili e con costi accettabili sia per il singolo che per la collettività. Il ruolo delle triplette a base di platino rimane ancora controverso in questo gruppo di soggetti, mentre sono ormai disponibili farmaci per via orale che potranno ulteriormente migliorare la *compliance* dei pazienti ai trattamenti.

Bibliografia

1. Souhamni RL, Stewart LA, Arriagada R, *et al*. A data base meta-analysis of randomized trials of chemotherapy versus best supportive care in advanced non small cell lung cancer. Lung Cancer 1994; 24-5.
2. Non Small Cell Lung Cancer Collaborative Group. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomized clinical trials. BMJ 1995; 311: 899-909.
3. Frontini L, Candido P, Filipazzi V, *et al*. Cisplatin-vinorelbine combination chemotherapy in locally advanced NSCLC. Tumori 1996; 82: 57-60.
4. De Pierre A, Chastang C, Quoix E, *et al*. Vinorelbine versus vinorelbine and cisplatinum in advanced NSCLC. Ann Oncol 1994; 5: 37-42.
5. Gil Deza E, Balbiani L, Coppola F, *et al*. A phase III study of navelbine plus cisplatin in NSCLC stage IIIb or IV. Proceedings ASCO 1996; 15: abstract 1193.
6. Colucci G, Gebbia V, Galetta D, *et al*. Cisplatin and vinorelbine followed by ifosfamide plus epirubicin *vs* the opposite sequence in advanced NSCLC. A prospective randomized trial of GOIM. Br J Cancer 1997; 76: 1509-17.

7. Martoni A, Guaraldi M, Piana E, *et al*. Multicenter randomized clinical trial on high dose epirubicin plus cisplatinum versus vinorelbine plus cisplatin in advanced NSCLC. Lung Cancer 1998; 22: 31-8.
8. Wozniack AJ, Crowlei JJ, Balcerzack SP, *et al*. Randomized trial comparing cisplatin with cisplatin plus vinorelbine in the treatment of advanced NSCLC. A SWOG study. J Clin Oncol 1998; 16: 2459-65.
9. Tan H, Lee CT, Fung KJ, *et al*. Final results of prospective randomized phase III trial comparing vinorelbine and cisplatin versus vinorelbine, ifosfamide and cisplatin in metastatic NSCLC patients. Proceedings ASCO 2001; 21: abstract 1299.
10. Kakolyris S, Giatramanolaki A, Kouroussis C, *et al*. Preliminary results of a multicenter randomized phase III trial of docetaxel plus gemcitabine versus vinorelbine plus cisplatin in patients with advanced NSCLC. Proceedings ASCO 2002; 21: abstract 1182.
11. Martoni A, Marino A, Sperandi F, *et al*. Multicentre randomized phase III study comparing the same dose and schedule of cisplatin plus the same schedule of vinorelbine or gemcitabine in advanced non small cell lung cancer. Eur J Cancer 2005; 41: 81-92.
12. Scagliotti GV, De Marinis F, Rinaldi M, *et al*. A phase III randomized study comparing three platinum based doublets in advanced NSCLC. J Clin Oncol 2002; 1: 4285-91.

Eur. J. Oncol., vol. 10, n. 2, pp. 143-148, 2005

Mesotelioma pleurico da inusuale esposizione ad amianto. Resoconto di tre casi clinici

Pleural mesothelioma from unusual asbestos exposure. Report of three clinical cases

Arianna Binarelli*, Marinella Bertolotti**, Dario Mirabelli**, Stefano M. Candura*

* Scuola di Specializzazione in Medicina del Lavoro, Università degli Studi di Pavia e Divisione di Medicina del Lavoro, Fondazione Salvatore Maugeri, Clinica del Lavoro e della Riabilitazione, IRCCS, Istituto Scientifico di Pavia, Italia

** Servizio Universitario di Epidemiologia dei Tumori e Registro dei Mesoteliomi Maligni del Piemonte, CPO Piemonte Azienda Ospedaliera S. Giovanni Battista, Torino, Italia

Riassunto

***Finalità*. Richiamare l'attenzione sull'importanza dell'anamnesi lavorativa e ambientale per il corretto inquadramento eziologico del mesotelioma pleurico, neoplasia assai aggressiva che riconosce come principale fattore di rischio l'inalazione di fibre d'amianto. *Casi clinici*. Sono presentati tre casi, esemplificativi delle diverse modalità attraverso le quali si può realizzare un'esposizione inusuale, non strettamente professionale, all'amianto. Essi provengono da Casale Monferrato (Alessandria), dove dal 1907 al 1986 operò la principale industria italiana produttrice di manufatti in cemento-amianto e dove tuttora si registra un'alta incidenza di mesotelioma maligno. Il primo caso, una donna di 81 anni, è un mesotelioma pleurico da esposizione "professionale passiva", legato alla presenza di fibre nell'ambiente di lavoro e di vita. Il secondo caso, un uomo di 85 anni, è un mesotelioma da vicinanza, mentre il terzo, una donna di 77 anni, è un mesotelioma da esposizione domestica, per contatto con abiti di lavoro del coniuge. *Conclusioni*. Il mesotelioma pleurico può insorgere come conseguenza di esposizioni all'amianto inusuali e lontane nel tempo. In tali casi, un'accurata anamnesi lavorativa e ambientale è fondamentale ai fini della corretta diagnosi eziologica, alla quale conseguono complesse ricadute medico-legali. Eur. J. Oncol., 10 (2), 143-148, 2005**

***Parole chiave:* cancerogenesi ambientale, anamnesi lavorativa, immunoistochimica**

Summary

***Aim*. To highlight the importance of collecting detailed occupational and environmental histories for the correct aetiological assessment of pleural mesothelioma, a very aggressive malignancy that may be caused by the inhalation of asbestos fibres. *Case reports*. Three cases are presented, illustrative of the different modalities through which an unusual, not strictly occupational, asbestos exposure may occur. They come from Casale Monferrato (Piedmont, Italy), where from 1907 to 1986 the main Italian asbestos-cement factory operated, and where a high incidence of malignant mesothelioma is still recorded. The first case, an 81 year-old woman, is a pleural mesothelioma due to "passive occupational" exposure, related to the presence of fibres in the work and life environment. The second case, an 85 year-old man, is a neighbourhood mesothelioma, while the third, a 77 year-old woman, is a household exposure mesothelioma, due to contact with asbestos from the husband's working clothes. *Conclusions*. Pleural mesothelioma may develop as a consequence of unusual asbestos exposures even distant in time. In such cases, an in-depth occupational and environmental history is crucial for a complete aetiological assessment, upon which complex medical and legal consequences depend. Eur. J. Oncol., 10 (2), 143-148, 2005**

***Key words:* environmental carcinogenesis, occupational history, immunohistochemistry**

Received/Pervenuto 23.3.2005 - Accepted/Accettato 15.4.2005

Address/Indirizzo: Prof. Stefano M. Candura, Divisione di Medicina del Lavoro, Fondazione Maugeri, Via A. Ferrata 8, 27100 Pavia, Italia
Tel. 0039/0382/592740 – Fax 0039/0382/592701 - E-mail: scandura@fsm.it

Introduzione

Il mesotelioma pleurico è una neoplasia relativamente rara e assai aggressiva, che riconosce quale principale fattore di rischio l'inalazione di fibre di amianto (o asbesto)[1-3], materiale che ha trovato ampia utilizzazione in centinaia di processi industriali[4,5].

L'Italia (fino alla fine degli anni '80 secondo produttore mondiale di manufatti in amianto dopo l'ex Unione Sovietica) bandì estrazione, importazione/esportazione e impiego dell'asbesto (al pari degli USA e degli altri paesi dell'Europa occidentale) con la legge 27 marzo 1992, n. 257. L'amianto continua tuttavia a rappresentare un problema di salute pubblica per almeno tre ragioni[5,6]:

1) soggetti esposti in passato (specialmente in ambito lavorativo) sono tuttora a rischio (il mesotelioma può svilupparsi fino a 40 anni dopo l'esposizione[3,7]);
2) esiste tuttora una categoria di lavoratori esposti a rischio specifico, rappresentata dagli addetti a operazioni di smaltimento e bonifica, regolamentate dal DM 6 settembre 1994;
3) nei comuni ambienti di lavoro e di vita sono tuttora presenti manufatti in amianto che vanno inesorabilmente incontro a invecchiamento e disgregazione con liberazione di fibre nell'aria; si tratta di concentrazioni molto basse rispetto a quelle riscontrate in passato nei luoghi di lavoro; tuttavia queste dosi non possono essere trascurate in relazione sia al rischio neoplastico (che nel caso del mesotelioma è stocastico, ossia senza la possibilità di individuare con certezza una soglia di sicurezza), sia al concetto di dose cumulativa (le fibre inalate si accumulano nell'organismo accrescendo progressivamente il rischio per la salute)[1].

Il presente contributo si propone di richiamare l'attenzione sull'importanza dell'anamnesi lavorativa e ambientale per la corretta diagnosi eziologica del mesotelioma pleurico. Sono presentati tre casi clinici, esemplificativi delle diverse modalità attraverso le quali si può realizzare un'esposizione inusuale, non strettamente professionale, a fibre d'amianto. I pazienti provengono da Casale Monferrato (Alessandria), dove dal 1907 al 1986 operò la principale industria italiana produttrice di manufatti (lastre, ondulati, tubature) in cemento-amianto e dove tuttora si registra un'alta incidenza di mesotelioma maligno (pleurico e peritoneale) in entrambi i sessi[8]. I casi sono stati raccolti dal Registro dei Mesoteliomi Maligni (RMM) del Piemonte, che, per la raccolta dei dati anamnestici, utilizza un questionario strutturato[9] analogo al modello predisposto dall'ISPESL per il Registro Nazionale Mesoteliomi, cui il RMM afferisce[10].

Casi clinici

Caso n. 1

Il primo caso riguarda una donna di 81 anni, non fumatrice, giunta all'osservazione con astenia, dispnea ingravescente e abbondante (>1000 cc) versamento pleurico alla base polmonare sinistra, recidivante dopo toracentesi. L'anamnesi lavorativa e la storia residenziale rivelavano che la paziente aveva lavorato (con mansioni impiegatizie e segretariali) per 44 anni (dall'età di 17) presso un'azienda di autotrasporti di manufatti in cemento-amianto e di amianto sfuso, di proprietà del marito e adiacente alla loro abitazione. Il tetto dell'azienda (circa 370 m^2) era a sua volta in cemento-amianto e non aveva mai subito interventi o modifiche. Nel cortile prospicente l'azienda e l'abitazione erano lavati settimanalmente i mezzi di trasporto, con conseguente dispersione di fibre di asbesto.

L'analisi citologica del liquido pleurico evidenziava elementi cellulari atipici d'aspetto epitelioide; le indagini immunoistochimiche su campione bioptico fornivano un profilo compatibile con la diagnosi di mesotelioma: positività per citocheratine e HBME-1 (fig. 1); negatività per CEA, BerEP4 e LeuM1. La paziente fu trattata con due somministrazioni di interleuchina intrapleurica (3.000.000 UI) e con terapia palliativa. Dopo circa due anni e mezzo dalla diagnosi comparve un nodulo cutaneo ulcerato che l'esame istologico identificò come metastasi di mesotelioma. Le condizioni cliniche peggiorarono quindi rapidamente fino all'*exitus*.

Caso n. 2

Il secondo paziente è un uomo di 85 anni, non fumatore ed ex dirigente delle Ferrovie dello Stato, ricoverato per dispnea e astenia, residente dall'età di 36 anni a circa 150 metri in linea d'aria dalla citata fabbrica di manufatti in cemento-amianto (riferita la presenza, fino al 1986, di abbondante polvere bianca che si depositava all'interno dell'abitazione, sui davanzali e nel cortile); in precedenza il paziente aveva abitato nei pressi di una caserma con il tetto in cemento-amianto, mai sottoposto negli anni a interventi di bonifica.

La tomografia computerizzata (TC) del torace rivelava versamento pleurico alla base destra, parzialmente saccato, e, sempre a destra, diffuso ispessimento della pleura (mediastinica, laterale e diaframmatica), in alcuni punti irregolare e bottonuto (fig. 2). Nonostante l'esame citologico sul liquido pleurico non avesse evidenziato elementi cellulari neoplastici, il quadro tomografico e l'anamnesi residenziale furono ritenuti compatibili con la diagnosi di mesotelioma pleurico. Le condizioni cliniche peggiorarono nei sei mesi successivi, con aumento del versamento e retrazione dell'emitorace destro, fino all'*exitus*.

Caso n. 3

Il terzo caso è una donna di 77 anni, non fumatrice, ricoverata per dolore trafittivo alla base dell'emitorace sinistro, dispnea ingravescente e astenia. Dall'anamnesi lavorativa non si evidenziava esposizione a fibre di asbesto; tuttavia il coniuge aveva lavorato per dieci anni, dal 1956 al 1966, per una ditta che svolgeva lavori di manutenzione, verniciatura e sabbiatura all'interno della fabbrica di cemento-amianto, ed era solito tornare a casa con gli abiti da lavoro, che erano spazzolati e lavati dalla moglie due volte la settimana.

La TC del torace mostrava, a sinistra, cospicuo versamento pleurico con irregolari ispessimenti del contorno pleurico toraco-mediastinico e atelettasia da compressione (fig. 3). Le indagini microscopiche e immunoistochimiche condotte su biopsia pleurica portarono alla diagnosi di mesotelioma di tipo epiteliale: positività per calretinina (fig. 4), citocheratine e HBME-1; negatività per CEA, BerEP4 e cerbB2. La paziente è deceduta a circa 7 mesi dalla diagnosi.

Discussione

L'associazione tra esposizione ad asbesto e mesoteliomi maligni pleurici fu segnalata per la prima volta nel 1960, in seguito all'osservazione di un'alta incidenza di tali neoplasie tra i residenti nella North Western Cape Province del Sudafrica, un'area geografica circoscritta dove era estratta e trasportata la crocidolite (appartenente al gruppo degli anfiboli)[11]. Tale studio è di particolare interesse, non solo perché individuava la correlazione amianto-mesotelioma, ma anche perché evidenziava la possibilità di effetti avversi nella popolazione non lavorativa.

In Italia, su tale tema risultarono fondamentali le osservazioni cliniche ed epidemiologiche della Scuola torinese[12-14], con la dimostrazione di danni dovuti all'amianto (asbestosi "da vicinanza", placche e mesoteliomi pleurici) nei territori circostanti Balangero (sede di un importante giacimento di amianto crisotilo) e Casale Monferrato, città da dove provengono anche i tre casi di mesotelioma pleurico qui presentati, correlabili a pregressa, prolungata e inusuale esposizione ad amianto.

Il primo caso può essere definito da esposizione "professionale passiva", cioè legato alla presenza di fibre nell'ambiente di vita e di lavoro, anche se la mansione non prevedeva la manipolazione dei materiali (da sottolineare l'insolita comparsa di una metastasi cutanea e la sopravvivenza relativamente lunga). Il secondo caso è con ogni probabilità un mesotelioma pleurico da vicinanza, mentre il terzo può essere considerato da esposizione domestica, per contatto con abiti di lavoro contaminati portati a casa dal coniuge. In tutti i tre casi l'anamnesi ambientale e lavorativa, raccolta con l'ausilio di un questionario particolarmente dettagliato[9], è risultata fondamentale per la corretta diagnosi eziologica. Una valutazione dell'esposizione più tradizionale tipicamente sarebbe stata basata solo sulla raccolta della storia lavorativa e sull'identificazione come esposti dei soli addetti a tipiche lavorazioni dell'amianto (che tra l'altro nella zona di Casale Monferrato non mancano certo); questo tipo di valutazione dell'esposizione avrebbe portato a giudicare non esposti tutti e tre i casi qui presentati. A proposito degli ultimi due, ed a supporto della valutazione da noi proposta, vogliamo brevemente menzionare che: 1) il rischio di mesotelioma maligno della pleura da esposizione ambientale è stato ben documentato a Casale Monferrato[9,14]; 2) il rischio di morte per tumore maligno della pleura tra le mogli dei di-

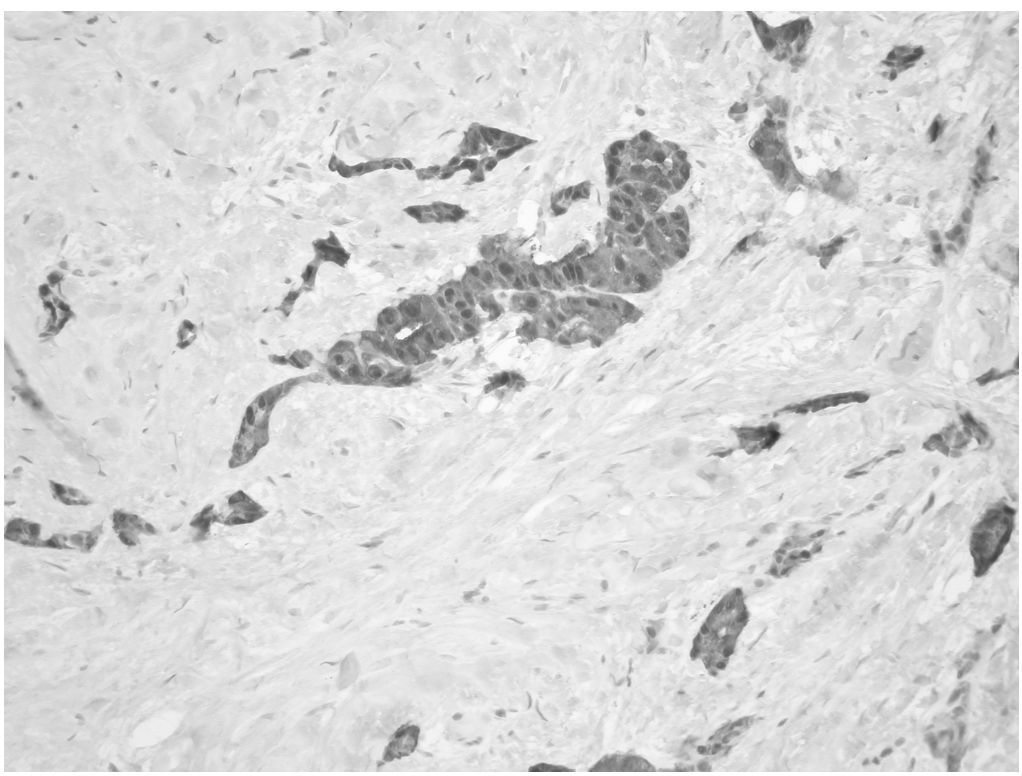

Fig. 1. Positività immunoistochimica per HBME-1, relativa a biopsia pleurica eseguita su caso di mesotelioma da esposizione "professionale passiva" ad amianto. 250X.

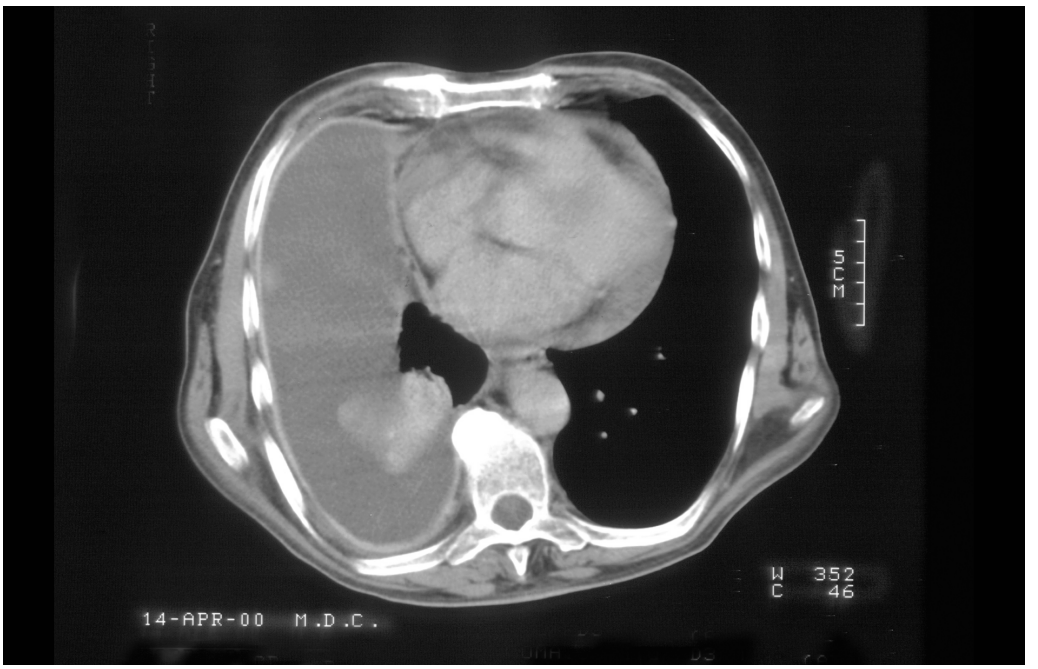

Fig. 2. TC del torace con mezzo di contrasto (mdc) (mesotelioma pleurico da vicinanza). A destra si apprezzano: ispessimento della pleura parietale con evidenza di retrazione del parenchima polmonare; ispessimento della pleura mediastinica; cospicuo versamento pleurico; versamento pericardico di lieve entità.

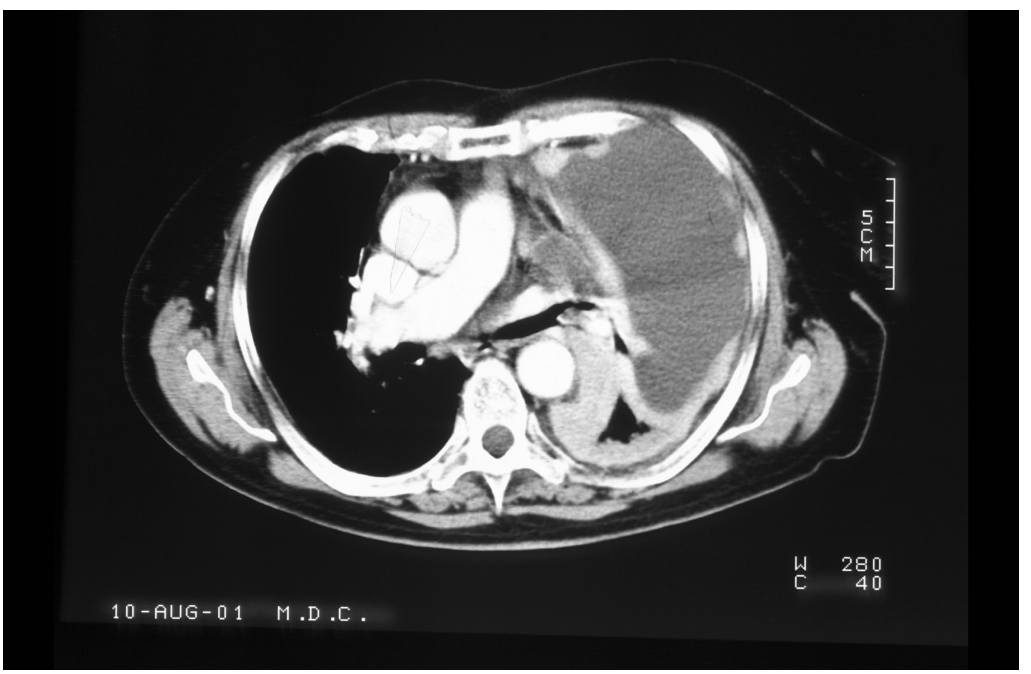

Fig. 3. TC del torace con mdc (mesotelioma pleurico da esposizione domestica ad amianto). A sinistra si apprezza cospicuo versamento pleurico che sottende la presenza di irregolari ispessimenti nodulari a carico della pleura parietale e viscerale. L'asse mediastinico risulta essere lievemente deviato a destra.

pendenti dello stabilimento del cemento-amianto è stato parimenti ben documentato[15].

I meccanismi responsabili dell'azione oncogena dell'asbesto sono noti solo in parte. L'amianto sembra comportarsi da promotore nella patogenesi del carcinoma broncogeno (agendo in sinergia con il fumo di tabacco), mentre nell'induzione del mesotelioma agisce come cancerogeno completo. In questo processo sembrano svolgere un ruolo centrale radicali liberi dell'ossigeno provvisti di azione genotossica sulle cellule mesoteliali[16, 17].

La diagnosi di mesotelioma pleurico può presentare difficoltà, per la relativa rarità della neoplasia, per l'aspecificità delle manifestazioni cliniche (dispnea, dolore toracico, versamento pleurico) e per l'estrema variabilità istopatologica. Al microscopio ottico il mesotelioma epiteliale (o epitelioide), la variante istologica più comune (50-75% dei casi), può presentare la stessa morfologia di metastasi pleuriche di natura carcinomatosa, mentre il più raro (15-20%) mesotelioma sarcomatoso (o sarcomatoide) può a sua volta simulare sarcomi; l'unico fenotipo patognomonico è il mesotelioma misto (o bifasico: 20-30%), dove cellule epitelioidi e sarcomatoidi coesistono;

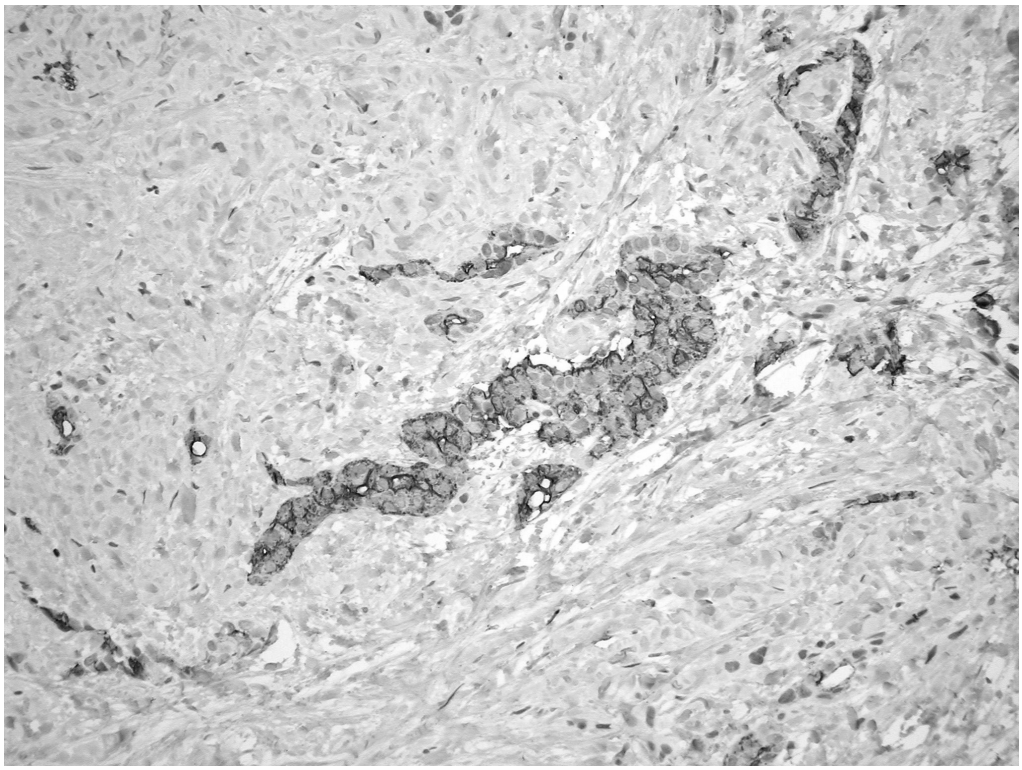

Fig. 4. Positività immunoistochimica per calretinina (biopsia pleurica del caso della fig. 3). 250X.

pure problematica risulta la diagnosi istologica delle varianti rare (a piccole cellule, desmoplastico, linfoistiocitoide)[16]. Per risolvere i dubbi diagnostici l'immunoistochimica (con applicazione di un pannello di anticorpi) spesso fornisce il contributo decisivo (come nel primo e nel terzo caso qui presentati): reazioni negative per l'antigene carcino-embrionario (CEA) e per le glicoproteine epiteliali Ber-EP4, LeuM1 e cerbB2 depongono contro la natura epiteliale della neoplasia, mentre positività per citocheratine, calretinina e HBME-1 indicano reattività del tessuto neoplastico con anticorpi che legano preferenzialmente cellule di origine mesoteliale[16, 18, 19]. Per queste ragioni l'inquadramento diagnostico del secondo caso come mesotelioma maligno è discutibile; tuttavia il caso, sulla base delle linee-guida approvate dall'ISPESL per il Registro Nazionale Mesoteliomi, è classificato dal RMM come "mesotelioma possibile"[20].

Secondo alcune proiezioni, la mortalità per mesotelioma nell'Europa occidentale potrebbe aumentare fino ad attorno il 2018[21], quando raggiungerebbe un valore circa doppio dell'attuale. 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: tra il 2012 e il 2024 è atteso un picco di mortalità per mesotelioma pleurico di circa 800 casi per anno[22]. Per tali ragioni, è indispensabile proseguire la sorveglianza sanitaria ed epidemiologica degli ex-esposti ad amianto, sia per identificare e seguire la patologia derivante dal passato sia per valutare l'effetto delle basse esposizioni verificatesi negli ultimi anni. Un contributo sostanziale in proposito, soprattutto al fine del riconoscimento di disomogeneità geografiche e di concentrazioni di casi, si è ottenuto grazie all'attuazione del registro nazionale dei mesoteliomi (ex art. 36 del D.lgs. 277/1991), costituito dall'insieme dei registri regionali, ulteriore passo verso l'identificazione di sorgenti non note di esposizione ad amianto[13] dopo l'esperienza dell'Atlante comunale di mortalità per tumore maligno della pleura[23]; inoltre, come strumento di sorveglianza epidemiologica, il registro potrà contribuire alla verifica dell'innocuità dei prodotti fibrosi entrati nella produzione come sostituti dell'amianto.

Conclusioni

I tre casi presentati indicano che il mesotelioma pleurico può insorgere in età avanzata come conseguenza di esposizioni all'amianto inusuali e lontane nel tempo, richiamando l'importanza di un'accurata anamnesi lavorativa e ambientale ai fini di una corretta diagnosi eziologica, alla quale conseguono complesse ricadute medico-legali in ambito penale, civile e assicurativo[24].

Ringraziamenti

Si ringraziano il Dott. Carlo Poletti e la Direzione Sanitaria del Presidio Ospedaliero S. Spirito di Casale Monferrato per avere

gentilmente fornito le immagini tomografiche. Si ringrazia inoltre la Dott.ssa Laura Villani, del Servizio di Anatomia Patologica della Fondazione Salvatore Maugeri di Pavia, per la revisione critica dei preparati istopatologici.

Bibliografia

1. Selikoff IJ, Lee DHK. Asbestos and disease. New York: Academic Press, 1978.
2. Antman KH. Natural history and epidemiology of malignant mesothelioma. Chest 1993; 103: 373S-6S.
3. Vogelzang NJ. Emerging insights into the biology and therapy of malignant mesothelioma. Semin Oncol 2002; 29 suppl 18: 35-42.
4. Candura F, Candura SM. Elementi di tecnologia industriale a uso dei cultori di medicina del lavoro. Piacenza: CELT, 2002.
5. American Thoracic Society Documents. Diagnosis and initial management of nonmalignant diseases related to asbestos. Am J Respir Crit Care Med 2004; 170: 691-715.
6. Chiappino G. Il problema amianto oggi. G Ital Med Lav Erg 1998; 20: 200-2.
7. Lanphear BP, Buncher CR. Latent period of malignant mesothelioma of occupational origin. J Occup Med 1992; 7: 718-22.
8. Ivaldi C, Dalmasso P, Nesti M, *et al*. Malignant mesothelioma registry from Piedmont. Incidence in 1990-1995. Epidemiol Prev 1999; 23: 85-6.
9. Magnani C, Agudo A, Gonzalez CA, *et al*. Multicentric study on malignant pleural mesothelioma and non-occupational exposure to asbestos. Br J Cancer 2000; 83: 104-11.
10. Nesti M, Marinaccio A, Silvestri S (curatori). Il registro nazionale dei mesoteliomi. Primo rapporto. Roma: ISPESL, 2002.
11. Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. Br J Ind Med 1960; 17: 260-71.
12. Rubino GF, Scansetti G, Donna A, *et al*. Epidemiology of pleural mesothelioma in North-western Italy. Br J Ind Med 1972; 29: 436-42.
13. Magnani C, Mollo F, Paoletti L, *et al*. Asbestos lung burden and asbestosis after occupational and environmental exposure in an asbestos cement manufacturing area: a necropsy study. Occup Environ Med 1998; 55: 840-6.
14. Magnani C, Dalmasso P, Biggeri A, *et al*. Increased risk of malignant mesothelioma of the pleura after residential or domestic exposure to asbestos: a case control study in Casale Monferrato, Italy. Environ Health Perspect 2001; 109: 915-9.
15. Magnani C, Terracini B, Ivaldi C, *et al*. A cohort study on mortality among wives of workers in the asbestos cement industry in Casale Monferrato, Italy. Br J Ind Med 1993; 50: 779-84.
16. Attanoos RL, Gibbs AR. Pathology of malignant mesothelioma. Histopathology 1997; 30: 403-18.
17. Walker C, Everitt J, Barret JC. Possible cellular and molecular mechanisms for asbestos carcinogenicity. Am J Ind Med 1992; 21: 253-73.
18. King JE, Hasleton PS. Immunohistochemistry and the diagnosis of malignant mesothelioma. Histopathology 2001; 38: 471-6.
19. Ordóñez NG. The immunohistochemical diagnosis of mesothelioma. A comparative study of epithelioid mesothelioma and lung adenocarcinoma. Am J Surg Pathol 2003; 27: 1031-51.
20. Moccaldi A, Palmi S, Nesti M. Linee guida per la rilevazione e la definizione dei casi di mesotelioma maligno e la trasmissione delle informazioni all'ISPESL da parte dei centri operativi regionali. Roma: ISPESL, 2003. In: http://www.ispesl.it/ispesl/sitorenam/index.asp
21. Peto J, Decarli A, La Vecchia C, *et al*. The European mesothelioma epidemic. Br J Cancer 1999; 79: 666-72.
22. Marinaccio A, Montanaro F, Mastrantonio M, *et al*. Predictions of mortality from pleural mesotelioma in Italy: a model based on asbestos consumption figures supports results from age-period-cohort models. Int J Cancer 2005; 115: 142-7.
23. Mastrantonio M, Belli S, Binazzi A, *et al*. La mortalità per tumore maligno della pleura nei comuni italiani (1988-1997). Istituto Superiore di Sanità, 2002. Rapporti Istisan, 02/12; 27 pp.
24. Neri G. Gli aspetti medico-legali delle patologie asbesto correlate. Difesa Sociale 2002; 81: 63-76.

Eur. J. Oncol., vol. 10, n. 2, pp. 149-153, 2005

Collegium Ramazzini
presents

FRAMING THE FUTURE IN LIGHT OF THE PAST: LIVING IN A CHEMICAL WORLD

3rd International
Scientific Conference
Bologna - Italy
September 18-21, 2005

Keynote Addresses

Cancer as a global problem, today and into the future
Peter Boyle, France

The relationship between prenatal environmental exposures and developmental outcomes, cancer risk and respiratory health in a prospective cohort study
Frederica Perera, USA

Human exposures during the World Trade Center aftermath: what we could and could not conclude and goals for the future
Paul Lioy, USA

The European Chemical Policy 2005: REACH and the Water Framework Directive
Steven Eisenreich, Italy

Interpreting cancer trends
Michael Thun, USA

EFSA's mission: harmonization of food and feed risk assessment approaches across Europe
Herman Koeter, Belgium

Scientific Sessions
I. IDENTIFYING AND PREVENTING HAZARDS IN THE ENVIRONMENT AND AT WORK

Endocrine Disruptors

Co-chairs Mary Wolff, USA & Eva Buiatti, Italy

- Risk assessment of endocrine disrupters: the role of toxicological studies
  Alberto Mantovani, Italy
- Biomonitoring exposure to emerging environmental chemicals
  Antonia Calafat, USA
- Phthalates and their impact on infant health
  Katharina Main, Denmark
- Session Summary
  Mary Wolff, USA

Air Pollution and Non-fibrous Particles

Co-chairs: Peter Wardenbach, Germany & Paul Lioy, USA

- Nanotoxicology: exposure routes, biokinetics and effects of nanosized particles
  Günter Oberdörster, USA

- Carcinogenicity studies with granular dusts in experimental animals
  Markus Roller, Germany
- Lung cancer risk of occupational exposure to diesel motor emissions and coal mine dust
  Karl-Heinz Jöckel & Barbara Hoffmann, Germany
- Semi-volatile and volatile mixtures emitted and deposited indoors: dynamics, chemistry and implications for human exposure and health
  Paul Lioy, USA
- WHO evaluation of long term effects of particulate air pollution
  Michal Krzyzanowski, Germany

Benzene and Other Gasoline Constituents/Additives
Co-chairs: Fiorella Belpoggi, Italy & Myron Mehlman, USA
- Benzene and multiple myeloma
  Peter Infante, USA
- Benzene and cancer risk in China
  Richard Hayes, USA
- The Health Watch case control study: the story so far
  Deborah Glass, Australia

Nanotechnology and Emerging Industries
Co-chairs: John Bucher & Michael Luster, USA
- Health and environmental implications of engineered nanoparticles
  Kevin Ausman, USA
- New advances in nanomedicine: from basic to clinical
  Chiming Wei, USA
- Getting it right the first time: developing nanotechnology while protecting workers, public health and the environment
  John Balbus, USA
- Nanoparticle research at the National Institute for Occupational Safety and Health: focus on nanotubes
  Michael Luster, USA
- The National Toxicology Program nanotechnology safety assessment initiative
  John Bucher, USA

Long Term Carcinogenesis Bioassays and Other Testing Strategies
Co-chairs: James Huff, USA & Morando Soffritti, Italy
- Milestones in chemical carcinogenesis bioassays
  James Huff, USA
- First experimental demonstration of the carcinogenic effects of aspartame administered in feed to Sprague-Dawley rats
  Fiorella Belpoggi, Italy
- Results of long term carcinogenesis bioassay on Sprague-Dawley rats exposed to sodium arsenite administered in drinking water
  Morando Soffritti, Italy
- Applications of magnetic resonance microscopy to the long term carcinogenesis bioassay
  Robert Maronpot, USA
- Use of long term carcinogenicity bioassays in the IARC Monographs
  Vincent James Cogliano, France

Asbestos and Man-made Mineral Fibers
Co-chairs: Tushar Joshi, India & Arthur Frank, USA
- Measurements of asbestos fibers in tissues
  Ronald Dodson, USA
- Health effects and future health risks among World Trade Center responders
  Steve Levin, USA
- Ban on asbestos diaphragms in the chlorine related chemical industry and efforts toward a world wide ban
  Fernanda Giannasi, Brazil
- Asbestos ban in India: challenges ahead
  Tushar Joshi, India

Use and Misuse of Mechanistic Data in Risk Assessment
Co-chairs: Lorenzo Tomatis, Italy & Ronald Melnick, USA
- Introduction to the use and misuse of mechanistic data in risk assessment
  Ronald Melnick, USA
- The difficult integration of mechanistic data into epidemiology
  Paolo Vineis, Italy
- Collision of basic and applied approaches to risk assessment of thyroid toxicants
  Tom Zoeller, USA
- Evaluation and prediction of risks of environmental estrogens
  Wade Welshons, USA
- Gene-environment interactions: uses and misuses of mechanistic data
  Kari Hemminki, Germany
- Manufactured uncertainty and public health
  David Michaels, USA

Radiation
Co-chairs: Anders Ahlbom, Sweden & David Hoel, USA
- Childhood cancer in relation to high voltage power lines: results from a recent study in England and Wales and comparison with previous studies
  Gerald Draper, UK
- Mobile telephony and tumor risk: assessment of current data
  Anders Ahlbom, Sweden
- Two year chronic bioassay study of rats exposed to a 1.6 GHz radio frequency signal
  Larry Anderson, USA
- An overview of recent epidemiological studies involving ionising radiation
  David Hoel, USA
- Results of long term carcinogenesis mega-experiment on Sprague-Dawley rats exposed to gamma-radiation at six weeks of age
  Morando Soffritti, Italy

II. REDUCING AND MANAGING RISK IN HIGH HAZARD SECTORS

Health Care
Co-chairs: Melissa McDiarmid, USA & Brigitte Froneberg, Switzerland
- Chemical hazards in health care: high hazard, high risk but low protection
  Melissa McDiarmid, USA
- Residual hazard assessment related to handling of antineoplastic drugs: safety system evolution and quality assurance of analytical measurement
  Roberta Turci, Italy
- Hazardous anticancer drugs in healthcare: environmental exposure assessment
  Tom Connor, USA
- Chemical safety and health conditions among Hungarian hospital nurses
  Anna Tompa, Hungary
- National and international response to healthcare hazards
  Brigitte Froneberg, Switzerland
- Environmental impact of the healthcare industry
  Lillian Corra, Argentina
- Beyond managing healthcare risks: the health promoting hospital initiative
  Carlos Santos-Burgoa, Mexico
- Handling anticancer drugs: from risk identification to risk management
  Marja Sorsa, Finland

Hazardous Waste
Co-chairs: Ellen Silbergeld, USA & Stanislaw Tarkowski, Poland
- Hazardous wastes: recognition of the problem and response
  Henrik Harjula, France
- Exposure pathways and assessment

Peter Lees, USA
- Heath effects of hazardous waste
  Tom Sinks, USA
- Developments in technology of waste reduction and disposal
  Philip Rushbrook, UK
- New directions in managing hazardous waste from the industry perspective
  Lynn Johnson, USA
- Cancer mortality in Campania, Italy: an area with multiple toxic dumping sites
  Pietro Comba, Italy
- Panel discussion: can we have international control of hazardous wastes?
  Ellen Silbergeld, USA & Stanislaw Tarkowski, Poland

Genetic, Environment and Effect Modulators
Co-Chairs: Kenneth Olden, USA & Massimo Crespi, Italy
- Toxicogenomics: new tools for studying pathways to disease
  Kenneth Olden, USA
  Transcriptional profiling and functional genomics reveals a role of Ahr transcription factor in nephrogenesis
  Kenneth Ramos, USA
- Toxicoproteomics in acute liver injury: differential expression in the liver and serum proteomes
  Alex Merrick, USA
- Gene expression alterations in immune system pathways following exposure to immuno suppressive chemicals
  Dori Germolec, USA

Evaluating Energy Technology Risks
Chair: J. Michael Davis, USA
- Systematic approach to evaluating trade-offs among fuel options: the lesson of MTBE
  J. Michael Davis, USA
- Global burden of disease from combustion mismanagement
  Kirk Smith, USA
- Environmental impact and costs of energy
  Ari Rabl, France

Child Health
Co-chairs: Philip Landrigan, USA & Jenny Pronczuk de Garbino, Switzerland
- New developments in children's environmental health in Europe
  Giorgio Tamburlini, Italy
- Environmental impacts on children's health in Southeast Asia
  Mathuros Ruchirawat, Thailand
- health and the environment in Latin America
  Raul Arjona Harari, Ecuador
- Only one chance to develop a brain: consequences of developmental neurotoxicity
  Philippe Grandjean, Denmark
- The epidemic of child obesity
  Richard Jackson, USA

Weapon Destruction
Co-Chairs: John Bailar, USA & Ralf Trapp, The Netherlands
- Worldwide governmental efforts to locate and destroy chemical weapons and weapon materials: minimizing risk in transport and destruction
  Ralf Trapp, The Netherlands
- Clean-up of Rocky Flats nuclear site: radiation and beyond
  Daniel Teitelbaum, USA
- Risks involved in the transport of toxic chemicals
  David Hoel, USA
- Health and environmental threats associated with the destruction of chemical weapons
  Jiri Matousek, Czech Republic
- Options for the destruction of chemical weapons and management of the associated risks
  Ron Manley, UK

III. TOOLS AND STRATEGIES TO REDUCE RISK: APPLYING SCIENCE TO ACHIEVE PREVENTION

Agriculture
Co-Chairs: Jane Hoppin, USA & Vittorio Silano, Italy
- The assessment of pesticide exposure among farmers and their children in Nicaragua using saliva biomonitoring
  Chensheng (Alex) Lu, USA
- Chemical hazard communication comprehensibility in South Africa: implications for the adoption of the Globally Harmonised System for Chemical Hazard Classification (GHS)
  Leslie London, South Africa
- Pesticide and adult respiratory outcomes
  Jane Hoppin, USA
- Characteristics of 4895 cases of pesticide poisoning in the North China countryside
  Zhao-lin Xia, China
- Cancer and pesticides: an overview and some results of the Italian multicentre case-control study on hematolymphopoietic malignancies
  Lucia Miligi, Italy
- Pesticides and Parkinson's disease
  Beate Ritz, USA
- Risk assessment of botanicals and botanical preparations widely used as food supplements and related products
  Vittorio Silano, Italy

Chlorinated Solvents
Co-chairs: David Ozonoff, USA & Raul Arjona Harari, Ecuador
- Potential health effects of chlorinated solvent exposure
  Avima Ruder, USA
- Science and policy risk assessment of chlorinated ethenes
  Cristina Rudèn, Sweden
- Induction of peroxisome proliferation by trichlorethylene and perchloroethylene: implications for risk assessment
  Ronald Melnick, USA

Construction
Co-chairs: Anders Englund, Sweden & Knut Ringen, USA
- Carcinogens in the construction trade
  Bengt Jarvholm, Sweden
- Exposure to solvents, epoxy resins and other chemicals in the painting and construction trades
  Cor van Duivenbooden, Netherlands
- Exposure to PAH dermal contamination in asphalt road pavers
  Vito Foà & Laura Campo, Italy
- Frequency and quality of radiation monitoring of construction workers at two gaseous diffusion plants
  Eula Bingham & Knut Ringen, USA

ROUNDTABLE SESSION

Social and Economic Perspectives on Environmental and Occupational Hazards
Co-chairs: Leslie Boden, USA & Andrew Watterson, UK
- Women and labor conditions in China
  Marina Thorborg, Sweden
- Valuing the adult health effects of air pollution in Chinese cities
  Robert Mead, USA
- Factors affecting the economic impacts of occupational injuries and illnessess on workers
  Les Boden, USA
- The economic costs of health service treatments for asbestos-related mesothelioma deaths
  Andrew Watterson, UK
- Applying cost analyses to drive policy that protects children: mercury as a case study
  Leo Trasande, USA

EUROPEAN JOURNAL OF ONCOLOGY

GIORNALE EUROPEO DI ONCOLOGIA

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

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

The journal is a quarterly publication

INFORMATION AND INSTRUCTIONS TO AUTHORS

INFORMATION

The European Journal of Oncology publishes contributions in the various areas of oncology: biology, epidemiology, pathology and clinical medicine.

Contributions may be in the form of:

- editorials;
- general reviews;
- original studies and research;
- clinical case reports;
- brief communications;
- letters to the Editors.

The official languages of the journal are Italian and English.

Acceptance of contributions for publication is subject to review by referees chosen from experts in the various fields. The Editors may require modifications to manuscripts, as suggested by the referees, and all adjustments necessary to maintain homogeneity of style in the journal. Editorials and critical reviews may be solicited by the Editors, or submitted without request. In all cases they must adhere to the instructions to Authors, and will be submitted to referees like other contributions.

Publications, once accepted, belong to the journal. No papers, or substantial parts thereof, may be published in other journals or books, without written permission from the Publisher.

The European Journal of Oncology assumes no responsibility for statements and opinions advanced by contributors to the journal.

INSTRUCTION FOR MANUSCRIPTS

Mailing

Manuscripts must be sent to the Editorial Office (at the Cancer Research Centre, Bentivoglio) in THREE HARD COPIES AND ON DISKETTE. Texts must be written in Word 6 (or earlier versions) for Windows or Mac and sent on floppy discs or CDs. Floppy discs or CDs must be labelled as follows: author's first name, surname and address and software used.

In the covering letter to the Editorial Staff, all relations that might raise a conflict of interests for the Authors with reference to the topic of the report must be clearly stated.

Texts

Texts must be typewritten or printed, double or triple-spaced, on good quality, 8.5 x 11 inch, white paper, with a margin of at least 1 inch.

Pages must be numbered sequentially.

EDITORIALS, GENERAL REVIEWS AND ORIGINAL STUDIES AND RESEARCH

Title page. The first page of the manuscript should contain the following information: 1) title of the report, in English and Italian; 2) complete Authors' names; 3) name of institution in which the work was done; 4) acknowledgments of collaboration and research support; 5) name and address of the Authors to whom communications regarding the manuscript should be directed and by whom reprints may be requested; and 6) running title of 45 characters or less.

Abstract. The page(s) following the title page is/are to contain an abstract of 250 words or less, with the following headings: 1) aim; 2) materials and methods, or patients and methods; 3) results; 4) conclusions. For all articles the abstract must be prepared in English and Italian, the first version being the one in the language in which the text is written.

Key words. These must be indicated after the abstract, in both languages, and must not exceed 5 in number.

Text. In the text the various sections must be clearly defined in the following order: introduction, materials and methods, results, discussion, conclusions. This is not the case for editorials and general reviews.

References. These should be numbered consecutively in the order in which they appear in the text. The list of references should be typed on separate sheets and numbered following the citation order, in accordance with the examples hereunder reported.

Bibliography style

Journal report, up to 3 Authors:

Sheibani K, Battifora H, Burke J. Antigenic phenotype of malignant mesotheliomas and pulmonary adenocarcinomas. Am J Pathol 1986; 123: 212-9.

Journal report, more than 3 Authors:

Fisher B, Costantino JP, Redmond CK, *et al*. Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. J Natl Cancer Inst 1994; 86: 527-37

Complete book:

Selikoff IJ, Lee DHK. Asbestos and disease. New York: Academic Press, 1978.

Chapter of book:

Freedman AS, Nadler LM. Non-Hodgkin's lymphomas. In Holland JF, Breast RC J, Morton DL , *et al*: Cancer Medicine, IV Ed, 2. Baltimore: Williams and Wilkins, 1997, 2757-95.

Chapter of book that forms the proceedings of a meeting:

Lipkin M. Current knowledge of the cancer latent period. Chemoprevention strategies during colonic cancer development. In Maltoni C, Soffritti M, Davis W. International Forum, The Scientific Bases of Cancer Chemoprevention, Amsterdam: Excerpta Medica, 1996, 61-71.

Abstract:

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

Supplement:

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

Editorial:

Morrow M. The natural history of ductal carcinoma in situ: implications for clinical decision making. Cancer 1995; 76: 1113-5 (editorial).

Letter to the Editor:

Peat IM, Madden FJF. Neurological assessment of high grade astrocytomas following high dose radiotherapy as sole treatment. Clin Oncol 1995; 7: 273 (letter).

Scientific or technical report:

Akutsu T. Total heart replacement device - Bethesda (MD): National Institute of Health, National Heart and Lung Institute; 1974 Apr. Report No.: NIH-NHLI-69-2185-4

Newspaper article:

Rensberger B, Specter B. CFCs may be destroyed by natural process. The Washington Post 1989 Aug 7; Sect. A:2 (col. 5).

Tables. Tables must be typewritten or printed separately on one or more sheets, and must be numbered progressively, at the top left, in arabic numerals, and as such quoted in the text. Each table must have a title heading, which must appear at the top, near the identification number. Legends should include specifications or abbreviations, and should be listed at the bottom of the table, identified with small letters. Tables should be inserted in the manuscript after the text and references.

Figures. Graphs, diagrams, drawings and photographs, macro and micro, are to be indicated as figures and identified with progressive arabic numerals as a separate category. The size should not be larger than that of journal pages, and further reduction may be necessary. Pictures must be of a high technical quality, must be sent unmounted and should not be retouched. On the back of each figure, the name of Authors, the progressive number and an indication of top and bottom should be lightly pencilled. Legends to figures should be collected, double-space typewritten or printed, on one or more pages, under the page title FIGURES. All legends to figures and the figures themselves must be inserted in the manuscript after the tables.
Files must be saved on PC or MAC formatted floppy discs, CDs or ZIP discs. Images must be saved as single files in 10x15 cm format. *DRAWINGS-GRAPHS-DIAGRAMS* (black & white) must have an 800 dpi resolution and must be saved in BMP (bit map) or TIFF format. *RADIOGRAPHIES* (shades of grey) and digital images (full colour) must have a 300 dpi resolution and they must be in JPEG format with medium compression.

CASE REPORTS

The text should follow the indications given for articles on original research and studies. The manuscript should have its various sections identified, i.e.: introduction, general data on cases, illustration of cases, discussion and conclusions.

BRIEF COMMUNICATIONS

Communications should deal with issues of particular originality and topicality. They must be double-space typewritten or printed, and not exceed 3 pages of text, without including references, tables or figures. The sequence of sections of the report (which however should not be labelled), references, tables and figures should follow the instructions given for the articles on original studies and research. If accepted, brief communications will be given special priority in publication.

LETTERS TO THE EDITOR

Letters to the Editor are welcome, and if their content is appropriate will be published. Letters must be double-space typewritten or printed, and should not exceed 2 pages. The letter should have a title and be signed by the Author, who must also state his/her affiliation. Letters should also include references (few and specific).

PROOF READING

The Authors will be sent galley proofs and are asked to correct typesetting errors. Minor changes are allowed: any Author making undue alterations to proofs will be charged with the cost. The galley proofs must be returned within 5 days from receiving them.

REPRINTS

Reprints should be requested by order form, which will be sent with the galley proofs. The same order form indicates the cost of reprints, which varies according to the number of pages and the number of copies requested. Readers who wish to obtain a reprint of an article appearing in the European Journal of Oncology, should contact the Author, at the address given on the first page of the article.

ANNOUNCEMENTS

Announcements of meetings, conferences and similar, which may be of interest to readers of the European Journal of Oncology, should be sent to the Editorial Staff at least 4 months before the event. A fee is charged for each announcement, and the cost will be based on the length of the announcement. Further details may be obtained by contacting the Publisher directly.

PUBLICITY

The European Journal of Oncology publishes advertisements dealing with fields of interest for the Readers. For ethical reasons, advertisements are submitted to the approval of the Editors. Enquiries concerning economic and administrative aspects should be directed to the publisher Mattioli 1885 - Casa Editrice.

TRANSFER OF AUTHOR COPYRIGHT

Please include a signed release of copyright to European Journal of Oncology with your manuscript. Include the title of the article being submitted, as well as the date. Include the signature of coauthors.

EUROPEAN JOURNAL OF ONCOLOGY

GIORNALE EUROPEO DI ONCOLOGIA

Il Giornale Europeo di Oncologia, Organo Ufficiale della
Società Italiana Tumori (SIT)/Prevenzione, Diagnosi e Terapia, è promosso dalla:
Fondazione Europea di Oncologia e Scienze Ambientali "B. Ramazzini"
via Guerrazzi, 18 - 40125 Bologna, Italia
telefono: per l'Italia 051/237286 - per l'estero 0039/051/237286
fax: per l'Italia 051/2911679 - per l'estero 0039/051/2911679
e pubblicato presso la:
Mattioli 1885 - Casa Editrice
via Coduro, 1/B - 43036 Fidenza (PR) - Italia
telefono: per l'Italia 0524/84547 - per l'estero 0039/0524/84547
fax: per l'Italia 0524/84751 - per l'estero 0039/0524/84751
Tutta la corrispondenza editoriale deve essere indirizzata a:
Redazione Giornale Europeo di Oncologia
Centro di Ricerca sul Cancro,
Castello di Bentivoglio, Via Saliceto 3, 40010 Bentivoglio (Bologna), Italia
telefono: per l'Italia 051/6640650-6640143 - per l'estero 0039/051/6640650-6640143
fax: per l'Italia 051/6640223 - per l'estero 0039/051/6640223

Tutta la corrispondenza amministrativa va invece inviata alla Mattioli 1885 - Casa Editrice.
Il Giornale Europeo di Oncologia viene inviato gratuitamente ai Soci della SIT in regola con le quote associative.

La rivista esce con periodicità trimestrale

INFORMAZIONI E ISTRUZIONI PER GLI AUTORI

INFORMAZIONI

Il Giornale Europeo di Oncologia pubblica contributi nei vari settori dell'oncologia: biologia, epidemiologia, patologia e clinica.

I lavori possono essere sotto forma di:
- editoriali;
- riviste generali;
- studi e ricerche originali;
- resoconti di casi clinici;
- comunicazioni brevi;
- lettere ai Direttori.

Le lingue ufficiali della rivista sono l'italiano e l'inglese.

L'accettazione per la pubblicazione dei lavori è subordinata al giudizio di revisori, scelti fra studiosi dell'argomento di volta in volta trattato. La Direzione si riserva di richiedere eventuali modifiche indicate dai revisori, e di apportare quelle che sono necessarie per mantenere una omogeneità di stile nella rivista. Editoriali e riviste critiche possono essere richiesti dalla Direzione della rivista, o essere inviati senza richiesta. In ogni caso devono essere preparati secondo le istruzioni per gli Autori, e verranno sottoposti ai revisori come tutti gli altri contributi.

Le pubblicazioni accettate diventano di proprietà della rivista. I lavori, o parti sostanziali di essi, non potranno essere pubblicati in altre riviste o libri, senza permesso scritto dell'Editore.

Il Giornale Europeo di Oncologia non assume alcuna responsabilità per affermazioni ed opinioni enunciate nel giornale dagli Autori.

ISTRUZIONI PER I MANOSCRITTI

Invio

I testi completi devono essere inviati alla Redazione (presso il Centro di Ricerca sul Cancro, Bentivoglio), in TRE COPIE CARTACEE E SU DISCHETTO. I testi inviati su supporto informatico devono essere in Word 6 (o versione inferiore) per Windows o Mac su dischetti o CD. Il dischetto o CD va etichettato con: nome e cognome, indirizzo dell'autore e indicazione del programma utilizzato.

Nella lettera di accompagnamento devono essere specificate le relazioni che possono porre conflitti di interesse per gli Autori in riferimento al contenuto dell'articolo.

Stesura

I testi devono essere dattilografati o stampati, a doppio o triplo spazio, su carta bianca di buona qualità, di cm 29,5 x 21, con un margine di almeno cm 2,5.

Le pagine devono essere numerate sequenzialmente.

EDITORIALI, RIVISTE GENERALI E STUDI E RICERCHE ORIGINALI

Pagina con titolo. La prima pagina del manoscritto deve contenere le seguenti informazioni: 1) titolo del lavoro, in italiano e in inglese; 2) nome per esteso degli autori; 3) nome dell'istituzione in cui la ricerca è stata fatta; 4) riconoscimenti per le collaborazioni e per i supporti economici della ricerca; 5) nome e indirizzo dell'autore a cui vanno indirizzate le comunicazioni relative al lavoro, e a cui vanno richiesti gli estratti; e 6) titolo abbreviato di testa che non deve superare i 45 caratteri.

Riassunto. Alla pagina con titolo segue un riassunto di massimo 250 parole, con i seguenti titoli: 1) finalità; 2) materiali e metodi o casistica e metodi, ecc; 3) risultati; 4) conclusioni. Per tutti gli articoli, il riassunto va preparato sia in lingua italiana che in lingua inglese, in pagine successive, mettendo per prima versione quella nella lingua in cui è scritto il testo.

Parole chiave. Vanno indicate dopo i riassunti nelle due versioni in misura non superiore a 5, in italiano ed in inglese.

Testo. Il lavoro deve avere chiaramente identificate le varie sezioni, e cioè nell'ordine: introduzione, materiali e metodi o equivalenti, risultati, discussione, conclusioni. Questa suddivisione non è necessaria per gli editoriali e le riviste generali.

Bibliografia. Le voci bibliografiche dovranno essere numerate in ordine di citazione ed il numero riportato nel testo tra parentesi. Tutta la bibliografia citata dovrà essere dattiloscritta su fogli separati e numerata secondo l'ordine di citazione, secondo gli esempi riportati di seguito.

Stile della bibliografia

Articolo in rivista, fino a 3 autori:

Sheibani K, Battifora H, Burke J. Antigenic phenotype of malignant mesotheliomas and pulmonary adenocarcinomas. Am J Pathol 1986; 123: 212-9.

Articolo in rivista, più di 3 Autori:

Fisher B, Costantino JP, Redmond CK, *et al*. Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Sur-gical Adjuvant Breast and Bowel Project (NSABP) B-14. J Natl Cancer Inst 1994; 86: 527-37

Libro completo:

Selikoff IJ, Lee DHK. Asbestos and disease. New York: Academic Press, 1978.

Capitolo di libro:

Freedman AS, Nadler LM. Non-Hodgkin's lymphomas. In Holland JF, Breast RC J, Morton DL, *et al*. Cancer Medicine, IV Ed, 2. Baltimore: Williams and Wilkins, 1997, 2757-95.

Capitolo di libro che costituisce gli atti di un convegno:

Lipkin M. Current knowledge of the cancer latent period. Chemoprevention strategies during colonic cancer development. In Maltoni C, Soffritti M, Davis W. International Forum, The Scientific Bases of Cancer Chemoprevention, Amsterdam: Excerpta Medica, 1996, 61-71.

Abstract:

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

Supplemento:

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

Editoriale:

Morrow M. The natural history of ductal carcinoma in situ: implications for clinical decision making. Cancer 1995; 76: 1113-5 (editorial).

Lettera all'Editore:

Peat IM, Madden FJF. Neurological assessment of high grade astrocytomas following high dose radiotherapy as sole treatment. Clin Oncol 1995; 7: 273 (letter).

Resoconto scientifico o tecnico:

Akutsu T. Total heart replacement device - Bethesda (MD): National Institute of Health, National Heart and Lung Institute; 1974 Apr. Report No.: NIH-NHLI-69-2185-4

Articolo di giornale:

Rensberger B, Specter B. CFCs may be destroyed by natural process. The Washington Post 1989 Aug 7; Sect. A:2 (col. 5).

Tabelle. Le tabelle devono essere dattiloscritte o stampate, ciascuna in un foglio o più fogli separati, e vanno numerate progressivamente, in alto a sinistra, con numeri arabi (e come tali citate nel testo). Ogni tabella va illustrata con un titolo che deve comparire in testa a fianco del numero di identificazione. Eventuali legende, che devono comprendere anche la specificazione delle abbreviazioni, vanno posizionate ai piedi della tabella, e identificate con lettere minuscole. Le tabelle vanno inserite nel lavoro dopo il testo e la bibliografia.

Figure. I grafici, gli schemi, i disegni, le foto (microfoto o macrofoto) vanno denominati come figure, e vanno identificate con numeri arabi, progressivamente, come un'unica categoria. Le loro dimensioni devono tenere conto di quelle della rivista, pur considerando l'eventualità di riduzioni. Le fotografie devono essere di alta qualità tecnica, e vanno inviate non montate nè ritoccate. In ciascuna fotografia sul retro, leggermente a matita, vanno scritti il nome dell'Autore ed il numero progressivo, e indicata la base. Le legende delle figure vanno riportate, in un foglio o più fogli a parte, dattilografate o stampate a doppio spazio, sotto il titolo di pagina FIGURE. Tutte le legende delle figure e le figure stesse vanno inserite dopo le tabelle.
Per quanto concerne il supporto informatico, i files devono essere salvati su dischetto, CD o ZIP formattati PC o MAC. Le immagini vanno salvate come singolo file in formato di 10x15 cm. I *DISEGNI-GRAFICI-DIAGRAMMI* (tratti bianco/nero) devono avere una risoluzione di 800 dpi ed essere salvati in formato BMP (bit map) o TIFF. Le *RADIOGRAFIE* (scala di grigio) e le immagini digitali (colore) devono avere una risoluzione di 300 dpi ed essere in formato JPEG con compressione media.

RESOCONTI SU CASI CLINICI

I testi devono essere preparati secondo le indicazioni fornite per gli articoli su studi e ricerche originali. Il resoconto deve avere chiaramente identificate le varie sezioni, e cioè: introduzione, materiali e metodi, dati generali sulla casistica, illustrazione della casistica, discussione e conclusioni.

COMUNICAZIONI BREVI

Le comunicazioni devono riguardare contributi di particolare novità ed attualità. Devono essere dattiloscritte o stampate in doppio spazio, e non devono essere più lunghe di 3 pagine, escludendo bibliografia, tabelle e figure. La sequenza delle sezioni (che tuttavia non vanno evidenziate), la bibliografia, le tabelle e le figure devono uniformarsi alle stesse istruzioni fornite per gli articoli su studi e ricerche originali. Se accettate, le comunicazioni brevi avranno una speciale priorità nella pubblicazione.

LETTERE ALL'EDITORE

Le lettere all'Editore sono ben accette e, se il loro contenuto è interessante, vengono pubblicate. Le lettere devono essere dattiloscritte o stampate in doppio spazio, non devono essere più lunghe di due pagine. L'Autore deve proporre un titolo, e deve sottoscriversi, indicando anche l'istituzione di appartenenza. La lettera può fare riferimento a voci bibliografiche, che devono essere contenute nel numero ed essere assolutamente specifiche.

CORREZIONE DELLE BOZZE

Gli Autori riceveranno le bozze per la lettura e la correzione degli errori di stampa. In sede di bozze sono ammesse soltanto minime modifiche del dattiloscritto: gli Autori che apporteranno eccessive modificazioni devono sostenerne il costo. Le bozze corrette devono essere inviate alla Redazione entro 5 giorni dal loro ricevimento.

ESTRATTI

Gli estratti dei lavori devono essere richiesti con l'apposito modulo, che viene inviato insieme alle bozze. Nello stesso modulo è indicato il costo degli estratti a seconda del numero delle pagine e del numero di copie. Coloro che desiderano avere estratti di un articolo comparso sul Giornale Europeo di Oncologia devono richiederlo all'Autore di riferimento, all'indirizzo indicato nella prima pagina del testo.

ANNUNCI

Gli annunci di convegni, congressi e simili, che possono interessare i Lettori del Giornale Europeo di Oncologia, possono essere inviati alla Redazione, almeno quattro mesi prima dell'evento. Gli annunci sono pubblicati a pagamento, ed il costo varierà a seconda della lunghezza dell'annuncio. Per ulteriori dettagli rivolgersi alla Casa Editrice Mattioli.

PUBBLICITÀ

Il Giornale Europeo di Oncologia pubblica inserzioni pubblicitarie inerenti a settori di interesse per i Lettori. La pubblicazione degli annunci pubblicitari è sottoposta ad approvazione dei Direttori della rivista. Per gli aspetti economici ed amministrativi rivolgersi alla Mattioli 1885 - Casa Editrice.

TRASFERIMENTO DEL COPYRIGHT

Si prega di accludere al manoscritto una dichiarazione di cessione del copyright al Giornale Europeo di Oncologia. Citare il titolo dell'articolo, la data di invio e il nome di tutti i co-autori con le firme autografe.