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Editorial/Editoriale

Judicial distortion of science and the handicapping of justice in US law

Distorsioni giudiziarie della scienza e ostacoli alla giustizia nella legislazione degli USA

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Summary

A number of US judicial decisions concerning science have distorted science and substantially handicapped the possibility of justice for those who have been injured by exposures to toxic substances. Too often judges have imposed artificial constraints on the kinds of scientific evidence that can support an expert's testimony needed in such cases, precluding some kinds of relevant evidence and privileging others. The result is that an expert may not be able to testify about what the weight of all the relevant scientific evidence shows about the case. This creates a "gulf" between the science that is permitted in some personal injury cases and the toxicology that scientists know and use for drawing inferences about likely causal properties of substances. Such tendencies by courts can be remedied but only if judges become knowledgeable, sensitive, thoughtful consumers of scientific studies and inferences, and learn to review scientific evidence as an integrated whole. In this, they can learn from the evidentiary patterns revealed by scientific committees at the International Agency for Research on Cancer and the US National Toxicology Program. This, combined with adopting a better heuristic for guiding their legal deliberations, would increase the possibility of justice in toxic tort cases. Eur. J. Oncol., 12 (4), 229-234, 2007

Key words: **tort law, toxic substances, carcinogens, scientific inferences, justice**

Riassunto

Un certo numero di sentenze giudiziarie negli USA su temi scientifici ha distorto la scienza ed ostacolato notevolmente la possibilità di giustizia per le vittime di esposizioni a sostanze tossiche. Troppo spesso i giudici hanno imposto vincoli artificiosi ai tipi di evidenza scientifica che possono sostenere la testimonianza di un esperto necessaria in questi casi, precludendo alcuni tipi di evidenza attinente e privilegiandone altri. Il risultato è che un esperto può non riuscire a testimoniare ciò che il peso di tutte le evidenze scientifiche attinenti mostra sul caso. Questo crea un abisso tra la scienza che è concessa in alcuni casi di danni personali e la tossicologia che gli scienziati conoscono ed usano per trarre conclusioni sul ruolo probabilmente causale delle sostanze. Tale orientamento dei tribunali può essere corretto, ma solo se i giudici diventano ben informati, sensibili, attenti utilizzatori di studi e conclusioni scientifiche, ed imparano a valutare l'evidenza scientifica come un insieme integrato. Per far questo, essi possono imparare dagli esempi probativi presentati dai comitati scientifici della *International Agency for Research on Cancer* e dell'US *National Toxicology Program*. Questo, insieme all'adozione di una migliore euristica per guidare le decisioni legali, aumenterebbe la possibilità di giustizia nei casi di danni da sostanze tossiche. Eur. J. Oncol., 12 (4), 229-234, 2007

Parole chiave: **legge sui torti, sostanze tossiche, cancerogeni, conclusioni scientifiche, giustizia**

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Introduction

Recent US judicial decisions concerning the scientific support for expert testimony have distorted science and substantially handicapped the possibility of justice for those who have been injured by exposures to toxic substances. Not infrequently judges have imposed artificial constraints on the kinds of scientific evidence that can support an expert's testimony needed in such cases. This has precluded some scientifically relevant data and privileged others. As a consequence an expert may not be able to testify about what the weight of all the relevant scientific evidence shows about the case. This creates a "gulf" between the science that is permitted in some personal injury cases and the toxicology that scientists know and use for drawing inferences about likely causal properties of substances[1]. Courts' inclinations in this regard can be remedied but only if judges become "knowledgeable, sensitive, thoughtful consumers of scientific studies and inferences", and learn to review scientific evidence as an integrated whole[2]. In this, they can learn from the evidentiary patterns revealed by scientific committees at the International Agency for Research on Cancer and the US National Toxicology Program. This, combined with adopting a better heuristic for guiding their legal deliberations, would increase the possibility of justice in toxic tort cases. I develop each of these points in what follows.

Background

When science and law are utilized in making social decisions about, for example, how human health or the environment should be protected from the effects of suspected toxic molecules, the policy outcome is a joint function, *inter alia*, of the pertinent law, how science is to be used in conjunction with the law, and how certain the science must be for the decisions. However, for these two intellectual areas to jointly function well, they must be utilized within a range of accuracy appropriate to the institution. This has not occurred when federal judges utilized science in personal injury or tort law within the United States.

In regulatory or administrative law settings[3], where the ideal aim is to preemptively protect human health from the toxic effects of substances, there is a comparatively obvious place for scientific evidence. Scientists within regulatory agencies review the pertinent science pertaining to an environmental or health issue, and then agency lawyers and scientists devise rules to protect the public or workplace health within the law and what is understood about the science.

The rôle of science in the tort or personal injury law, the subject of this comment, is somewhat less direct. The tort law provides a forum in which those who believe they have been wrongly injured by the actions or products of others may try to set matters right and receive compensation for their injuries[4]. Toxic tort law is a species of torts concerning harm from exposures to toxic substances such as carcinogens.

The science-law interaction is more complex in torts than in regulatory law, because the issue is, what evidentiary basis do scientific experts need to be permitted to testify before a jury? A jury of citizens then decides a case in view of the law and the scientific and other evidence with which it is presented. Although juries are the ultimate decision makers, judges have critical rôles in permitting or not permitting juries to hear expert witnesses whose testimony is based on scientific evidence.

Judges in conducting trials would typically review and rule on whether to permit different kinds of witnesses to present testimony to juries, with the common law insisting "upon the most reliable sources of information"[5]. For instance, witnesses to an automobile accident may testify to that fact, provided they have firsthand knowledge of the event. However, in order to testify they "*must have had an opportunity to observe, and must have actually observed the fact*"[5]. When an expert testifies as would be needed in toxic tort (and other) cases to assist a jury with technical material, an expert makes a different contribution.

"This is the power to draw inferences from the facts, which a jury would not be competent to draw. To warrant the use of expert testimony, two general elements are required. First, some courts state that the subject of inference must be so distinctively related to some science, profession, business or occupation as to be beyond the ken of laymen … Second, the witnesses must have sufficient skill, knowledge, or experience in or related to the pertinent field or calling as to make it appear that his opinion or inference will probably aid the trier in the search for the truth"[5].

For an expert (or any witnesses) to be allowed to testify, he or she must be officially "admitted" by the trial judge. Unless this occurs, the jury will not hear his or her testimony. In toxic tort cases the plaintiff has the burden of proof on causation (and other legally required elements) typically to show that it is more likely than not that a substance *can cause* injuries of the kind that a plaintiff suffered (general causation) and that it *did cause* the plaintiff's particular injuries (specific causation)[a].

[a] Some legal jurisdictions do not require a sharp distinction between general and specific causation.

Litigants ordinarily need knowledgeable experts to help juries understand arcane scientific issues.

How well courts decide issues concerning scientific evidence used in support of expert testimony profoundly affects justice between parties to a dispute. If court requirements for utilizing scientific evidence in testimony are too stringent, few injured parties will have access to lawyers and the courts, even fewer plaintiffs will have their day before a jury, and fewer still will receive compensation for any injuries suffered. Any deterrence provided by the tort law will also be reduced. If the requirements are too lenient, more plaintiffs will be able to bring their cases to court and a jury, but some of them may be undeservedly compensated and some defendants will mistakenly be required to pay compensation when their actions or products caused no harm. The result will be over-deterrence, sometimes increasing the costs of products or driving them from the market. The admissibility of experts in principle affects both plaintiffs and defendants. However, it is of greater significance to plaintiffs because they have the burden of presenting evidence and persuading the jury.

From 1993-1999 the US Supreme Court issued three rulings on legal issues concerning scientific or other technical experts' testimony in a jury trial[6-8]. These decisions gave trial judges both heightened responsibilities to review expert testimony[6] and wide discretion in doing so (their decisions could only be overturned on appeal if the judges "abused their discretion")[7]. Two of the three cases were toxic torts[6,7].

The Court held that a judge must review the testimony of a scientific expert to ensure it is grounded "in the methods and procedures of science", which "connotes more than subjective belief or unsupported speculation"[6], although "the subject of scientific testimony [need not] be 'known' to a certainty …"[6]. Consequently, they held:

> *the trial judge must … [conduct] … a preliminary assessment of whether the reasoning or methodology underlying the testimony is scientifically valid and of whether that reasoning or methodology properly can be applied to the facts in issue*[6].

A trial judge is not to decide the correctness of expert testimony; that is the jury's task[6]. Instead, a judge should review testimony to ensure that it is sufficiently "reliable" for a jury to consider it.

Courts' struggles with scientific testimony

The Supreme Court, as well as trial courts and intermediate appellate courts implementing the Supreme Court's decisions, have struggled in addressing the science needed to support expert testimony. Some courts have made it virtually a necessary condition that before an expert can testify that a toxicant causes disease, he or she must base testimony on human epidemiological studies[2]. While scientists consider epidemiological studies quite important and possibly among the best kinds of evidence for human harm, such studies are not scientifically necessary for concluding that substances cause human diseases and harm.

Other judges have placed special conditions on epidemiological studies that might appear to have a patina of scientific or legal respectability-requiring that studies show a relative risk greater than two or have a particular level of statistical significance, demanding that most of Austin Bradford Hill's considerations are met, and so on[2]. However, these are scientifically misleading and legally questionable as some courts have utilized them[2]. Still other courts probably have not recognized the importance of sample size or duration of studies or the limitations of "no effect" results. They may be unaware that "there is no evidence of an effect", does not imply "there is evidence of no effect"[2].

In addition, some courts have precluded experts from testifying if their testimony was based on certain kinds of non-human studies-various combinations of animal studies, chemical structure-biological activity relationships, mechanistic data and so on. They have denigrated combinations of non-human evidence as a foundation of testimony, even though scientists themselves would regard all such evidence as presumptively scientifically relevant[2].

Finally, other courts, including the US Supreme Court in *General Electric v. Joiner* endorsed an especially unscientific procedure for reviewing the basis of an expert's testimony. The *Joiner* court permitted judges to review each piece of evidence individually for whether it "reliably" supported an expert's testimony[7]. By permitting litigants to attack each piece of evidence for reliability, it did not permit experts to rely upon all the *scientifically relevant and integrated evidence* for their testimony[9]. Perhaps many of these errors could have been avoided if judges had had a better understanding of the non-deductive reasoning that is central to science and the variety of on-the-ground judgements that scientists make in assessing the toxicity of substances.

Judicial naiveté about science is not the only concern, however. Typically, judges learn from litigants as well as from short courses and colleagues. The defense bar and affected companies have been quick to teach judges that human epidemiological studies are required and even to insist on special constraints on them. By urging courts to require notoriously insensitive epidemiological studies and denigrating other forms of evidence, this helps to mislead the judiciary.

Scientific remedies

Unfortunately, courts have not always appreciated how different kinds of evidence can be scientifically relevant to and appropriately integrated with other evidence to support judgments that a substance can cause human harm. Consequently, they could usefully learn from patterns of scientific evidence employed by scientific committees such as the International Agency for Research on Cancer (IARC) or the US National Toxicology Program (NTP). These committees have utilized many different patterns of evidence in determining whether a substance causes or contributes to human cancer. They rely upon human epidemiological studies, if they are available and of appropriate quality. They also rely upon experimental animal studies, human case reports, chemical similarities between substances, mechanistic data, and so on[10].

Moreover, courts themselves can find more specific guidance about how scientists reason about the toxicity of substances by reviewing the texts of consensus scientific committees' opinions about why, for example, they judged that CCNU (an anti-cancer drug), dioxin, or neutron radiation were known or likely human carcinogens. Let us consider some examples.

For a large majority of substances identified as known human carcinogens, IARC has had available human epidemiological studies and often multiple epidemiological studies in addition to other evidence. These were easy scientific cases by the time IARC classified the substances because of the large amount of accumulated evidence and because of substantial human data. However, for a small number of known human carcinogens IARC either had no direct human evidence or inadequate human evidence, but still concluded that substances were known human carcinogens by making inferences from animal, molecular, or mechanistic evidence together with background knowledge they had of the disease, similar toxic effects produced by structurally similar substances and so on. Such combinations of non-direct human evidence provide counter-examples to courts' assertions that certain kinds, or even combinations, of evidence could not provide an appropriate basis of expert testimony that substances could cause or contribute to, for example, cancer in humans.

For example, for two known human carcinogens – ethylene oxide (ETO)[11] and dioxin (2,3,7,8-tetrachlorodibenzo-para-dioxin)[12] – there was only "limited" human evidence (when they were so classified) that these substances caused cancer in humans (i.e., "chance, bias or confounding could not be ruled out with reasonable confidence" in the relevant studies)[11]. However, for both there was "sufficient" evidence of carcinogenicity in experimental animal studies plus additional supporting evidence that led the committees to conclude that these substances were indeed human carcinogens. ETO is an alkylating agent[11]. Dioxin is a multisite carcinogen in experimental animals that acts through a receptor-mediated mechanism in cells that is believed to be common to animals and humans and preserved through evolution – the "aryl-hydrocarbon receptor"[12].

For four other known human carcinogens – neutron radiation[13], Direct Black 38, Direct Blue 6 and Direct Brown 95 (benzidine-based commercial dyes) – there were no adequate human epidemiological studies at all. However, there was sufficient evidence of carcinogenicity in animals plus other supporting evidence that led NTP to conclude that these substances were human carcinogens.

> *Dyes that are metabolized to benzidine are known to be human carcinogens based on the following evidence: (1) benzidine is known to be a human carcinogen, (2) metabolism of benzidine-based dyes results in the release of free benzidine in humans and in all experimental animal species studied, . . . and (3) benzidine exposure from exposure to benzidine-based dyes is equivalent to exposure to equimolar doses of benzidine…*[14]

Perhaps more important for the toxic tort law, IARC judges that a substance is a "probable human carcinogen" and NTP judges that a substance is "reasonably anticipated to be a carcinogen" by utilizing patterns of evidence that do not include predominantly (or in some cases no) human evidence. For about 65-70 substances on IARC's list of "probable human carcinogens" approximately 60% had inadequate or limited evidence of carcinogenicity in human studies[15]. IARC supported its scientific inferences by experimental animal studies and "other data relevant to the evaluation of carcinogenicity and its mechanisms"[15].

The data for the NTP are more dramatic. NTP has identified about 185 substances as probable human carcinogens. This group of substances is so classified largely on the basis of animal and other non-human data.

Examples such as neutron radiation, benzidine-based dyes, and dioxin as well as the patterns of evidence IARC and NTP utilize to classify substances as probable human carcinogens can provide useful and nuanced examples to guide judges' thinking about scientific evidence in cases before them. The examples show that working scientists come to conclusions about which substances are certainly or are highly likely to cause cancer in humans without in many cases relying upon good human data. If courts, following the Supreme Court's mandate, aim to better incorporate the science of our technological society into

legal cases, they should permit scientists to reason about these matters utilizing all the scientifically relevant and integrated evidence as they would in ordinary scientific contexts.

Legal remedies

Courts have also been misled in how they think about scientific issues by some language in the Supreme Court's *Joiner*[7] decision, suggesting that a court may permissibly review each piece of a litigant's evidence for whether it reliably supports the litigant's scientific conclusion. Such a review procedure is contrary to scientific practice. Scientists typically assess each individual piece of evidence for whether it is scientifically relevant, that is whether it would make even the slightest difference to a scientist's conclusion[16-18]. They then assess the totality of the body of evidence to try to determine which explanation (if any) it best supports[9].

The Supreme Court in *Kumho Tire v. Carmichael*[8] provided two useful heuristics for reviewing scientific testimony for admissibility. The first is that the court should ensure "that an expert … employs in the courtroom the same level of intellectual rigor that characterizes the practice of an expert in the relevant field"[8]. The Court amplified its view in application by indicating that a court may exclude evidence if it finds that an expert's testimony falls "outside the range where experts might reasonably differ, and where the jury must decide among the conflicting views of different experts, even though the evidence is 'shaky'"[8]. Both heuristics are something of sociological guidelines for judges. How does this expert's testimony on the issue in question compare to the standards of the profession (the first) or to other reasonable experts in the field (the second)?

The second heuristic is especially helpful in the law where the aim should be for a judge merely to determine whether experts have based their testimony on the kinds of evidence about which reasonable experts could disagree. After that it is the jury's responsibility to determine the facts of a case and apply the law to the facts that it finds. The second heuristic embodies fairness toward reasonable scientific views appropriate for litigation. When responsible, respectable experts disagree, this is precisely the kind of issue juries, not judges, should decide.

Conclusion

Regrettably, some US trial judges and appellate courts utilizing simplified guides are mistakenly constraining expert testimony with asymmetrical adverse effects on injured parties. This keeps good scientific evidence and expert testimony from court, denies some meritorious victims the possibility of public trials and precludes their possibilities for justice.

In order to conduct fair admissibility reviews and avoid being manipulated by litigants, judges should fully recognize the complexity of scientific evidence, consider expert testimony based on all an expert's integrated evidence, and then rule on admissibility based on whether he or she proposes to testify within the range where reasonable experts would disagree. To accomplish this, courts must recognize the variety of explanatory paths to causal conclusions and the varied patterns of evidence that support reasonable causal inferences. In turn this would move courts closer to the goal that seems to underlie the *Daubert*[6] trilogy of cases: ensuring that legal decisions were reasonably supported by the science pertinent to the issues of the case while also ensuring the possibility of justice between parties.

However, even if the conduct of trials were impeccable on these dimensions, if the courts require appropriate science for social decisions largely at the end of a series of social events – from the creation of products to production, to commercialization, to distribution, to contributing to harm to citizens – it is likely to be too little, too late[2]. Typically, too little health (and environmental) research is produced early in the social lives of substances before commercialization. The consequence is considerable ignorance about thousands of substances in commerce[19]. Because the tort law is remedial, the science produced in this venue is too late for regulatory action and too late to prevent harm to the public or the workforce. Moreover, courts' demanding good scientific evidence in support of expert testimony so late in the sequence of events creates counterproductive incentives for companies to refrain from testing their products, thus multiplying mistakes and harm. Companies have incentives to remain in the dark about the toxicity of their products and try to defeat toxic tort cases for lack of sufficient scientific support. They often succeed. The workforce, the larger public and the environment then become guinea pigs for the productive capacities of commerce. Courts need to improve their review of science and legislatures need to institute broader legal changes to uncover the toxicity of products earlier in their commercial lives.

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Surveillance of Gulf War I veterans exposed to depleted uranium: 15 years of follow-up

Sorveglianza dei veterani della prima Guerra del Golfo esposti ad uranio impoverito: 15 anni di follow-up

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Summary

***Aim*. To provide a summary of selected findings from the Depleted Uranium (DU) Follow-Up Program surveillance fifteen years after exposure to DU during the Gulf War. *Patients and methods*. A dynamic cohort of seventy-four 1991 Gulf War soldiers with known exposure to DU resulting from their involvement in "friendly-fire" incidents with DU munitions is being followed by the Baltimore Veterans Affairs Medical Center. Biennial medical surveillance visits designed to identify uranium-related changes in health have been conducted since the early 1990s. Ongoing systemic exposure to DU in veterans with embedded metal fragments is indicated by elevated urine uranium (U) excretion at concentrations up to 1,000-fold higher than that seen in the normal population. Typically, urine U concentration is determined at the time of each surveillance visit. During the 2005 visit, a cumulative measure of U exposure was also calculated based on each veteran's past urine U concentrations since first exposure in 1991. *Results*. Using either exposure metric, results continue to show no evidence of clinically significant uranium-related health effects. Particular interest in proximal tubule and other renal parameters, the presumed critical organ targets for U toxicity, have not shown any differences when examined by stratifying the cohort into high-versus-low (normal) U groups. There is**

Riassunto

***Scopo*. Fornire un sommario dei risultati più significativi della sorveglianza nell'ambito del programma di *follow-up* sull'uranio impoverito (DU) 15 anni dopo l'esposizione a DU durante la Guerra del Golfo. *Pazienti e metodi*. Una coorte dinamica di 74 militari della Guerra del Golfo del 1991, con esposizione nota a DU dovuta a coinvolgimento in incidenti da "fuoco amico" con munizioni contenenti DU, viene seguita dal *Baltimore Veterans Affairs Medical Center*. Visite biennali di sorveglianza medica, programmate per individuare problemi di salute dovuti all'uranio, sono state eseguite dall'inizio degli anni '90. La perdurante esposizione sistemica a DU nei veterani con frammenti metallici ritenuti viene indicata da una elevata escrezione urinaria di uranio a concentrazioni fino a 1000 volte quelle osservate nella popolazione generale. Tipicamente la concentrazione urinaria di uranio viene determinata al momento di ciascuna visita di controllo. Durante la visita del 2005 è stata calcolata anche l'entità totale dell'esposizione ad uranio sulla base delle concentrazioni urinarie pregresse di uranio nei singoli veterani a partire dalla prima esposizione nel 1991. *Risultati*. Usando entrambe le misure di esposizione, i risultati continuano a non mostrare evidenza di effetti sanitari clinicamente significativi correlati all'uranio. Studi particolari sul tubulo prossimale ed altri parametri renali, i presumibili organi**

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some evidence of a weak genotoxic effect from the ongoing DU exposure as measured at the HPRT (hypoxanthine-guanine phosphoribosyl transferase) locus, and as suggested by the fluorescent *in-situ* hybridization (FISH) results in peripheral blood, that recommends the need for continued surveillance of this population. *Conclusions*. The continued evidence of genotoxic effects, albeit relatively weak effects, recommends the need for continued surveillance of this population. Eur. J. Oncol., 12 (4), 235-242, 2007

Key words: **depleted uranium, health monitoring, genotoxic effects, HPRT, FISH**

bersaglio critici per la tossicità da uranio, non hanno mostrato differenze quando valutati stratificando la coorte in gruppi ad alta *vs* bassa (normale) esposizione ad uranio. C'è qualche evidenza di un debole effetto genotossico da esposizione in corso a DU misurata nel locus HPRT (ipoxantina-guanina fosforibosil transferasi) e suggerita dai risultati dell'ibridizzazione a fluorescenza *in situ* (FISH) nel sangue periferico. Tali risultati suggeriscono la necessità di una continua sorveglianza di questa popolazione. *Conclusioni*. La perdurante evidenza di effetti genotossici, sebbene relativamente modesti, indica la necessità di una continua sorveglianza di questa popolazione. Eur. J. Oncol., 12 (4), 235-242, 2007

Parole chiave: **uranio impoverito, monitoraggio sanitario, effetti genotossici, HPRT, FISH**

Introduction

The constellation of symptoms and health complaints in returned Gulf War troops, described in the more than 15 years since that war's end, has been termed, among other names, "unexplained illness", and has received the bulk of both scientific and public attention. However, an additional small collection of "explained" adverse health outcomes have also been reported, related to environmental exposures in that conflict.

Certainly the best characterized, and possibly the most controversial environmental hazard described in relation to Gulf War service, is that to depleted uranium (DU). While many veterans have had concern about potential exposure to DU and it has been suggested as a possible cause of unexplained illnesses and many other hypothesized health outcomes, clinically significant exposure to DU probably occurred only to a relatively small number of soldiers. The most significant exposures occurred among a cluster of soldiers who were victims of "friendly-fire" incidents. During one two-day period in February 1991, approximately 100 US tank crew members on twenty-one vehicles were mistakenly fired upon by other US forces, using DU penetrators. DU possesses high density, lending it armour-piercing capability. Upon striking a target, the DU penetrator's pyrophoric character and high temperature promote small particulate to ignite. Upon impact, small shards (spall) of the target's armour are also produced. This DU-metal dust then deposits on and within a target vehicle[1,2].

Over ten fatalities resulted from these incidents and an additional 50 soldiers required medical attention. Traumatic injuries were the primary consequence, although wound contamination and inhalation exposures to DU dust also accrued to both tank crew members and rescuers.

Toxicity

Uranium (U) is a radioactive heavy metal emitting primarily alpha radiation (though a small fraction of beta and gamma radiation is also present). DU is a waste product of the uranium enrichment process, whereby there is a removal of much of the U^{235} and U^{234}. Hence, DU, as the name implies, possesses only about 60% of the radioactivity of natural uranium[1].

Because uranium decays primarily by high-energy emission of alpha particles, which travel very short distances in tissues, the principal radiological hazard is to tissues in immediate contact with DU, which may have been internalized as small particles or fragments. The dose is a function of contact time, particle solubility and rate of elimination[1,3].

Health effects

The exposure scenarios of primary concern from the Gulf War involve those related to the friendly fire incidents. The majority of these exposures were of short duration and involved inhalation of aerosolized DU particulate that was primarily made up of uranium oxide. DU particulate may have also contaminated wounds and may have been ingested after coughing to clear an airway. The other and most significant exposure scenario involves that of retained DU metal fragments (shrapnel) that are embedded in a victim's soft tissue. Over time,

these fragments are oxidizing *in situ* and act as a depot for ongoing systemic absorption of DU.

The long-term health effects of concern derive both from uranium's radiologic and its chemical, heavy metal character. While natural uranium, and to a lesser extent, DU are radioactive, they do not appear to be highly carcinogenic. There is poor evidence of an excess cancer risk, specifically of lung, bone or kidney, the most likely targets in occupational cohorts[4,5] whose exposures in both intensity and duration were greater than those of the Gulf War soldiers. The lung cancer excess observed in uranium miners has been attributed primarily to radon exposure, the decay progeny of uranium, as well as to other toxicants in the mines[6,7]. Radon is about 10,000 times more intensely radioactive than natural uranium[8,9]. DU is a man-made product, with little to no decay products beyond U^{234} present, having been separated during the processing of uranium ore. As well, new, post-U^{234} decay products have not had sufficient time to form, since the DU was processed due to the 10,000 year half-life of thorium-230, the initial decay product of U^{234} [10].

Radiation dose estimates for some Gulf War veterans with retained DU shrapnel were calculated from whole-body radiation counting, using the ICRP 30 Biokinetic model for uranium, and have been published previously[11]. These data demonstrated fairly low whole body radiation at 0.1 rem/year for an upper limit, which is also the allowable public dose limit; and 5.3 rem/50 years (for comparison, the annual occupational exposure limit is 5 rem/year). Due to these radiation estimates and the occupational epidemiology available, the primary mechanism of concern from DU exposure has been its chemical toxicity.

Long-term surveillance has been conducted on 74 of the approximately 100 surviving friendly-fire cohort members. To date, these soldiers have been evaluated six times over the last 15 years. The cohort includes veterans who had primarily an inhalation exposure to DU during the friendly-fire incidents, but about one third of the cohort possess retained DU metal fragments in soft tissue, which have not be removed due to the surgical morbidity related to their removal. We report here a summary of the findings over the more than 15 years of follow-up since first exposure in 1991.

Methods

Clinical surveillance of friendly fire Gulf War I victims

Members of the DU-exposed friendly-fire cohort are invited to the Baltimore Veterans Affairs (VA) Medical Center every two years for a three-day surveillance visit. The data elements obtained during this visit are displayed in Table 1. A 24-hour urine uranium determination is used as the exposure metric and an isotopic uranium determination is made to distinguish depleted from natural uranium by measuring the U^{235}/U^{238} ratio, using an inductively coupled plasma dynamic reaction cell mass spectrometer (ICP/DRC/MS) technique described previously[12].

Table 1 - Elements of the biennial clinical surveillance evaluation

Complete history
Medical
Social
Family
Reproductive
Occupational exposure
Partner health and reproductive history
Extensive laboratory studies
Haematology
Renal
Neuroendocrine
Reproductive
Genotoxicological
Neurocognitive
Skeletal/bone density
Shrapnel evaluation (X-rays done every 6 years)
Focus group/risk communication

While a general assessment of overall health status is made, specific target organs of chronic U toxicity are emphasized, including renal effects[13-15]. Also serially followed have been measures of genotoxic insult, due to concerns about ongoing exposure to this radioactive heavy metal in the tissue environment immediately surrounding retained metal fragments. Laboratory methods for renal and genotoxic measures have been described in detail previously[16-18].

Urine uranium screening of veterans of Gulf War I or Operation Iraqi Freedom (OIF)

In addition to the intensive surveillance performed on the friendly-fire cohort, our programme also provides urine uranium screening for any veteran of any conflict who is concerned about possible DU exposure. This programme includes a detailed health examination performed at the veteran's local VA medical centre and a 24-hour urine uranium determination for uranium on a sample delivered to our laboratories via overnight mail. An isotopic uranium measurement is also made and results are reported to the veteran and their care provider.

Table 2 - Demographic characteristics of the 2005 participants[a] compared to all participants[b]

	2005 cohort		All participants	
	N.	%	N.	%
Race				
African American	9	26.4	24	32.4
Asian American	1	2.9	1	1.4
Caucasian	21	61.8	40	54.1
Hispanic	2	5.9	8	10.1
Native American	1	2.9	1	1.4
Age[c]	40.8±5.7 years		39.2±5.0 years	

[a] N. = 34
[b] All participants enrolled in DU Follow-up Program (N. = 74)
[c] Mean age at time of 2005 evaluation (±standard deviation)

Data analysis

Clinical outcomes (dependent variables) are assessed during each surveillance visit in light of the veteran's DU exposure as measured by their urine U concentrations. Data are stratified into a "low U exposure group" (urine U concentration <0.1 μg U/g creatinine) and a "high U exposure group" (urine U concentration ≥0.1 μg U/g creatinine).

Results

The demographics of this dynamic cohort are displayed in Table 2, showing the data for the most recent biennial surveillance visit in 2005 where N=34, but compared to the total cohort of N=74. Although the specific veterans' participations in each visit vary from year to year, depending on their ability to travel to the surveillance site, most cohort members have participated in at least three surveillance evaluations.

Urine uranium measurements

Friendly-fire cohort

The distribution of the 24-hour total urine U analysis for the 74 members of this cohort is presented in fig. 1. Mean uranium concentrations for this group ranged from (0.002 to 36.659 μg U/g creatinine). Values at or above a cut point of 0.1 μg U/g creatinine were from veterans with known retained shrapnel fragments and U isotopic signatures indicative of DU.

Expanded cohort (mail-in)

Table 3 displays the urine U measurements in mailed 24-hour urine samples from veterans of both Gulf War I

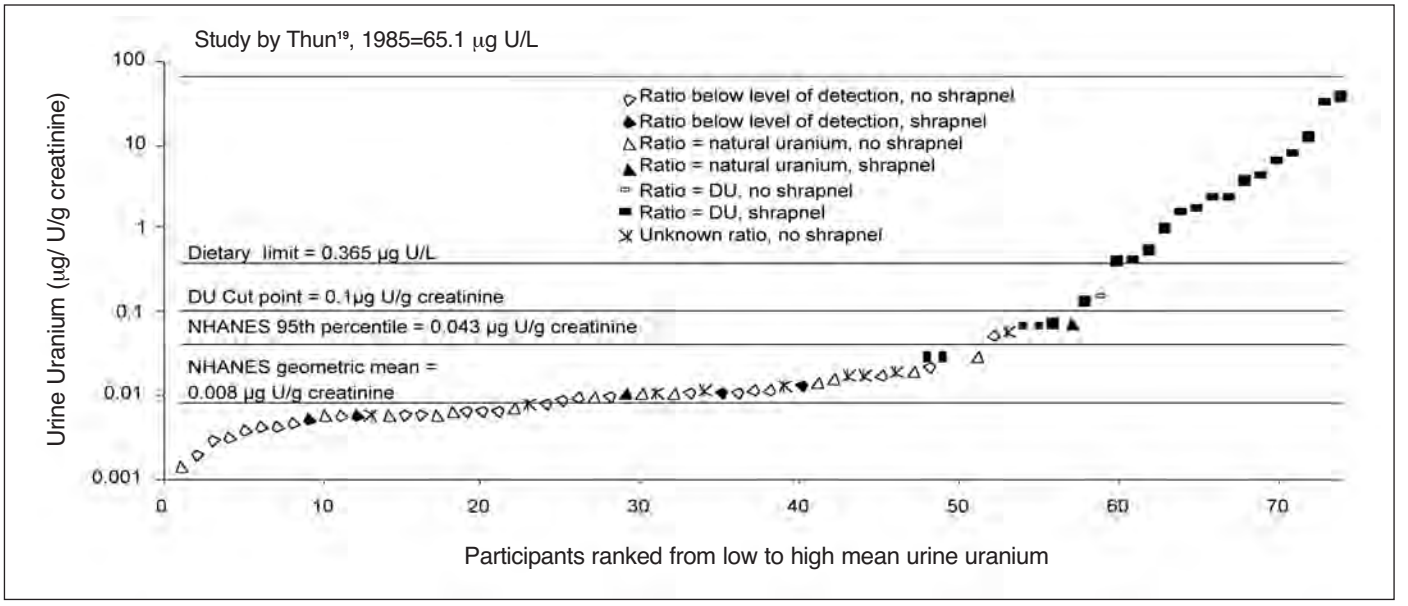

Fig. 1. Mean urine uranium values for all participants (N. = 74)
The top horizontal line (65.1 μg U/L) represents the mean total urine uranium found in a sub-cohort of uranium fabrication workers in 1975, as reported in a study by Thun *et al*[19]. The second line (0.365 μg U/L) is an upper limit for the dietary contribution of uranium in urine for a general population from drinking water[20]. This value was calculated by dividing the upper limit for 24-h uranium excretion for "reference man" by 1.4 L/24 h. It is assumed that corrections per gram creatinine and per litre are generally equal for "reference man" and for this group of veterans with normal renal function. The third line (0.1 μg U/g creatinine) indicates the cut point established by the DU Follow-up Program to identify low *vs* high urine uranium concentration[11]. The fourth line (0.043 μg U/g creatinine) depicts the 95th percentile for urine uranium concentration for adults from the NHANES Survey (2001-2002 data)[21, 22]. The last line depicts the geometric mean for urine uranium from the same NHANES cohort[21, 22]

Table 3 - Urine uranium concentration (μg U/g creatinine) in mailed samples from veterans of Gulf War I (1991) and OIF[a] (2002 - present) compared to NHANES results

	GWI[b]	OIF[b]	NHANES (2001-2)[c]
# of analyzed samples	768	1107	1559
Samples measured for DU	230	1107	
Samples with DU present by isotopic analysis	0	0	
Mean urine uranium ± SD.	0.018 ± 0.110	0.009 ± 0.053	0.008 (0.007-0.010)[d]
Range of values	0.000 - 2.895	0.000 - 1.686	
95th percentile			0.043 (0.030-0.063)[e]

[a] Operation Iraqi Freedom
[b] Results as of 20/7/2007
[c] National Health and Nutrition Examination Survey, 1999-2002[20]
[d] Geometric mean with 95% confidence interval
[e] 95% confidence interval

and the present conflict in Iraq (OIF), who had concerns about possible DU exposure: these included, primarily, inhalation exposure while driving by a burning tank or in handling DU rounds (transporting or loading) and in the salvage and clean-up of damaged tanks. Mean urine U concentrations for both of these groups are comparable to the US national mean as determined in the National Health and Nutrition Examination Survey[22] and well below the 95th percentile cut off of the national distribution. Individual values above 0.05 μg U/g creatinine are repeated and since the early 2000s, when a more reliable measure of U isotopic ratios could be made at very low (10 μg U/g creatinine) total U concentrations, all samples also have U isotopic ratios measured.

Clinical measurements

The clinical parameters described in Table 1 and measured in each surveillance visit have recently been summarized elsewhere[23] and, therefore, we will highlight here only renal and genotoxicity results as these remain of high interest.

Renal measurements

Overall, standard clinical measurements of renal function (serum creatinine, serum uric acid and urine creatinine) continue to show results within the normal range, with no evidence of acute or progressive renal damage, when comparing veterans in the low U, *versus* the higher U exposure groups (Table 4). During the past six surveil-

Table 4 - Renal parameter comparison of low *vs* high urine uranium groups for 2005 cohort

Laboratory test (normal range)	Low uranium group[a] (mean ± SD)	High uranium group[b] (mean ± SD)	Mann-Whitney *p*
Urine creatinine (1.5-2.6 g/24hr)	2.03 ± 0.09	1.91 ± 0.29	0.24
Creatinine clearance (97-137 ml/min)	130.21 ± 7.14	135.21 ± 12.13	0.70
Urine calcium (100-300 mg/24hr)	158.90 ± 21.83	199.04 ± 43.75	0.59
Urine PO_4 (0.4-1.3 g/24hr)	3.04 ± 2.01	1.11 ± 0.34	0.29
Urine glucose (0-0.5 g/24hr)	34.45 ± 33.37	0.26 ± 0.14	0.59
Urine β_2 microglobulin (0-160 μg/g creatinine)	63.25 ± 6.75	71.05 ± 11.56	0.22
Urine intestinal alkaline phosphatase (IAP) (<2 U/g creatinine)	0.34 ± 0.11	0.46 ± 0.15	0.67
Urine *N*-acetyl -β-glucosaminidase (NAG) (<5 U/g creatinine)	1.51 ± 0.27	1.24 ± 0.15	0.96
Urine total protein (1-150 mg/24h)	122.75 ± 26.80	89.30 ± 17.79	0.50
Urine micro-albumin (<25 mg/g creatinine)	15.17 ± 9.88	3.55 ± 0.62	0.40
Urine retinol binding protein (<610 μg/g creatinine)	64.73 ± 6.59	71.45 ± 11.49	0.25
Glucose (70-105 mg/dl)	107.00 ± 6.92	109.20 ± 7.41	0.67
Serum creatinine (0-1.4 mg/dl)	1.06 ± 0.05	0.99 ± 0.03	0.72
Serum calcium (8.4-10.2 mg/dl)	9.32 ± 0.06	9.46 ± 0.09	0.24
Serum PO_4 (2.7-4.5 mg/dl)	3.52 ± 0.15	3.47 ± 0.15	0.70
Serum uric acid (3.4-7 mg/dl)	6.14 ± 0.27	5.35 ± 0.45	0.10

[a] < 0.10 μg/g creatinine (n=24)
[b] ≥ 0.10 μg/g creatinine (n=10)

Table 5 - Summary of differences in renal parameters across evaluations

Renal parameter	Evaluation year					
	1994	1997	1999	2001	2003	2005
Urine creatinine	ns[a]	ns	l>h[b] (p=0.07)	ns	ns	ns
Urine calcium				ns	ns	ns
Urine PO_4				ns	ns	l>h (p=0.10)
Urine β-2 microglobulin	ns	ns	ns	ns	ns	ns
Urine intestinal alkaline phosphatase (IAP)			ns	ns	ns	ns
Urine N-acetyl-β-glucosa-minidase (NAG)			ns	ns	ns	ns
Urine total protein		ns	ns	H[c]>L[d]	l>h(p=0.21)	ns
Urine microalbumin					ns	ns
Retinol binding protein (RBP)		ns	ns	h>l[a] (p=0.06)	h>lns[e]	ns
Serum creatinine	ns	ns	ns	L>H	ns	ns
Serum calcium				ns	ns	ns
Serum PO_4				ns	H>L	l>h ns
Serum uric acid	ns	ns	ns	ns	ns	ns

[a] ns = no significant differences between groups
[b] lower case letters = non-significant findings; upper case letters = significant findings
[c] H = High urine uranium group (U ≥ 0.1 μg/g creatinine)
[d] L = Low urine uranium group (U < 0.1 μg/g creatinine)
[e] High uranium group 80.5 ± 51.4, low uranium group 27.3 ± 3.1, p=0.54

lance visits, a particular laboratory parameter on occasion has shown a small statistical difference when compared between exposure categories; but these have not been observed consistently, in the same parameter over subsequent visits, or in the expected "direction" (i.e., an excursion indicating a worse effect in the higher U group) and typically not outside the normal clinical range of that parameter[18, 23].

Measurements of proximal tubular function (a specific target of U) have been followed serially over the years (Table 5) and only retinol-binding protein (RBP) has appeared to approach statistical difference, with higher values in the high U group compared to the low group[17, 24]. This effect appears to be dampening however, as can be seen from the most recent 2005 results (Table 5). We are continuing to follow RBP, in search of a possible "sentinel" marker of U-induced proximal tubular effects.

Genotoxicity measurements

Table 6 displays summary results for the markers of genotoxic effect employed over the course of this longitudinal surveillance. Sister chromatid exchange (SCE) and chromosomal aberrations (CAs) have been measured in blood lymphocytes since 1997 and have generally shown mixed results (in both magnitude and direction) as seen in the Table. In light of both *in vitro* genotoxicity work[25-31] and *in vivo* carcinogenesis studies[32-35] the hypoxanthine-guanine phosphoribosyl transferase (HPRT)

Table 6 - Summary of differences in genotoxicity parameters across evaluations

Genotoxicity parameter	Evaluation year					
	1994	1997	1999	2001	2003	2005
Sister chromatid exchange (SCE)		l>h[a] ns	H[b]>L[c]	l>h ns	ns[d]	-
Chromosomal aberrations (CA)		ns	ns	H>L	ns	ns
Hypoxanthine-guanine phosphoribosyl transferase (HPRT) mutation frequency (MF)				h>l ns	h>l ns	h>l ns
Fluorescent *in-situ* hybridization (FISH); Mean number of total mutations per subject in chromosomes 5, 7, 11, and 13						h>l p=.08

[a] lower case letters = non-significant findings; upper case letters = significant findings
[b] H = High urine uranium group (U ≥ 0.1 μg/g creatinine)
[c] L = Low urine uranium group (U < 0.1 μg/g creatinine)
[d] ns = no significant differences between groups

mutation assay was added to the protocol in 2001. Results have shown a consistent, but non-statistically significant trend of higher mutation frequencies in the higher U group compared to the low U group. However, the intensity of this difference has dampened in the most recent 2005 measures[18]. Most recently, fluorescent *in-situ* hybridization (FISH) has been added to detect mutations in chromosomes 5,7,11 and 13. No differences in mutation frequencies in individual chromosomes have been observed between low and higher U veterans, but when total mutations are summed, a non-statistically significant difference in frequency is seen, with the higher U group having a greater frequency of mutations.

Discussion

The principal finding from this study of more than 15 years of follow-up of veterans exposed to DU is that urine U excretion is significantly higher in veterans with retained metal fragments in soft tissue compared to either those DU-exposed veterans without fragments[11, 23, 36, 37] or a comparison population of Gulf War deployed, but not DU-exposed veterans[11]. This observation displayed in fig. 1 is also supported by data from Table 6, showing results for close to 2000 veterans of both Gulf War conflicts with very low total urine U and none with the isotopic signature of DU. The disadvantage to this mailed specimen surveillance is that the specimen is usually sent months after return from deployment, and any uranium excursion, if it occurred, would not be captured, due to uranium elimination in urine. However, because toxicity is a function of duration as well as dose, assurances to veterans have been possible even where normal results were (and usually are) documented.

Renal parameter results and the summary of differences in these parameters between the low and high U groups illustrate little evidence of impact of DU exposure on renal function at this time. This was true when using the usual U exposure metric, the 24-hour U value, and when using an integrated measurement based on an "area under the curve" (AUC) calculation (data not shown)[18].

Despite the increasing evidence for the genotoxicity of DU in *in vitro* studies (cited above), the weak genotoxicity results we have observed in several different measurements employed are consistent with the low risk of increased cancer predicted by The Royal Society[13, 14] for Gulf War exposures. They also are consistent with over sixty years of occupational epidemiology studies of U miners and factory workers[5, 19] and with radiation dose estimates measured for Gulf War veterans with shrapnel, which were close to the annual occupational exposure limit of 5 rem/year[11]. Nonetheless, continued monitoring of general health parameters and for genotoxic effects is warranted given the weak (but dampening) HPRT results and the FISH mutation frequency excesses. This is especially true for the veterans with retained shrapnel fragments as their exposure is ongoing.

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Prognostic value of CD99, CD117, p53 and bcl-2 in Ewing sarcoma family tumours

Valore prognostico di CD99, CD117, p53 e bcl-2 nei tumori della famiglia del sarcoma di Ewing

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Summary

***Aim*. In the past the expression of CD99 (mic2 gene), CD117 (*c-kit*) proto-oncogene, bcl-2 protein and p53 gene has been studied in various types of members of Ewing sarcoma family tumours (ESFT), especially for diagnostic purposes. However, little is known about their prognostic value in combination with the clinico-pathological data of such patients. *Materials and methods*. In the current retrospective study, we investigated the immunohistochemical expression of p53, bcl-2, CD99 and CD117 on formalin-fixed, paraffin-embedded material of 72 patients with various types of ESFT, using a tissue microarray method. *Results*. Patient age ranged from 2 to 59 years. The results of immunohistochemistry were compared with clinico-pathological data using Survival Analysis (Cox regression): bcl-2 expression was detected in 70.1%, p53 in 66.6%, CD117 in 75.8% and CD99 in 90% of cases. No immunoreactivity was observed in normal tissues around the tumours. *Conclusions*. We found that the expression of CD99 (p=0.140), CD117 (p=0.612), p53 (p=0.540) or bcl-2 (p=0.382) has no statistically significant impact on survival. Patient age at the time of**

Riassunto

***Finalità*. In passato l'espressione del CD99 (gene mic2), del proto-oncogene CD117 (*c-kit*), della proteina bcl-2 e del gene p53 è stata studiata in diversi tipi di tumori appartenenti alla famiglia del sarcoma di Ewing (TSE), soprattutto ai fini prognostici. Ciononostante si sa poco sul loro valore prognostico in combinazione con i dati clinico-patologici dei pazienti. *Materiali e metodi*. In questo studio retrospettivo abbiamo valutato l'espressione immunoistochimica di p53, bcl-2, CD99 e CD117 in materiale fissato in formalina ed incluso in paraffina proveniente da 72 pazienti con vari tipi di TSE usando una metodica di *microarray* tissutale. *Risultati*. L'età dei pazienti variava da 2 a 59 anni. I risultati immunoistochimici sono stati confrontati con i dati clinicopatologici usando l'Analisi di Sopravvivenza (regressione di Cox): l'espressione di bcl-2 è stata osservata nel 70,1% dei casi, quella del p53 nel 66,6%, quella del CD117 nel 75,8% e quella del CD99 nel 90%. Non è stata osservata nessuna immunoreattività nei tessuti normali intorno ai tumori. *Conclusioni*. Abbiamo trovato che l'espressione di CD99 (p=0,140), CD117**

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diagnosis (*p*=0.008) and presence of tumour necrosis (*p*=0.033) are the only significant prognostic factors in our study. Tumour location turned out not to be a significant prognostic factor (*p*=0.380). Eur. J. Oncol., 12 (4), 243-253, 2007

(p=0,612), p53 (p=0,540) o bcl-2 (p=0,382) non ha un impatto statisticamente significativo sulla sopravvivenza. L'età dei pazienti al momento della diagnosi (p=0,008) e la presenza di necrosi tumorale (p=0,033) sono i soli fattori prognostici significativi nel nostro studio. La sede del tumore non si è rivelata un fattore prognostico significativo (p=0,380). Eur. J. Oncol., 12 (4), 243-253, 2007

Key words: **Ewing sarcoma family tumours (ESFT), immunohistochemistry, prognostic factors, survival analysis, tissue array method**

Parole chiave: **tumori della famiglia del sarcoma di Ewing (TSE), immunoistochimica, fattori prognostici, analisi della sopravvivenza, metodi di saggio tissutale**

Introduction

Ewing sarcoma family tumours (ESFT) are aggressive malignant tumours arising primarily in bone or soft tissue and belonging to the group of malignant small round-cell tumours (MSRCT)[1]. Within ESFT, typical undifferentiated Ewing's sarcoma (ES) lies at one end of the spectrum and peripheral primitive neuroectodermal tumour (pPNET) with clear evidence of neural differentiation at the other[2]. Almost all (90-95%) ESFT share the same non-random t (11; 22)(q24; q12) chromosome rearrangement, and in view of the same cytogenetic, molecular genetic and biochemical characteristics it is now believed that they have a common neural histogenesis[3-6].

ES is the second most common malignant bone tumour of children and young adults and accounts for 10-15% of all primary bone tumours, while pPNET represents 20% of soft tissue malignancy in childhood[7]. The peak age group for ES is in the second decade, the tumour showing predilection for males (ratio 1.5:1), and approximately 60% of cases occur in the pelvic girdle and lower extremities, with the femur being the most frequently affected bone[8].

The first symptom is increasing local pain, with fever, weight loss and elevated sedimentation rate. The X-ray of the affected bone shows an extensive, destructive, sometimes permeative and poorly circumscribed lytic lesion. There is often prominent periosteal new bone formation, giving rise to an onionskin appearance. The bone lesion is often accompanied by a soft tissue mass and it is sometimes difficult to determine the primary site of the tumour. CT and MRI imaging studies help in defining the extent of the bone and soft tissue lesions. When histologically compared to conventional ES, pPNETs tend to show hyperchromasia and a greater degree of nuclear pleomorphism, lobular growth pattern and higher mitotic activity[9]. There is general consensus that the presence of rosette formations is a histological criterion necessary for diagnosing pPNET.

Since the prognosis and treatment of ES and pPNET are the same, it is not critical to make this histological distinction as was once proposed[10].

Adequate clinical information, recognition of morphological, immunohistochemical and at times ultrastructural features help us to distinguish ESFT from other MSRCT[11]. Immunohistochemical studies are helpful in making the diagnosis of ESFT. Up to 90% tumours show strong membrane staining for CD99. The expression of CD99 in ESFT is not specific[11, 12].

Cytogenetic and molecular genetic studies can be useful tools in diagnosing ESFT. Approximately 90-95% ESFT have t (11; 22)(q24; q12), whereas 5-10% contain the t (21; 22)(q22; q12) chromosomal translocation. However, all those translocations are not specific for ESFT. EWS/FLI-1 fusions have been recently described in other sarcomas, for instance in alveolar rhabdomyosarcoma, desmoplastic small round-cell tumour and even neuroblastoma[3, 6, 13, 14].

Accurate diagnosis of ESFT is crucial for the most appropriate clinical management of patients[15].

At the time of diagnosis, almost 13-30% of patients with ESFT already have clinical signs of metastatic disease. In fact, practically all patients with ESFT have occult tumour cells in the peripheral blood and micrometastases in bone marrow at the time of diagnosis[16-18]. Because of the strong adverse effect of metastases on the survival of patients with ESFT, local treatment should be followed by adjuvant chemotherapy and radiotherapy, since ESFT are highly radio- and chemosensitive[17-19]. Treatment is individualized, using various combinations of chemotherapy, surgery and radiation[20]. However, such combination therapy has a spec-

trum of side effects, including the appearance of secondary malignancies, and is also relatively expensive[21-25]. Therefore the question emerges of whether such aggressive therapy is justified in all patients with ESFT.

Improved outcome may be achieved by stratifying patients for treatment according to risk. Recently a systematic review of prognostic tumour markers in ESFT was undertaken. The most frequently investigated prognostic tumour marker in the past was CD99 (mic2)[11, 12], while FLI-1[2], p53[20, 26], IGF-1 and IGF-3[27], bcl-2[28], Pgp[29], Her2[30] and CD117 (*c-kit*) were far less investigated[31, 32].

Very few prognostic markers are routinely used in everyday practice in ESFT, due to the small number of studies and patients evaluated for clinical outcome, and because of the controversial results.

The aim of this study was to assess CD99, p53, CD117 and bcl-2 expression patterns in a series of ESFT cases, and to determine the prognostic value of CD99, p53, CD117 and bcl-2 in these patients.

CD99 (mic2)

The mic2 gene (CD99) is located on the pseudoautosomal region of both chromosomes X and Y. The product of the mic2 gene is a membranocytoplasmic glycoprotein with a molecular weight of approximately 28,000-32,000 kd, and it appears to be involved in cell adhesion processes[33]. It is expressed in virtually all ESFT. The specificity of CD99 antibodies for neuroectodermal tumours is not absolute, because they may also label 90% of the lymphoblastic lymphomas and less than 15% of alveolar rhabdomyosarcomas. In 20% of neuroendocrine carcinomas the positive reaction of CD99 is also observed. Several normal tissues can also show significant reactivity for the CD99 antibody[34].

CD117 (*c-kit*)

C-kit is a proto-oncogene encoding a transmembrane receptor (CD117) with an internal thyrosine kinase component that is structurally related to the platelet-derived growth factor receptor and the receptor for colony stimulating factor-1[35]. The ligand for *c-kit* is the stem cell factor (SCF), also known as cell growth factor, responsible for a number of different processes in development and adulthood, including cell survival and proliferation, differentiation, migration and homing[36]. *C-kit* expression has been studied in gastrointestinal stromal tumours (GIST)[37], Ewing sarcomas[38], melanomas[39], angiosarcomas[40], small cell carcinoma of the lung[41], acute myelogenous leukaemias[42], and mast cell disease[43]. The *c-kit* expression in these tumours, especially in GIST, was seen as a strong, diffuse cytoplasmatic and membranous staining.

bcl-2

Proteins of the bcl-2 family - of which bcl-2 and Bax are the best known - are regulators of apoptosis, displaying cell death promoting or inhibiting effects. Altered expression of these proteins occurs in many human tumours, leading to neoplastic cell division by suppression of programmed cell death and extension of the tumour cell life span. The apoptosis-suppressing protein bcl-2 is selectively expressed in normal tissues and tumours and was originally discovered as a proto-oncogene in low-grade B-cell lymphomas[44].

p53

The p53 gene has a critical rôle in cell cycle regulation and tumour suppression as guardian of the genome. It is a nuclear protein that binds to and modulates the expression of genes important for DNA repair, cell division and cell death by apoptosis[45]. Mutation of the p53 gene is the most common genetic abnormality associated with malignancy. Numerous studies have shown a close correlation between mutations of p53 and overexpression of p53 protein in association with high histological grade and adverse prognosis in different malignancies[2]. There is an accumulation of mutated p53 in the nucleus of the tumour cell, the levels of which can be determined by using immunohistochemical methods[46].

Materials and methods

Patients

Approval was acquired from the Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia prior to the beginning of data collection for this retrospective study.

We studied the surgical pathology records at the Institute of Oncology and the Cancer Registry of the Republic of Slovenia for the years 1972-2002. In 72 patients with various types of ESFT we could trace formalin-fixed paraffin-embedded biopsy specimens. The patients either had diagnostic biopsy and treatment at the Institute of Oncology or confirmed histologic diagnosis in the tissue

blocks from other institutions and treatment at the Institute. These patients had a median age of 19.4 (range 2-59) years at the time of diagnosis. Data for gender, survival time, treatment, stage of disease and site of metastases were collected as well. Unfortunately we were not able to study the X-ray and CT images of the tumours and could therefore not determine their size and volume. We reviewed histopathological reports filed under the diagnosis of ES, PNET, and Askin tumour. The review of the original histological slides stained with haematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) showed that all the tumours were composed of sheets of small round cells with pale, scanty cytoplasm, heterogeneously rich in glycogen, with small round to oval nuclei with inconspicuous nucleoli. Mitotic figures were frequent. The presence of necrosis in tumours was recorded. We also reviewed the original immunohistochemically-stained sections of the tumours.

Treatment of the primary tumour

Therapy of the primary tumour in our patients was designed according to the stage of disease, possibility of eradicating local disease and maintaining the best possible function. The period in which the patient was observed and treated most probably also influenced the choice of local treatment.

Local therapy was individualized and consisted of radical surgery followed by radiotherapy. The decision for the local treatment was taken by considering the following parameters: 1) resectability of the tumour, even if with "marginal surgery", and 2) location of the tumour (in an expendable bone, where resection does not produce severe malfunction i.e. rib, clavicle, scapula or in a skeletal articular segment, where resection can be repaired with a prosthesis).

Neoadjuvant chemotherapy was introduced in Slovenia after 1982.

Paediatric patients received chemotherapy which included doxorubicin (Adria), cyclophosphamide (C), actinomycin D (A), and vincristine (V) according to T2, T6, T9 or T11 protocols[47]. During the 1990s, cyclophosphamide was replaced by ifosphamide (Ifos) (VAIA protocol). In high risk patients, etoposide was added (EVAIA protocol). Older patients have been treated at the Institute of Oncology and received different combinations of either etoposide, ifosphamide, vincristine and farmarubicin, platinol, adriamycin, and/or epidoxorubicin.

Patients from the Paediatric Haemato-oncology Department were followed there until the age of 18 years. After that follow-up continued at the Institute of Oncology, where the patients were evaluated for late effects of treatment. After 1991, some patients were lost to follow-up, when Slovenia became an independent state of former the Yugoslavia. For the same reason, the data of disease stage were not available in 13 cases and of therapy in 19 cases. Stage of disease and treatment are summarized in Table 1.

Tissue microarray (TMA) construction

Tumour samples were arrayed as previously described[48, 49]. In short, by reviewing the original H&E stained sections, the representative tumour regions were defined and marked on the "donor" tissue block. Three core tissue biopsies with a diameter of 0.6 mm were then punched out from the selected areas of each "donor" block using a manual custom-made precision instrument (Beecher instruments, Silver Spring, MD, USA) and arranged in a new "recipient" paraffin block (tissue array block). Four "recipient" blocks were made in this way.

Immunohistochemistry

Sections from each of the "recipient" blocks were used for immunostaining. Sections 5 μm thick were cut, dried, deparaffinized, and rehydrated as usual. For antigen retrieval, tissue sections were cooked in a Milestone Micromed T/T Mega microwave oven (Milestone, Italy) for CD117, bcl-2 and p53, and in a Miele type M 752 microwave oven (Miele, Germany) for CD99. Immunostaining was performed with the Dako Tech-Mate automated immunohistochemistry system (Dako, Denmark) using an En-vision detection kit (Dako, Denmark). The primary antibodies, dilutions and pretreatment are presented in Table 2.

Appropriate negative and positive controls were included.

Positive immunoreaction for bcl-2 was identified as cytoplasmatic staining, for p53 as nuclear staining, for

Table 1 - Stage and treatment of the disease

Therapy	Stage of disease				
	1	2	3	4	Unknown
S[a]			1		
ChT[b]		3		1	
RT[c]		1			
ChT+S	1	5	2		
ChT+RT	2	8	6	2	1
ChT+S+RT	1	10	2	6	
Unknown	1		3	3	13

[a] S = surgery
[b] ChT = chemotherapy
[c] RT = radiotherapy

Table 2 - Antibodies, dilutions and pretreatment used in this study

Antibody	Clone	Pretreatment	Temperature, time	Antibody dilution	Source
CD99	12E7	Citric buffer; pH 6	850 W, 15 minutes	1:75	Dako, Glostrup, Denmark
CD117	Polyclonal	Tris-EDTA, 0.01 M, pH 8	98°C, 10 minutes	1:25	Dako, Glostrup, Denmark
bcl-2	124	Tris-EDTA, 0.01 M, pH 8	98°C, 10 minutes	1:40	Dako, Glostrup, Denmark
p53	DO-7	Tris-EDTA, 0.01 M, pH 8	98°C, 10 minutes	1:200	Dako, Glostrup, Denmark

CD117 (*c-kit*) as diffuse/granular staining in cytoplasm with accentuation of the cell membrane, and for CD99 (mic2) as strong-diffuse, membrane-predominant staining pattern. The immunostaining results were assessed in a semiquantitative way.

No staining or very exceptional positive cells were considered a negative result. Staining in <25% of tumour cells was considered as slightly positive (1+), staining in 25-50% of the tumour cells as moderately positive (2+) and in >50% of the tumour cells as strongly positive immunoreaction (3+). Because of the small tissue samples, no distinction between focal and diffuse positive staining was recorded.

To confirm the diagnosis of ESFT immunohistochemically, we also tested 5 μm tissue sections from constructed "recipient tissue blocks" against a panel of other antibodies such as desmin, sm actin, S-100 protein, CD45, CD79a, EMA, TdT, CK MNF116 and NSE.

Statistical analysis

Survival analysis was performed using the univariate and multivariate statistic methods (Cox proportional hazard regression model, analysis of variance, bi-serial correlation coefficient and chi-square [χ^2] test)[50]. The overall survival was defined as the time from diagnosis to the date of review or date of death. The results were considered statistically significant at $p<0.05$.

Results

Seventy-two patients with ESFT were studied, 46 males and 26 females. The mean age at diagnosis was 19.4 years (range 2-59 years).

Conservative surgery, followed by radiation (35-45 Gy to the tumour bed and remaining bone) and combined with chemotherapy was carried out in 19 patients. In 2 patients surgery or radiotherapy was the only mode of treatment.

Nineteen patients were treated with combined radiation therapy and chemotherapy and 4 patients with chemotherapy alone. In 8 surgically treated patients chemotherapy started 4-8 days after surgery.

Five patients had disease in stage 1, 27 patients in stage 2, 14 patients in stage 3 and 12 patients in stage 4. The stage of disease for 14 patients was not known.

Thirty-five patients developed metastatic disease after the initial diagnosis and treatment. Forty-seven patients were already dead at the time of our study and the remaining 25 patients were either alive or the data of their status were not available. The former group was regarded as "uncensored cases" (event) and the latter group as "censored" one.

The minimum survival time was 2 and maximum survival time was 203 months. The age, presence of necrosis, gender, censored and uncensored cases, and the results of immunohistochemical expression of CD99, CD117, bcl-2 and p53 are shown in Table 3.

Cases labelled as NA (not available) had very thin original tumour tissue in the tissue blocks (tissue blocks were already cut-off profoundly for reaching the primary diagnosis). In these cases, the tissue cores prepared for tissue microarray were very short and therefore some tissue cores were not in the same level with other, longer tissue cores. Consequently, some of the cores did not appear in the whole tissue microarray section.

CD99 was detected as membrane-type diffuse expression in 90% (63 out of 70), CD117 in 75.8% (47 out of 62), bcl-2 in 70.1% (47 out of 67) and p53 in 66.6% (44 out of 66) of the ESFT studied (Table 4).

Survival analysis and CD99, CD117, bcl-2 and p53 expression

Survival analysis was performed in 72 patients for CD99, in 62 patients for CD117, in 67 patients for bcl-2, and in 66 patients for p53 expression.

According to the Cox regression analysis, the expression of all four immunohistochemical markers turned out to be a statistically non-significant prognostic survival factor (CD99: $p=0.140$; CD117: $p=0.612$; bcl-2: $p=0.382$; p53: $p=0.540$) in patients with ESFT. The calculated regression coefficient exponent showed that the risk of death is higher in patients with stronger expression of CD117 by 9.2%, of bcl-2 by 2.2% and of p53 by 11.8%. On the contrary, the risk of death is lower in patients with

Table 3 - Summary of immunohistochemical staining results and clinicopathological features of ESFT

Case N.	Age	Status[a]	Gender[b]	Necrosis[c]	CD99[d]	CD117[d]	p53[d]	bcl2[d]
1	19	1	F	+	0	0	NA	0
2	26	0	F	+	2	1	1	2
3	8	1	F	+	2	0	1	0
4	12	1	M	+	3	NA	3	0
5	2	0	F	+	3	1	1	0
6	6	1	M	+	3	1	2	3
7	12	1	F	+	1	1	1	3
8	13	1	M	+	1	3	2	0
9	17	1	M	+	3	3	2	3
10	11	0	M	0	3	3	2	2
11	11	1	F	+	3	1	0	0
12	28	1	M	+	3	0	3	2
13	21	1	M	+	3	0	2	0
14	3	0	F	0	3	1	2	1
15	20	0	F	0	3	2	2	0
16	30	1	M	+	1	1	0	1
17	14	1	M	+	3	2	1	2
18	20	1	F	+	NA	NA	NA	NA
19	13	0	F	+	3	1	1	2
20	32	1	F	+	3	0	0	0
21	22	0	M	+	1	2	1	2
22	22	1	F	+	3	0	1	0
23	11	0	M	0	3	1	0	1
24	14	0	F	0	3	1	1	1
25	27	0	F	+	2	1	2	3
26	7	0	F	+	0	NA	0	0
27	18	1	M	+	3	1	0	3
28	26	0	M	+	3	3	0	2
29	16	1	M	+	3	2	0	2
30	16	1	M	+	3	1	1	3
31	29	1	M	+	2	1	1	2
32	26	1	F	+	1	1	2	2
33	20	1	M	0	0	NA	3	0
34	12	1	M	+	1	1	NA	NA
35	18	1	M	+	2	1	1	1
36	13	1	F	0	3	3	0	1
37	11	0	M	+	3	0	0	0
38	12	0	M	0	2	1	1	0
39	3	0	M	+	3	0	0	0
40	40	1	M	+	0	0	NA	NA
41	19	0	M	+	1	0	NA	0
42	12	0	M	+	2	1	1	0
43	22	0	M	+	1	NA	NA	NA
44	13	0	F	+	1	1	1	1
45	29	0	M	0	3	1	2	2
46	12	1	F	+	3	2	0	1
47	8	0	M	0	3	3	0	1
48	13	1	F	+	3	3	0	3
49	16	1	M	0	0	0	0	3
50	12	1	M	+	1	1	1	1
51	36	1	F	+	3	0	2	2
52	8	0	M	+	NA	NA	0	3
53	31	1	M	+	1	1	3	3

(footnotes next page)

(to be continued on the next page)

Table 3 - continued

Case N.	Age	Status[a]	Gender[b]	Necrosis[c]	CD99[d]	CD117[d]	p53[d]	bcl2[d]
54	47	1	M	0	2	NA	1	2
55	19	1	M	+	3	2	2	3
56	37	1	M	+	2	NA	1	0
57	11	1	M	0	3	0	0	2
58	59	1	M	0	3	1	2	3
59	54	1	F	+	3	2	1	1
60	24	1	M	+	2	1	1	1
61	18	1	M	0	2	2	1	1
62	22	0	M	0	0	0	0	2
63	36	1	F	+	3	2	2	2
64	16	1	M	+	2	1	1	3
65	18	1	F	+	3	1	1	1
66	26	1	M	+	1	1	0	0
67	5	1	F	+	2	NA	0	NA
68	9	0	M	0	3	0	1	2
69	16	0	M	+	0	1	1	1
70	34	1	M	+	3	1	2	1
71	19	1	F	+	3	1	2	1
72	14	1	M	+	1	NA	0	0

[a] Status: 1 - event, 0 - censored
[b] Gender: M - male, F - female
[c] Necrosis: + positive, 0 negative
[d] Immunohistochemistry: NA = not available, 0 = negative immunoreaction, 1 = slight immunoreaction, 2 = moderate immunoreaction, 3 = strong immunoreaction

Table 4 - CD99, CD117, bcl-2 and p53 expression pattern in ESFT

Expression pattern	NA (not available)	Negative - 0 (%)	Positive - 1 (%)	Positive - 2 (%)	Positive - 3 (%)	Positive - total (%)
CD99	2	7 (10.0%)	13 (18.6%)	13 (18.6%)	37 (52.9%)	63 (90%)
CD117	10	15 (24.2%)	31 (50.0%)	9 (14.5%)	7 (11.3%)	47 (75.8%)
bcl-2	5	20 (29.9%)	17 (25.4%)	17 (25.4%)	13 (19.4%)	47 (70.1%)
p53	6	22 (33.3%)	25 (37.8%)	15 (22.7%)	4 (6.1%)	44 (66.6%)

stronger expression of CD99 by 19% as well. The survival frequencies according to predictor values are presented in figs. 1-4.

Stage of disease, therapy and expression of CD99, CD117, p53 and bcl-2

The analysis of variance of the mean values of the CD99, CD117, p53 and bcl-2 expression and the given therapy showed no statistic significance for survival ($p>0.05$) for any of these markers. The mean values of marker expression were highest in all cases, if the combined therapy (surgery, chemotherapy and radiotherapy) was performed. The last statement was true particularly for bcl-2 (Table 5).

The expression of the studied markers in tumours at different stages of disease was also statistically not significant for survival.

Discussion

In the current retrospective analysis, the tissue arrays and immunohistochemistry were used to study the expression of CD99, CD117, bcl-2 and p53 proteins in 72 patients with various types of ESFT. In the past all those antibodies were already applied for the purposes of diagnosing ESFT. However, we know little about the prognostic value of their expression in combination with clinicopathological data. The present study was made possible with the use of the tissue array method, which

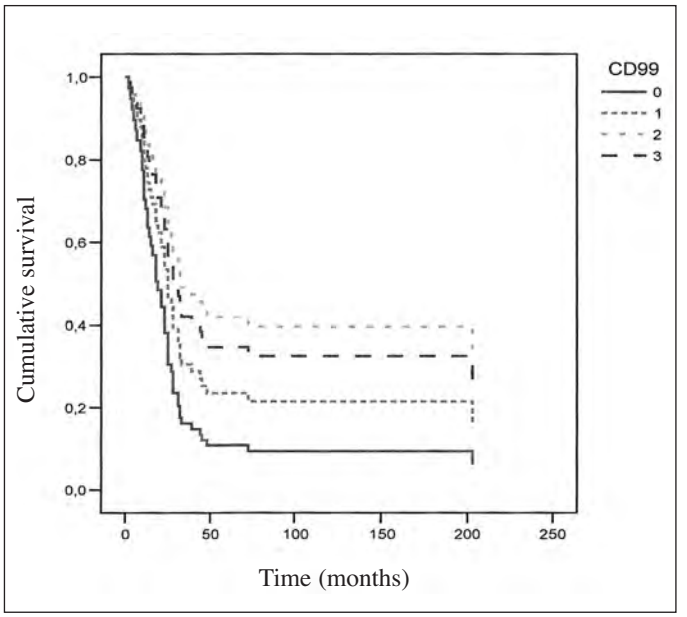

Fig. 1. Overall survival according to CD99 expression
p=0.140

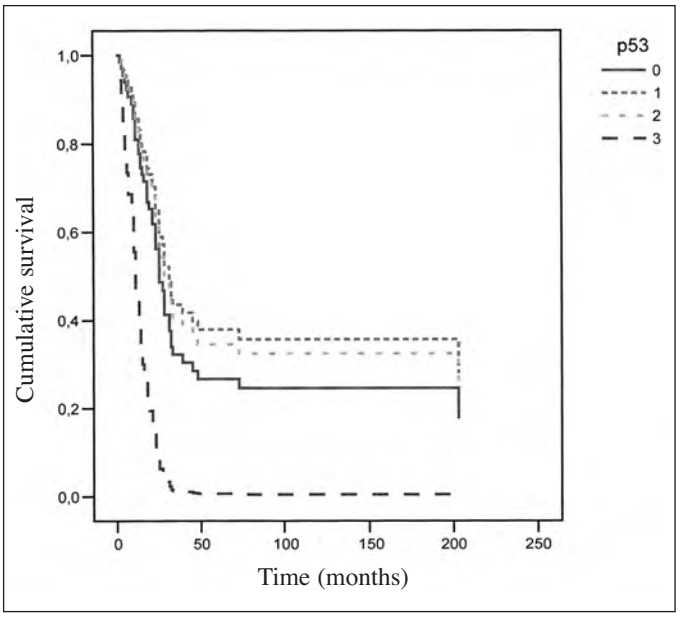

Fig. 3. Overall survival according to p53 expression
p=0.540

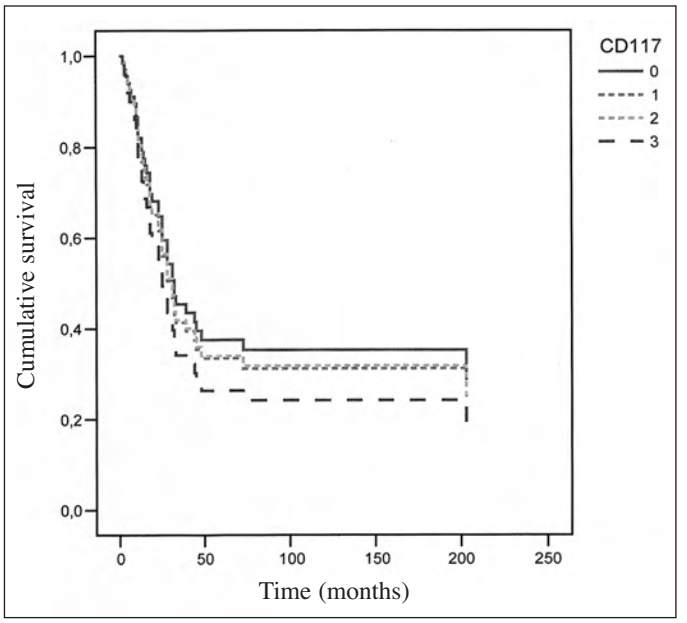

Fig. 2. Survival curves for CD117
p=0.612

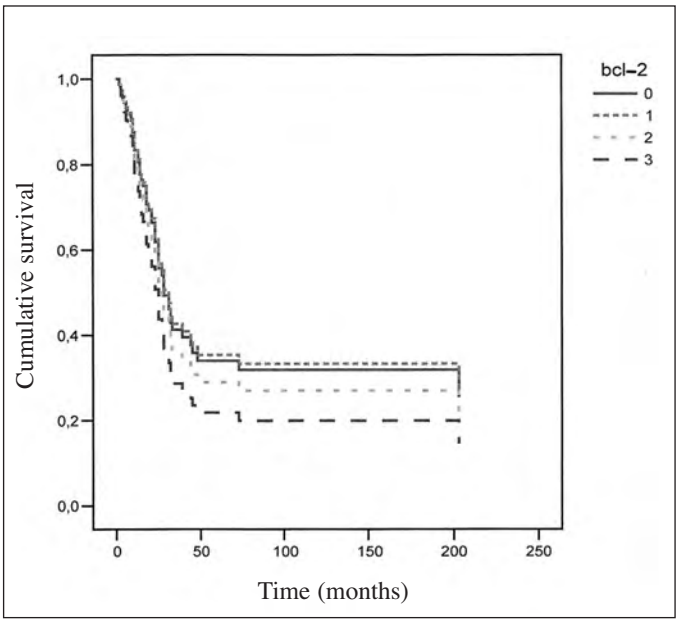

Fig. 4. Survival curves for bcl-2
p=0.382

enables the immunohistochemical analysis of the expression of different antibodies in a large number of ESFT.

The potential limitations of this method are mainly associated with the acquisition of information from only a tiny area in each tumour that has already been studied. In order to emphasize the influence of tumour heterogeneity and to evaluate the ability of the tissue array method to yield information on the prognostic values of biomarkers, multiple replicate tissue array blocks were constructed or multiple tissue cores were punched out from the donor tissue block and put in the same recipient tissue block, both by us and by other researchers, as well[51]. In all of these studies, the data from each replica array were almost identical. The prognostic associations of the markers were always as good as, or better, when measured from the tissue array slides, than the analysis of individual large tumour sections.

In our study, immunohistochemical data were compared with clinicopathological data using the Cox proportional hazard regression model.

Table 5 - Therapy and CD99, CD117, p53 and bcl-2 expression[a]

Markers	Therapy	Patients (N.)	Mean	Standard Deviation	F	p
CD99	(S+ChT)	8	2.25	0.886		
	(ChT+RT)	17	2.12	1.166	0.107	0.899
	(S+ChT+RT)	18	2.28	1.018		
CD117	(S+ChT)	6	0.67	0.516		
	(ChT+RT)	14	0.93	0.917	0.528	0.595
	(S+ChT+RT)	16	1.13	1.088		
p53	(S+ChT)	7	0.57	0.535		
	(ChT+RT)	15	0.67	0.816	1.365	0.268
	(S+ChT+RT)	18	1.11	1.079		
bcl-2	(S+ChT)	8	0.88	1.356		
	(ChT+RT)	15	0.87	0.834	2.457	0.100
	(S+ChT+RT)	17	1.65	1.169		

[a] Markers were not studied in all patients

Analysing the results of the immunohistochemical testing, we found that neither the expression of CD99 (p=0.140), CD117 (p=0.612), p53 (p=0.540) nor bcl-2 (p=0.382) has statistically significant impact on the survival in patients with ESFT.

The data from the literature show similar immunoreactivity of ESFT, with CD99 ranging from 82% to 100%, compared with our result of 90% (63 out of 70 cases)[2]. The calculated regression coefficient exponent for CD99 [exp(b)=0.810] showed that the mortality of the patients with ESFT in our study was rising inversely proportionately to the expression of the marker, i.e., it was 19% higher in cases with lower values as compared to the cases with higher values of the expressed CD99.

In the present study, the CD117 expression was detected in 75.8% (47 out of 62 cases) of the ESFT studied - a result comparable with the data from other researchers. But the strong and diffuse immunoreactivity of CD117 in our study was present only in 11.3% (7 out of 62 cases) of the studied tumours and is lower than reported in the literature[52]. The influence of the *c-kit* pathway to tumorigenesis in ESFT is not clear yet, although some previous studies suggest possible autocrine or paracrine influence[40]. The strong and diffuse pattern of CD117 immunoreactivity is typical for GIST. Tumours other than GIST, including ESFT, with strong, diffuse staining for CD117 in a pattern similar to GIST, may represent suitable targets for new therapeutic drugs, i.e. oral agent STI-571 (Gleevec, Novartis). STI-571 is a specific inhibitor of some thyrosine kinases (*bcr/abl*, *abl*, *c-kit* and platelet-related growth factor receptor). This agent has already shown good results in the management of patients with GIST and chronic myelogenous leukaemia.

Despite the fact that CD117 expression in the tumours of our patients was not statistically significant for survival, the calculated regression coefficient exponent was >1 [exp(b)=1.092] and therefore we were able to confirm the 9.2% higher risk for death in the patients with ESFT showing stronger CD117 antigen expression.

The p53 over-expression in our study was present in 66.6% (44 out of 66) of the ESFT studied. These data contradict previous reports of minimal positive p53 over-expression in ESFT, i.e. 15% and 43%[2,53]. However, they are very similar to the data from aforementioned reports about worse prognosis regarding the overall survival of patients with ESFT. In our study, the calculated regression coefficient exponent was >1 [exp(b)=1.118]. Consequently, the risk of death for patients with over-expression of p53 in ESFT is higher by 11.8%.

Bcl-2 expression was detected in 70.1% (47 out of 67) of studied ESFT. The result matches with the data from the literature[27]. Although no statistically significant difference was found in the survival of patients with bcl-2 positive *vs* negative tumours, there may be a trend for worse survival of the former. The calculated regression coefficient exponent [exp(b)=1.112] showed that the risk of death is higher by 12.2% in patients with stronger expression of bcl-2.

The presence of tumour necrosis, little or abundant, was a statistically significant adverse prognostic factor (p=0.033), confirming the data from the literature[54]. On the other hand, the patients younger than 20 years of age at the time of the diagnosis, have a statistically significantly better prognosis than the older ones (p=0.008), a result consistent with the data from other reports[1,55].

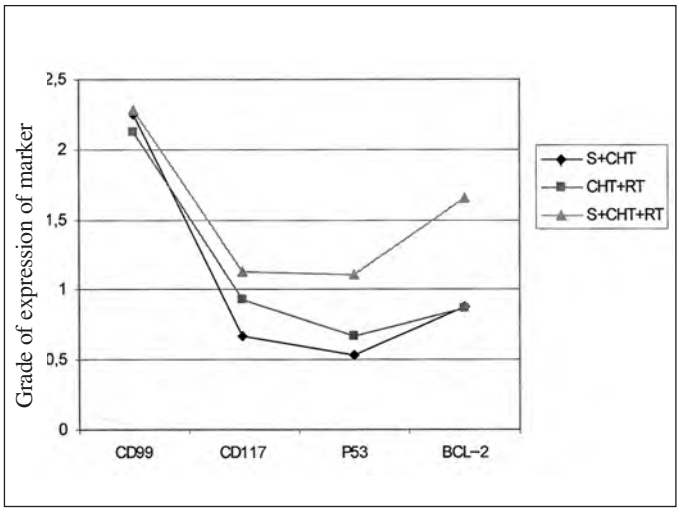

Fig. 5. Expression of CD99, CD117, p53 and bcl-2 in tumours treated with different treatment modalities

The application of combined therapy (surgery, chemotherapy and/or radiation) has a more favourable impact on survival than chemotherapy, radiation or surgery alone.

In the management of the primary tumour, high expression of CD99, CD117, p53 and bcl-2 in the tumour at the time of the diagnosis may serve as an indication to the clinicians for the application of dual local therapy (surgery and radiation) combined with neoadjuvant chemotherapy (fig. 5).

Conclusion

In summary, the report presents an immunohistochemical expression study performed on 72 ESFT patients using the tissue array method. The expression of CD99, CD117, bcl-2 and p53 in these tumours is not statistically significantly associated with patient survival and has only a minor impact in determining patient survival. Further immunohistochemical studies investigating new possible prognostic antibodies may provide answers to the question: which immunohistochemical marker could be useful not only in establishing the diagnosis of ESFT but also in predicting the survival prognosis of the patient with ESFT?

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Malignant mesothelioma of the peritoneum and heavy exposure to asbestos

Mesotelioma maligno del peritoneo ed intensa esposizione all'asbesto

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Summary

***Aim*. The purpose of this study is to analyse and discuss the relationship between development of malignant peritoneal mesothelioma and heavy exposure to asbestos. *Patients and methods*. Six cases of malignant peritoneal mesothelioma, seen in five insulators and one picker in the Monfalcone area, Italy (population about 60,000), between 1992-2007, were reviewed. The diagnosis was based on/or confirmed by necropsy on five cases. In one case, mesothelioma was diagnosed by histological examination of peritoneal samples obtained at surgery. Occupational data were obtained from the patients themselves or from their relatives by means of personal interviews. In four cases asbestos bodies were isolated from the lung tissue following the Smith-Naylor method. *Results*. The group included six men aged between 49 and 74 years. Among insulators, two patients had been exposed to asbestos only in insulation work, and three also in other settings (two as plumbers in shipyards, and one as a worker in a paper mill). The latency periods (time intervals between first exposure to asbestos and diagnosis of mesothelioma) ranged from 30 to 47 years (mean 38.0 years). Lung asbestos bodies varied between 150,000 and 822,000 per gram of dried tissue. In one case an additional malignancy (squamous cell carcinoma of the lung) was found at necropsy. *Conclusions*. In accordance with various data from the literature, experience gained in the Monfalcone area suggests that, when exposure to**

Riassunto

***Finalità*. Scopo del lavoro è quello di analizzare e discutere le relazioni esistenti tra insorgenza del mesotelioma maligno del peritoneo ed esposizione intensa all'asbesto. *Pazienti e metodi*. Vengono riesaminati sei casi di mesotelioma maligno del peritoneo, osservati in cinque coibentatori e in un picchettino nell'area di Monfalcone, Italia (popolazione totale circa 60.000 abitanti), tra il 1992 e il 2007. In cinque casi la diagnosi era basata sull'autopsia o confermata dai reperti autoptici. In un caso il mesotelioma fu diagnosticato con l'esame istologico di campioni di tessuto peritoneale ottenuti all'intervento chirurgico. Le storie professionali furono raccolte attraverso interviste personali dei pazienti stessi o dei loro familiari. In quattro casi i corpi dell'asbesto furono isolati dal tessuto polmonare secondo il metodo di Smith-Naylor. *Risultati*. Il gruppo comprendeva sei pazienti di sesso maschile, di età variabile tra 49 e 74 anni. Dei pazienti isolatori, due erano stati esposti all'asbesto soltanto in lavori di coibentazione, mentre gli altri tre avevano subito esposizione anche in altri ambienti di lavoro (due nei cantieri navali con mansioni di tubisti, uno come operaio in una cartiera). I periodi di latenza, definiti come intervalli di tempo intercorsi tra inizio dell'esposizione all'asbesto e diagnosi di mesotelioma, variavano da 30 a 47 anni (media 38,0 anni). I corpi dell'asbesto isolati dal tessuto polmonare variavano fra 150.000 e 822.000 per grammo di tessuto secco. In un caso venne riscontrato all'autopsia anche un carcinoma squa-**

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asbestos has been very heavy in intensity, the peritoneum is a preferential target. Such conclusion has implications on the problem of the effects of asbestos fibre ingestion. Eur. J. Oncol., 12 (4), 255-258, 2007

Key words: **peritoneum, insulation, asbestos, asbestos ingestion, lung asbestos bodies, multiple malignancies**

moso del polmone. *Conclusioni*. In accordo con vari dati della letteratura, i risultati ottenuti nell'area di Monfalcone suggeriscono che, quando l'esposizione all'asbesto è stata di forte intensità, il peritoneo diventa una sede preferenziale di mesotelioma. Tale conclusione ha delle implicazioni sul problema dei possibili effetti da ingestione di fibre di asbesto. Eur. J. Oncol., 12 (4), 255-258, 2007

Parole chiave: **peritoneo, coibentazione, asbesto, ingestione di asbesto, corpi dell'asbesto polmonari, tumori multipli**

Introduction

Malignant mesothelioma arising in the peritoneum shows strong similarities with mesothelioma originating in the pleura. Asbestos[1, 2], erionite[1], and radiotherapy[3] are well recognised aetiologic agents for both these tumours, with asbestos being by far the principal cause. Peritoneal mesothelioma, however, differs from its pleural counterpart for a markedly lower incidence[1, 4].

The epidemiology of peritoneal mesothelioma presents many grey areas due to the major difficulties encountered both in its diagnosis, and in its registration[4].

A recent review[1] concludes that the relationship of peritoneal mesothelioma with asbestos is not so strong as that of pleural mesothelioma. This statement seems to be in contrast with studies showing high numbers of peritoneal mesotheliomas precisely in those occupational categories with the heaviest exposure to asbestos, such as for instance the insulators[5]. In the present study, a small group of peritoneal mesotheliomas, observed in Monfalcone among people with heavy exposure to asbestos, were reviewed.

Patients and methods

Six patients, seen at the Hospital of Monfalcone and/or at our Centre between 1992 and 2007, were included in the study. In five cases, the diagnosis was based on, or confirmed by necropsy findings. In one case, large biopsies of peritoneum, obtained at surgery, were early interpreted as a malignant tumour of undetermined origin. Review of the histological slides and of clinical data led later to a diagnosis of peritoneal primary tumour. Detailed occupational histories were obtained from the patients themselves, or from their relatives, by means of personal interviews. The work booklet, a personal document in which all the occupations and the employers are listed, was also consulted. In four cases, lung asbestos bodies were isolated and counted after chemical digestion of pulmonary tissue, obtained at necropsy, following the Smith-Naylor method[6].

Results

The group included six men, aged between 49 and 74 years (mean 62.5 years). The principal features of the cases are reported in Table 1. Four patients were resident in the Monfalcone municipality (total population about 30,000), and the remaining two patients in the San Canzian d'Isonzo municipality (total population about 5,000). A clinical diagnosis of peritoneal mesothelioma had been made in four cases (cases 1-3, and 5). Exposure to asbestos had begun between 1957 and 1968. Three patients were exposed to asbestos partly in insulation work, and partly in other settings. The duration of employment does not necessarily mean duration of exposure to asbestos, since alternative materials began to be used in Monfalcone in around 1980. Latency periods (time intervals between first exposure to asbestos and diagnosis of mesothelioma), ranged between 30 and 47 years (mean 38.0 years). Lung asbestos body burdens varied from 150,000 to 822,000 bodies per gram of dried tissue. In the case 6, a squamous cell carcinoma of the lung co-existed with peritoneal mesothelioma.

Discussion

Peritoneal mesotheliomas represent a small proportion of total mesotheliomas. The ratio of pleural to peritoneal mesothelioma varies widely from one country to another, being 13:1 in Australia[7], 8:1 in Japan[8], 7.3:1 in Sweden[9, 10].

Table 1 - Exposure to asbestos in six people with malignant mesothelioma of the peritoneum, Monfalcone, Italy, 1992-2007

Case N.	Sex	Age	Mesothelioma incidence year	Trade	First asbestos exposure	Employment duration	Latency period	Lung asbestos body burden/g dried tissue
1	M	60	1992	Insulator	1957	31	35	800,000
2	M	51	1993	Picker	1963	30	30	822,000
3	M	49	1998	Insulator[a]	1964	9	34	174,000
4	M	70	2001	Insulator[b]	1957	26	44	not done
5	M	74	2004	Insulator[c]	1957	26	47	not done
6	M	71	2007	Insulator	1968	26	38	150,000

[a] The patient had worked for 11 months as an insulator, and 8 years as a shipyard plumber
[b] The patient had worked for 30 months as an insulator, and then as a worker in a paper mill
[c] The patient had worked for 13 years as an insulator, and for a further 13 years as a shipyard plumber

In Italy, of a series of 5,173 mesotheliomas collected by the National Mesothelioma Registry, peritoneal primaries represented about 6%[11]. In this context, it is noteworthy that substantially different ratios have been observed in some studies on insulation workers. In a mortality study regarding 162 insulators in Belfast, Northern Ireland, eight deaths from pleural mesothelioma and five deaths from peritoneal mesothelioma were observed[12]. However, some doubts remained in this investigation about the sure distinction between gastrointestinal malignancies and peritoneal mesotheliomas. In studies on the mortality of insulators in the United States and Canada, a high proportion of peritoneal mesothelioma was found[5]. A cohort comprising 17,800 people was followed from 1967 to 1987. Of 4,591 total deaths, 285 were due to peritoneal mesothelioma, and 173 to pleural mesothelioma. In a study of insulators in Sweden, cancer morbidity and cause of death were investigated in 248 workers[13]. Seven cases of peritoneal mesothelioma and no cases of pleural mesothelioma were observed. However, in an investigation on cancer incidence among 1,116 Norwegian insulators, nine pleural and two peritoneal mesotheliomas were seen[14]. In a study regarding mortality from peritoneal cancer in the United States, the occupations of people who had died due to peritoneal malignancy were compared with those of people who had died for non-malignant disease[15]. Elevated odds ratios (OR), comprised between 1.5 and 5.1, were observed for different occupations. OR for insulators reached the value of 180.

As a whole, the above data are strongly suggestive of a strict relationship between heavy exposure to asbestos and mesothelioma of the peritoneum. The experience gained in the Monfalcone area is in accordance with this view.

The Monfalcone district has been characterized as an area with a very high incidence of mesothelioma[16, 17]. However, in such an area, peritoneal primaries are not frequent in comparison with the pleural ones[18]. Of 92 mesotheliomas diagnosed at the Monfalcone Hospital in the period from October 1979 to April 1992, 89 were pleural and three peritoneal[16]. Between 1982 and 2003, a group of 15 peritoneal mesotheliomas were treated at the Monfalcone Hospital[18]. In addition, it should be noted that in the Monfalcone area insulators represent a small percentage of the total workforce. A series of 182 mesotheliomas of the pleura, diagnosed among men in Monfalcone in the period from October 1979 to June 2002 included five insulation workers (2.75%)[17].

The findings obtained in the present study demonstrate that asbestos exposure had been very heavy in the four patients, three insulators and one picker, for whom asbestos body counts were available. The duration of exposure varied markedly in the cases we examined. While the year of the first exposure may be determined exactly, the duration of exposure cannot be established with precision, since in Monfalcone asbestos began to be substituted with other materials in around 1980. Also latency periods showed substantial variations, going from 31 to 47 years. The rule of an inverse relationship between intensity of exposure and length of the latency periods[19-21] seems to be only partially observed in the present group. Despite a history of intense exposure in case 5, the latency period was of 47 years.

An additional malignancy, a squamous cell carcinoma of the lung, was detected at necropsy in case 6. Multiple malignancies are not rare in cases of pleural mesothelioma[22]. Some studies suggest a higher vulnerability to cancer in general among mesothelioma patients[23]. Additional cancers in insulators with peritoneal mesothelioma have also been reported. For example, the squamous cell carcinoma of the lung in the case described by Bianchi *et al*[24], and the colorectal carcinoma in the case reported by Attanoos *et al*[25] were both cases of associated malignancy.

Conclusions

Various data suggest that when exposure to asbestos is very heavy, the peritoneum becomes a frequent site of mesothelioma. The reasons for this fact are not clear. The possibility that ingestion of fibres is the way of entrance has to be considered. In fact, if asbestos fibres could arrive only through the respiratory system, it is difficult to explain why the pleura is not the target.

The interest of the question is not merely scientific, being related to a great problem of public health. Given the frequent presence of asbestos fibres in drinking water, the problem of asbestos ingestion remains of concern. Studies regarding the possible effects of asbestos in drinking water and beverages have a long history, starting back in the early 1970s[26]. These studies did not lead to univocal conclusions, and the question remains a matter of debate[27]. A recent investigation, conducted in Woodstock, New York, did not find a link between ingestion of water contaminated by asbestos and cancer[28]. Such findings, however, do not detract from some previous positive results. In this field, indications coming from the experience gained with people severely exposed to asbestos should be taken into account.

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Fattori prognostici e predittivi nella storia clinica del carcinoma metastatico della mammella: uno studio di esito

Prognostic and predictive factors related to metastatic breast cancer clinical history: an outcome study

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Riassunto

***Premesse*. I fattori prognostici condizionano l'esito finale, cioè la sopravvivenza, in modo indipendente dal trattamento oncologico; i fattori predittivi, invece, sono correlati più direttamente all'efficacia del trattamento di una neoplasia. Gli studi di *outcome* (esito) valutano i parametri più attendibili che possono esprimere il risultato a lungo termine delle diverse strategie terapeutiche. *Pazienti e metodi*. I dati clinici delle pazienti affette da neoplasia della mammella afferite all'U.O. di Oncologia Clinica dell'Azienda Ospedaliero-Universitaria S. Anna di Ferrara, con prima visita compresa tra il 1 gennaio 1999 e il 31 dicembre 2005, sono stati inseriti in una scheda informatizzata e quindi elaborati statisticamente nell'ambito di un studio di tipo retrospettivo-osservazionale, al fine di identificare i fattori prognostici e predittivi nel tumore metastatico della mammella. *Risultati*. Complessivamente sono state analizzate 1227 pazienti, di cui 164 presentavano malattia metastatica (98 decedute, 65 viventi con metastasi e 1 persa al *follow-up*). Quattro variabili sono risultate in grado di condizionare la sopravvivenza relativa all'analisi univariata: stato recettoriale sia per gli estrogeni (p=0,0041) che per il progesterone (p=0,0424), presenza di metastasi epatiche (p=0,0161) e**

Summary

***Introduction*. Prognostic factors correlate to the final therapeutic outcome, i.e. survival, independently of the choice of oncological treatment; predictive factors are able to correlate more directly with the efficacy of cancer therapy. Outcome studies evaluate the most reliable parameters that may define the long term result of different treatment strategies. *Patients and methods*. The clinical data of patients with breast cancer who were visited at the Oncology Unit of the University Hospital, Azienda Ospedaliero-Universitaria S. Anna, Ferrara between 1st January 1999 and 31st December 2005 were registered in a computer database, and then statistically examined in a retrospective-observational study, with the aim of identifying prognostic-predictive factors of metastatic breast cancer. *Results*. The database included 1227 patients. Among these, 164 were metastatic (98 deceased, 65 alive with metastases and 1 lost during follow-up). Four variables were shown to condition relative survival at univariate analysis: oestrogenic receptor (p=0.0041), progesterone receptor (p=0.0424), liver metastases (p=0.0161) and cerebral metastases (p<0.0001); at multivariate analysis, only cerebral metastases have been demonstrated to be a negative**

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metastasi cerebrali (p<0,0001); all'analisi multivariata, solamente le metastasi al SNC si sono dimostrate un fattore prognostico-predittivo negativo (p=0,006). ***Conclusioni*****. Studi come quello descritto, analizzando casistiche non selezionate, permettono di valutare l'impatto in termini di *outcome* di una serie di fattori prognostico-predittivi, al fine di pianificare studi prospettici, e possono avere anche ricadute di tipo assistenziale condizionando scelte di politica sanitaria. Eur. J. Oncol., 12 (4), 259-266, 2007**

Parole chiave: **carcinoma metastatico della mammella, fattori prognostico-predittivi, studio di *outcome***

prognostic-predictive factor (p=0.006). ***Conclusions*****. Studies such as this are able to evaluate the outcome impact of a series of prognostic-predictive factors, through the analysis of non selected case series. They also permit the planning of prospective studies and may influence public health policy decisions. Eur. J. Oncol., 12 (4), 259-266, 2007**

Key words: **metastatic breast cancer, prognostic-predictive factors, outcome study**

Introduzione

I fattori prognostici di una neoplasia, siano essi clinici, biologici o molecolari, condizionano l'esito finale, cioè la sopravvivenza, in modo indipendente dal trattamento oncologico, quindi permettono un'accurata stratificazione delle pazienti in base al rischio. I fattori predittivi invece sono più direttamente correlati all'efficacia del trattamento di una neoplasia. Tra essi non sempre vi è corrispondenza, in quanto pur essendo molti fattori prognostici anche predittivi, essi possono non concordare dal punto di vista del significato clinico: cioè un fattore prognostico negativo può avere un significato predittivo positivo, o viceversa[1].

I dati presenti in letteratura riguardanti fattori prognostici e predittivi nel tumore metastatico della mammella sono senza dubbio molti, ma l'attenzione generale si è concentrata su un numero limitato di essi. In letteratura esistono infatti studi focalizzati o solo ai fattori prognostici o solo ai fattori predittivi, ma difficilmente ad entrambi in modo contemporaneo[2-7].

Vista la difficoltà ad oggettivare, specie in studi retrospettivi, la risposta clinica, si preferisce utilizzare come parametro di efficacia e di efficienza di una strategia terapeutica la valutazione della sopravvivenza attraverso studi di *outcome* (esito) i quali hanno come obiettivo principale la valutazione dei parametri più attendibili che possono esprimere il risultato a lungo termine delle diverse scelte terapeutiche[8].

Il presente studio osservazionale retrospettivo si poneva come obiettivo principale quello di identificare nella casistica reale i fattori prognostici e predittivi nel tumore metastatico della mammella, con l'intento di correlare tali fattori con l'esito finale, inteso come sopravvivenza relativa.

Pazienti e metodi

Si trattava di uno studio di tipo retrospettivo-osservazionale, condotto su una casistica di pazienti affette da tumore della mammella, afferite alla U.O. di Oncologia Clinica dell'Azienda Ospedaliero-Universitaria S. Anna di Ferrara tra il 1 gennaio 1999 e il 31 dicembre 2005, le cui cartelle cliniche fossero reperibili in archivio. Sono state escluse le pazienti con tumore non metastatico, o con *follow-up* minore di 1 mese o le pazienti perse di vista con data di ultimo *follow-up* di oltre 1 anno. Per la raccolta dei dati è stata utilizzata una scheda informatizzata, creata in ambiente FileMaker Pro® v.8, che per ogni paziente inserita prevedeva la registrazione di oltre 200 informazioni, elaborata nell'ambito del Percorso Senologico dell'Ospedale S. Anna di Ferrara. I dati sono stati dapprima trascritti su schede cartacee e successivamente riportati nel database informatizzato integrandoli ove necessario con i dati dell'archivio dell'Anatomia Patologica dello stesso ospedale.

Nella raccolta dei dati sono stati utilizzati i seguenti criteri:

a) in caso di assenza di informazioni sull'insorgenza della menopausa, le pazienti sono state registrate come post-menopausali se di età superiore a 65 anni alla prima visita;
b) nel caso la data di decesso non fosse precisata in cartella, questa è stata sostituita con la data di ultimo *follow-up* risultante dal diario clinico;
c) il *performance status*, se non fossero reperibili riferimenti diretti, è stato desunto dalle indicazioni riportate nel diario clinico.

Il consenso al trattamento dei dati non è stato chiesto esplicitamente alle pazienti, in quanto già da loro precedentemente espresso al momento della prima visita, co-

munque a tutela della *privacy* i cognomi e i nomi non sono stati riportati per esteso per l'analisi statistica. Inoltre, l'accesso al *database* era protetto, al fine di garantire la sicurezza e la segretezza.

L'analisi statistica è stata svolta tramite l'utilizzo del programma SPSS® 12.0 per Windows.

L'analisi di sopravvivenza è stata effettuata utilizzando il metodo di Kaplan-Meier. La sopravvivenza relativa è stata calcolata come differenza tra la data di decesso o dell'ultimo *follow-up* e la data di riscontro della malattia metastatica.

E' stata dapprima effettuata una analisi univariata utilizzando il test del Log Rank con un limite di significatività a 0,05, considerando tutte le variabili che potenzialmente potessero correlarsi con la sopravvivenza relativa. Quindi è stata effettuata una analisi di regressione logistica multivariata, secondo il metodo di Cox, al fine di controllare la stima del rischio e l'impatto sulla sopravvivenza relativa delle diverse variabili prognostiche e predittive che avessero raggiunto una significatività statistica all'analisi univariata.

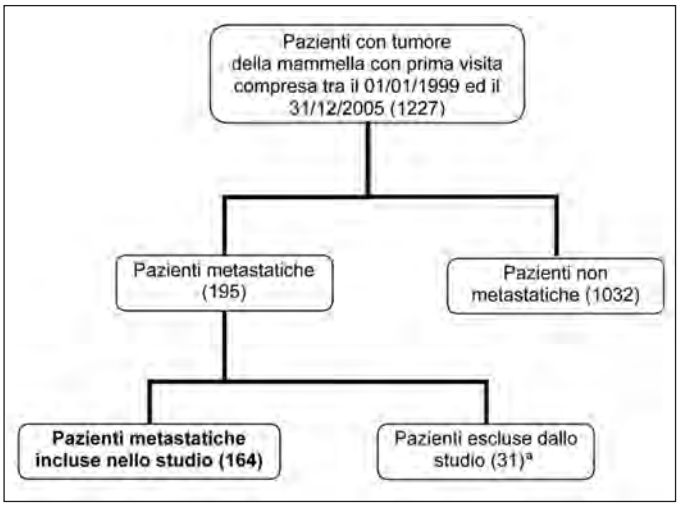

Fig. 1. Definizione della casistica utilizzata
[a] di cui
– 20 pazienti con *follow-up* <1 mese
– 11 pazienti perse di vista con data di ultimo *follow-up* risalente >1 anno

Risultati

Complessivamente sono state analizzate 1227 pazienti affette da carcinoma della mammella. Nella fig. 1 è riportata la definizione della casistica analizzata, che è descritta in dettaglio nella Tabella 1. L'età mediana delle 164 pazienti considerate, che presentavano malattia metastatica, era 61 anni (*range* 34-87). Come si può notare, nella maggior parte dei casi le pazienti erano in post-menopausa, il tipo di intervento chirurgico era prevalentemente demolitivo, il diametro del tumore primitivo era

Tabella 1 - Descrizione della casistica (164 pazienti)

Variabile	Valori	N.	%	Variabile	Valori	N.	%
Età	< 65 anni	96	58,5	Tipo istologico	CDI	118	72,0
	≥ 65 anni	68	41,5		Altro	31	18,9
	Mancanti	0	-		Mancanti	15	9,1
Menopausa	No	22	13,4	pT	pT1	49	29,9
	Sì	127	77,4		pT2, pT3, pT4	85	51,8
	Mancanti	15	9,1		Mancanti	30	18,3
PS_1[a]	0	66	40,2	LN+ alla diagnosi[c]	0	32	19,5
	1	5	3,0		1-3	34	20,7
	2	4	2,4		≥4	59	36,0
	Mancanti	89	54,3		Mancanti	39	23,8
PS_2[b]	0	76	46,3	*Grading*	1	4	2,4
	1	11	6,7		2	56	34,1
	2	4	2,4		3	62	37,8
	Mancanti	73	44,5		Mancanti	42	25,6
Tipo I intervento chirurgico	Demolitivo	103	62,8	MIB1[d]	Positivo	105	64,0
	Conservativo	46	28		Negativo	25	15,2
	Mancanti	15	9,1		Mancanti	34	20,7

(note alla pagina seguente)

(continua alla pagina seguente)

Tabella 1 - continua

Variabile	Valori	N.	%	Variabile	Valori	N.	%
ER[e]	Positivo	93	56,7	Metastasi polmone	No	115	70,1
	Negativo	45	27,4		Sì	47	28,7
	Mancanti	26	15,9		Mancanti	2	1,2
PgR[f]	Positivo	73	44,5	Metastasi epatiche	No	123	75,0
	Negativo	64	39,0		Sì	39	23,8
	Mancanti	27	16,5		Mancanti	2	1,2
HER 2	Positivo	46	28,0	Metastasi retrop.	No	159	97,0
	Negativo	87	53,0		Sì	3	1,8
	Mancanti	31	18,9		Mancanti	2	1,2
Recidiva	No	137	83,5	Metastasi SNC	No	146	89,0
	Sì	27	16,5		Sì	16	9,8
	Mancanti	0	-		Mancanti	2	1,2
CT adj.[g]	No	87	53,0	Tipo CT M+[j]	Taxani	39	23,8
	Mancanti	42	25,6		Antracicline	12	7,3
	Sì	77	47,0		Combinazione	10	6,1
	Mancanti	0	-		Altri	39	23,8
					Mancanti	64	39,0
OT adj.[h]	No	105	64,0				
	Sì	59	36,0	N. linee CT M+[k]	1 linea	42	25,6
	Mancanti	0	-		2 linee	30	18,3
					3 linee	14	8,5
RTE adj.[i]	No	97	59,1		4 linee	13	7,9
	Sì	67	40,9		Mancanti	65	39,6
	Mancanti	0	-				
				N. linee OT M+[l]	1 linea	71	43,3
N. siti M+	1	96	58,5		2 linee	13	7,9
	2	51	31,1		3 linee	7	4,3
	3	7	10,9		Mancanti	73	44,5
	4	2	1,2				
	Mancanti	2	1,2	Trastuzumab M+[m]	No	121	73,8
					Sì	28	17,1
Metastasi ossee	No	67	40,9		Mancanti	15	9,1
	Sì	95	57,9				
	Mancanti	2	1,2				
Metastasi tessuti molli	No	120	73,2				
	Sì	42	25,6				
	Mancanti	2	1,2				

[a] *Performance status* al momento della diagnosi
[b] *Performance status* al momento del riscontro delle metastasi
[c] Numero di linfonodi positivi al momento della diagnosi
[d] MIB1 = positivo se >13%, negativo se ≤13%
[e] ER = stato del recettore per gli estrogeni (positivo se >10%, negativo se ≤10%)
[f] PgR = stato del recettore per il progesterone (positivo se >10%, negativo se ≤10%)
[g] Chemioterapia adiuvante
[h] Ormonoterapia adiuvante
[i] Radioterapia adiuvante
[j] Tipo di chemioterapia utilizzata nella fase metastatica
[k] Numero di linee di chemioterapia utilizzata nella fase metastatica
[l] Numero di linee di ormonoterapia della fase metastatica
[m] Uso del Trastuzumab nella fase metastatica

maggiore di 1 cm (pT>1), i linfonodi ascellari erano prevalentemente interessati, il *grading* era G1-G2, i recettori ormonali (ER, PgR) positivi, il MIB1 >13%, l'HER2 (espressione c-erbB2) era negativo, non vi era stata recidiva locale, non era stata eseguita chemio-ormonoterapia o radioterapia adiuvante, la sede metastatica era unica, il *performance status* ECOG era 0-1 anche al momento della comparsa di metastasi.

Al momento dell'analisi (16/02/07), 65 pazienti risultavano viventi con metastasi, 98 decedute, e solamente 1 persa di vista. Il tempo mediano di *follow-up* è di 24,5 mesi (*range* 1-85,7); la mediana dell'intervallo libero è di 17 mesi (*range* 0-386); la sopravvivenza relativa è di 13,5 mesi (*range* <1-173), e l'intervallo libero da progressione di 5 mesi (*range* 0-65).

L'analisi univariata della sopravvivenza relativa, effettuata con il metodo di Kaplan-Meier (Tabella 2) ha tenuto in considerazione le diverse variabili analizzate nella descrizione della casistica, "dicotomizzandole" ove possibile al fine di valutarne con chiarezza l'impatto.

Sono risultati significativi con tale analisi: la positività dei recettori per gli estrogeni e per il progesterone, la presenza di metastasi epatiche, e la presenza di metastasi cerebrali (fig. 2).

Nell'analisi di regressione logistica multivariata (Tabella 3) si sono valutate le 4 variabili suddette, e solo la

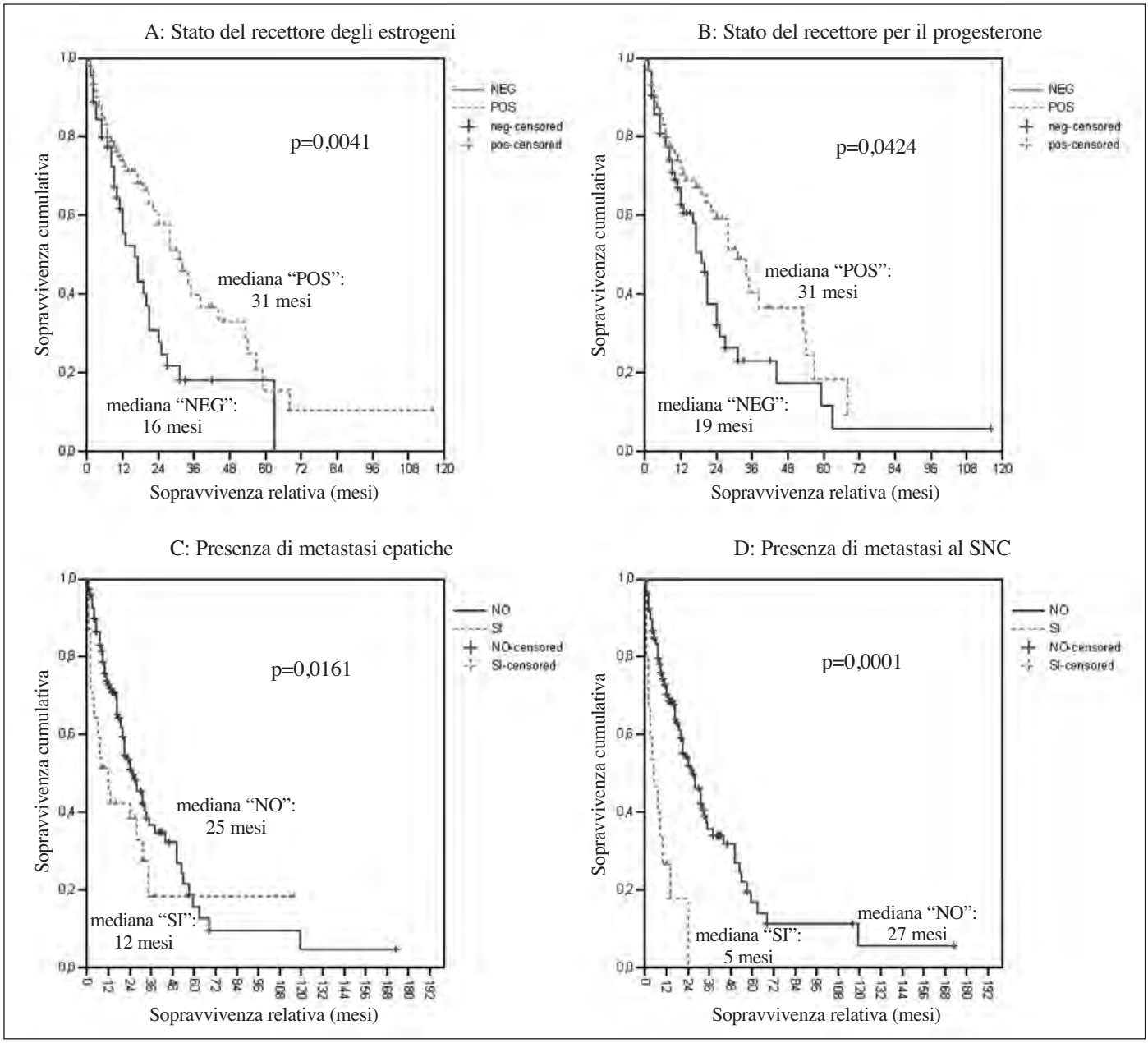

Fig. 2 (A-D). Curve di sopravvivenza delle variabili significative all'analisi univariata: (A) recettore estrogeno, (B) recettore progestinico, (C) metastasi epatiche, (D) metastasi al SNC

Tabella 2 - Analisi univariata: fattori associati alla sopravvivenza relativa

Variabili	N.	Mediana (mesi)	p	Variabili	N.	Mediana (mesi)	p
Età				Recidiva			
< 65 anni	96	22	0,8335	No	137	22	0,4893
≥ 65 anni	68	24		Sì	27	28	
Menopausa				CT adj.[g]			
No	22	28	0,9038	No	87	21	0,2469
Sì	127	22		Sì	77	25	
PS_1[a]				OT adj.[h]			
0-1	71	28	0,7550	No	105	24	0,7981
≥ 2	4	5		Sì	59	21	
PS_2[b]				RTE adj.[i]			
0-1	87	31	0,2873	No	97	21	0,0941
≥ 2	4	5		Sì	67	31	
Tipo I intervento chirurgico				N. siti M+			
Demolitivo	103	25	0,4506	1	89	28	0,0731
Conservativo	46	21		≥ 2	69	19	
Tipo istologico				Metastasi ossee			
CDI	118	24	0,7535	No	67	21	0,4167
Altro	31	21		Sì	95	25	
pT				Metastasi tess.molli			
pT1	49	19	0,1596	No	120	24	0,1133
pT2, pT3, pT4	85	28		Sì	42	21	
LN + alla diagnosi				Metastasi polmone			
Sì	93	28	0,2774	No	115	24	0,3382
No	32	21		Sì	47	21	
N. linfonodi + alla diagnosi[c]				Metastasi epatiche			
≤ 3	34	19	0,6223	No	123	25	0,0161
> 3	59	28		Sì	39	12	
Grading				Metastasi retrop.			
1-2	60	28	0,1685	No	159	24	0,3830
3	62	19		Sì	3	—	
MIB1[d]				Metastasi SNC			
Positivo	121	24	0,2239	No	146	27	<0,0001
Negativo	27	24		Sì	16	5	
ER[e]				Tipo CT M+[j]			
Positivo	93	31	0,0041	Taxani	39	31	
Negativo	45	16		Antracicline	12	20	>0,05[k]
PgR[f]				Combinazione	10	—	
Positivo	73	31	0,0424	Altri	39	24	
Negativo	64	19		Trast. M+[l]			
HER 2				No	121	21	0,1401
Positivo	46	25	0,7799	Sì	28	28	
Negativo	87	21					

[a] *Performance status* al momento della diagnosi
[b] *Performance status* al momento del riscontro delle metastasi
[c] Numero di linfonodi positivi al momento della diagnosi
[d] MIB1 = positivo se >13%, negativo se ≤13%
[e] ER = stato del recettore per gli estrogeni (positivo se >10%, negativo se ≤10%)
[f] PgR = stato del recettore per il progesterone (positivo se >10%, negativo se ≤10%)
[g] Chemioterapia adiuvante
[h] Ormonoterapia adiuvante
[i] Radioterapia adiuvante
[j] Tipo di chemioterapia utilizzata nella fase metastatica
[k] Confronto tra ciascun trattamento e ciascuno degli altri
[l] Uso del Trastuzumab nella fase metastatica

Tabella 3 - Analisi multivariata: modello di Cox

Variabili	HR[a]	IC 95%[b]		p
		Inferiore	Superiore	
Positività ER[c]	0,581	0,298	1,131	0,110
Positività PgR[d]	0,942	0,488	1,821	0,860
Metastasi epatiche	1,357	0,791	2,326	0,267
Metastasi SNC[e]	2,917	1,351	6,298	0,006

[a] *Hazard ratio*
[b] Indice di confidenza al 95%
[c] Recettore estrogeno
[d] Recettore progestinico
[e] SNC = sistema nervoso centrale

presenza di metastasi al sistema nervoso centrale (SNC) ha dimostrato avere significato prognostico negativo.

Discussione

Lo studio riportato ha considerato una casistica consecutiva di 7 anni, con un *follow-up* mediano di circa due anni: la mediana di età (61 anni) si avvicina a quella riportata dai principali registri tumori nazionali[9]. Anche i dati riguardanti la prevalenza di pazienti in post-menopausa e la caratterizzazione anatomo-patologica sono in linea con i dati della letteratura, con elevata incidenza del carcinoma duttale infiltrante (>50% del totale). Tuttavia, a differenza dei dati riportati da altri[10-14], non emerge dalla nostra casistica l'importanza in senso prognostico dello stato linfonodale, della dimensione della componente infiltrante del tumore, e del ruolo di HER2 quale fattore predittivo di risposta. Peraltro anche nel nostro studio risulta il ruolo prognostico favorevole della positività recettoriale, in accordo con i dati della letteratura[15], che mostrano che la positività dei recettori per gli estrogeni, specie se associata alla contemporanea espressione dei recettori per i progestinici, rappresenta un fattore significativo sia sotto il profilo prognostico che predittivo di risposta all'endocrinoterapia. Il nostro studio sembrerebbe quindi confermare che lo stato recettoriale è legato non solo ad una modalità di crescita della neoplasia ma anche alla presenza di un potenziale metastatico.

Il ruolo in termini prognostici della terapia adiuvante invece non sembra essere significativo nella nostra casistica, a differenza di quanto dimostrato in altri studi[16-18].

Anche il numero di siti metastatici non sembra influenzare significativamente la sopravvivenza, in contrasto con i dati di altri Autori[19]. Inoltre, mentre i dati della letteratura dimostrano che il numero di metastasi ossee avrebbe un valore prognostico[20] e che le pazienti con un numero minore di metastasi ossee (1 o 2 sedi) avrebbero un vantaggio in termini di sopravvivenza rispetto alle pazienti con 3 o più sedi, nella nostra analisi la presenza o assenza di metastasi ossee non sembra influire sulla sopravvivenza. Analoga conclusione per la presenza di metastasi ai tessuti molli, che altri Autori[21] hanno considerato fattore prognostico "positivo", e fattore predittivo della maggiore responsività alla terapia[21], in quanto indicherebbe una malattia più localizzata rispetto ad altre sedi metastatiche e quindi con una minore aggressività.

Per quanto concerne l'interessamento metastatico del fegato invece, i dati ottenuti sono in sintonia con la letteratura, infatti la presenza di metastasi epatiche è indice di una patologia aggressiva, più che di una diffusione metastatica ad altri organi[22]. In altre casistiche la presenza di metastasi epatiche è anche in grado di determinare la predittività nei confronti di alcune linee di trattamento, condizionando la resistenza alle antracicline[23]. Anche la presenza di metastasi al SNC, caratteristica della fase avanzata di malattia, conferma un ruolo prognostico-predittivo negativo. Verosimilmente, la significatività raggiunta solo da questo cofattore all'analisi col modello di regressione di Cox indicherebbe che le metastasi cerebrali sono in grado di condizionare la prognosi in maniera molto più significativa rispetto ad altre localizzazioni metastatiche.

Per quanto concerne il trattamento effettuato nella fase metastatica, nella nostra esperienza, come anche da altri riportato[24], il trattamento contenente taxani, pur non raggiungendo la significatività statistica, comporta una mediana di sopravvivenza superiore rispetto agli altri tipi di chemioterapia nella malattia avanzata.

I limiti di uno studio retrospettivo, in cui la popolazione non può essere selezionata in modo rigido e che necessiterebbe di numeri elevati per evidenziare fini differenze di impatto prognostico di una o più variabili, sono evidenti nello studio da noi riportato. Tuttavia, uno studio come quello in esame offre la possibilità di osservare lo stato reale della coorte esaminata, considerando la casistica "di tutti i giorni" al di fuori degli studi clinici, e permette quindi di verificare l'impatto di una serie di fattori potenzialmente utili per progettare in tempo successivo studi prospettici, ed in definitiva è in grado di indirizzare scelte di politica sanitaria e di allocazione delle risorse.

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An unusual case of Cushing's syndrome

Un caso insolito di sindrome di Cushing

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Summary

Cholangiocarcinoma is a usually fatal cancer with an unclear pathogenesis and with a rising incidence worldwide. Only a single case of ectopic corticotropin secretion (ECS) due to cholangiocarcinoma has been previously reported in the literature and the effectiveness of imaging studies (CT, MRI, octreoscan, FDG-PET) in detecting this particular corticotropin-secreting tumour is unknown, as is the effect of medical treatment. A 37 year old man presented with the clinical and biochemical features of Cushing's syndrome. High plasma levels of corticotropin and secondary hypercortisolism were detected. Imaging studies revealed a liver tumour and a biopsy demonstrated diffuse infiltration of a cholangiocarcinoma with endocrine differentiation. Immunostaining for corticotropin demonstrated a scattered strong positivity. It is of interest to note that both octreoscan and FDG-PET showed no uptake at all. Since liver transplantation was not an option, the patient was started on dopamine-agonist therapy with cabergoline to inhibit ECS. The treatment reduced corticotropin levels but did not significantly reduce cortisol secretion. Thus, we report, for the first time in the literature, the utility of CT, MRI, octreoscan and FDG-PET in detecting an intrahepatic cholangiocarcinoma with immunohistochemically demonstrated endocrine differentiation, and the effect of cabergoline treatment

Riassunto

Il colangiocarcinoma è un tumore maligno di solito fatale a patogenesi ancora incerta e con crescente diffusione nel mondo. In letteratura è stato riportato solamente un caso di secrezione ectopica di corticotropina da parte di un colangiocarcinoma, e la reale utilità degli esami radiologici (CT, RMN, octreoscan, FDG-PET) nella diagnostica di questa rara forma di tumore secernente corticotropina è ancora incerta, così come l'efficacia della terapia medica. Un maschio di 37 anni si è rivolto alla nostra attenzione per un quadro clinico e bioumorale compatibile con sindrome di Cushing e confermato dagli alti livelli circolanti di corticotropina e dall'ipercortisolismo secondario. Le indagini radiologiche hanno mostrato la presenza di un tumore epatico che istologicamente si è rivelato un colangiocarcinoma diffusamente infiltrante, con differenziazione endocrina e con immunofissazione fortemente positiva per corticotropina. Curiosamente, sia l'octreoscan che la FDG-PET erano totalmente negative. Valutata la non-operabilità del caso, è stata intrapresa terapia medica con cabergolina al fine di inibire la secrezione ectopica di corticotropina. Pur riducendo i livelli circolanti di corticotropina, il farmaco non è stato in grado di ridurre in modo significativo la produzione di cortisolo. In sintesi, viene qui riportata per la prima volta in letteratura l'utilità di: CT, RMN, octreoscan e FDG-PET nello studio del colangiocarci-

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in reducing ectopic secretion of corticotropine and (secondary) cortisol in this rare form of tumour. Eur. J. Oncol., 12 (4), 267-271, 2007

Key words: **cholangiocarcinoma, Cushing's syndrome, corticotropin, neuroendocrine tumour**

noma intraepatico differenziato in senso endocrino, e viene riportata l'utilità della cabergolina nel controllo della secrezione ectopica di corticotropina e (secondaria) di cortisolo in questa rara forma tumorale. Eur. J. Oncol., 12 (4), 267-271, 2007

Parole chiave: **colangiocarcinoma, sindrome di Cushing, corticotropina, tumore neuroendocrino**

Introduction

Ectopic corticotropin secretion (ECS) from a nonpituitary tumour, first reported in 1963[1], accounts for about 10% of cases of Cushing's syndrome overall[2,3]. The commonest causes of ECS are pulmonary carcinoid, thymic carcinoid (which are extremely rare) and neuroendocrine tumours, whereas the corticotropin source remains unknown in about 18% of ECS cases[2,4]. Cholangiocarcinoma is a relatively rare tumour, representing the second commonest primary hepatic tumor and accounting for 3% of all gastrointestinal cancers[5]. In the last decades the incidence of intrahepatic cholangiocarcinoma is increasing worldwide[6], but the reasons for this rise are still unclear.

Intrahepatic cholangiocarcinoma is associated with a very poor prognosis (overall survival rate is less than 5% at 5 years[7]), mainly because it presents late and is difficult to diagnose. Moreover, surgical resection is the only chance for cure, and chemotherapy and radiotherapy have been reported to be mostly ineffective.

Only one case of ECS due to cholangiocarcinoma has been published before[8]. In this report we describe a patient with intrahepatic cholangiocarcinoma presenting with Cushing's syndrome, for whom surgery was not feasible and medical treatment was required to reduce ECS.

Case report

A 37 year-old man with history of obesity and grade II hypertension developed, 6 months before admission, muscle weakness, dizziness and malaise and, more recently, jaundice. At admission (1st September 2005) clinical examination showed jaundice, flapping tremor, obesity (BMI=34 Kg/m^2), violaceous striae, oedema of the ankles, and hyperpigmentation. High blood pressure was present (BP=170/100 mmHg) notwithstanding polytreatment with canrenoate (400 mg/*die*), hydrochlorothiazide (25 mg/*die*), bisoprolol (6.25 mg/*die*), doxazosine (8 mg/*die*) and nifedipine (60 mg/*die*).

Laboratory tests showed diabetes, hypokalaemia (1.7 mmol/L), increase in transaminases (7 x n.v), ALP (2 x n.v.), gGT (40 x n.v.), bilirubin, cholesterol and ammoniemia, and a slight decrease in albumin and protrombin time. Common tumour markers elevation included: tissue polypeptide antigen (TPA), carbohydrate antigen (CA) 19-9, cytokeratin fragment 21.1 (Cyfra 21.1), neuron-specific enolase (NSE) and gastrine. Carcinoembryonic antigen (CEA), serum calcitonine, a-feto protein (AFP), prostate-specific antigen (PSA) and urinary 24-h 5-hydroxyindoleacetic acid, were normal. Hormonal measurements with dynamic testing showed: elevated 24-h urine cortisol excretion (759 nmol/24h, n.v. <270 nmol/24h), elevated plasma corticotropin concentration (adenocorticotrophic hormone (ACTH) 8.00 a.m.=124 ng/l and 18.00 p.m.=80 ng/l) after nocturnal suppression test with dexamethasone 2 mg (fig. 1). The renin/angiotensin/aldosterone system was suppressed.

Imaging studies, including chest X-ray, computed tomography (CT) of the chest, magnetic resonance imaging (MRI) of the brain, scintigraphy (total body) with ^{99m}Tc-HDP and gastroscopy, were normal. Colonoscopy identified two little intestinal polyps which proved to be adenomatous. Octreoscan (total body) at the dose of 185 Mbq and [^{18}F] Fluorodeoxyglucose (FDG) positron emission tomography (PET) at the dose of 440 Mbq, showed no uptake at all.

CT of the abdomen/pelvis and cholangio-MRI showed: enlarged inhomogeneous liver with irregular margins, with severe hypotrophy of the left lobe, which appeared to be replaced by a slight hyperintense lesion extending to the hilus, as shown on T2-weighted images. No biliary dilatation was evident.

A liver biopsy demonstrated diffuse infiltration of neoplastic cells consistent with possible cholangiocarcinoma (cytokeratin CK7 positive at immunostaining). At laparotomy the whole liver appeared involved, with multiple lesions of variable size, the largest of which totally replacing segment IV; the left lateral segment was remarkably atrophic. Such an unexpected massive tumour involvement precluded any surgical treatment. Multiple biopsies from both lobes were performed and revealed a neoplastic proliferation of small ducts type destroying the parenchyma (fig. 2, A). At immunohistochemistry, the small ducts were shown to be widely positive for both cytokeratins CK7 and AE1 (fig. 2, B), and also for chromogranin A and synaptophysin (fig. 2, C). Immunostaining for corticotropin demonstrated a scattered strong positivity (fig. 2, D). Based on the above findings, the diagnosis of intrahepatic cholangiocarcinoma with endocrine differentiation and ECS was made. Cytological examination on samples of peritoneal fluid, obtained as soon as the abdominal cavity was entered, was also positive for neoplastic cells. Since liver transplantation was also

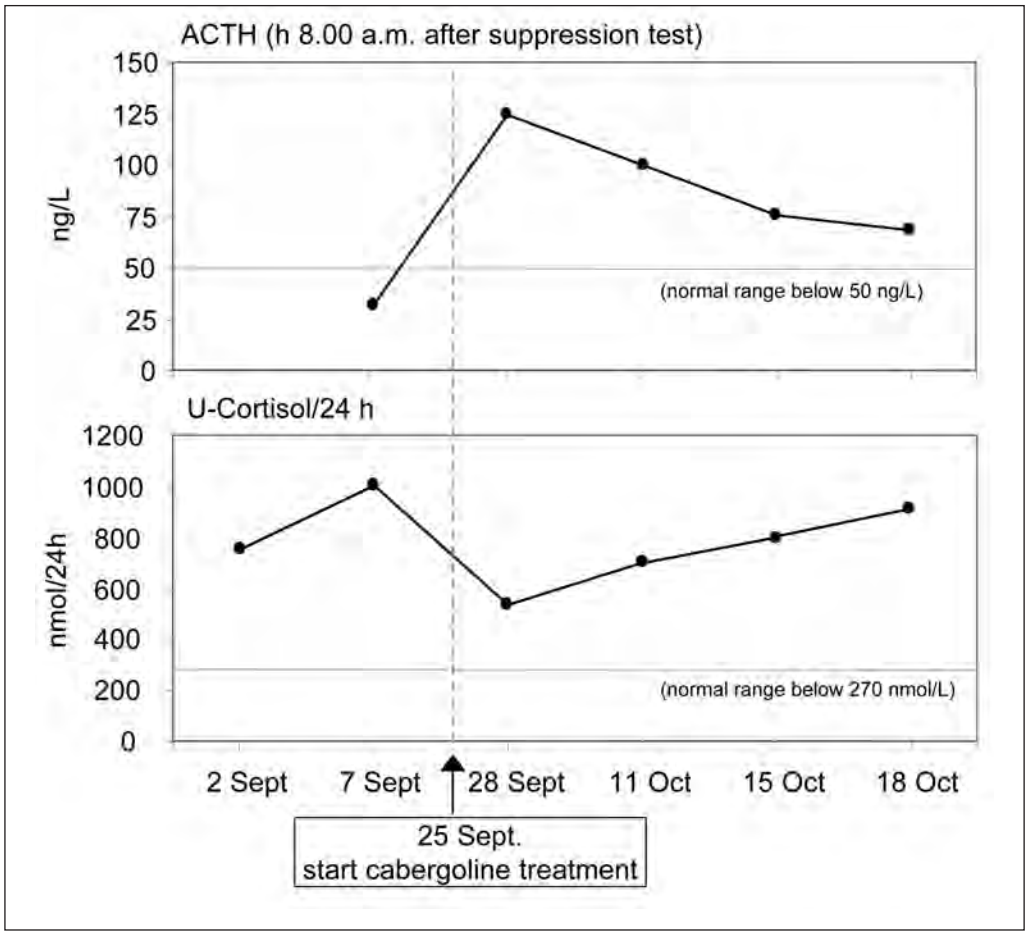

Fig. 1. Corticotropin plasma levels (assessed at 8.00 a.m., after suppression test) and urinary cortisol levels and their variation in response to the cabergoline treatment (which was started on 25th September)

not an option, palliative medical treatment to inhibit adrenal cortisol secretion was required, and the patient was started on dopamine-agonist therapy with cabergoline (2 mg and subsequently 3.5 mg weekly) as previously reported[9, 10]. A significant decrease in corticotropin secretion was obtained after few days of treatment, without concomitant decrease in cortisol secretion which remained significantly high (fig. 1).

Discussion

In this case report we describe an extensive intrahepatic cholangiocarcinoma with immunohistochemically demonstrated endocrine differentiation, accounting for a high-grade ECS and thus presenting with Cushing's syndrome. This tumour (which is rare in individuals younger than 50 years of age) is associated with a high mortality, because it presents late and it is notoriously difficult to diagnose. Moreover, no tumour markers specific for cholangiocarcinoma are available, both CA 19-9 and CEA being considered useful only as a diagnostic guide. In our patients we also found an elevation of TPA, Cyfra 21.1, NSE and gastrine. Imaging studies are the cornerstone for tumour localization in patients with Cushing's syndrome due to ECS. In general, CT and/or MRI localize the corticotropin-secreting tumour only in about 70% of the patients[2, 11]. In our patient, CT of the abdomen/pelvis and cholangio-MRI clearly identified the liver tumour. It is of interest to note that both high-dose octreoscan (total body) and FDG-PET showed no uptake at all, but the sensitivity of these examinations in the detection of neuroendocrine tumours is debated. Since the sensitivity of the octreoscan depends on multiple factors, including the doses of radionuclide, lesion size, type and/or degree of somatostatin receptor expression, the evidence of its utility in detecting neuroendocrine tumours is controversial[12-14]. In a series of patients with Cushing's syndrome caused by ECS, the sensitivity of octreoscan in localizing the corticotropin-secreting tumour was 53% (95% CI: 29-76%) and similar to that of CT[11].

FDG-PET is an emerging staging technique for many cancers, particularly with high proliferative activity. In a retrospective study in which FDG-PET was used in 21 patients with intrahepatic cholangiocarcinoma (both hilar and peripheral), an intensely increased FDG uptake was observed in all peripheral cholangiocarcinomas, in which FDG-PET detected unsuspected distant metastases[15]. Nevertheless, in a subset of 10 patients with hilar cholan-

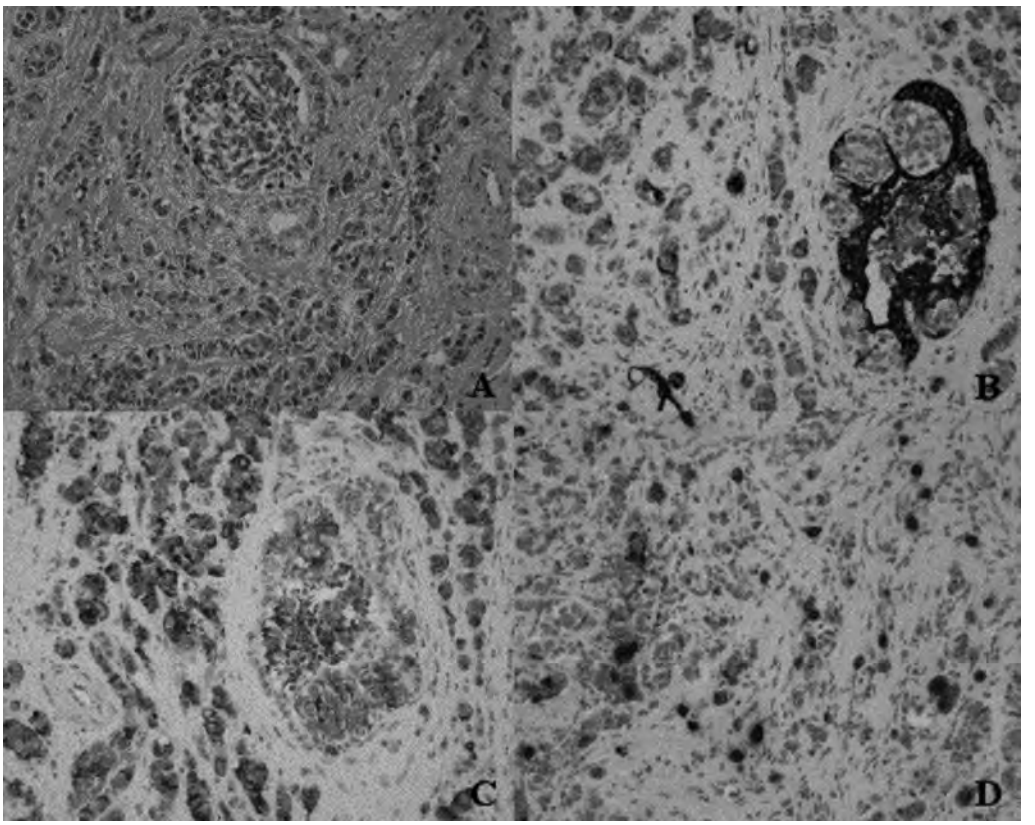

Fig. 2. (**A**) morphological features of the neoplastic proliferation (H&E x200); (**B**) widely positive immunostaining for AE1 (x200); (**C**) widely positive immunostaining for chromogranin A (x200); (**D**) scattered positive immunostaining for corticotropin (x200)

giocarcinomas, FDG uptake was intense in only 2 of them, and was slightly higher than that of the hepatic parenchyma in 8 patients[15]. Therefore, whilst this technique can detect nodular cholangiocarcinomas and unsuspected distant metastases, it seems less helpful in the detection of diffuse infiltrating tumours, such as the one presented in this case report. Moreover, even if only few endocrine tumours have been studied with FDG-PET, there is evidence of a not significant uptake of FDG in well differentiated neuroendocrine tumours with low proliferative activity. In fact, in a series of patients with Cushing's syndrome due to ECS, the sensitivity of FDG-PET in localizing the corticotropin-secreting tumour was only 35% (95% CI: 15-61%)[11].

The massive tumour involvement of the liver precluded any surgical approach. However, medical treatment to inhibit steroidogenesis with ketoconazole and/or RU 486 was severely limited because of the progressive liver failure, and because other medications (metyrapone, aminoglutethimide, etomidate)[16, 17] are not available in Italy.

In ectopic corticotropin-producing tumours which express somatostatin receptors, octreotide therapy can produce a rapid and sustained reduction of ECS and cortisol levels, sometimes representing the only long-term therapy possibile[18]. Unfortunately, in our patient octreoscan showed no uptake at all and thus somatostatin analogues could not be used. Thus, despite the lack of evidence of dopamine-receptor expression in the tumour, the patient was started on dopamine-agonist therapy with cabergoline, which decreased corticotropin secretion after a few days of treatment, but without concomitant decrease in cortisol secretion which remained significantly high (fig. 1).

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Sustaining life on Earth: environmental and human health through global governance

La salvaguardia della vita sulla Terra: salute ambientale e umana grazie ad una tutela globale

edited by / a cura di
Colin L. Soskolne

A lion crouching by a waterhole can foresee (salivate and probably taste) its future soon-to-arrive prey, but not the impact of its kill on prey populations (nor perhaps on its own population). A unique feature of being human is our ability to foresee larger and longer consequences of actions – our individual and collective actions. We therefore can feel guilt if our current actions predict consequences that we don't like or approve of. This is one basis for morality, and those who deny that we currently have any moral responsibility for the world we leave to future generations are not just short-sighted, but fail to experience guilt. That is the definition of a sociopath. And there are many sociopaths among us including regrettably in leadership positions. Heilbruner's[1] essay "What has posterity ever done for me" reminds us that we cannot rely on rationality alone because a rationale view does not automatically value future generations, anymore than our ancestors a century ago worried enough about the world of violence and inequity that they bequeathed to their own offspring.

This book that Colin Soskolne and colleagues provide is therefore avowedly anti-sociopathic and guardedly optimistic. In the interest of self-disclosure let me say at the outset of this review that any mention of "sustainable" or "sustainability" immediately alerts me to some phenomenon or condition that is most likely not sustainable. Trained as an ecologist I believe in the finite carrying capacity that any ecosystem, small or large, has for any organism, big or small. A Petri culture dish can only support so many billions of bacteria for so long. They multiply rapidly, eating themselves out of house and home, and eventually exhausting their resources, the entire culture succumbs. Migratory locusts devastate and move, living a short, merry, non-sustainable life style. And humans too, with burgeoning populations, particularly in the poorest countries, outstrip the resources they need to support the barest essentials of life. Faced by shortages and overcrowding they must stagger long distances to fetch water or firewood, or forage for food – imitating in many cases and places the hunter-gatherer life of the earliest humans. Small, soil-depleted farms can support only so many family members, forcing others to migrate to cities which will soon contain more than half the world's population. Famine, war, and genocide have ecological underpinnings. In the face of sustained population growth, health is not sustainable. There is no infinite lunch.

In the past, discussion of sustainability has focussed on expanding the carrying capacity – building infrastructure, converting forest to farms, investing in technology, crop enhancement, and new agricultural techniques. The much heralded Green Revolution provided a blip to the carrying capacity, much as new breeds of rice and other crops will hopefully do. Joel Cohen[2] attempted to estimate how many people the Earth can support, by examining estimates of exploitable land and water, but found the question perplexing. What does "support" mean, with what lifestyle. In exploring the earth's carrying capacity he concluded that the "caring capacity" or responsiveness of social institutions, was a critical determinant of human well-being.

Demographers have for decades projected population growth into the future, their estimates remarkably precise. Thus, frustrated by reading many articles and books on climate, energy, food, health, and sustainability, which eschew any reference to population growth and control, I did not have high hopes for "Sustaining Life on Earth",

C.L. Soskolne. Sustaining life on Earth: environmental and human health through global governance. Rowan and Littlefield Publishers, New York and London, 2007

suspecting it might be more of the same. My apocalyptic vision of humans sustaining themselves by alternating bouts of war and famine, was pleasantly disrupted by this volume. It is not a blueprint, exactly, but a guidance for optimism, at least guarded optimism. Soskolne and colleagues argue that there are things that we can do, that we should do, that we must do. To be sure there is no easy way out, no free lunch. While the politicians and economists argue about carbon trading, for example, or the merits of subsidizing corn, the contributors to "Sustaining Life on Earth" extol an approach to ecological integrity based on natural ethics, moral responsibilitity, and a global ethic – *The Earth Charter.* So this book is important reading, needs a widespread audience, and should be widely taught. It is not more of the same.

Firstly I was glad to learn that there is a Global Ecological Integrity Group and that, unlike some political organizations, that is not a misnomer for its opposite. The list of chapter authors reveals widespread representation of relevant disciplines including basic science, ecology, engineering, policy, law and ethics. Anthony McMichael (Australian expert on climate change and health) sets the stage in the Foreword by identifying *"a prime focus on… the health of ecosystems and of the human species"* and their interdependence. Human health, well-being, satisfaction, and economy are inter-related with environmental quality and the services that an intact global ecosystem provides: air, water, soil, and food. And, most gratifyingly, there on the first page is the mention of "human numbers", so often shunned by conservationists, climatologists, nutritionists and all. And McMichael emphasizes that *"sustainability is not a destination. It is an endless journey"*. So far so good: he has my attention.

I accept the Brundtland Commission[3] definition of sustainable development: *"meeting the needs of the current generation without compromising the needs of future generations"* or, in the words of an anonymous bumpersticker: *"we borrow the future from our children"*. The bumpersticker is only half right: we do inherit the earth from our ancestors and it is obvious what an awful hash they have made of it – centuries of wanton resource exploitation and pollution – ignoring the global commons. So also in the interest of disclosure, I have been steeped in the "commons" tradition ever since reading Garrett Hardin's 1968 seminal paper "The Tragedy of the Commons"[4]. It is interesting that Soskolne and colleagues cover some of the same ground, focussing on ecosystem integrity, rather than the more resource management oriented "commons" theme illustrated by Burger and colleagues[5].

Colin Soskolne's preface identifies the objective of changing the past irrational ("dumb") behaviour and recognizing and fulfilling *"our duty to protect the earth's capacity to sustain life"*. He emphasizes the *Earth Charter* which becomes the centerpiece or focal point of the book. We are accustomed to an anthropocentric view of the earth and universe, while this book gives credibility to an ecocentric view as well. We must train ourselves to see the world as many other species (on which we depend) see it. The emphasis throughout is on *ecological integrity.*

The ensuing 27 chapters, sorted into eight parts, each with a non-specialist summary, represent a broad range of perspectives and experiences focussed on the many dimensions of human-environment interaction. It is human ecology writ large (and long). This is a book for non-specialists, that many specialists will find stimulating, reassuring, and providing at least some basis for optimism regarding reversing the seemingly inexorable 20th Century "progress" towards widespread environmental degradation.

There isn't room, short of another monograph, to characterize each chapter and reveal its contribution to the future integrity of the human environment. Part I brings together government with ecology and economy as support for human well-being. Not much optimism here. Our current models of government are poorly chosen to protect the global commons.

Part II addresses globalization and its impact on the human condition and human rights. More self-destruction and non-sustainable activities herein. The global economy, spearheaded by multi-national corporations and international finance institutions, are achieving a form of supra-national governance bent on rapid resource extraction and short-term amortization of investment. In grade school we subscribed to the "Weekly Reader" with its polyanna-ish predictions for our great society, extolling the virtues of renewable resources. As children we were taught that forests and fisheries were being managed sustainably. Not so. It was sobering later to learn that fishery economics encouraged you to fish out the resource, make a big profit quickly, and sell your boats to some other nation so they could do the same.

Part III pursues these themes, examining governance alternatives, reminding me of Aristotle's Politics, in which he compared and contrasted individual (home manager) *versus* governmental (king) relationships to resource acquisition and management.

Part IV examines treaties and covenants and sets forth the *Earth Charter*, adoption of which will move mankind towards sustainability and peace. Part V itself is divided into four parts, offering far-ranging discussion of ideology related to alternative governance, with emphasis on the Kyoto Protocol and failures to fulfill it, and on access to

food and water. Here I must emphasize the Pimentels' important chapter on the human population. At 6.5 billion people, we already have at least half that number in poverty, undernourished or malnourished, many flirting with frank starvation. While Americans "*expect the most advanced and effective diagnosis and therapies for disease, no matter the cost*"[6] the other half of the world has minimal access to modern health care, preventive or therapeutic. We in the developed countries, now engaged in self-flagellation over our profligate and disproportionate use of resources, do not clearly articulate that we maintain our lifestyles not only at the expense of our environment, but at the expense of those who must go hungry. They may go hungry even when surrounded by food too dear to purchase, while we stuff ourselves with food that remains relatively cheap by our inflated standards.

Optimists such as Joel Cohen[2] predict that population may stabilize at 9.5 billion, perhaps as early as the mid-century. At the other extreme, a population of 13 billion, not stabilized, may occur. As our World population doubles, possibly by 2070, we will leave further behind most of the new additions who will be born into poor countries with poor food security. We will be lucky if they have the strength to cut more forest or gut more mangroves, to plant food for our own growing population (since our land is becoming too valuable to merely farm it, except perhaps for subsidized biofuel). I don't know whether I am an optimist or a pessimist, because starvation and poverty are already bad enough. There doesn't seem to be a viable plan for making them better now. This book offers a path of optimism for keeping them from getting a whole lot worse in the future. But it will take commitment.

Bosselmann's chapter promoting global governance independent of nation-states, appeals to the idealist in me, sounding much like visions we discussed as undergraduates. Unfortunately, although the growing power of transnational corporations and international finance is already undermining national sovereignty, it is trending in the opposite direction, leading to fragmentation rather than cohesiveness.

In my school days (late 1940s) my father worked at the very new United Nations, and I was raised with an intense conviction that that world organization would literally save the world with its focus on all dimensions: peace, justice, self-governance, food and agriculture, and health. Perhaps frustrated belief in World Governance makes me a sceptic. Bosselmann sees globalization as opportunity and invokes the *Earth Charter* as guidance for protecting ecological integrity.

So what is the *Earth Charter*? Is it sound? Does it work? Could it work? Is it precautionary? It consists of three sections: a Preamble focussing on our Earth ecosystem, the challenges for nurturing it (and ourselves), and our "universal responsibility". The second section lists 16 principles (Table 1), with 59 subheadings. The entire document is available at www.earthcharter.org.

Finally there is "The Way Forward" which requires a fundamental change of head and heart, and a new sense of "universal responsibility". We must "*harmonize diversity with unity*", and I am particularly pleased to note the exhortation for nations to renew commitment to the United Nations. The UN is by no means perfect, but it embodies the principles represented throughout the book and has agencies which could recreate themselves to foster interdependence, clean air and water, good health and nutrition, and population regulation, leading in turn

Table 1 - The sixteen principles of the Earth Charter

1. Respect earth and life in all its diversity
2. Care for the community of life with understanding, compassion, and love
3. Build democratic societies that are just, participatory, sustainable, and peaceful
4. Secure Earth's bounty and beauty for present and future generations
5. Protect and restore the integrity of Earth's ecological systems with special concern for its biological diversity and the natural processes that sustain life
6. Prevent harm as the best method of environmental protection and, when knowledge is limited, apply a precautionary approach
7. Adopt patterns of production, consumption, and reproduction that safeguard Earth's regenerative capacities, human rights, and community well-being
8. Advance the study of ecological sustainability and promote the open exchange and wide application of the knowledge acquired
9. Eradicate poverty as an ethical, social, and environmental imperative
10. Ensure that economic activities and institutions at all levels promote human development in an equitable and sustainable manner
11. Affirm gender equality and equity as prerequisites to sustainable development and ensure universal access to education, health care and economic opportunity
12. Uphold the right of all, without discrimination, to a natural and social environment supportive of human dignity, bodily health, and spiritual well-being with special attention to the rights of indigenous peoples and minorities
13. Strengthen democratic institutions at all levels, and provide transparency and accountability in governance, inclusive participation in decision making, and access to justice
14. Integrate into formal education and life-long learning the knowledge, values and skills needed for a sustainable way of life
15. Treat all living beings with respect and consideration
16. Promote a culture of tolerance, non-violence and peace

to reduced resource exploitation, reuse and recycling of materials, and reduction of the human footprint. And pragmatically, judging by the failure of leading nations to sign on to many recent environmental treaties, there is little likelihood of creating an alternative infrastructure in the foreseeable future. So by all means let's make good use of the UN and its resources. So the *Earth Charter* makes sense in its clarity, could work, and is precautionary. Can it be marketed? This is another question.

It is easy for sceptics and sociopaths (among which I include many international leaders) to deny apocalyptic predictions, because they see malnutrition (afflicting half the world's children today) and war as natural consequences of poverty, arguing that expanding investment would provide jobs, technologies and new food crops, thereby beating the odds on poverty. Secretly or not so secretly they interpret "poverty" to mean "cheap labour". Large scale famines and genocides occur intermittently and far away and economists seem able to ignore these.

It is helpful to see this book in context. It reminds me of Tom Emmel's "Global Perspectives on Ecology"[7], which had more ecology and cataclysm chapters, but ended grappling with similar issues on decisions, policy and global governance, notably Maurice Strong's chapter "A Global Imperative for the Environment"[8]. Strong exhorted us to *"evolve a strategy for global environmental security – a planetary policy to avoid disaster"* facing *"spaceship Earth"*. His six elements were 1) population stabilization (policy-based rather than relying on war, famine or disease), 2) conservation of scarce resources and development of technologies and consumption patterns that are less energy-intensive, 3) new models for social and economic progress, 4) resource transfer from rich to poor to provide basic social services to combat poverty, 5) science and technology to reduce ignorance about environment, resources, and population, aimed at improving rather than degrading the human condition, and 6) placing the ocean resources under international control, requiring new dimensions of international cooperation and an expanded rôle for a "new internationalism". The *Earth Charter* is a more mature exposition of these themes.

Strong emphasized that this was "*not a utopian dream but an objective necessity…well within our reach*" requiring "*the community of nations*" and "*political wisdom*". Since these are not new themes, we have an opportunity to assess how well they have performed since the 1970s. And the present volume examines some of these trends during the intervening generation. Have we moved closer or away from global governance? Have we seen the strengthening or weakening of the United Nations as the pre-eminent institution poised to move forward? Can we infer that trans-national corporations, international treaties, and financial institutions, by weakening national jurisdiction over resources and labour, have helped us toward ecologic integrity or undermined it? Lamentably, green advertising and propaganda notwithstanding, the evidence is opposite. Accelerated exploitation of resources, short-term profitability, the cheapening of life and labour, the broadening gap between rich and poor, all point to the devastating impact of these dynamics.

At the other extreme, in a book ironically and aptly titled "A Poverty of Reason"[9], economist Wilfred Beckerman argues that we don't even need to worry about development being sustainable. *"Not every need of the present generation is being met, so why should future generations be any different?"* Beckerman's starting premise is that "needs" are not an objective reality. *"Although billions of people today suffer appalling environmental conditions – such as lack of clean water and sanitation, and deteriorating ecosystems – these problems are caused predominantly by poverty, not 'unsustainable' development"*. Like many economists Beckerman believes that there are free market mechanisms to deal with shortages, whereas many environmentalists (among which I proudly include myself) think that our problems, including widespread poverty, stem largely from free market failures – globalization and transnational corporate exploitation of resources and labour being glowing examples.

Even if the authors of "Sustaining Life" started from separate viewpoints they converge and expand on these themes from a generation once-removed. Climate change, or rather accelerated climate change due to anthropogenic atmospheric modification, is hardly a new concept. The World Resources Institute[10] featured it nearly 20 years ago, and it wasn't new then. I abhor the cumulative disregard of climatologic science, but it isn't sea level rise that I worry about. Despite the increasing concentration of population in coastal cities on all continents, people don't really fear the rising water. It is the already evident destabilization of climate regimes, particularly temperature and rainfall that will impact agricultural productivity and disease patterns. Nor is our carbon footprint going to catch up to us as quickly as air pollution and energy depletion. Already half the world doesn't have access to basic energy requirements (cooking and heating), much less to land and food.

Those of us who believe in democracy might hope that democratic institutions and public realization of the importance of ecologic integrity (at least on a national scale) will lead to better decisions. How can one extract optimism from the chaos and the negative indicators? I force myself to remember that when environmentalists

first began to extol the virtues of recycling, it didn't occur to us that anyone would listen, much less that it would become the norm and even the law in many lands. Likewise, cleaning up hazardous waste seemed out of the question in the 1970s: just stop new waste from accumulating. Then along comes Superfund, and money gets spent, and hazardous waste gets removed, treated, contained. We climate change spokespersons have been hammering the issue since the mid-1980s, and it is now front page news. So the Malthusian and the World Government view could very well become the establishment view in the next generation – let us hope it is not too late. "Sustaining Life on Earth" directs us there.

In conclusion, this book reminds us of Garrett Hardin's perceptiveness, for in "The Tragedy of the Commons"[4] he emphasized that the problem facing society was one for which there was "*no technical solution*", and this revelation amazed or puzzled readers and leaders in a generation which revered technical approaches to virtually everything[11]. Many chapters in "Sustaining Life on Earth" likewise emphasize that we are suffering the consequences of relying on failed technical rather than untried social approaches. The two intertwined themes of the book are "duty" and "ecological integrity" as articulated by Soskolne in the preface. "Duty" is a key word. Readers who feel this duty will learn a lot from this book on changing duty into action. But equally important will be readers who become convinced that they have such a duty. This book needs to be read widely, discussed widely, and the *Earth Charter* needs to become as familiar today, as the *Four Freedoms* annunciated by Franklyn D. Roosevelt was more than sixty years ago.

Ironically, Norman Rockwell's illustration of the *Four Freedoms* on a US postage stamp in 1943 proved an effective marketing tool for selling War Bonds. Maybe an international postage stamp campaign in which all countries could advertise the *Earth Charter* could be a first step in marketing the book's theme on a large scale.

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Professor Le Van Trung
(1934-2007)

We regret to inform that Professor Le Van Trung, the President of the Vietnam Association of Occupational Health, the Former Director of the Vietnam National Institute of Occupational and Environmental Health (NIOEH), and the Former Director of the WHO Collaborating Center on Occupational Health, passed away on June 14th, 2007 at age 74.

Professor Le Van Trung was a long-time collaboration partner of the International Commission on Occupational Health. He was Director of the first Fogarty grant with NIOEH.

Professor Le Van Trung was born in 1934 in Hanoi, Vietnam. He graduated as Medical Doctor at Hanoi Medical University in 1960 and as Ph.D. diplomat of occupational diseases in 1984. From 1991 to 2002, he was Director of the National Institute of Occupational and Environmental Health, WHO Collaborating Center for Occupational Health.

After retiring in 2002, he was the President of the Vietnam Association of Occupational Health.

During more than 40 years of working in the field occupational and environmental health, he contributed greatly to the development of preventive medicine, and occupational and environmental health in Vietnam and internationally. His distinguished publications include textbooks on occupational diseases, and numerous chapters and articles on silicosis, other diseases of miners, pesticide poisoning, and other health issues.

He was the first person to lay the foundation for the development and to expand the international collaboration of occupational and environmental health in Vietnam.

Thanks to his great contribution, the Vietnamese Government conferred upon him the Third-class Labour Medal and the Second–class Resistance War Medal.

Professor Le Van Trung had been elected to Fellowship of the Collegium Ramazzini in October 2000. In November 2002, the Occupational Health and Safety Section of the American Public Health Association also conferred the International Award upon him in recognition of his work in occupational health and safety internationally.

We pray for his soul to be peaceful in the heaven.

Nguyen Khac Hai
Professor and Director
The Vietnamese National Institute of Occupational and Environmental Health, and
Vice President
Vietnam Occupational Health Association

Eur. J. Oncol., vol. 12, n. 4, pp. 281-282, 2007

Professor Dame Anne McLaren
(1927-2007)

Professor Dame Anne McLaren died, in a car accident, in England, on 7th July 2007. She was an internationally known scientist respected for her unstinting commitment in the field of mammalian genetics. This unexpected loss was a great shock to her many colleagues and friends worldwide.

Anne Laura Dorinthea McLaren was born on 26th April 1927, the daughter of Henry McLaren, second Lord Aberconway, and his wife Christabel McNaughton. Having gained a zoology degree at Lady Margaret Hall, Oxford, she went on to obtain her doctorate on murine neurotropic viruses, in 1952.

From 1952 to 1955, she worked, with her husband Donald Michie, at University College London and, from 1955 to 1959, at the Royal Veterinary College, London, studying the variation in the number of lumbar vertebrae in mice as a function of the maternal environment.

Anne McLaren continued her work on mammalian fertility at the Institute of Animal Genetics in Edinburgh, where, from 1959 to 1974, she studied various aspects of fertility, development and genetics in mice, including work on immuno-contraception, on DNA hybridisation, and on chimeras.

In 1974 she left Edinburgh to become Director of the Medical Research Council Mammalian Development Unit at University College London, where she studied the development differentiation of mammalian primordial germ cells.

Upon her official retirement in 1992, Anne McLaren was appointed Principal Research Associate at the Wellcome Trust/Cancer Research UK Gurdon Institute in Cambridge. She was also a Research Fellow of King's College, Cambridge.

She was elected a Fellow of the Royal Society in 1975 and, from 1991 to 1996, was the Society's Foreign Secretary: the first woman in 330 years to become an officer of the Royal Society.

Indeed, throughout her working life she endeavoured to promote the careers of women in the sciences, and in 1995 became President of the Association for Women in Science and Engineering.

Among the many honours she received, Anne McLaren was appointed DBE in 1993; she still preferred, however, to be called Doctor, rather than Dame or, indeed, Professor.

In 1986 she was made a Fellow of the Royal College of Obstetricians and Gynaecologists for her outstanding contribution to the field of fertility. In 1991 she became a Founder Fellow in the Academy of Medical Sciences and the Fullerian Professor of Physiology at the Royal Institution. She was also a Trustee of the Natural History Museum London, from 1994 to 2003.

Anne McLaren published two academic books and more than 300 scientific papers.

We had the pleasure of meeting Anne McLaren, during the International Meeting of National Academies, held under the auspices of the Collegium Ramazzini, the Town of Bologna and the University of Bologna, which she attended, here, in 1989. Subsequent to this, Dr Anne McLaren was elected to Fellowship of the Collegium Ramazzini in 1990. She acquired Emeritus status in the Collegium in 1994.

During her last few years Dr McLaren served as Trustee of the Frozen Ark Project which she had co-founded. This aims to preserve the DNA and viable cells of the world's endangered animal species before they become extinct.

In the car crash in which Anne McLaren died, her former husband and scientific colleague, Donald Michie also lost his life. They are survived by their three children, two daughters and a son.

A Memorial Fund for studentships and fellowships for scientific research for young women scientists has been established in Anne McLaren's name. Information on the Fund may be requested from Jonathan Michie, The Garden House, Apley Park, Norton, Shropshire WV15 5NE, England.

Jill Victoria Brazier
National Ramazzini Institute
Bologna, Italy

Doctor Lorenzo Tomatis
(1929-2007)

Lorenzo Tomatis was a good man. He did much to advance the cause of public health and the health of workers. He was a true follower of Ramazzini.

He was a physician and scientist who took his medicine and his science into the public arena.

Having obtained his medical degree in 1953 at the University of Turin, he went on to specialize in preventive medicine and occupational health. He served clinically as a pathologist in Turin and then in Chicago. In 1967, Dr Tomatis embarked formally upon his lifelong quest to understand and conquer the preventable causes of cancer, when he joined the International Agency for Research on Cancer (IARC) as Chief of the Unit of Chemical Carcinogenesis. He laboured at IARC for nearly 30 years, and for 12 of those years, he served with enormous distinction as Director of IARC, the cancer agency of the World Health Organization. Dr Tomatis expanded the reach and respect of IARC. He stood firmly for prevention. He was tenaciously heroic in the face of strong and well-organized opposition.

In 2005, Lorenzo Tomatis received the Ramazzini Award for his outstanding contribution to the prevention of cancer, in particular the identification of industrial agents.

Doctor Tomatis died on 21st September 2007.

I had the privilege to serve on two IARC Monograph Working Groups while Lorenzo was Director - the Benzene volume (#29) and the Silica volume (#42). What I remember most about those experiences was Lorenzo's strong sense of responsibility, his recognition that we absolutely had to get the science right, but that we also needed to present our findings in a way that would protect workers. I remember especially in the silica deliberations when the pressure on Lorenzo was fierce to declare silica to be not a human carcinogen. In response, he looked carefully at the data, he saw clearly that silica is indeed a carcinogen, and he resisted the pressure and made the proper declaration. That's what being a follower of Ramazzini is all about.

Philip J. Landrigan
President, Collegium Ramazzini
Professor and Chairman
Department of Community and Preventive Medicine
Professor of Pediatrics
Mount Sinai School of Medicine
New York, NY, USA

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

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Bernardino Ramazzini
Biography and bibliography
Biografia e bibliografia

Pericle Di Pietro

First volume of the "Ramazzini Library" series. Dedicated to the life and work of Bernardino Ramazzini, acknowledged founder of occupational medicine, by Professor Pericle Di Pietro, authority on the Master from Carpi. English and Italian Texts.

Primo volume della collana "Ramazzini Library". Dedicata alla vita e alle opere di Bernardino Ramazzini, riconosciuto fondatore della medicina del lavoro, è opera del Professor Pericle di Pietro, illustre conoscitore del grande Maestro carpigiano. In lingua italiana e in inglese.

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Foreword

The European Journal of Oncology is publishing a monograph dedicated to Bernardino Ramazzini (biography and bibliography) (GEO/ EJO Library, vol. 1) by Professor Pericle Di Pietro, the well-known authority on the life, works and cultural legacy of the Maestro from Carpi. This monograph was commissioned from the eminent scholar by the Collegium Ramazzini.

The text is in Italian with a parallel English translation, and is illustrated with plates giving the original documentation in essential form.

The Scientific Editors of the journal are indebted to Professor Di Pietro for his generosity in undertaking a text, which will surely remain a landmark in the historiography of Bernardino Ramazzini.

Ramazzini's contribution stands as a milestone in the history of medicine. Not only is he the acknowledged founder of Occupational Medicine; he it is who clarified the fact that many diseases are exogenous in origin and hence preventable; he it is to whose vigour and originality we owe the notion that "Longe praestantius est praeservare quam curare, sicut satius est tempestatem praevidere ac illam effugere quam ab ipsa evadere", which is to say "it pays far more to prevent than to treat, since it is easier to anticipate and avoid the disease than to shake it off".

The lesson of Ramazzini remains acutely topical, as happens with the work of the great pioneers and anticipators of history.

There are many reasons why an oncology journal like ours should think fit, as a duty even, to publish a monograph on Bernardino Ramazzini.

Firstly, tumours are now a largely exogenous disease. The epidemiological proportions they have taken on in our times are due not only to population ageing, but to environmental pollution and a characteristic industrial era lifestyle.

In the present day scenario, moreover, many of the populations most at risk of cancer are from the categories of industrial worker.

Again, as many tumours are caused by exogenous agents, which are hence removable, they form a paradigm case of a disease in controlling which prevention is the main and most incisive course of action.

It should not, lastly, be forgotten that Ramazzini's specific contribution to oncology – in what may be seen as the first "aetiological epidemiology" research in the history of the discipline – that is, the observation of an increased risk of mammary cancers among nuns, anticipates the scientific evidence on the subject by some two and a half centuries: he makes a strict connection of these tumours with women's reproductive and hormonal patterns and, even more specifically, with the fact that the nullipara belongs to a bracket of the population at risk.

Written in the year 2000

Cesare Maltoni†
Scientific Director of the
European Ramazzini Foundation and
Secretary General of the Collegium Ramazzini

Presentazione

Il Giornale Europeo di Oncologia pubblica questa monografia dedicata a Bernardino Ramazzini (biografia e bibliografia) (GEO/EJO Library, Vol. 1), ad opera del Professor Pericle Di Pietro, illustre conoscitore della vita, delle opere e della eredità culturale del grande Maestro carpigiano. Questa monografia era stata richiesta all'illustre studioso dal Collegium Ramazzini.

Il testo esce in lingua italiana e in traduzione inglese, ed è illustrato da alcune figure che riproducono in maniera essenziale una documentazione originale.

I Direttori Scientifici della Rivista sono profondamente grati al Professor Di Pietro per l'impegno profuso a scrivere un testo, che certamente rimarrà un punto di riferimento nella storiografia di Bernardino Ramazzini.

Il contributo di Ramazzini rappresenta una grande pietra miliare nella storia della medicina. Ramazzini non solo è il riconosciuto fondatore della Medicina del Lavoro; è anche Colui che con precisione ha indicato che molte malattie sono di origine esogena e perciò prevenibili, e che ha introdotto con assoluta originalità e con forza il concetto che "Longe praestantius est praeservare quam curare, sicut satius est tempestatem praevidere ac illam effugere quam ab ipsa evadere", cioè che "è di gran lunga più conveniente prevenire che curare, poiché è più agevole prevedere la malattia ed evitarla, che liberarsi da essa".

La lezione di Ramazzini rimane oggi di grande attualità come capita per l'opera dei grandi pionieri che hanno anticipato la storia.

Le ragioni per cui una rivista di oncologia, come è la nostra, ha ritenuto opportuno, ed anzi un suo compito preciso, pubblicare la monografia "Bernardino Ramazzini" sono molteplici.

Innanzitutto i tumori sono oggi una malattia in larga misura di origine esogena: infatti la dimensione epidemiologica che essi hanno assunto nei nostri tempi è dovuta, oltre che all'invecchiamento delle popolazioni, anche all'inquinamento ambientale e agli stili di vita, caratteristici dell'era industriale.

Inoltre nell'attuale scenario molte delle popolazioni a maggiore rischio di cancro sono proprio categorie di lavoratori dell'industria.

Ancora, siccome molti tumori sono causati da agenti esogeni e quindi rimovibili, essi rappresentano un esempio emblematico di malattia per il controllo della quale la prevenzione rappresenta il maggiore e più incisivo intervento.

Non va dimenticato, infine, il contributo specifico di Ramazzini all'Oncologia, con quella che può essere considerata storicamente la prima ricerca "epidemiologica eziologica" oncologica, e cioè l'osservazione di un aumento del rischio di cancro mammario nelle monache, anticipando così di circa due secoli e mezzo l'evidenza scientifica che il rischio mammario è strettamente connesso alla storia riproduttiva e ormonale della donna e, in maniera più specifica, che le nullipare rappresentano una fascia di popolazione a maggiore rischio.

Edito nell'anno 2000

Cesare Maltoni†
Direttore Scientifico della Fondazione
Europea Ramazzini e
Segretario Generale del Collegium Ramazzini

The Precautionary Principle
Implications for research and prevention in environmental and occupational health

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

Second volume of the "Ramazzini Library" series. Proceedings of the International Conference "The Precautionary Principle. Implications for Research and Prevention in Environmental and Occupational Health", held in Bologna, Italy, in October 2002. English text

Secondo volume della collana "Ramazzini Library". Raccoglie gli atti del Convegno Internazionale "Il Principio di Precauzione. Implicazioni per la Ricerca e la Prevenzione nella Medicina Ambientale e del Lavoro", svoltosi a Bologna nell'ottobre del 2002. In lingua inglese

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Preface

By his effort over the millenia, man has surely been the motor driving what is called "development", by which we mean productive growth, technological progress, innovation, welfare and availability of goods.

Human effort on this planet of ours has been the chief factor determining the quality of the environment in which man has lived. In its turn, the quality of the environment has conditioned the gamut of pathologies that have progressively set in and, as a result, the state of human health.

But whereas man in the past ages had to defend himself from nature's aggressions in order to survive on the planet, nowadays, if he is to go on living, and above all give future generations a chance of doing so, he must take steps to protect nature from himself.

Such positive action will only be possible if one bears in mind certain inescapable starting assumptions: 1) our planet is in all likelihood unique as a biological scenario; 2) it is finite and so are its raw material and environmental resources; 3) there is a legitimate escalation in human demands.

But if such assumptions cannot be gainsaid, we must ask ourselves: is the current development model compatible with finite resources and legitimate growing human demands? And if the current development model is not compatible, what other form might it take and, above all, how might this be achieved?

To answer these questions we must rise above the ambiguities and irresponsibilities upon which our present development model is based, namely:

– first, that of taking it for granted that the answers to the main problems are essentially technological, economic and political in nature;
– second, that of assuming this development model to be unique, or at least a lesser evil, and that only its internal mechanisms may be adjusted, while the overall design of it cannot be changed, not to mention replaced by alternative models;
– lastly, the failure to recognize that what we hail as progress and creative expression by modern man has brought with it an artificial expansion of production and consumerism, aptly summed up in the fad for "disposable" wares.

Under the social, economic and political impact of the problem of reconciling development, environment and health, dare we claim nowadays to possess the right cognitive tools to guide our decisions towards so-called compat-

ible development? The answer may be yes. We do today possess a lot of scientific tools which can be used to predict (rather than observing later) the effects of development strategies which are mainly (though not entirely) geared to maximizing profits and petty group interests.

One classic case is the long-term carcinogenicity trials on experimental animals to identify carcinogens. When properly planned and conducted, above all when closely reproducing human exposure scenarios, such trials can give precise indications as to agent carcinogenicity and environmental risks conditions, the time-scale being relatively short (2-3 years). The results of such studies can be extrapolated to man, in both qualitative and quantitative terms, and thus form the most effective instrument for predicting the carcinogenic hazard of such agents.

Safeguarding the environment, public health and the quality of life is a planet-wide issue, an integral part of any strategy to achieve a more physiological development model, harbouring resources whilst more fairly satisfying the legitimate claims of the whole world population.

It is quite true that many mistakes have been made, many disasters caused: our society today is justly disoriented. But all is lost? Certainly not. If man takes the situation in hand, without leaving the initiative to egoism or letting things slide, there is clearly hope.

Science can make an important contribution, bringing about a change of course.

But science must be free to identify the problems, set priorities, decide its own programmes, assess the results as they come in; above all it must establish relations with society, and society must make use of its contribution. These relations, however, must not undermine its independence: in other words, the relationship is one of interaction and interdependence, not dependency.

Is all feasible? Maybe it is, probably the time is ripe. At all events, we must strive to think the answer is yes.

In this context, the Precautionary Principle approach to governing the regaining of a just equilibrium between development, environment and health represents an adequate approach augured by many.

The Collegium Ramazzini is grateful to Professor Philippe Grandjean for promoting and organizing the Workshop on the “Precautionary Principle: implications for research and prevention in environmental and occupational health”. This has reviewed what scientific basis we possess today for properly applying the Precautionary Principle to safeguarding the environment and public health.

The Collegium Ramazzini is also grateful to the Emilia-Romagna Region, the European Environmental Agency, the Ramazzini Foundation, the World Health Organization, the National Institute of Environmental Health Sciences, the National Institute of Occupational Safety and Prevention, the Regional Agency for Health Prevention and Environmental Protection in the Emilia-Romagna Region, the Province of Bologna and the Municipality of Bentivoglio, as well as to industry. Our thanks to all of them for their generous support.

Morando Soffritti
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Ban on asbestos in Europe
Messa al bando dell'amianto in Europa

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

Third volume of the "Ramazzini Library" series. Proceedings of the International Conference "Ban on Asbestos in Europe", held in Monfalcone, Italy, in February 2003. English text, with summaries in English and Italian

Terzo volume della collana "Ramazzini Library". Raccoglie gli atti del Convegno Internazionale "Messa al Bando dell'Amianto in Europa", svoltosi a Monfalcone nel febbraio del 2003. In lingua inglese, con riassunti in inglese e italiano

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Presentation

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

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

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

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

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

Presentazione

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

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

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

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

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

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Genetic testing at work
Ethical and legal implications
Test genetici nell'ambito lavorativo
Implicazioni etiche e legali

K. Van Damme, M. Sorsa, M. Soffritti,
F. Minardi, J.V. Brazier (Editors)

Fourth volume of the "Ramazzini Library" series. Proceedings of the International Conference "Genetic Testing at Work. Ethical and Legal Implications", held in Carpi, Italy, in October 2003. English text, with summaries in English and Italian.

Quarto volume della collana "Ramazzini Library". Raccoglie gli atti del Convegno Internazionale "Test Genetici nell'ambito Lavorativo. Implicazioni etiche e legali", svoltosi a Carpi nell'ottobre del 2003. In lingua inglese, con riassunti in inglese e italiano

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Introductory remarks

The Committee on Ethics of the Collegium Ramazzini decided some time ago to organize a special workshop on ethical issues related to occupational genetic testing as part of the Collegium's Annual Ramazzini Days 2003. With the generous support of the Collegium Secretariat in Bentivoglio, the endeavour was realized as a one-day symposium on 24th October at the former Convent of San Rocco in the historical town of Carpi, birthplace of Bernardino Ramazzini.

The timeliness of the topic relates to the rapid developments of molecular genetic techniques which allow potential applications in various biomedical fields with societal impact and interest. This concerns also the workplaces, with the possibilities that employers, either current or prospective, might be interested in applying the new genetic methods to select the best, fittest and healthiest job applicants or employees on the basis of their molecular genetic profile. This presumption is based on the idea that genetic tests might predict our future health and potential need for sick leave, thus leading to lower work efficacy and less economic profits. Such presumptive thinking needs to be openly discussed and analysed within the Collegium Ramazzini to bridge the science with the social and political impact, within the objective of the Collegium to conserve life and prevent disease.

The symposium comprised excellent lectures by guest speakers and Collegium Fellows followed by lively debates among all international participants. This proceedings volume collects the keynote lectures, but unfortunately leaves the comments and discussions for the imagination of the readers. A long and complex chain of potentially "ethically risky" behaviour is identified in the papers included. The true predictivity of present tests is questionable, both scientifically and statistically; the principle of free and informed consent is often dubious; and the ethical principles of beneficence, non-maleficence and respect for autonomy are vulnerable in global commercialisation and mass marketing of genetic tests. Legal rules and ethical codes have been established and this development should be encouraged to protect the well-being of workers.

This proceedings volume calls for further ethical discussions among its readers, persons involved with occupational safety and health issues and researchers on genetic susceptibility at work.

The organisers and the General Secretariat of the Collegium Ramazzini sincerely thank all authors and participants of the Carpi symposium for their valuable contributions.

Karel Van Damme, Marja Sorsa
Collegium Ramazzini Committee on Ethics

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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

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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.

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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.

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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.

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

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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

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