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Editorial/Editoriale

Experimental chemical carcinogenesis: fundamental and predictive rôle in protecting human health in the 1930s-1970s

Cancerogenesi chimica sperimentale: il suo ruolo fondamentale e predittivo nella protezione della salute dell'uomo negli anni 1930-1970

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Summary

The purpose of this review is to provide evidence for the fundamental and predictive rôle of experimental chemical carcinogenesis in protecting human health between the 1930s and 1970s. On the background of the evolution of experimental chemical carcinogenesis and of the understanding of some of the mechanisms underlying the process of carcinogenicity, of the availability of data related to the significance, relevance and predictive value of results of long-term carcinogenicity tests of environmental chemicals, and of the availability of information on the rules and regulations regarding carcinogens, as well as of guidelines for the conduct of long-term carcinogenicity tests, it is hard to believe that anybody with any sense of responsibility and with an even only minimal interest in public health could have discarded, or ignored, or belittled the rôle that the results of long-term carcinogenicity tests had, or could have had, in the years 1950s to 1970s in the adoption of measures of primary prevention for the protection of human health. Eur. J. Oncol., 11 (1), 5-13, 2006

Key words: **carcinogenicity tests, protection of human health**

Riassunto

Lo scopo di questo editoriale è di dimostrare il ruolo fondamentale e predittivo della cancerogenesi chimica sperimentale nella protezione della salute dell'uomo negli anni 1930-1970. Sullo sfondo dell'evoluzione della cancerogenesi chimica sperimentale e delle conoscenze di alcuni dei meccanismi inerenti al processo di cancerogenesi, della disponibilità di dati sul significato, pertinenza e valore predittivo dei risultati dei saggi di cancerogenicità su composti chimici ambientali, e della disponibilità di informazioni sulla regolamentazione degli agenti cancerogeni, come pure delle linee guida per la condotta dei saggi di cancerogenicità a lungo termine, è ben difficile poter giustificare come chiunque avesse avuto senso di responsabilità ed un minimo interesse per la salute pubblica abbia potuto ignorare, disattendere, o sottovalutare il ruolo che negli anni 1950-1970 hanno avuto, o avrebbero potuto avere, i risultati dei saggi di cancerogenicità sulla messa in atto di misure di prevenzione primaria per la protezione della salute dell'uomo. Eur. J. Oncol., 11 (1), 5-13, 2006

Parole chiave: **saggi di cancerogenicità, protezione della salute dell'uomo**

Introduction

Cancer research developed for centuries in two main directions: cure and prevention, the first aiming at the treatment and control of the disease, and the second at the reduction of its incidence (primary prevention) or its early detection (secondary prevention). Primary prevention uses two different approaches: 1) the reporting of

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cases and the clinical/epidemiological approach of verifying different incidences of cancer in different sections of the population in the attempt to identify the determinants of cancer; 2) the identification by experimental means of carcinogenic agents present in our environment with the purpose of drastically reducing or eliminating exposure to them. The availability in the early 1940s of spectrophotometers, the introduction of paper chromatography and the use of C_{14}-labelled carcinogens a few years later, made considerable advances in biochemistry possible. Starting from the 1950s, with the acquisition of new techniques in molecular biology, molecular genetics and immunology, further progress was made in the understanding of the mechanisms underlying the carcinogenesis process[1, 2]. Primary prevention of cancer, however, was implemented following the evidence of a causal relationship between exposure and cancer that was taking into consideration biological plausibility, but did not depend on the degree of understanding of the underlying mechanisms. The history of public health teaches us the lesson that prevention can be implemented before reaching a full understanding of mechanisms that could confirm or explain causality.

Early reports

In ancient times a largely shared view was that cancer was an expression of a divine punishment, toward which the only possible protection was the recognition of one's sin and repentance, hoping that this would open the path to God's forgiveness. From a medical point of view a prevailing hypothesis was that cancer was the local manifestation of a generalized unbalanced humoural condition. It was believed that there were four body humours: blood, phlegm, yellow bile and black bile, and that cancer was related to an excess of black bile. This belief was generally combined with the attitude that, except for those situations in which the cancerous lesion was superficial and well delimited and could be totally extirpated, it was much better not to intervene. A recommended, and rather reasonable rule at that time, was that *"quiescente cancro, medico quiescendum"* (when cancer is quiet, also the doctor should keep quiet).

A consistent change in the way cancer was looked at occurred in the XVIII Century and was related to the first reporting of the association between exposure to environmental agents and human cancer. In 1761 nasal tumours were reported to be associated with the use of tobacco snuff[3]; in 1775 Percival Pott reported the association between cancer of the scrotum in chimney sweeps and exposure to soot[4], and in 1795 the occurrence of cancer of the lip was reported to be associated with pipe smoking[5]. In the following century the therapeutic use of arsenic in the Fowler's solution, mainly although not exclusively employed to cure psoriasis, was found to be associated with skin cancer[6], and in 1895 a high incidence of urinary bladder cancers was found to be causally associated with occupational exposure of workers to aniline dyes[7]. In that same year, 1895, Roentgen discovered X-rays[8], the use of which spread rapidly and widely in the medical practice. Ionizing radiation was reported as a cause of malignant skin tumours seven years later[9, 10], a short delay compared with the generally longer periods required for the recognition of the carcinogenicity of certain chemicals. At the beginning of the XX Century, therefore, clinical observations provided evidence that cancer could be caused by exogenous chemical agents, such as tobacco, soot, arsenic and aromatic amines, and by ionizing radiation. The rôle of viruses in the experimental induction of tumours was reported by Peyton Rous in 1911[11], but his finding, for which he received a Nobel Prize in 1966, did not attract much attention for several decades.

Experimental chemical carcinogenesis

During the second half of the XIX Century experimental cancer research consisted mainly of studies on tumour transmission and tumour growth[12], but at the beginning of the XX Century various attempts were made in several institutions around the world to induce tumours in laboratory animals. The first to be fully successful in the experimental induction of tumours were two Japanese scientists, Yamagiwa and Ichikawa, who induced benign and malignant skin tumours by painting coal tar on rabbit's skin[13]. The choice of painting the tar on the inner, instead of the outer side of the ear, where the rabbit could not scratch it away and the tar could stay therefore long enough to damage the cells, favoured their success. Their experiments, carried out between 1914 and 1916, are generally considered as the beginning of experimental chemical carcinogenesis in modern terms. Tsutsui[14], a pupil of Ichikawa, developed in 1918 a method for the induction of skin tumours in mice, thus showing that the mouse could substitute the rabbit as a model animal. His method was used successfully in UK by Murray[15], and then by Passey[16, 17] who, in 1922, using the Tsutsui Method, was the first to induce malignant tumours of the skin with soot extracts.

Passey's results were considered to provide the final confirmation of the clinical observations made by Pott a century-and-a-half earlier about the origin of scrotal cancer in chimney sweeps. Such considerations implied

that clinical observations did need experimental confirmation in order to be fully accepted, marking in this way the beginning of an era of predominance of experimental chemical carcinogenesis in cancer research that lasted for several decades. The early part of this era was called by Shimkin[18] the period of the "tar tumours". The first chemical carcinogen of defined chemical structure was identified in 1930. It was initially described as 1,2,7,8-dibenzanthracene and later identified as 1,2,5,6-dibenzanthracene[2, 19, 20].

Further strength to the dominating rôle of experimental chemical carcinogenesis was provided by the evidence that carcinogens can exert their effect distant from their site of entry into the body. Murphy and Sturm[21] in fact reported the induction of lung tumours in mice following the cutaneous application of coal tar and, a few years later, Sasaki and Yoshida[22] reported the induction of liver cancer in rats following the oral administration of o-aminoazotoluene.

In the late 1930s it was therefore clear that: 1) a chemical mixture like soot could induce the same type of tumours in humans, in the rabbit and in the mouse; 2) the same chemical(s) could induce cancer in different animal species, even if not necessarily the same type of cancer; 3) the ability to induce cancer was not limited to a particular group of chemicals, and chemicals of quite different chemical structure could be carcinogenic; 4) carcinogens could induce cancer in organs distant from the carcinogen's point of entry.

In his autobiographical essay of 1977, Berenblum[23] mentions the Second International Cancer Congress held in Brussels in 1936, as the most momentous Cancer Congress ever held. It was attended by over 400 participants, a quite modest number if compared to the attendance of today's congresses, but huge for that time. At that Congress were presented for the first time comprehensive reports on several basic issues: 1) the carcinogenic properties of polycyclic aromatic hydrocarbons and many derivatives; 2) the induction of liver tumours by means of aminoazo compounds; 3) the induction of mammary tumours by oestrogenic compounds, the first report of which was published by Antoine Lacassagne in 1932[24]. In addition the results of early studies on the metabolism of carcinogens and their distribution in the body, and the new statistical approaches to the study of cancer in humans were also presented.

The two-stage carcinogenesis model

A significant contribution of experimental chemical carcinogenesis to the understanding of mechanisms of malignant transformation was made by the development of the two-stage carcinogenesis model. At the root of it is the early work of Berenblum[25], Rous and Kidd[26], Friedewald and Rous[27], Mottram[28] and Berenblum and Shubik[29, 30]. According to this model, cells exposed to a carcinogen may not attain a complete neoplastic transformation and may therefore remain latent until they are exposed to a further stimulus. Friedewald and Rous[27] proposed the term of 'initiation' for the first stage, where normal cells are converted in latent or dormant cells, and the term 'promotion' for the process stimulating the neoplastic growth of the initiated cells. Initiation was considered essentially irreversible, while promotion was considered largely reversible, at least in the initial phase[29]. The final phase of promotion, leading to the point of no return of the carcinogenesis process, and characterized by a higher degree of malignancy, invasiveness and metastatic growth, was also called 'progression'[31, 32].

Studies on the metabolism of chemical carcinogens

Results of relevant studies on the metabolism of polycyclic compounds were reported in the 1930s. Boyland[33] proposed in the early 1930s that toxic hydrocarbons are dealt with in either two ways: "*the toxic compounds might either be converted into more active pathogenic substances or be detoxicated by conversion into some harmless compound*". He further suggested that the metabolism of polycyclic hydrocarbons could be associated with carcinogenesis and that reactive metabolites could form reversible complexes with DNA[34, 35].

The list of chemical carcinogens continued to expand and, between the 1940s and the 1950s, included chemicals belonging to more than a dozen chemical classes, with quite different chemical structures. It became gradually evident, however, that most of them had "*a common metabolic feature*"[36, 37]. Using as a model the dye 4-dimethylaminoazobenzene given to rats, a firm binding to proteins in the liver was shown, providing further evidence that carcinogens "*could combine with amino acids constituents of proteins presumably through activated metabolites*"[2]. The covalent binding of carcinogens was then confirmed by the evidence of the binding to nucleic acids *in vitro* and *in vivo*. Alkylating agents, like nitrogen mustard, could bind directly, without requiring metabolism to be reactive, while aminoazodyes, polycyclic hydrocarbons and other chemical carcinogens do bind covalently to cellular DNA through reactive metabolites derived from the non-reactive parent compounds[36, 37]. As evidence that already in the 1930s experimental evidence of carcinogenicity obtained in long-term animal

tests was held as predictive of a similar effects in humans, it is worth noting that the use of 4-dimethylaminoazobenzene, known also as 'butter yellow', was forbidden as a food additive, because of the evidence of its carcinogenicity in rats provided by the experiments of Kinosita in 1937[38].

Studies on metabolism were, later on, focussed more on aromatic amines than on hydrocarbons. Evidence that a metabolite was more potent than its parent compound was thus first obtained with 2-acetylaminofluorene (AAF)[2,39].

The Delaney Amendment and the Toxic Substances Control Act

In 1958 the United States Congress approved an amendment to the US Food, Drug and Cosmetic Act, proposed by congressman James Delaney of New York, that became widely known as "The Delaney Amendment". The text of the amendment reads as follows: "*no additive should be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal*".

It was stressed, through a testimony of the National Cancer Institute, that the estimate of the possible toxic properties of chemicals added to food for human consumption should include consideration of their carcinogenic properties in laboratory animals. The Congress, in approving the Delaney Amendment, did not seem excessively concerned by the anomaly created by the different standards of safety applied to pesticide residues and to intentional food additives. Pesticide residues were considered quite differently from food additives. Food additives were perceived as optional, used either to make food more appealing or to provide "other marketing advantage"[40]. The resulting ambiguity in the interpretation of the text of the Delaney Amendment reveals the compromise made between the desire to guarantee absolute safety and the recognition of the rôle of pesticides in guaranteeing the nation's food supply.

The ambiguity is the result of the coexistence of sections 408 and 409 of the Federal Drug and Cosmetic Act of 1954. Section 408 recognized that the use of pesticides implied benefits and risks and that both had to be considered in setting raw commodity tolerances for pesticide residues. Section 409 stipulated that residues must be proven safe, meaning that there must be the "reasonable certainty" that no harm would result from the food additive, while no consideration of benefits was authorized. The Delaney clause, added to section 409, prohibits the use of a food additive that has been found to induce cancer in humans or animals, or for which the results of appropriate tests provide evidence that it may induce cancer in humans or animals.

The text of the Delaney clause was therefore in keeping with the public health-oriented attitude that measures of primary prevention are taken on the basis of what is recognized as causative factors of human cancer.

In 1960 Congress passed the "Color Additives Amendment", prohibiting the approval of any colour shown to induced cancer in humans and animals.

There were then, of course, as there are now, dissident opinions on the extent to which the Delaney clause could contribute in preventing human cancers[41]. Without going into a detailed discussion of the controversies that the approval of the amendment did generate, it is pertinent, however, to note that, for the purposes of the law, approved by Congress, the evidence of carcinogenicity provided by results of long-term animal tests was considered as a valid prediction of similar effects in humans.

In 1969 the Council on Environmental Quality (CEQ) was established as an agency within the Executive Office of the President with the purpose of studying the potential dangers to human health caused by metals and synthetic organic chemicals. The CEQ produced in 1970 a draft report of the study, published in 1971, and which was in effect the first version of the Toxic Substances Control Act (TSCA). In the report it was stressed that for most substances there were few data available and mainly related to acute effects, while knowledge of long-term effects was largely inadequate. The conclusion of the CEQ report was that to ensure proper protection of human health and of the environment, regulation was critical. Congress also realized that "*the most desirable time to determine the health and environmental effects of the substance, and to take action to protect against any potential adverse effects, occurs before commercial production begins*". The text of the TSCA was debated for years at the House of Representatives and the Senate. The TSCA was finally signed by President Ford on October 11, 1976[42].

The 1964 WHO Report on Prevention of Cancer

The relevance of experimental animals studies in predicting similar adverse effects in humans was stressed in the report prepared by the World Health Organization (WHO) on Prevention of Cancer in 1964[43]. In the section of the report dealing with food additives and contaminants it is stated that "…*it is essential that all substances*

proposed for addition to the human diet should first be thoroughly tested in the laboratory animal. In most countries it is the view that any agent proving to be carcinogenic in such tests should not be used at any level". While this statement appears to stand in favour of guaranteeing absolute safety, in tune with what Congressman James Delaney was claiming, the section ends by saying: "*It is of course recognized that many benefits result from the use of certain additives and pesticides and that the increasing economic well-being of man is in many ways a result of these agents. In all instances, therefore, action taken to ban any substance must take into account not only the results of animal experimentation but also the nature of the use and benefits of the compound in question*". It is clear therefore that, without questioning the relevance of the results obtained in laboratory animals in predicting possible adverse effects in humans, conspicuous weight was given to economic factors, held almost as important as the defence of public health.

In the section on industrial cancer hazards, the WHO report states that "*with certain exceptions, identical cancers have been produced experimentally, and that in some instances incriminating evidence was first obtained in experimental animals before corresponding cancers were detected in man*". In the section on medicaments it is stated that: "*it would be prudent to ensure that all drugs, but more particularly those administered over a long period, and especially in childhood, receive thorough carcinogenicity testing*".

It appears, therefore, that in spite of the rather cautious wording, the relevance of long-term carcinogenicity tests to predict similar effects in humans was again authoritatively confirmed in 1964. The double standards in dealing with food additives and pesticide residues, both in the interpretation of the Delaney Amendment and in the text of the WHO report, merely stress the heavy rôle played by economic interests in conditioning the implementation of primary prevention following the principles of public health. The WHO expert committee that drafted the report was chaired by Sir Richard Doll and included some of the world's greatest experts on cancer at that time.

The confirmatory and predictive value of long-term experimental animal tests

Long-term carcinogenicity tests were first employed to prove or disprove that chemicals or chemical mixtures suspected of producing cancer in humans could induce tumours in experimental animals, the induction of tumours in experimental animals being required to confirm the suspected carcinogenic activity of the chemical in humans. Long-term tests were thereafter used to verify, independently from observations in humans, that a chemical could produce cancer in an animal model, the main end point of the tests being therefore a qualitative one[44].

The case of 2-naphtylamine shows that the apparent lack of a carcinogenic effect in experimental animals was used as an argument to contrast the evidence of carcinogenicity based on several reports of human tumours, as well as against the definition of human carcinogens that the International Labour Office (ILO)[45] gave in 1921 of 2-naphtylamine and benzidine. The argument crumbled when Hueper[46] succeeded in inducing urinary bladder tumours in dogs given 2-naphtylamine.

In the case of 4-dimethylaminobenzene (butter` yellow) and 2- acetylaminofluorene (2-AAF), the results of long-term carcinogenicity tests showing their carcinogenicity, respectively in 1938 and in 1941[38, 47] were held as sufficient warning for not starting their commercial production and use, the first chemical as a food colour and the second as an insecticide.

A further example where the evidence of carcinogenicity provided by the results of long-term animal tests prevented the production and use of a chemical carcinogen, was that of 4-aminobiphenyl. Produced and used in the US since 1935, the UK was considering beginning its production in the early 1950s. However the evidence of carcinogenicity provided by results of a long-term bioassay in rats, confirmed two years later by a study on dogs[48, 49] was considered sufficiently convincing to decide not to produce or use that compound in the UK. In the US the first report of cases of urinary bladder cancer associated with occupational exposure to 4-aminobyphenyl was published in 1955 and shortly thereafter the production of the chemical was finally stopped[50, 51].

Some of the first chemicals tested as potential chemotherapeutic agents, such as urethane, chloroethylamines (nitrogen mustards), and sulphonamide derivatives[34], were known to be carcinogenic in experimental animals, but of many others the possible long-term toxicity was totally unknown. To assess their therapeutic efficacy as well as their possible adverse effect(s) they were systematically submitted to long-term animal tests, held as predictors of similar effects in humans.

The vital importance of animal experimentation for studying the mechanisms of action of carcinogens as well as for testing chemicals for their possible carcinogenic action, is widely documented by a large series of publications in the US. Significant examples are the well known text of Greenstein[52] and the numerous reports published in

the Journal of The National Cancer Institute and other peer reviewed journals[53-56].

The British Medical Bulletin, at that time one of the most prestigious periodicals on biomedical science, published in 1958 a monographic issue "Causation of Cancer"[57] with the contribution of several of the world's best known cancer experts. The essential rôle of animal experimentation for both the research on mechanisms of action of chemical carcinogens and the prediction of possible carcinogenic effects in humans was thoroughly documented. Of particular relevance in this respect are the contributions of Boyland[58], Walpole and Williams[59], Bonser and Clayson[60], and Barnes and Schoental[61].

In 1959 Graffi and Bielka[62] published a text on the problems of experimental cancer research that, in spite of being in German, had a wide circulation and was widely quoted. In 1964 Hueper and Conway[63] published "Chemical Carcinogenesis and Cancer" that remained for many years the most widely known and frequently quoted source of condensed information on chemical carcinogens. The significance of animal experimentation and the predictive value of long-term carcinogenicity tests were amply documented, and detailed instructions were given on how to run long-term carcinogenicity tests.

The relevance of experimental animal data for the identification of potential adverse effects in humans is obviously not limited to cancer. A pertinent example is that of thalidomide[64] which was not approved for use in the US because a careful and competent FDA scientist, Dr. Frances Kelsey, in 1961 considered the available experimental animal data unsatisfactory and requested further studies. During the wait for the requested additional data the first report appeared in Germany on the association between thalidomide and phocomelia[65]. The resistance of Dr. Kelsey, to the pressure of the company producing thalidomide, spared the US from the birth of thousands of malformed babies as did instead happen in the UK and in Germany.

In the 1969 Report of the US Secretary's Commission on Pesticides[66] it is stated (p. 467) that: *"any substance which is shown conclusively to cause cancers in animals […] should be considered potentially carcinogenic for man and therefore not innocuous for human consumption"*[66, 67].

In the Report to the Surgeon General prepared by the Ad Hoc Committee on the Evaluation of Low Levels of Environmental Chemical Carcinogens of the National Cancer Institute, April, 22,1970[68, 69], it is stated that:

"*No chemical substance should be evaluated safe for human consumption without proper negative lifetime biological assays of adequate size. The minimum requirements for carcinogenesis bioassays should provide for: adequate number of animals of at least two species and both sexes with adequate controls, subjected for their lifetime to the administration of a suitable dose range, including the highest tolerated dose, of the test material by routes of administration that include those by which man is exposed*".

Further on it is stated: "*The implication of potential carcinogenicity should be drawn both from tests resulting in the induction of benign tumors and those resulting in tumors which are more obviously malignant*".

All chemicals that are known to be carcinogenic to humans, and that have been submitted to a long-term carcinogenicity tests, have been shown to be carcinogenic to at least one, and in most cases to more than one animal species. The only exceptions for a while were benzene and arsenic, but both were eventually proven to be potent animal carcinogens[70, 71]. A comparison of organ specificities indicates that chemical carcinogens can have the same target organ(s) in humans and in at least one of the experimental animal species tested. Chances of finding similar target organs may be greater if similar routes of exposure are used. However, chemicals which are carcinogenic in both humans and experimental animals are found to be carcinogenic in the latter also when administered by routes different from the one by which humans are exposed (e.g. BCME)[72-76].

The experimental evidence of the carcinogenicity of many chemicals preceded the evidence of their carcinogenic activity in humans and has or would have allowed an earlier implementation of preventive measures[44, 77]. This was for instance the case for aflatoxins, 4-aminobiphenyl, 1,3-butadiene, diethylstilbestrol, formaldehyde, melphalan, mustard gas and vinyl chloride.

Conclusion

The rôle of the experimental approach for the identification of environmental agents carcinogenic to humans has continued to grow following the successful experiment of Yamagiwa and Ichikawa of 1915[13]. In 1922 Passey[17] was able to induce cancer in the mouse with soot extracts, confirming in this way experimentally the clinical observations of the association between exposure to soot and human cancer. Thereafter long-term carcinogenicity tests were widely employed with the purpose of either confirming some suspected evidence of carcinogenicity of a chemical or chemical mixture based on reports of human cancer cases, or of verifying, in the absence of human data, whether they possessed any carcinogenic activity.

Between the 1930s and 1970s considerable progress

was made in the understanding of the mechanisms underlying the carcinogenesis process, thanks to the availability of` new tools and techniques in biochemistry and molecular biology, and to the invaluable aid provided by the experimental animal models[1, 2, 78].

In 1951, under the auspices of the Public Health Service, the first collection of data was published on all chemicals which had been tested for their possible carcinogenicity[79]; this was subsequently regularly updated[80, 81].

In 1958 Congress passed the Delaney Amendment prohibiting the approval of food additives found to "*induce cancer in man or animals*", and, in 1960, passed the "Color Additives Amendment" prohibiting the approval of any colour shown to induce cancer in humans and animals. In 1971 a draft report was published which was actually the first version of the TSCA

In 1964 a widely circulated and well known WHO Report on Prevention of Cancer[43] confirmed the need for testing in laboratory animals all substances proposed for addition to the human diet, and of submitting to thorough carcinogenicity tests all medical drugs. In the report it was also stressed that the evidence of carcinogenicity of several industrial products was first obtained in experimental animals before cancer cases were detected in humans.

The relevance given to results of long-term tests to predict similar effects in humans was shown by the cases of 4-dimethylaminobenzene (butter yellow) and 2-acetylaminofluorene (2-AAF), respectively proposed as a food colour and as an insecticide, and which were never marketed because of the experimental evidence of carcinogenicity (provided for butter yellow in 1937[38], and for 2-AAF in 1941[47], as well as by the case of 4-aminobiphenyl (experimental evidence of carcinogenicity provided in 1952 and 1954[48, 49]). In addition, all human carcinogens suitable to being submitted to long-term carcinogenicity tests have been shown to be carcinogenic in at least one, and often more than one animal species, and in many instances experimental evidence of carcinogenicity has preceded the human evidence and could have allowed an earlier adoption of preventive measures.

The International Agency for Research on Cancer in 1971 began a programme that basically consisted in the thorough and critical analysis of all the available data for each agent considered and in an evaluation of the evidence of their carcinogenicity published as individual Monographs[82]. Noting that for many of the chemicals evaluated there was sufficient experimental evidence of carcinogenicity, but data relating to carcinogenicity in humans were either insufficient or nonexistent, in the Preamble to each Monograph it is stated that IARC considers that "*in the absence of adequate data on humans, it is reasonable, for practical purposes, to regard such chemicals as if they presented a carcinogenic risk to humans*".

Although animal studies do have drawbacks, their primary rôle being to provide a qualitative evidence of carcinogenicity, it was and is widely accepted and is fundamental to all toxicological research, including therefore the experimental observation of carcinogenic effects, that results from animal tests are applicable to humans. In the years 1950-1970 the use of experimental methods was definitely recognized as adequate for the identification of carcinogens by providing evidence that a chemical can interact with target cells to induce neoplastic growth. Long-term animal tests were given high visibility in the well known text "The Origins of Human Cancer"[83], published by the Cold Spring Harbor Laboratory, and several Guidelines for the conduct of long-term carcinogenicity tests were available[84, 87].

On the background of the evolution of experimental chemical carcinogenesis and of the understanding of some of the mechanisms underlying the carcinogenesis process from the 1930s to the 1970s, of the availability of data related to the significance, relevance and predictive value of results of long-term carcinogenicity tests of environmental chemicals, and of the availability of information on rules and regulations of carcinogens, as well as of guidelines for the conduct of long-term carcinogenicity tests, it is hard to believe that anybody with any sense of responsibility and with an even only minimal interest in public health could have discarded or ignored or belittled, the rôle that long-term carcinogenicity tests had, or could have had, in the years 1950s to 1970s in the adoption of preventive measures for the protection of human health.

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Leukaemia and low level benzene concentration: revisited

Leucemia e bassi livelli di concentrazione del benzene: una revisione

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Summary

Benzene has been defined as a known human carcinogen. Prior studies have claimed that benzene-related leukaemia and haematotoxicity occur only at high levels of exposure, in the tens to hundreds ppm. This article reviews epidemiological human studies, biomarkers studies, and experimental animal studies supporting the opinions that: 1) benzene is haematotoxic and leukaemogenic at levels just above zero, ranging in the order of ppb to less than 10 ppm; 2) benzene is a non-threshold haematotoxin and leukaemogen; and 3) dosimetric risk assessment of benzene exposure should take into account intensity and peak levels of exposure as well as cumulative exposure when applicable. Eur. J. Oncol., 11 (1), 15-24, 2006

Key words: **benzene, biomarkers, DNA adducts, dose, epidemiology, gasoline stations, haematopoietic malignancies, leukaemia, low levels**

Riassunto

Il benzene è stato classificato come cancerogeno noto per l'uomo. Studi passati affermavano che le leucemie e l'ematotossicità correlate al benzene insorgono solo a elevati livelli di esposizione, dalle decine alle centinaia di ppm. Questo articolo passa in rassegna gli studi epidemiologici sull'uomo, gli studi sui biomarcatori e gli studi sperimentali sugli animali, che supportano l'ipotesi che: 1) il benzene è ematotossico e leucemogeno a livelli appena superiori a zero, con un intervallo che va dall'ordine delle parti per miliardo (ppb) a meno di 10 ppm; 2) non vi è soglia per l'ematotossicità e la leucemogenicità del benzene; e 3) la valutazione dosimetrica del rischio da esposizione a benzene dovrebbe considerare, quando pertinenti, l'intensità e i livelli massimi dell'esposizione oltre all'esposizione cumulativa. Eur. J. Oncol., 11 (1), 15-24, 2006

Parole chiave: **benzene, biomarcatori, addotti del DNA, dose, epidemiologia, distributori di benzina, tumori ematopoietici, leucemia, bassi livelli**

Introduction

Benzene is classified as a Group 1 human carcinogen by the International Agency for Research on Cancer (IARC). While the general consensus indicates that benzene can cause leukaemia, arguments regarding the levels of exposure that causes leukaemia have been raging for years[1-9]. Several studies support a causal nexus at low levels of exposure. Arguments that leukaemia only occurs at high levels of exposure have been based among others on claims that 'epidemiologic studies have underestimated levels of exposed workers' and that there is 'a threshold level of benzene' below which leukaemia does not occur.

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A number of governmental agencies and organizations prepare recommendations for exposure levels to toxic chemicals as industrial and public health guidelines. The recommendations for benzene exposure limits are as follows:

1) Agency for Toxic Substances and Disease Registry (ATSDR) Acute Inhalation Minimal Risk Level: 0.05 parts per million (ppm), Intermediate Inhalation Minimal Risk Level: 0.004 ppm;
2) American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value: 0.5 ppm, Short-term Exposure Limit: 2.5 ppm;
3) California Environmental Protection Agency (Cal EPA) No Significant Risk Level: 6.4 μg/day (oral) and 13 μg/day (inhalation);
4) National Institute for Occupational Safety and Health (NIOSH): Recommended Exposure Limit: 0.1 ppm, Short-term Exposure Limit: 1 ppm;
5) Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit: 1 ppm, Short-term Exposure Limit: 5 ppm[10-12].

The Scientific Committee on Occupational Exposure Limits (formerly the Scientific Expert Group) of the European Union has recommended that the limit value be below 1 ppm[13].

The Collegium Ramazzini recently appealed to reduce the standard for occupational exposures to benzene, well below the ACGIH standard[14].

This manuscript demonstrates that human, experimental animal and *in vitro* studies further support the scientific basis for the position of the Collegium Ramazzini and are in line with the recent scientific review by Mehlman[15] that benzene is a non-threshold carcinogen at levels above zero.

Human epidemiological studies

Several recent studies have demonstrated that exposure to benzene at low levels does increase the risk of leukaemia. The Australian Institute of Petroleum[16] has published findings from an epidemiological health surveillance programme of petroleum industry employees. Workers were followed for 12.8 years, where average benzene exposure was 200 parts per billion (ppb). Investigators found a 50% increase in incidence of leukaemia among this population (standardized incidence ratio, SIR = 1.50; 95% confidence interval, CI = 1.02 to 2.15). According to Mehlman[15], the increased risk equates to an additional 3.5 leukaemia cases per 1,000 population over a lifetime (life background risk calculated at 7 leukaemia deaths per 100,000 population). By comparison, OSHA's significant risk level is 1 per 1,000 for a benzene exposure of 45 years. The increased risk of 3.5 deaths from leukaemia per 1,000 over 12.8 years compared to OSHA's risk level translates to an observable effect level at 16 ppb[15].

It is only in the last few years that epidemiologists have begun using intermittent, peak and intensity exposure metrics in evaluating benzene-exposed workers for excess leukaemia risk. Researchers from Solutia (formerly Monsanto) examined lymphohaematopoietic cancer mortality among 4,417 workers at a chemical plant using low cumulative and short-term peak benzene exposure since toxicological data indicate that dose-rate is important. These investigators stated: "*We found little evidence of increasing risk with increasing cumulative exposure for all leukaemias or acute non-lymphocytic leukaemia… However, when peak exposures over 100 ppm for forty or more days are considered all leukaemias (standardized mortality ratio, SMR = 2.7, 95% confidence interval, CI = 0.5-14.9… are elevated*". The authors therefore concluded: "*We find that a high number of peak exposure to benzene is a better predictor of risk than cumulative exposure*"[17].

The epidemiologic study by Australian investigators produced similar findings[16]. Overall, the study demonstrated a significant increase in the incidence of leukaemia among this cohort of petroleum industry workers, whose average intensity of benzene exposure was only 0.2 ppm and equal to or less than 0.50 ppm for 90% of the subjects studied. Within this cohort of petroleum industry workers, a nested case-control study was carried out to examine the dose-response relationship between benzene exposure and lymphohematopoietic cancers. Data were analyzed by both intensity of exposure and by cumulative benzene exposure. Based on analyses by intensity of exposure, the authors stated: "*unmatched analysis suggested an effect from exposure intensity for leukemia… and conditional logistic regression confirmed these findings and showed a significant exposure intensity-response relationship for leukaemia, particularly for the highest exposure job*". The exposure intensity results were noteworthy, especially because the maximum exposure in the highest exposure job category was just 4.88 ppm. Those workers who had geometric "high intensity" exposures between 0.80 and 1.56 ppm had a 7-fold increased leukaemia risk (odds ratio, OR = 7.09, 95% CI = 1.24 to 34.1), and those workers who had geometric "high intensity" exposures between 3.49 and 4.8 ppm had a 26-fold increased leukaemia risk (95% CI = 2.00 to 338.7); all these results being statistically significant. An analysis by intensity of exposure indicated that workers who had jobs with potential for the highest intensity of exposure demonstrated a greater risk of leukaemia

Table 1 - Association of leukaemia and non-Hodgkin's lymphoma/multiple myeloma (NHL/MM) by benzene exposure group[a, b]

Cumulative lifetime benzene exposure (ppm-years)	Leukaemia OR (95% CI)	NHL/MM OR (95% CI)
≤ 1[c]	1.0	1.0
> 1-2	3.9 (0.9-17.1)	1.1 (0.4-2.9)
> 2-4	6.1 (1.4-26.0)	1.2 (0.5-3.0)
> 4-8	2.4 (0.4-13.6)	1.3 (0.5-3.2)
> 8-16	5.9 (1.3-27.0)	0.8 (0.3-2.6)
> 16	98.2 (8.8-109.0)	1.1 (0.3-4.5)

[a] From Glass *et al* [19]
[b] From Conditional Logistic Regression Analysis
[c] Reference category

than non-exposed subjects with similar cumulative benzene exposure. This observation supports the premise that opportunity for intermittent peak benzene exposure carries with it a greater risk of developing leukaemia than the same cumulative benzene dose accumulated by a more even mode of exposure[18].

A recent cohort study of Australian refinery workers examined the risk for leukaemia at low levels of benzene exposure, and compared cumulative exposure to intensity of exposure[19]. In analyzing cumulative benzene exposure (expressed as ppm years), the investigators found a six-fold increased risk for leukaemia (OR = 6.1, 95% CI = 1.4 to 26), at levels above 2 ppm-years. The increased risk of leukaemia at benzene exposure above 2 ppm-years is depicted in Table 1. Exposure intensity in the highest exposed job was strongly related to leukaemia risk, the increased risk starting at about 0.8 to 1.6 ppm, with the highest exposure category being nearly 20 times more likely to develop leukaemia than those who were unexposed. This study further demonstrates increased risk of leukaemia at benzene levels less than 2 ppm-years (cumulative exposure), and 0.8 to 1.6 ppm (intensity of exposure). This relationship is depicted in fig. 1.

The National Cancer Institute sponsored a cohort study by Hayes *et al*[9] that examined 74,828 benzene-exposed and 35,805 unexposed workers employed from 1972 to 1987, in 12 cities in China. For workers historically exposed to benzene at average levels of less than 10 ppm, the relative risk (RR) for all haematopoietic neoplasms combined was 2.2 (95% CI = 1.1 to 4.2). The study concludes that the risks for haematological malignancies are elevated at average benzene exposure levels of less than 10 ppm. A review of a Dow Chemical workers study demonstrated an increased incidence of leukaemia in workers exposed to benzene levels as low as 0.6 ppm-years[15, 20]. The summary of the study is depicted in Table 2. Rinsky *et al*[21] and Yin *et al*[22] reported haematological malignancies in benzene-exposed workers at low levels of 0.1 ppm-years (Tables 3 and 4).

Cigarette smoking is associated with an increased risk of leukaemia. The IARC[23] indicates sufficient evidence to support a causal relationship between smoking and leukaemia. Benzene is an important human carcinogen emitted from cigarette smoke. While cigarettes contain other carcinogens, benzene is the most probable carcinogen associated with leukaemia. Active smoking was reported recently as a significant risk factor for

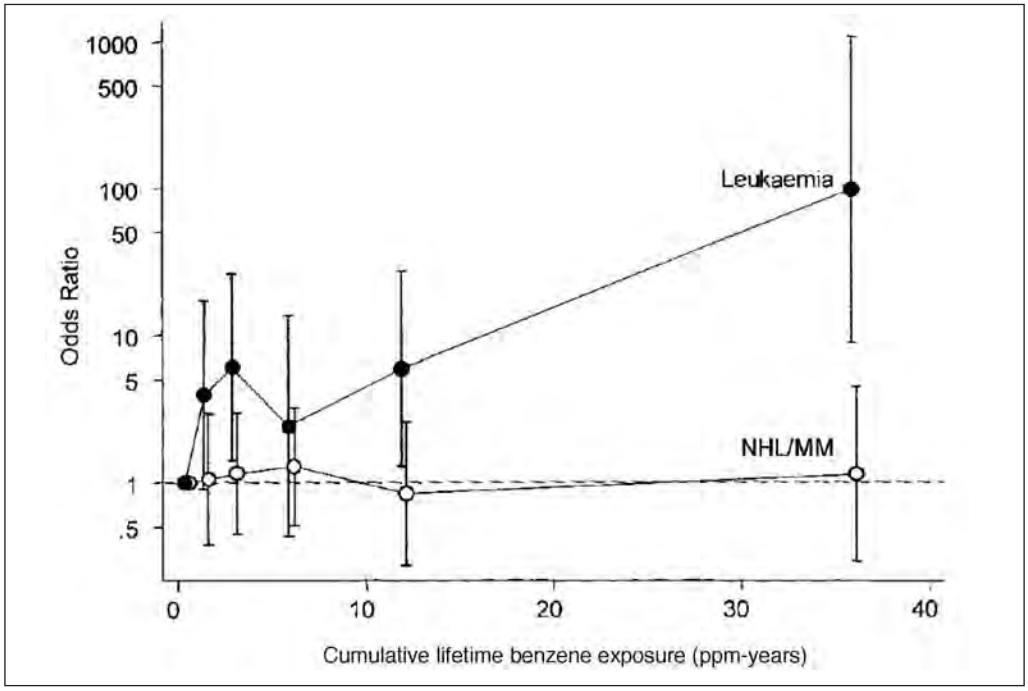

Fig. 1. Leukaemia and non-Hodgkin's lymphoma/multiple myeloma (NHL/MM) odds ratios by geometric benzene exposure groups (ppm-years) displayed at the midpoint of the exposure group (circles indicate odds ratios; vertical lines depict confidence intervals)
From Glass *et al* [19]

Table 2 - Deaths from lymphatic and haematopoietic cancers[a]

Case	Cancer	ppm-years[b]
4	Chronic lymphocytic leukaemia	43.6
6	Chronic myelogenous leukaemia	10.0
11	Chronic myelogenous leukaemia	0.6
8	Myeloma	1.2
16	Chronic lymphocytic leukaemia	-

[a] From Wong[20] and Mehlman[15]
[b] Low level: < 1 ppm TWA; medium level: 1-10 ppm TWA; high level: 11-50 ppm TWA, RR = 3.93 ($p < 0.02$) for leukaemia and aleukaemia: RR = 4.12 ($p < 0.06$) for non-Hodgkin's lymphopoietic cancers

Table 3 - Deaths from leukaemia and multiple myeloma in workers exposed to benzene[a]

Case	Cancer	ppm-years[b]
1	Monocytic leukaemia	49.99
2	Chronic myelogenous leukaemia	0.1
8	Myelogenous leukaemia	10.16
10	Multiple myeloma	19.50
11	Multiple myeloma	0.11
13	Multiple myeloma	7.75

[a] From Rinsky *et al*[21] and Mehlman[15]
[b] SMR = 5.6 for leukaemia ($p < 0.001$)

Table 4 - Leukaemias in benzene-exposed workers[a]

Case	Disease	ppm-years[b]
3	Acute monocytic leukaemia (AMoL)	89.1
7	Acute myelocytic leukaemia (AML)	96.0
8	Acute myelocytic leukaemia (AML)	27.9
9	Chronic myelocytic leukaemia (CML)	11.7
11	Acute myelocytic leukaemia (AML)	34.2
15	Chronic myelocytic leukaemia (CML)	16.2
16	Acute myelocytic leukaemia (AML)	28.8
18	Acute myelocytic leukaemia (AML)	12.4
26	Acute monocytic leukaemia (AMoL)	29.8
27	Acute myelocytic leukaemia (AML)	10.2

[a] From Yin *et al*[22] and Mehlman[15]
[b] SMRs for AML = 4.96 ($p < 0.001$); AMoL = 4.54 ($p < 0.05$); CML = 4.24 ($p < 0.1$)

leukaemia (OR = 1.5, 95% CI = 1.1 to 2.0)[24]. The levels of benzene exposure in active smokers have been reported at a range of 7.2 to 17.8 ppb[25]. These low concentrations indicate that benzene is a human leukemogenic agent at very low levels, in the order of ppb.

Zauli Sajani *et al*[26] examined the leukaemia risk due to air pollutant benzene for a population living within an urban area of Italy. The leukaemia risk at benzene levels of 2.16 ppb for the year 2000 was compared to benzene levels of 15.7 ppb for the year 1988. The leukaemia risk assessment was calculated at $4x10^{-2}$ based on cumulative benzene exposure. This study is in agreement with the risk assessment measures demonstrated by Mehlman[15], OSHA[11], and the California Environmental Protection Agency[12]. Parodi *et al*[27] investigated the risk of leukaemia for a population exposed to benzene emitted mainly from the neighbouring coke oven plant. The authors reported significant increased risks for all lymphohaematopoietic cancers and leukaemia in the male population (OR = 1.7, 95% CI = 1.3 to 2.4; and 2.4, 95% CI = 1.3 to 4.5, respectively). Mean benzene concentration was 3.18 ppb, with a range of 0.86 to 6.33 ppb.

The American Petroleum Institute (API)[28] has stated that benzene exposure at low levels is a carcinogen in susceptible populations. Since human susceptibility is not predictable, the API concluded that a safe level of exposure to benzene is no exposure. From toxicological and epidemiological point of views, children are considered a susceptible population group. Crosignani *et al*[29] demonstrated that the risk of childhood leukaemia was significantly higher at annual benzene exposure levels of 3.08 ppb (OR = 3.91, 95% CI = 1.36 to 11.27). These studies further support the notion that benzene is a leukemogenic agent at concentrations in the order of ppb.

These epidemiological and population studies described above question the opinions of Wong *et al*[7] and Schnatter *et al*[8], that there is no increase in leukaemia risk below 40 ppm-years. As a matter of fact, Wong *et al* demonstrated that case no.11 and case no. 8 of their study developed haematological malignancies at benzene concentrations of 0.5 ppm for 1.2 years and 2.3 years, respectively (Table 2)[20].

Haematological biomarker studies of workers exposed to benzene

Lan *et al*[30] examined white blood cell, platelet counts and progenitor cell colony formation in 250 workers exposed to benzene, compared to 140 controls. The investigators demonstrated haematotoxicity from exposure to benzene occurring at ambient levels of 1 ppm or less. This study is also important because it showed that benzene exposure reduced colony formation of myeloid progenitor cells, and that these progenitors were more sensitive to benzene toxicity than mature white blood cells. The statistically significant reduction in haematological markers and benzene exposure levels less than 1 ppm is depicted in Table 5.

Ward *et al*[31] examined red and white blood cells (WBC) from workers exposed to benzene and found a strong exposure response. The authors concluded that

Table 5 - Peripheral blood cell counts in relation to benzene exposure level[a]

Subject category (n)[b]	Controls (140)	<1 ppm (109)	1 to <10 ppm (110)	≥10 ppm (31)	p for <1 ppm *vs* controls[c]	p_{trend}, all subjects[d, e]
Benzene exposure						
Benzene air level (ppm)[f]	<0.05	0.57 (0.24)	2.85 (2.11)	28.73 (20.74)		
Benzene urine (μg/litre)[g]	0.382 (1.24)	13.4 (18.3)	86.0 (130)	847 (1250)		
Peripheral blood cell counts[h]						
White blood cells (WBC)[i]	6480 (1710)	5540 (1220)	5660 (1500)	4770 (892)	<0.0001	<0.0001
Granulocytes	4110 (1410)	3360 (948)	3480 (1170)	2790 (750)	<0.0001	<0.0001
Lymphocytes[l]	2130 (577)	1960 (541)	1960 (533)	1800 (392)	0.018	0.0014
$CD4^+$-T cells	742 (262)	635 (187)	623 (177)	576 (188)	0.003	0.019
$CD8^+$-T cells	553 (208)	543 (212)	564 (229)	549 (160)	0.75	0.97
$CD4^+/CD8^+$ ratio	1.46 (0.58)	1.26 (0.41)	1.22 (0.45)	1.09 (0.35)	0.015	0.024
B cells	218 (94)	186 (95)	170 (75)	140 (101)	0.003	0.0002
NK cells	586 (318)	558 (299)	566 (271)	415 (188)	0.56	0.0044
Monocytes	241 (92)	217 (97)	224 (93)	179 (74)	0.018	0.28
Platelets	230 (59.7) x 10^3	214 (48.8) x 10^3	200 (53.4) x 10^3	172 (44.8) x 10^3	0.023	0.0002
Haemoglobin (g/dl)	14.5 (1.6)	14.7 (1.5)	14.5 (1.7)	13.6 (1.6)	0.12	0.29

[a] There are up to 418 observations on 390 unique subjects (140 controls and 250 benzene-exposed workers). Data were obtained from 28 exposed subjects in both years (2000 and 2001) and are treated as independent observations in the summary data shown, distributing into benzene category on the basis of exposure level in the year that the blood sample was collected. Data shown here are from all subjects except one benzene-exposed subject with data for only CD4+/CD8+ ratio; two benzene-exposed subjects and one control with no benzene urine data; and two controls with no BMI data. From Lan *et al* [30]

[b] The 28 subjects studied in both the first (2000) and second (2001) year of the study are categorized based on the exposure assessment in 2000

[c] Controls *vs* exposed <1 ppm, by linear regression on ln of each endpoint

[d] p_{trend} using ln air benzene as a continuous variable. All statistically significant endpoints were inversely associated with benzene exposure

[e] Comparison of subjects ≥10 ppm *vs* controls for endpoints without statistically significant trends: $CD8^+$–T cells, $p = 0.31$; monocytes, $p = 0.0006$; haemoglobin $p < 0.0001$.

[f] Benzene air level is the arithmetic mean (TSD) of an average of two measurements per subject collected during the month before phlebotomy. This time period was chosen because granulocytes have relatively short half-lives in peripheral blood

[g] Urinary benzene (mean TSD) and mean individual air levels of benzene were strongly correlated (Spearman $r = 0.88$, $p < 0.0001$)

[h] Unadjusted mean (±SD) cells/μl blood

[i] Supplementary analyses are shown in the SOM Text

[l] Absolute count

there was no evidence of threshold for the haematological effects of benzene exposure, suggesting that even exposure at low levels of benzene (e.g., less than 5 ppm) may result in haematologic suppression.

Zhang[32] examined WBC from workers exposed to benzene at an average concentration of 4.57±6.16 mg/m^3 (1.43±1.93 ppm). Four hundred and thirty-seven workers were compared to 150 controls. With the increase in benzene concentration, Zhang found a lowering of WBC counts and an increased proportion of lymphocytic micronucleus, and concluded that low levels of benzene exposure are harmful to the exposed workers.

Qu *et al* [33] examined haematological indices in 130 exposed workers. Workers were monitored for benzene exposure; urinary metabolites and albumin adducts were among the biomarkers of exposure. An exposure-dependent decline in both red blood cells and WBC was demonstrated at a benzene concentration as low as 0.06 ppm (median of 3.2 ppm). At 0.5 ppm, the authors demonstrated significantly lower red blood cells and WBC counts. The corresponding individual cumulative exposure duration and intensity (yearly average exposure level) were 14 years and 2.68 ppm. These studies demonstrate the haematotoxicity of benzene at both low levels of exposure and intensity of exposure (Table 6).

Genotoxic effects in workers exposed to benzene: DNA and urinary levels of oxidative DNA studies

Nilsson *et al* [34] examined genotoxic effects in workers exposed to benzene at 0.1 ppm. This study compared

Table 6 - Means and standard deviations for blood cell counts, grouped according to average lifetime benzene exposure intensity[a, b]

Variable	Unexposed	ppm per year			
		>0 - <5	5 - <15	15 - <40	≥40
No. of subjects	51	54	36	29	11
Female (%)	53	54	53	48	55
Smokers (% reported + cotinine)	31	35	42	45	27
Mean age	33.3±7.4	35.2±7.6	39.3±5.2	36.3±8.7	31.4±8.6
Mean cumulative benzene exposure (ppm-years)	0	32±21	74±51	123±65	237±188
Mean duration of benzene exposure (years)	0	11.2±6.4	9.8±6.0	4.4±1.9	4.0±3.2
Mean current 4-week benzene exposure (ppm)	0.004±0.003	3.07±2.9	5.89±4.8	17.4±15.5	50.6±55.4
Red blood cells (x 10^{10}/l)[c]	463±52	403±62	396±57	404±51	391±39
Hematocrit (year 2 only)	44.2±5.3	43.0±3.7	42.7±4.7	43.1±3.3	-
Platelets (x 10^{9}/l; year 2 only)	277±43	271±69	292±72	288±38	-
White blood cells (x 10^{6}/l)[c]	6,671±1,502	6,383±1,330	6,089±1,455	6,103±1,560	4,727±548
Lymphocytes (x 10^{6}/l)	2,205±789	2,551±797	2,136±779	2,169±559	1,956±395
Neutrophils (x 10^{6}/l)[c]	4,006±1,108	3,377±868	3,491±1,121	3,501±1,314	2,480±451

[a] From Qu *et al* [33]

[b] Means and standard deviations reported are the raw variables, but for the monocytes, eosinophils, and basophils, the statistical tests were performed on the log transformed data

[c] $p \leq 0.001$, test for exposure-response trend

single strand breaks (SSB) in DNA of leukocytes and urinary levels of oxidative DNA adduct 8-hydroxy-deoxyguanosine (8OHdG) of 33 men occupationally exposed to benzene from gasoline with a like number of controls. The 8-hour time weighted average (TWA) exposure to benzene was only 0.13 ppm. Exposed workers had a significantly increased SSB over the shift compared with the controls. The investigators performed multiple linear regression analyses (adjusting for smoking habits) and showed a significant association between the exposure level of benzene during the shift and the increase of 8OHdG in urine over the shift among exposed workers. The authors concluded that the findings indicate genotoxic effect in humans of relatively low exposure levels of benzene of about 0.1 ppm.

Confounding by tobacco smoking was unlikely with regression analysis adjusting for cigarette consumption during the shift. Nilsson *et al* [34] acknowledged that benzene is one of several components of gasoline and that other components may have contributed to the genotoxic effect to some extent. However, benzene is the most well-established carcinogen in gasoline[35]. Measurements have shown considerable variation in exposure to benzene even though the TWA is rather low; short-term measurements of 5 to 15 minutes indicated concentrations of about 10 ppm or higher at loading operations on ships, railroads and road tankers in garages, further supporting the importance of intensity of exposure to benzene over a short period of time[36].

Rodent DNA studies

Several studies utilizing accelerator mass spectrometry have shown the development of DNA and haemoglobin adducts from benzene and/or its metabolites at nanomolar concentrations.

Creek *et al* [37] examined tissue distribution in macromolecular binding of benzene over a dose range spanning nine orders of magnitude, to determine the nature of the dose response, and established benzene's internal dosimetry at doses encompassing human environmental exposure. The study was conducted with labelled C_{14} benzene administered to male mice. Tissue, DNA and protein were analyzed for radioactive labelled-benzene. The uptake and distribution of benzene labelled metabolites, by liver DNA and bone marrow DNA, are demonstrated in fig. 2. Dose response relationships are demonstrated in Table 7; dose and its effect on liver DNA and bone marrow DNA is described in fig. 3. The authors concluded that, regardless of the mechanism, while the distribution of total benzene metabolites to the tissue does not appear to be indicative of target organ toxicity, a very different picture is obtained when peak benzene adduct levels or the time integrated adduct concentration among tissue are compared. The study showed that utilizing the technique of accelerator mass spectrometry with C_{14} benzene labelling, benzene and its metabolites are rapidly absorbed and cleared in the bone marrow of mice at lower doses. The investigators state: "*We find that benzene's*

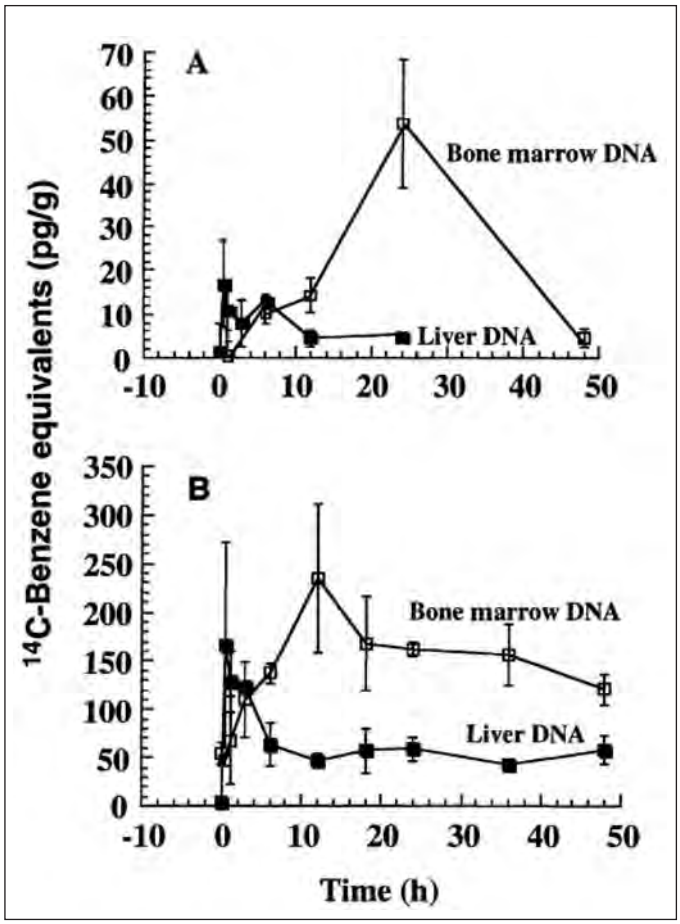

Fig. 2. Uptake and distribution of [C_{14}]-benzene equivalents in male $B_6C_3F_1$ mice by liver DNA and bone marrow DNA. Dosing was by i.p. administration of 628 ng (A) and 5 μg/kg (B) [C_{14}]-benzene in corn oil. Data represent mean ± SD of three animals/time point. From Creek *et al*[37]

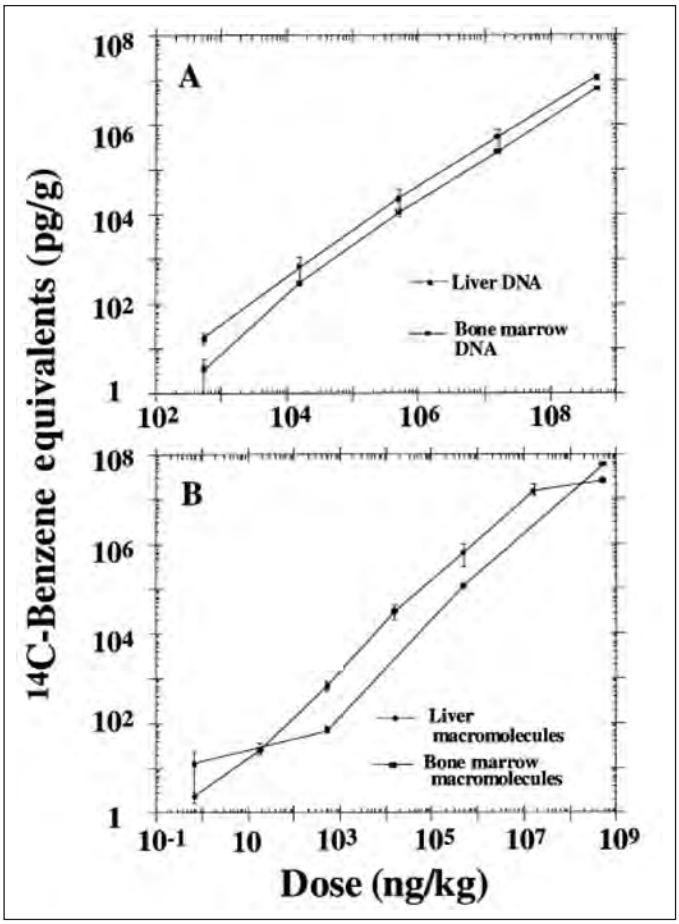

Fig. 3. Dose–response relationship of [C_{14}]-benzene equivalents in liver and bone marrow DNA (A) and macromolecules (B) from male $B_6C_3F_1$ mice 1 h after i.p. administration of various doses of [C_{14}]-benzene. Values represent mean ± SD of three animals/time point, with the exception of liver DNA (500 mg/kg dose, $n = 2$) and bone marrow DNA (15.78 μg/kg dose, $n = 2$). Bone marrow macromolecules showed significant nonlinearity at the lowest dose, but the most likely explanation for this is inadvertent carbon contamination. Statistical tests for equality of slopes show that the slope for bone marrow DNA is marginally significantly different from liver DNA when a multivariate regression approach is used ($p < 0.05$). From Creek *et al*[37]

total dose to the tissues and the bioactive component of that dose (estimated from macromolecular adduct levels) will scale linearly with administered dose below doses of 20 mg/kg of body weight to doses in the low ng/kg body weight range… this dose response range encompasses human environmental exposure levels and the doses used in rodent bioassays… additionally, these data show that greater levels of bioactive benzene intermediates are present at low dose in the primary response organ for benzene toxicity, bone marrow, relative to a non-target tissue (liver), where significant amounts of metabolites are generated"[37]. This study demonstrates the importance of peak levels and intensity levels of biological exposure to benzene at very low levels, and its primary response on the bone marrow. Furthermore, the study validates the hypothesis that benzene toxicity of the bone marrow occurs at peak levels and a scale of ppb.

Mani *et al*[38] examined the macromolecular binding of extremely low doses of labelled benzene in rodents utilizing accelerated mass spectrometry. Utilizing this methodology, these investigators concluded that studying labelled benzene in rat and mouse strains at environmentally relevant doses, demonstrated that total benzene adducts in bone marrow parallel sensitivity to benzene metabolism. The authors observed that even at low doses, the capacity to bioactivate benzene (based on adduct) is related to its toxicity in rodents at levels which are environmentally relevant[25].

Albumin adducts in benzene-exposed workers

Rappaport *et al*[39] examined albumin adducts of benzene oxide and 1,4-benzoquinone as measures of

Table 7 - Dose-response relationship of [C_{14}]-benzene equivalents in male $B_6C_3F_1$ mice 1 h after i.p. administration[a]

[C_{14}]-benzene equivalents, pg/g tissue[b, c, d, e]				
Dose (ng/kg)	Liver	Kidney	Blood	Bone marrow[f]
500064020.5	59820347.1±5592447.0	46302189.2±9563868.3	22859794.7±5051111.3	2520159.1±566265.1
15782020.5	15836406.7±3206380.4	18467986.6±13298040.2	3510364.0±860217.5	n/a
499263.9	458372.1±251031.3	435342.6±126233.0	138810.9±87890.0	44196.8±16418.9
15782.0	18746.9±2487.2	14526.3±1360.9	5325.4±2828.2	n/a
540.3	543.5±92.5	512.4±191.4	139.6±58.3	36.0±9.4
18.3	27.2±5.6	32.1±21.9	15.7±14.5	0.9±0.2
0.7	0.4±0.2	0.4±0.0	0.0±0.0	n/a
[C_{14}]-benzene equivalents, pg/g tissue[b, c, d, e]				
Dose (ng/kg)	Lung	Spleen	Thymus	Heart
500064020.5	19055766.7±899845.2	8394521.9±958579.2	5399735.6±599461.2	6835393.1±1122843.2
15782020.5	2320340.1±326711.1	1414067.0±246721.6	1030709.1±288312.9	984392.4±95114.1
499263.9	89604.9±18701.3	47818.7±15152.0	47225.2±7167.8	45547.8±20206.8
15782.0	3117.8±510.6	1892.3±231.6	1164.3±37.6	1480.4±162.4
540.3	90.1±26.3	62.1±25.0	21.3±12.3	35.7±5.8
18.3	5.5±3.3	2.4±0.5	2.0±0.4	2.7±2.3
0.7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

[a] From Creek *et al* [37]

[b] Data represent the mean of three animals ± SD, with the exception of thymus (628 ng/kg, $n = 2$). Values for 18.3 ng/kg dose represent data from two independent experiments ($n = 8$ and $n = 9$). Values for 0.7 ng/kg dose represent four animals/dose. For the data shown the background contribution of radiocarbon (i.e. the amount of radiocarbon in undosed animals) has been subtracted

[c] Regression parameters were fit to the model $y = a(x)^b$, where a = intercept and b = slope. Parameters were generated using all data except for the omission of the highest data point, where saturation was evident. Individual parameters for each tissue are as follows (intercept, slope): liver (1.155, 0.9895), kidney (1.207, 0.9825), blood (0.467, 0.9553), bone marrow (0.044, 1.0515), lung (0.271, 0.9630), spleen (0.171, 0.9553), thymus (0.140, 0.9460) and heart (0.101, 0.9795)

[d] Statistical tests of whether the regression slopes were equal to 1 were not rejected except for bone marrow, which had a slope significantly >1 and lung, spleen and thymus, which had slopes significantly <1

[e] Regression goodness-of-fit tests showed significant non-linearity for liver, blood and thymus, but the practical size of this effect is quite small, as evidenced by plots of the data

[f] Values for bone marrow tissue were obtained from independently replicated sets of experiments. All other measurements were made from the same animals

benzene metabolism among 134 benzene-exposed workers, with range of exposure from 0.7 to 46.6 ppm, and 51 unexposed subjects in Tianjin, China. The results demonstrated a supralinear dose-response relationship for albumin adducts from benzene oxide at concentrations <1 ppm. Presumably, Rappaport *et al* suggest that the true risk may be substantially larger than current predictions of leukaemia risk based on linear kinetics.

Conclusion

The studies described in this manuscript support the opinions that: 1) benzene is haematotoxic at low levels <1 ppm; 2) there is no evidence for threshold for benzene haematotoxicity; 3) where applicable, benzene haematotoxicity and leukaemia risk must be addressed at intensity levels, peak exposure levels and cumulative exposure levels; and 4) the human epidemiological studies *in vivo* and experimental animal studies support the recent Collegium Ramazzini appeal *"to reduce the standard for occupational exposure to benzene well below the standard recommended by ACGIH (0.5 ppm in 1997) and the current OSHA 1 ppm (TLV-TWA) standard"* [14].

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Disclosure

Some of the comments in this manuscript have been presented in civil court. Nachman Brautbar, MD serves from time to time as an expert in litigation related to benzene exposures. This manu-

script was not authored for litigation process. The opinions expressed in this manuscript do not necessarily reflect that of any organizations the authors are affiliated with.

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Pattern molecolare nella malattia da esostosi multiple ereditarie

Molecular pattern in hereditary multiple exostosis disease

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Riassunto

L'Esostosi Multipla Ereditaria (Hereditary Multiple Exostosis = HME) è caratterizzata dalla crescita di osteocondromi in corrispondenza delle diafisi delle ossa lunghe di derivazione encondrale. E' una malattia ereditaria, a trasmissione autosomica dominante ed i loci coinvolti sono almeno due, EXT1 ed EXT2, geni che codificano per due proteine, dette esostosine, correlate con la biosintesi dell'eparansolfato (HS), molecola fondamentale nella regolazione del processo di maturazione dei condrociti. Numerose molecole, tra cui l'*Indian Hedgehog* (Ihh) e il *Parathyroid hormone-related hormone* (Pthlh) interagiscono in una complessa rete di segnali per regolare gli specifici passaggi di tale processo differenziativo. Mutazioni in EXT determinano una riduzione della sintesi di HS; tale condizione favorisce la distribuzione del segnale di Ihh conducendo alla formazione delle esostosi. Il riconoscimento del preciso modello patogenetico permetterà di individuare nuove strategie terapeutiche: la ciclopamina, inibendo il segnale di Ihh, potrebbe avere un ruolo nel trattamento degli osteocondromi. Eur. J. Oncol., 11 (1), 25-31, 2006

Parole chiave: **HME, Indian Hedgehog, osteocondromi, EXT**

Summary

Hereditary Multiple Exostosis (HME) is characterized by the growth of osteochondromas in the diaphyses of long bones of enchondral origin. It is an hereditary disease, with autosomal dominant transmission, and the loci involved are at least two, EXT1 and EXT2, genes which codify for two proteins, called exostosins, related with the biosynthesis of heparan sulphate (HS), a fundamental molecule in the regulation of the maturation process of chondrocytes. Several molecules, among which the Indian Hedgehog (Ihh) and the Parathyroid hormone-related hormone (Pthlh), interact in a complex network of signals, in order to regulate the specific steps of this differentiation process. EXT mutations determine a reduced synthesis of HS; this condition increases the distribution of the Ihh signal, leading to the formation of exostoses. The identification of the precise pathogenic model will permit the determination of new therapeutic strategies: cyclopamine, by inhibiting the Ihh signal, could play a rôle in the treatment of osteochondromas. Eur. J. Oncol., 11 (1), 25-31, 2006

Key words: **HME, Indian Hedgehog, osteochondromas, EXT**

Introduzione

L'Esostosi Multipla Ereditaria (Hereditary Multiple Exostosis = HME) è una malattia ereditaria caratterizzata dalla crescita di tumori benigni, ricoperti da cartilagine (detti esostosi o osteocondromi), in corrispondenza delle estremità delle diafisi delle ossa lunghe di derivazione encondrale[1].

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Attualmente l'HME è la patologia ossea neoplastica in assoluto più comune, con una prevalenza di 1/50.000[2,3] in Europa ed 1/100.000 negli USA[4].

Nessuna predilezione di sesso è finora stata dimostrata, sebbene sembri che i maschi siano affetti in maniera più grave o comunque ricorrano più frequentemente alle cure mediche[5], suggerendo una potenziale influenza degli ormoni sessuali sulla crescita tumorale.

Si tratta di una patologia diagnosticata in età prettamente pediatrica, con un'età media di insorgenza stimata intorno ai 4 ± 1 anni, senza differenze tra i due sessi. Tipicamente è possibile lo sviluppo di nuove esostosi fino alla chiusura delle cartilagini di accrescimento, sottolineando il ruolo di una disregolazione della differenziazione di condrociti nello sviluppo delle esostosi.

Genetica

La HME presenta una trasmissione genetica di tipo mendeliano autosomico dominante, con penetranza verosimilmente del 100%[6]. Si tratta di una patologia geneticamente eterogenea ed i loci coinvolti sono almeno due: esostosina 1 (EXT1), localizzata sul cromosoma 8q24.1[7], e l'esostosina 2 (EXT2), localizzata sul cromosoma 11p11-p13[8]. La maggior parte dei pazienti presenta mutazioni in EXT1, gene coinvolto nel 60-70% dei casi, mentre il 30-40% presenta mutazioni in EXT2. Le mutazioni più frequenti sono di tipo *missense* (si tratta di sostituzioni non sinonime che danno origine ad un codone che specifica un aminoacido diverso da quello originario), o di tipo *frameshift,* nelle quali si verifica uno slittamento del modulo di lettura e traduzione, introducendo un codone di terminazione precoce e determinando la perdita dell'espressione genica. Il risultato nella maggior parte dei casi consiste nella produzione di proteine tronche[9,10].

In letteratura è riportato che EXT1 e EXT2 sono coinvolti nella maggioranza dei casi di HME[9]. Essi sono stati identificati come geni oncosoppressori e la inattivazione di entrambe le coppie alleliche (*loss of heterozygosity*) è associata con la trasformazione maligna che avviene in circa il 5% dei casi[3, 11-13]. Tra questi, nel 90% dei casi l'evoluzione maligna avviene verso la formazione di condrosarcomi all'interno della capsula cartilaginea; nei restanti casi l'evoluzione avviene verso altre forme sarcomatose[14].

Patogenesi

Le proteine codificate dai geni EXT, denominate esostosine, sono correlate con la biosintesi dell'eparansolfato (HS)[15], molecola fondamentale nella regolazione del processo di maturazione dei condrociti.

Ossificazione encondrale

Durante lo sviluppo embrionale lo scheletro assiale ed appendicolare e la maggior parte delle ossa facciali vanno incontro ad un processo a più fasi chiamato ossificazione encondrale. Questo processo comincia con la condensazione delle cellule mesenchimali che portano allo sviluppo di due tipi di cellule: i condrociti, che formano gli elementi della cartilagine, e le cellule pericondriali che circondano la cartilagine formata. Il processo di ossificazione inizia dalla porzione centrale dell'osso ed i condrociti vanno incontro ad una serie di passaggi maturativi: da condrociti proliferanti si differenziano in cellule preipertrofiche, poi ipertrofiche ed infine cellule ipertrofiche terminali, che in ultimo sono sostituite da osso e midollo osseo[16].

Numerose molecole, tra cui l'*Indian Hedgehog* (Ihh), il *Parathyroid hormone-related hormone* (Pthlh) ed i *Fibroblast growth factors* (FGFs), interagiscono in una complessa rete di segnali per regolare gli specifici passaggi del processo differenziativo suddetto.

Indian Hedgehog (Ihh) e Parathyroid hormone-related hormone (Pthlh)

L'inizio della differenziazione ipertrofica è controllata da un *feedback* negativo tra l'Ihh e il Pthlh[16]:

– l'Ihh, che è espresso dai condrociti preipertrofici, attiva l'espressione del Pthlh nella regione periarticolare;
– il Pthlh, a sua volta, inibisce la differenziazione dei condrociti da preipertrofici ad ipertrofici (fig. 1).

I proteoglicani eparansolfati (HSPGs) sono a loro volta implicati nella diffusione e nel legame recettoriale di alcune proteine appartenenti alle famiglie dei FGFs e proteine Ihh: la presenza degli HSPGs offrirebbe una maggiore resistenza alla diffusione di questi fattori di crescita attraverso la matrice extracellulare[15].

EXT e sintesi di eparansolfato (heparan sulphate = HS)

Gli HSPGs sono costituiti da una proteina centrale della famiglia dei glicani o dei sindecani, legata a lunghe catene di HS. Le catene di HS sono sintetizzate a partire da un tetrasaccaride e l'allungamento della catena è catalizzato da un complesso eteromerico di glicosiltransferasi, quali le esostosine EXT1 ed EXT2, che aggiungono in modo alternato unità di N-acetilglucosamina e acido glucuronico. Successivi passaggi di deacetilazione, sulfatazione ed epimerizzazione risultano in un ampio spettro di differenti catene di HS. È stato dimostrato che proprio la sulfatazione delle catene di HS è fondamentale per il legame con i fattori di crescita suddetti[17].

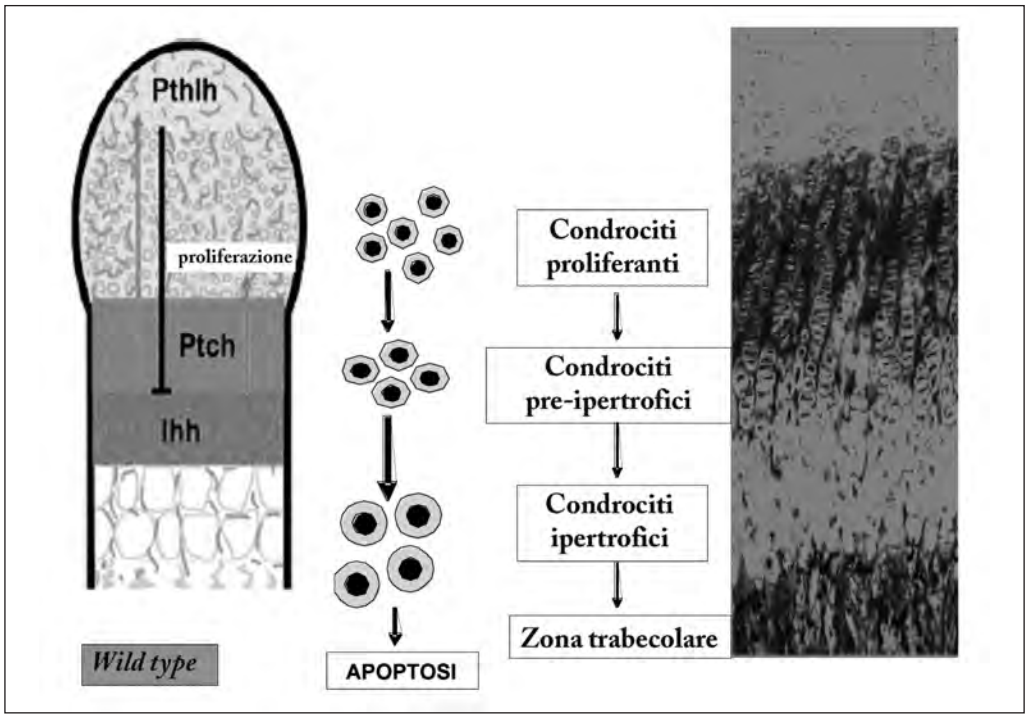

Fig. 1. Il segnale di Ihh è espresso dai condrociti ed attiva l'espressione di Pthlh mediante un *feedback* negativo, regolando la differenziazione dei condrociti

EXT 1 ed EXT 2 nella regolazione della proliferazione encondrale

I geni EXT sono fondamentali nella regolazione dei segnali di proliferazione e differenziazione dei condrociti.

Numerose analisi genetiche supportano il ruolo di EXT[18] nella regolazione della diffusione delle proteine *Hedgehog*: nel topo, la delezione selettiva di EXT1 conduce ad una completa assenza della sintesi di HS e del mesoderma e pertanto non è compatibile con la sopravvivenza postnatale. Nella *Drosophila* invece la perdita di *tout velu* (ttv), omologo di EXT1, porta all'inibizione della diffusione di *Hedgehog*, facendo ipotizzare che gli HS prodotti da ttv siano necessari per il trasporto del segnale di Ihh. È stata dunque ipotizzata una correlazione tra EXT1 e la propagazione del segnale di Ihh per spiegare le anomalie ossee osservate nell'HME. Per dimostrare tale correlazione è stata creata una linea di topi portatori di un allele ipomorfico di EXT1 ($Ext1^{Gt/Gt}$). Ne è risultata non una totale assenza, ma solo una riduzione significativa dei livelli di HS.

In contrasto rispetto a quanto osservato negli studi sulla *Drosophila*, nei mutanti $Ext1^{Gt/Gt}$ è stata riscontrata una diminuzione dell'espressione di Ihh, a seguito della riduzione della sintesi di HS. Questi stessi topi hanno mostrato un ritardo nella differenziazione ipertrofica dei condrociti. Si potrebbe pensare quindi che la riduzione dell'espressione di Ihh acceleri l'inizio della differenziazione ipertrofica, ma in realtà quello che si osserva è un ritardo della differenziazione[18].

Pthlh è l'effettiva molecola a valle del segnale di Ihh nel regolare l'inizio della differenziazione ipertrofica. Sorprendentemente, a tutti gli stadi analizzati, l'espressione di Pthlh è *upregolata*: questo potrebbe far supporre l'intervento di altri fattori di crescita nel regolare l'espressione di Pthlh indipendentemente da Ihh. In alternativa il segnale di Ihh potrebbe essere aumentato dal ridotto numero di HS, condizione dunque che ne favorirebbe la diffusione.

Riassumendo, l'inespressione di EXT determina una riduzione della sintesi di HS; tale condizione favorirebbe la distribuzione del segnale di Ihh e l'aumento del segnale di Ihh sarebbe responsabile del ritardo della differenziazione ipertrofica.

Quantità ridotte di HS determinano un aumentato *range* del segnale di Ihh; di conseguenza aumentate concentrazione di HS potrebbero restringere il segnale di Ihh ed accelerare l'inizio della differenziazione. Sembra pertanto che HS regoli il segnale di Ihh restringendo la distribuzione piuttosto che inibendo la recezione del segnale (figg. 2, 3)[19].

EXT e Ihh nell'HME

Nell'HME l'inespressione di EXT e la conseguente amplificazione del segnale di Ihh potrebbero allo stesso modo essere responsabili dell'*overproliferazione* di *clusters* di condrociti ed i gruppi di cellule proliferanti in stretto contatto con il pericondrio potrebbero essere in grado di sfuggire alla regolazione della differenziazione all'interno della placca di accrescimento ed erompere attraverso il pericondrio inducendo lo sviluppo dell'esostosi[20].

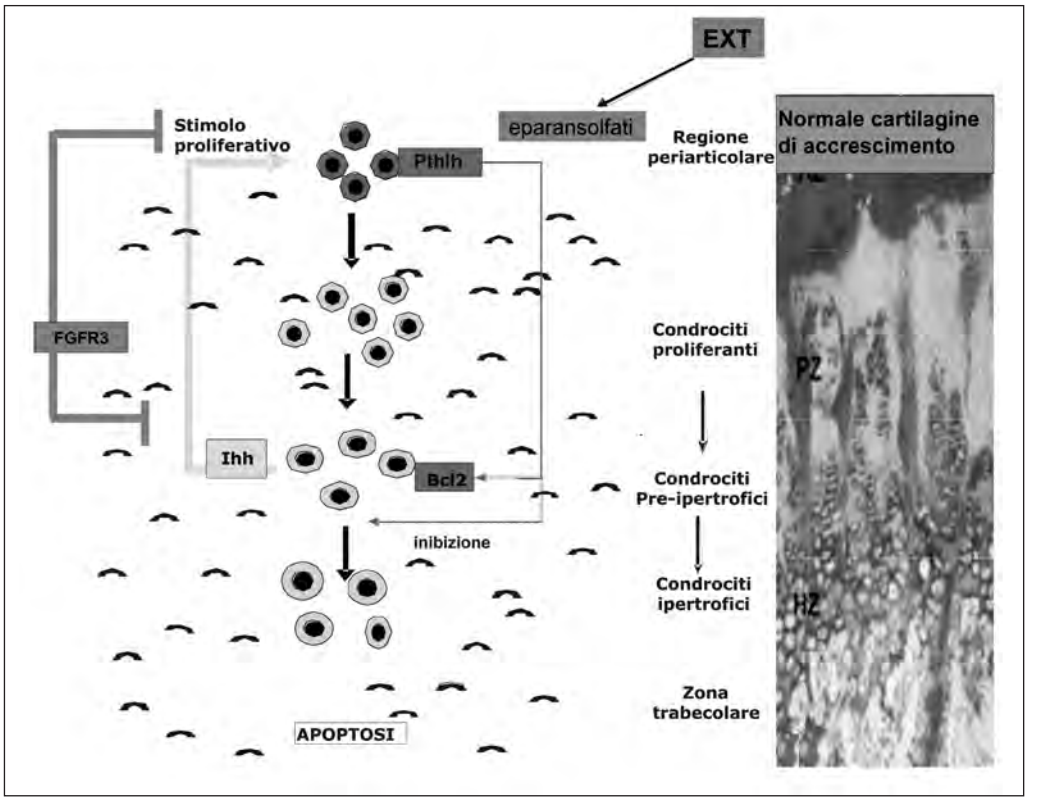

Fig. 2. Normale processo differenziativo dei condrociti

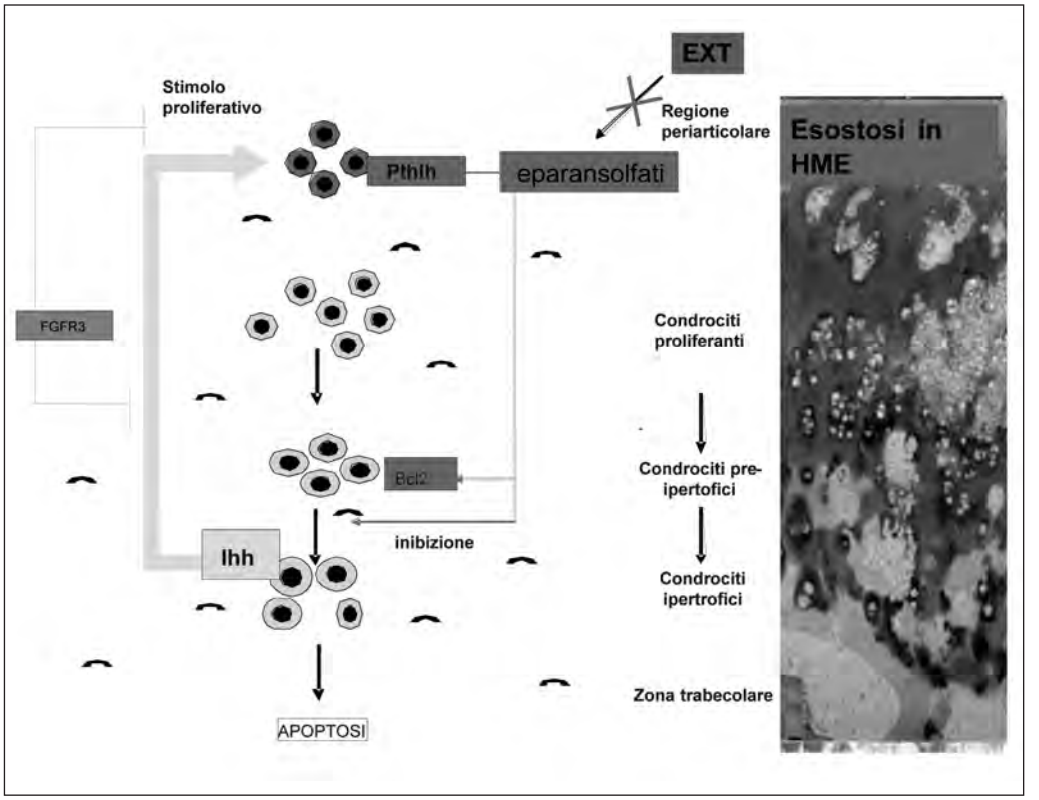

Fig. 3. Mutazioni in EXT alterano il segnale di Ihh e conducono alla formazione delle esostosi

Ruolo del FGF nella proliferazione e differenziazione dei condrociti

Gli FGFs appartengono ad una famiglia di 12 geni codificanti essenzialmente per proteine strutturali. Gli FGFs ed i loro recettori (*fibroblast growth factor receptor* = FGFR) regolano lo sviluppo dell'ossificazione encondrale e la loro espressione varia a secondo dello stadio maturativo dei condrociti[21].

FGFR3 è espresso nella zona proliferativa dell'osso

suggerendo un ruolo diretto di FGFR3 nella regolazione della proliferazione dei condrociti[22]; al contrario l'espressione di FGFR1 nei condrociti ipertrofici suggerisce la possibile implicazione di FGFR1 nel determinare la sopravvivenza dei condrociti ipertrofici, nella regolazione del *feedback* che ne controlla la differenziazione o nel segnale che ne indurrebbe l'apoptosi[22-24].

Diversi sono gli FGFs prodotti durante lo sviluppo dell'osso encondrale. L'analisi di mutazioni a carico di FGFR3 con perdita di funzione della proteina recettoriale ha mostrato come la diretta conseguenza del segnale trasmesso attraverso tale recettore sia l'inibizione della proliferazione e differenziazione dei condrociti[21, 25]. Questo effetto è mediato in parte direttamente sui condrociti[26], in parte indirettamente attraverso la via di segnale di Ihh e Pthlh[27, 28].

La correlazione tra la via di segnale del FGF e la presenza di HS è stata oggetto di numerosi studi[29].

EXT e FGF nell'HME

È stato ipotizzato che le mutazioni a carico di EXT1 ed EXT2, riducendo la sintesi di HS possano indurre una diminuzione del segnale di FGF; questa condizione potrebbe contribuire nel determinare l'abnorme proliferazione dei condrociti in corrispondenza dei siti di formazione delle esostosi.

Caratteristiche cliniche

Gli osteocondromi solitari vengono di solito diagnosticati nella tarda adolescenza e nella prima età adulta, mentre le forme multiple si manifestano durante la fanciullezza: in una percentuale di casi compresa tra il 65% ed il 90%[30] la sintomatologia inizia a presentarsi prima dei sei anni d'età. Le prime lesioni solitamente compaiono in corrispondenza della tibia e della scapola, probabilmente perché sono le regioni maggiormente sviluppate nel bambino[5].

Le esostosi continuano a crescere fino alla chiusura delle cartilagini di accrescimento; non se ne formano di nuove dopo la pubertà, sebbene siano possibili recidive dopo asportazione chirurgica[31-33].

Distribuzione delle esostosi

Le esostosi originano di solito in corrispondenza della metafisi, a livello della placca di accrescimento delle ossa lunghe. La diagnosi radiografica permette la localizzazione esatta delle lesioni. Le lesioni sono spesso patognomoniche dal punto di vista radiologico.

HME: aspetti clinici e psicologici

Diverse sono le caratteristiche cliniche dell'HME:
- il 75% dei pazienti mostra almeno una lesione clinicamente evidente;
- il 50% dimostra deformità degli arti;
- il 45% presenta deformità a carico della caviglia;
- il 20% presenta deformità a carico del ginocchio;
- il 40% degli affetti mostra una bassa statura.

Dal punto di vista clinico gli osteocondromi si presentano come masse in lento accrescimento e possono causare un'importante sintomatologia dolorosa se vanno a comprimere un nervo o se il peduncolo va incontro a frattura. Nell'HME l'osso sottostante può essere arcuato e risultare quindi accorciato, riflettendo un difetto dell'accrescimento epifisario. Le esostosi possono produrre deformità come curvatura dell'avambraccio, limitazione della prono-supinazione, tibia valga, inclinazione dalla metafisi distale della tibia con accorciamento del perone relativo. La sintomatologia conseguentemente risulterà variabile a seconda del numero e della sede delle lesioni. E' evidente, oltremodo, come tutto ciò possa assumere particolare importanza in un bambino in accrescimento: nei casi più gravi può essere necessaria l'assunzione di una terapia antidolorifica anche quotidiana. La limitazione dei movimenti in un bambino può significare una grave frattura nel suo relazionarsi ai compagni, con conseguente tendenza all'isolamento; ne può derivare un'importante compromissione del gioco e di altre attività ricreative, con conseguente ritardo della crescita relazionale del bambino. La sua malattia può dunque essere motivo di stress psicologico oltre che fisico. Il dolore notturno può portare a deprivazione di sonno, con conseguente facile stancabilità diurna e diminuzione del rendimento. Le deformità e la bassa statura possono essere motivo di derisione.

I bambini affetti sono spesso costretti ad utilizzare sostegni, a sopportare gessature anche per lunghi periodi e sono spesso sottoposti a numerosi interventi chirurgici, i quali, oltre a costituire essi stessi un importante fattore di stress, sono spesso causa di riduzioni staturali; gli stessi interventi possono essere ancora complicati da lesioni a carico di nervi, e ne possono residuare invalidità che vanno dal piede cadente alla monoplegia, o lesioni a carico di arterie.

Trasformazione maligna

Tra le complicanze della malattia la più temibile è senz'altro la trasformazione in condrosarcoma per degenerazione neoplastica della componente cartilaginea. La trasformazione maligna è stata correlata con la inattiva-

zione di entrambe le copie alleliche (*loss of heterozygosity*) in EXT1 ed EXT2 e si verifica in circa il 5% dei casi[12, 13].

Il picco di incidenza si verifica tra la seconda e la terza decade nei soggetti affetti da HME. Approssimativamente circa il 15% di tutti i condrosarcomi insorgono nelle regioni periferiche dello scheletro per trasformazione di precursori benigni, nel restante 85% dei casi crescono *ex novo* e generalmente nelle porzioni centrali dello scheletro, incluse pelvi, spalle e coste. In presenza di tumori di basso grado raramente si riscontrano metastasi a distanza alla diagnosi, mentre il 70% dei tumori di alto grado viene diagnosticato quando la malattia è già metastatica. Quando vanno incontro a metastatizzazione, diffondono preferenzialmente ai polmoni e allo scheletro. Un importante fattore prognostico è rappresentato dalle dimensioni della lesione: tumori maggiori di dieci centimetri hanno un comportamento più aggressivo.

Il tasso di sopravvivenza a cinque anni dei pazienti con condrosarcoma di grado 1, 2, 3 è stimato intorno al 90%, 81% e 43% rispettivamente.

Caratteristiche radiografiche che devono far sospettare la trasformazione maligna sono: la crescita di una lesione che sembrava stabilizzata, la presenza di una superficie irregolare, il riscontro di zone di mineralizzazione all'interno della lesione o di fenomeni di erosione e distruzione dell'osso adiacente[12, 34, 35].

Terapia

La terapia chirurgica è riservata oggi più alla correzione delle deformità che all'escissione delle esostosi ed è associata ad un rischio di recidiva pari al 2%[36].

È importante effettuare una resezione completa del pericondrio per diminuire tale rischio di recidiva. La resezione chirurgica, alla quale ricorre comunque il 13% dei pazienti affetti da HME, non è priva di complicanze[37] quali le neuropatie, le lacerazioni di arterie, sindromi compartimentali e fratture.

Riguardo alla terapia medica, preponderante è l'uso di analgesici. Il controllo della sintomatologia dolorosa nei bambini affetti deve essere un *target* sempre presente.

Ciclopamina: un farmaco per il futuro?

La ciclopamina è una sostanza alcaloide estratta dal fiore di giglio selvatico. Nel 2002 sono state dimostrate *in vivo* su modelli animali le sue potenzialità antitumorali. In particolare, la sua azione è stata correlata all'inibizione del segnale di Ihh, il quale risulta iperespresso in numerose neoplasie. In più studi è stato dimostrato come linee cellulari di adenoma e carcinoma del colon retto, tumori nei quali risulta amplificata la via di segnale di Ihh, abbiano risposto al trattamento con ciclopamina. In altri tumori del tratto gastrointestinale, in particolare del pancreas, ma anche in alcuni tumori cerebrali, soprattutto medulloblastoma, la proliferazione cellulare maligna è stata correlata con l'aumento dell'espressione del segnale di Ihh. La ciclopamina potrebbe essere dunque considerata come un potenziale agente terapeutico *mechanism-based* per il trattamento di questi tumori. Le dimostrazioni *in vivo* ed *in vitro* sull'efficacia di tale sostanza sono ad oggi considerevoli, tali da legittimare l'investimento nella creazione di una molecola dalle caratteristiche simili[38].

Anche nell'esostosi multipla ereditaria, come precedentemente spiegato, il segnale di Ihh è alla base dell'abnorme proliferazione condrocitaria responsabile della formazione delle esostosi. L'acquisizione di conoscenze di genetica e di patogenesi molecolare deve essere incoraggiata perché da esse potranno partire nuove possibilità terapeutiche.

Conclusioni

La malattia da HME rappresenta una patologia neoplastica di frequenza elevata soprattutto in età pediatrica.

Tradizionalmente descritta come displasia scheletrica, la HME è stata solo recentemente caratterizzata negli aspetti genetici e molecolari responsabili dello sviluppo delle lesioni ossee, delle complicanze e della potenziale trasformazione neoplastica.

L'acquisizione dei meccanismi coinvolti nella regolazione del processo di maturazione dei condrociti renderà possibile l'applicazione di nuovi e specifici approcci terapeutici migliorando le possibilità di cura dell'HME, attualmente basata solo su interventi sintomatici.

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Impatto delle cure palliative sull'assistenza dei pazienti oncologici in fase terminale in provincia di Ferrara nel 2002

The effect of palliative care on the assistance of terminally ill cancer patients in the Province of Ferrara in 2002

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Riassunto

***Finalità*. Il presente studio si propone di valutare in termini di efficienza l'impatto dell'Assistenza Domiciliare Integrata (ADI) nei confronti del tradizionale ricorso all'Assistenza Ospedaliera. *Materiali e metodi*. Sull'intera coorte di pazienti deceduti per neoplasia maligna in provincia di Ferrara nell'anno 2002 (1.460 casi) è stata condotta un'analisi sulle differenze di assistenza ospedaliera e di luogo del decesso tra pazienti seguiti e non con ADI. Sono stati analizzati i tassi di ospedalizzazione (ricoveri e giornate di degenza) degli ultimi 90 giorni di vita, insieme alla probabilità di decesso intra- rispetto ad extra-ospedaliero. Sesso, età, residenza e raggruppamento prognostico dei tumori sono stati assunti come possibili modificatori di effetto o confondenti. *Risultati*. I pazienti seguiti con ADI attivata prima dell'ultimo trimestre di vita hanno presentato tassi di ricovero inferiori a quelli con assistenza tradizionale (IRR globale 0,78; IC 95% 0,67-0,91), assieme ad una netta diminuzione delle giornate di degenza, progressivamente più consistente all'aumentare della durata del programma (IRR globale 0,58; IC 95% 0,55-0,60) con modificazione di effetto da parte della prognosi, età, genere e residenza. Gli stessi pazienti seguiti con ADI hanno mostrato una netta ten-**

Summary

***Aim*. The present study compares the efficacy of an home care programme with that of conventional hospital care. *Materials and methods*. An analysis was carried out on the whole cohort of patients who died of cancer in the Province of Ferrara in 2002 (1460 cases), evalutating the differences in hospital admittance and place of death, between patients who had or had not been followed in a home-care regimen. The rates and the length of hospital admittance during the last 90 days of life, and the probability of death inside or outside the hospital, were analysed. Sex, age, place of residence and tumour groups according to prognosis were considered for possible interactions or as confounding factors. *Results*. The patients followed in an home-care programme activated before the last 3 months of life had a lower hospitalization rate than those followed in a conventional way (overall IRR 0.78; IC 95% 0.67-0.91), together with a significant reduction of the hospitalization period, proportional to the duration of the home-care programme (overall IRR 0.58; IC 95% 0.55-0.60), with interactions due to prognosis, age, gender and residence. Patients in home-care showed a clear trend for a death outside the hospital, increasingly so if the programme was**

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denza al decesso in sede extraospedaliera, maggiore per le assistenze attivate tardivamente (OR 0,13; IC 95% 0,07-0,23 nell'ultima settimana *versus* OR 0,48; IC 95% 0,36-0,58 da più di tre mesi). *Conclusioni.* L'ADI, protratta per tempi sufficientemente lunghi, sembra influenzare, con un minor ricorso al ricovero ospedaliero, il percorso assistenziale terminale, mentre i programmi attivati a ridosso del decesso sembrano consentire l'alternativa della morte al di fuori dell'ospedale. Eur. J. Oncol., 11 (1), 33-39, 2006

Parole chiave: **cure palliative, assistenza domiciliare, assistenza ospedaliera**

activated in the late period (OR 0.13; IC 95% 0.07-0.23 in the last week *versus* OR 0.48; IC 95% 0.36-0.58 > 90 days). *Conclusion*. The home-care programme, if protracted for a fairly long period, seems to influence the terminal phase in terms of assistance, with a lesser need of hospital stay, while the late activation of home care seems to allow death outside the hospital. Eur. J. Oncol., 11 (1), 33-39, 2006

Key words: **palliative care, home care, hospital care**

Introduzione

La letteratura riguardante la valutazione delle cure palliative è caratterizzata da notevole eterogeneità tra le casistiche esaminate, relativa difformità dei criteri di valutazione e da ampia variabilità dei parametri studiati. Tutto ciò riflette notevoli differenze nei risultati ottenuti; tuttavia è possibile cogliere un comune denominatore rappresentato dalla preferenza dei pazienti per l'assistenza domiciliare: morire nella propria abitazione in un contesto familiare rappresenta di per sé una cura palliativa in grado di rassicurare il paziente e di restituirgli dignità.

Infatti questa preferenza per la gestione domestica, nonostante la scarsa autosufficienza dei pazienti, compare in diversi studi[1-3] con maggiore razionalizzazione di utilizzo delle risorse finanziarie destinate all'assistenza domiciliare[4,5].

L'analisi dei dati ottenuti dall'Azienda USL di Ferrara mette in evidenza una elevata percentuale di pazienti deceduti per neoplasia non inseriti in un programma di assistenza domiciliare (>60%) e questo rispecchia l'andamento nazionale in un contesto di estrema variabilità del fenomeno (40-90%), influenzato dalle esperienze che garantiscono un forte supporto familiare ed un'assistenza continuativa di buona qualità[6,7].

Alle considerazioni di tipo assistenziale deve essere affiancata una valutazione comparativa dei costi rispetto alla tradizionale assistenza di tipo ospedaliero dei pazienti neoplastici terminali: si è documentata di recente anche in Italia la tendenza alla riduzione del numero di giorni di ospedalizzazione per i pazienti con assistenza domiciliare attivata (>40%)[8].

In provincia di Ferrara l'assistenza domiciliare oncologica ha avuto inizio nel 1989 come servizio rivolto a pazienti in fase terminale, disagiati nell'accedere alla struttura ospedaliera e richiedenti terapia antalgica e di supporto. Il Servizio di Assistenza Domiciliare (Ospedale S. Anna) si avvaleva di consulenze specialistiche periodiche e vere e proprie forme di ospedalizzazione domiciliare, integrate eventualmente da ricoveri ospedalieri ordinari.

Nel marzo 2002 è stata attivata anche a Ferrara, nell'ambito del Sistema Sanitario Regionale (SSR), l'Assistenza Domiciliare Integrata (ADI), in attuazione della Deliberazione Regionale n. 124 dell'8 febbraio 1999 e della Legge Regionale 29/94, il cui obiettivo principale era la riorganizzazione su base distrettuale dell'assistenza domiciliare integrata, attraverso:

1) la valutazione multidimensionale dello stato funzionale del paziente;
2) la predisposizione di un piano personalizzato di assistenza;
3) l'erogazione di assistenza da parte di una *équipe* multiprofessionale, coordinata dal Medico di Medicina Generale (MMG), il quale resta responsabile terapeutico del caso, e/o da medici di associazioni di volontariato territoriali in convenzione con la Azienda USL di Ferrara.

Nel caso di pazienti affetti da patologie neoplastiche terminali, era previsto il livello assistenziale ADI3, che comportava più accessi alla settimana di tipo medico, infermieristico e di altre figure professionali (consulenza specialistica oncologica), garantendo la pronta disponibilità diurna medica e infermieristica. L' ADI3 veniva attivata su richiesta del MMG, per il quale erano previsti rimborsi da parte del SSR.

Parallelamente è stato attivato un *hospice* territoriale del distretto di Ferrara (12 posti letto). Questo modello rispecchia il nuovo programma di *"rete di cure palliative oncologiche"*, il cui scopo è di garantire il miglioramento della qualità di vita del malato e della propria famiglia, e la programmazione di ulteriori servizi e strutture di riferimento. Nell'ambito di tale rete l'assistenza domicilia-

re, l'*hospice* e la struttura ospedaliera sono in continua interrelazione tra di loro.

Il confronto tra i dati di mortalità per tumore nell'anno 2002 in provincia di Ferrara con i dati dei pazienti assistiti a domicilio nel biennio 1999-2001 mostra una notevole quota di pazienti non raggiunti da questo tipo di assistenza, per motivi che necessitano certamente di un approfondimento. Il presente studio si propone di affrontare, sia pur parzialmente, questa problematica attraverso l'analisi dell'impatto che l'ADI esercita sulla tradizionale assistenza ospedaliera per i pazienti oncologici terminali residenti in provincia di Ferrara e deceduti nel corso dell'anno 2002, con l' obiettivo di valutare le differenze di ricorso al ricovero ospedaliero tra i pazienti inseriti in ADI e quelli che percorrono i convenzionali *iter* assistenziali, ed infine di analizzare la sede del decesso.

Materiali e metodi

Sono stati inclusi nello studio tutti i pazienti, residenti in provincia di Ferrara, deceduti nell'anno 2002 per neoplasia maligna. Si trattava di una coorte di 1.460 pazienti di cui si è ricostruito l'*iter* assistenziale attraverso un *linkage* tra le schede di dimissione ospedaliera (SDO) e i dati relativi all'assistenza domiciliare, inclusi la data di inizio, la durata e il tipo di assistenza erogata (domiciliare, *hospice*, combinata). È stato considerato il *periodo terminale* del paziente, cioè il percorso seguito negli ultimi 90 giorni di vita. Tra i fattori determinanti sono stati inclusi la *durata di attivazione dell'ADI* (<7, 8-30, 31-90, >90 giorni), ed il *tipo di assistenza* erogata (domiciliare, *hospice*, combinata). Come possibili modificatori di effetto, o in alternativa come confondenti, sono stati considerati il sesso (M/F), l'età (<70, 71-80, >80 anni), la residenza suddivisa in area Est (comprendente i distretti di Copparo, Portomaggiore e Codigoro) e area Ovest (comprendente i distretti di Cento e Ferrara)[9], le diverse sedi neoplastiche sulla base dell'aspettativa di vita complessiva dalla diagnosi al decesso, desunta dai dati della letteratura[10], nell'ipotesi di una relazione tra la durata della storia clinica e la strategia dei percorsi assistenziali. Questi ultimi criteri hanno individuato neoplasie (per sede anatomica) con mediana di sopravvivenza (OS) < 1 anno (neoplasie dell'esofago, stomaco, fegato, polmone, pleura, SNC); fra 1 e 5 anni (neoplasie testa-collo, ovaio, mieloma); oltre i 5 anni (neoplasie colon-retto, mammella, prostata, utero, rene, vescica, ecc.).

Per l'elaborazione dei dati sono stati utilizzati i programmi SPSS 8.0 e Stata 8.0. Sono stati calcolati i tassi grezzi di incidenza dei ricoveri e le giornate di degenza (IR=eventi/gg.) ed il loro rapporto (*incidence rate ratio* = IRR) nonché l'"*odds ratio*" (OR) tra probabilità di decesso in ambito ospedaliero o a domicilio, in funzione delle due tipologie di assistenza (assistenza domiciliare ed ospedaliera). L'analisi dei "modificatori di effetto" e l'aggiustamento per i "confondenti" è stata effettuata mediante un modello di regressione di Poisson (IRR dell'ospedalizzazione) ed un modello di regressione logistica (OR della probabilità di morte in ospedale), con intervalli di confidenza sempre riferiti al 95%.

Risultati

In provincia di Ferrara nell'anno 2002 sono deceduti 1.460 pazienti per neoplasia maligna: di questi, 514 hanno usufruito di un regime di ADI. Complessivamente sono stati effettuati, negli ultimi 90 giorni di vita, 2.254 ricoveri ospedalieri, per un totale di 28.554 giornate di degenza (Tabella 1).

Dall'analisi dei dati di ospedalizzazione sulla base della presenza o assenza di ADI e della sua durata, aggiustati per i confondenti, emerge che, quando è attivata un'assistenza domiciliare per un periodo superiore all'ultimo trimestre di vita, si realizza una reale riduzione del numero di ricoveri (-22%) rispetto ai pazienti seguiti in modo convenzionale (Tabella 2). Tale fenomeno è altrettanto evidente sia per i ricoveri ordinari che per quelli in *Day Hospital*.

Tabella 1 - Casistica globale in studio

	Maschi	Femmine	Totali
Decessi osservati	892	568	1.460
Assistenza convenzionale	589	357	946
ADI ≤ 7 gg	39	10	49
ADI 8-30 gg	76	65	141
ADI 31-90 gg	95	72	167
ADI > 90 gg	93	64	157
Età ≤ 64 anni	188	106	294
Età > 64 anni	704	462	1.166
Residenti area Ovest	519	347	866
Residenti area Est	373	221	594
Ricoveri totali	1.447	807	2.254
Ricoveri totali ordinari	1.188	695	1.883
Ricoveri *day hospital*	259	112	371
Giornate di degenza totali	17.365	11.189	28.554
Giornate di degenza ordinaria	16.260	10.608	26.940
Giornate di degenza *day hospital*	1.105	509	1.614

Tabella 2 - Ricoveri in rapporto alla durata dell'assistenza domiciliare, regressione di Poisson

	ADI – *Incidence Rate Ratio* (IC 95%)[a, b]				
	Nessuna	≤ 7 gg	8-30 gg	31-90 gg	> 90 gg
Ricoveri totali	1,00	1,19 (0,97-1,46)	0,99 (0,86-1,14)	0,96 (0,84-1,09)	**0,78** (0,67-0,91)
Ricoveri ordinari	1,00	1,12 (0,88-1,41)	0,95 (0,81-1,11)	0,93 (0,81-1,08)	**0,79** (0,67-0,94)
Ricoveri *day hospital*	1,00	1,54 (0,97-2,43)	1,27 (0,93-1,74)	1,07 (0,79-1,46)	0,73 (0,48-1,10)

[a] IRR aggiustati per genere ed età
[b] **In neretto:** IRR significativi

Tabella 3 - Giornate di degenza in rapporto alla durata dell'assistenza domiciliare

	ADI – *Incidence Rate Ratio* (IC 95%)[a, b]				
	Nessuna	≤ 7 gg	8-30 gg	31-90 gg	> 90 gg
Totale	1,00	1,03 (0,97-1,09)	**0,78** (0,75-0,82)	**0,73** (0,70-0,76)	**0,58** (0,55-0,60)
Maschi	1,00	1,03 (0,96-1,10)	**0,77** (0,72-0,81)	**0,74** (0,70-0,78)	**0,68** (0,65-0,72)
Femmine	**1,10** (1,07-1,13)	**1,16** (1,02-1,32)	**0,86** (0,81-0,92)	**0,78** (0,73-0,82)	**0,50** (0,43-0,51)
Età					
≤ 70 anni	1,00	**1,29** (1,17-1,41)	**0,89** (0,84-0,95)	**0,68** (0,64-0,72)	**0,60** (0,55-0,65)
71-80 anni	0,98 (0,94-1,01)	**0,84** (0,76-0,93)	**0,68** (0,63-0,73)	**0,77** (0,72-0,83)	**0,62** (0,57-0,66)
> 80 anni	**0,79** (0,76-0,81)	**0,77** (0,67-0,90)	**0,54** (0,49-0,77)	**0,59** (0,54-0,65)	**0,37** (0,34-0,41)
Residenza ovest	1,00	1,03 (0,96-1,11)	**0,74** (0,70-0,78)	**0,62** (0,59-0,66)	**0,76** (0,73-0,78)
Residenza est	**1,15** (1,12-1,18)	**1,13** (1,01-1,27)	0,96 (0,90-1,02)	0,97 (0,92-1,03)	**0,77** (0,72-0,82)
OS < 1 anno	1,00 (1,07-1,13)	0,96 (0,98-1,04)	**0,78** (0,73-0,83)	**0,82** (0,77-0,86)	**0,45** (0,41-0,49)
OS 1-5 anni	0,99 (0,93-1,05)	1,10 (0,83-1,46)	**0,80** (0,66-0,97)	**0,84** (0,73-0,97)	**0,82** (0,71-0,95)
OS > 5 anni	**0,96** (0,93-0,99)	**1,18** (1,06-1,32)	**0,85** (0,80-0,90)	**0,67** (0,62-0,71)	**0,73** (0,69-0,77)
Sede maldefinita	0,99 (0,94-1,04)	0,83 (0,67-1,03)	**0,39** (0,33-0,47)	**0,24** (0,19-0,30)	**0,07** (0,05-0,10)

[a] IRR aggiustati per le variabili non considerate come modificatori
[b] **In neretto** IRR significativi

Per i pazienti affetti da neoplasia a prognosi più infausta, l'attivazione assistenziale ADI si traduce in un notevole risparmio dei ricoveri ospedalieri anche quando vengano seguiti per almeno una settimana. Nello specifico (Tabella 3), si è osservata una riduzione delle giornate totali di degenza con un IRR quasi prossimo al 50% per i

Tabella 4 - Ricoveri in rapporto alla tipologia dell'assistenza domiciliare

	ADI – *Incidence Rate Ratio* (IC 95%)[a, b]			
	Nessuna	Domiciliare	*Hospice*	Dom. + *hosp*.
Totale	1,00	**0,73** (0,72-0,76)	**0,78** (0,74-0,82)	**0,55** (0,51-0,59)
Maschi	1,00	**0,76** (0,73-0,79)	**0,88** (0,83-0,94)	**0,62** (0,56-0,67)
Femmine	**1,10** (1,07-1,14)	**0,78** (0,75-0,82)	**0,73** (0,67-0,79)	**0,51** (0,45-0,57)
Età:				
≤ 70 anni	1,00	**0,80** (0,76-0,84)	**0,83** (0,77-0,89)	**0,55** (0,50-0,61)
71-80 anni	0,97 (0,94-1,01)	**0,75** (0,73-0,79)	**0,66** (0,60-0,72)	**0,53** (0,47-0,60)
> 80 anni	**0,80** (0,76-0,81)	**0,47** (0,44-0,50)	**0,73** (0,65-0,82)	**0,45** (0,39-0,53)
Residenza est	1,00	**0,67** (0,64-0,70)	**0,76** (0,72-0,80)	**0,52** (0,48-0,56)
Residenza ovest	**1,15** (1,12-1,18)	**0,92** (0,88-0,96)	0,95 (0,84-1,09)	**0,81** (0,68-0,96)

[a] IRR aggiustati per genere ed età
[b] **In neretto** IRR significativi

pazienti seguiti da oltre 90 giorni: infatti l'aumento della durata dell'ADI comporta una diminuzione progressiva delle giornate di degenza rispetto al gruppo di controllo senza ADI.

Inoltre, l'analisi per sottogruppi mostra nei maschi una proporzione inversa tra durata ADI e numero dei giorni di degenza, anche se poi, per probabile "effetto livellamento", si ha una inversione dei dati con una maggiore riduzione dei giorni di ricovero per le femmine rispetto ai maschi. In base alla residenza dei pazienti, coloro che risiedono nell'area Est mostrano una riduzione dei ricoveri ordinari al progressivo aumento della durata dell'ADI, uguagliando la situazione dell'area Ovest per durata ADI >90 giorni. In relazione all'età dei pazienti, per tutte le classi esaminate si evidenzia una tendenza alla diminuzione delle giornate di degenza all'aumentare della durata ADI, più evidente per i pazienti con età >80 anni. In relazione alla tipologia neoplastica, si osserva una riduzione dell'IRR più evidente per pazienti con neoplasie a prognosi peggiore (OS <1 anno o tipologia non definita).

Analizzando il rischio di ospedalizzazione in rapporto alla tipologia dell'ADI (Tabella 4), si osserva una notevole riduzione delle giornate di degenza per l'assistenza combinata (*hospice*+assistenza domiciliare, -45% rispetto ai controlli). Tale fenomeno è più evidente per i pazienti con età >80 anni e per i residenti nell'area Est.

Esaminando il rapporto tra luogo del decesso e durata dell' ADI (Tabella 5) si evidenzia una maggiore tendenza al decesso in luogo extraospedaliero per i pazienti con ADI, sia osservando il fenomeno senza interazioni, sia considerando l'effetto di modificazione legato al sesso. In particolare, rispetto alla probabilità di morire in ospedale per i pazienti non seguiti da ADI, gli *Odds Ratio* più bassi si osservano nei programmi ADI instaurati a ridosso del decesso (ultimi 30 giorni), pur mantenendo un marcato e significativo effetto in tutte le classi di durata dell'ADI. Per il sesso femminile, il fenomeno appare più evidente nelle classi di ADI rispettivamente a durata più breve (<7 giorni) e più prolungata (>90 giorni), rispetto ai corrispettivi valori riferiti dai maschi. Considerando infine la correlazione del luogo di decesso con la tipologia di assistenza domiciliare, risulta evidente una correlazione del decesso extra-ospedaliero con l' assistenza in ADI (IRR ridotto del 45%), più significativa per l' assistenza in *hospice* (con o senza ADI, IRR <10%).

Discussione

Per quanto riguarda gli aspetti più strettamente legati all'analisi effettuata, è possibile osservare come all'interno della provincia di Ferrara, che mostra livelli di mortalità

Tabella 5 - Mortalità intraospedaliera in rapporto all'ADI[a, b]

	ADI – *Odds Ratio* (IC 95%)				
	Nessuna	≤ 7 gg	8-30 gg	31-90 gg	> 90 gg
Totale	1,00	**0,13** (0,07-0,23)	**0,24** (0,17-0,35)	**0,38** (0,27-0,54)	**0,48** (0,36-0,58)
Maschi	1,00	**0,15** (0,07-0,31)	**0,18** (0,10-0,30)	**0,33** (0,21-0,51)	**0,63** (0,40-0,99)
Femmine	**0,81** (0,61-1,08)	**0,05** (0,01-0,41)	**0,28** (0,17-0,48)	**0,39** (0,24-0,64)	**0,26** (0,15-0,44)

	ADI – *Incidence Rate Ratio* (IC 95%)			
	Nessuna	Domiciliare	*Hospice*	Dom + *hosp*.
Totale	1,00	**0,55** (0,42-0,72)	**0,08** (0,05-0,14)	**0,09** (0,05-0,18)

[a] OR aggiustati per le variabili non considerate come modificatori
[b] **In neretto** OR e IRR significativi

per tumore in linea con quanto precedentemente osservato in studi di confronto geografico[11], l'accesso ad un programma di ADI sia associato ad un risparmio di ricoveri ospedalieri se attivato in tempo (sufficientemente lungo) utile a produrre un significativo impatto sulle strategie assistenziali globali dei pazienti. Viceversa, il ricorso all'ADI limitato alla fase preterminale o terminale della vita, tenderebbe a indurre una sovrapposizione con i canali di assistenza ospedaliera, con un aumento di ospedalizzazioni di cui non è al momento possibile analizzare l'impatto sulla qualità globale dell'assistenza. In particolare, valutando l'impatto della durata dell'ADI sul carico delle giornate di degenza di ogni paziente, è possibile osservare un effetto costante di diminuzione dell'ospedalizzazione al progressivo aumentare della durata del programma di Assistenza Domiciliare, sia considerando il totale dei deceduti, sia i diversi sottogruppi suggeriti dal modello di analisi.

Per quanto riguarda le giornate di degenza in regime ordinario, vale la pena di sottolineare l'impatto dell'ADI sulla progressiva riduzione della degenza, particolarmente a carico delle categorie a maggior consumo tendenziale (donne e pazienti residenti nella zona orientale della provincia) per le quali, stante la preoccupazione di garantire ottimali livelli di assistenza, è possibile conseguire un maggior risparmio nei confronti di forme assistenziali (ricoveri ospedalieri) sicuramente più disagevoli anche per il paziente, oltre che più onerosi per la comunità. L'ADI comporta anche una riduzione delle giornate di degenza in regime di *Day Hospital*, progressivamente più evidente all'aumentare del periodo di assistenza a domicilio. Questo aspetto del fenomeno assume un valore ancor più importante proprio in alcune fasce di popolazione (pazienti più anziani) meno inclini ad accedere a questa forma di ospedalizzazione, per fattori legati alla probabile maggior quota di patologie concomitanti ed alla maggior difficoltà a raggiungere quotidianamente l'ospedale e perciò più orientate verso il ricovero ordinario. L'ottimizzazione dell'offerta di queste risorse, in rapporto anche a fattori socio-ambientali (realtà rurale), appare promettente per una offerta sanitaria più puntuale e mirata alle reali esigenze dei malati.

È infine assai evidente l'impatto dell'ADI in rapporto al luogo del decesso e delle ultimissime fasi della vita. A questo riguardo i dati che emergono dall'analisi suggeriscono due possibili e sovrapposte tendenze nell'ambito della casistica osservata. I regimi di ADI instaurati a stretto ridosso del decesso appaiono infatti orientati prevalentemente a consentire l'alternativa del decesso in un luogo fuori dall'ospedale, potenzialmente più consono alle esigenze del paziente e dei familiari. I regimi di assistenza domiciliare di più lunga durata appaiono invece in grado di perseguire lo stesso obiettivo finale attraverso una più incisiva ed organizzata influenza su tutto il percorso assistenziale della fase terminale della vita dei pazienti oncologici e, a questo proposito, si rendono ancor più urgenti studi mirati in grado di valutare, con maggiore sensibilità, anche parametri in grado di documentare la qualità del percorso per le esigenze dei pazienti e non solo l'impatto sui macro-parametri assistenziali.

In ogni caso è evidente la necessità di una valutazione più globale del fenomeno, includendo anche l'analisi di altri determinanti del percorso assistenziale dei pazienti (prescrizioni farmaceutiche, accesso alla medicina specialistica), in grado di illustrare più compiutamente l'impatto dei diversi modelli sull'efficacia dell'assistenza, in rapporto alle esigenze dei pazienti, e sulla sua efficienza, in rapporto alle esigenze di economicità e flessibilità dell'organizzazione sanitaria in campo oncologico.

Conclusioni

Il settore delle cure palliative, a differenza di altri settori dell'oncologia, non è stato finora oggetto di attenzione scientifica di pari grado da parte dei ricercatori, e quindi si ritiene che in futuro vi debba essere un sempre maggiore interesse per questo settore.

È certamente auspicabile, nell' ambito di programmi dipartimentali, una sempre più stretta attenzione al "governo clinico" dei pazienti oncologici in fase avanzata, attraverso l'implementazione di modelli di dimissione protetta sempre più efficienti e possibilmente estesi a tutto il territorio provinciale.

Inoltre, il problema dell'analisi costo-beneficio, generalmente complesso nell'ambito della gestione della medicina, diventa una sfida ancor più imprescindibile per l'oncologia, in considerazione dell'alta incidenza neoplastica nella provincia e della considerevole quota di popolazione anziana.

Sono altresì da considerare le possibili diversità dei profili assistenziali nell'ambito, pur geograficamente limitato, del territorio provinciale, al fine di una più efficace gestione delle politiche di assistenza e di copertura della popolazione.

Per tutto ciò occorre proseguire nel miglioramento dei flussi informativi sanitari operanti sul territorio, attraverso gli strumenti più idonei disponibili ed una loro sempre più stretta integrazione.

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Lymph node status in neoadjuvant chemo-radiotherapy and surgery for rectal cancer: prognostic implications

Implicazioni prognostiche della valutazione della componente linfonodale nel cancro del retto trattato con il protocollo neoadiuvante combinato di radiochemioterapia e chirurgia

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Summary

***Aim*. Many studies report lymph node involvement as an important prognostic factor for local recurrence of rectal cancer, even in neoadjuvant chemoradiotherapy protocols. A precise mesorectal dissection provides adequate excision of the regional lymph node pathways, resulting in good local control with low rates of local recurrence even in cases of node-positive disease. The purpose of this study is to evaluate the prognostic implications of lymph node status in rectal cancer treated by neoadjuvant chemo-radiotherapy followed by surgery. *Patients and methods*. From 1994 to 2003, 58 patients with a primary diagnosis of rectal cancer were evaluated at our department and enrolled in a single centre, not randomized study based on 5 weeks of daily sessions of radiotherapy associated with a 30-day 5-FU continuous infusion, followed by surgical resection. All patients enrolled in the study were re-evaluated three times during the first year after resection and twice in the following years. The mean follow-up was 55.25±28.06 months (mean±SD; range 5-108 months). *Results*. At histological evaluation, 65.5% of patients were classified N_0. pN+**

Riassunto

***Finalità*. Molti lavori definiscono il ruolo della componente linfonodale come un importante fattore prognostico per lo sviluppo di recidiva locale da cancro del retto, anche nei protocolli neoadiuvanti radiochemioterapici. Una precisa dissezione del mesoretto consente un'adeguata asportazione della rete linfonodale regionale; il risultato è un buon controllo locale della malattia con una ridotta incidenza di recidiva locale anche nei pazienti che presentano metastasi linfonodali regionali alla diagnosi. Lo scopo dello studio che presentiamo è valutare il valore prognostico dell'interessamento linfonodale nel cancro del retto trattato con radiochemioterapia neoadiuvante combinata alla chirurgia. *Pazienti e metodi*. Dal 1994 al 2003, 58 pazienti affetti da cancro del retto sono stati valutati presso la nostra Clinica e arruolati nel protocollo neoadiuvante, monocentrico, non randomizzato, che prevede 5 settimane di sessioni quotidiane di radioterapia associate a 30 giorni di infusione continua di 5-FU, seguite dal trattamento chirurgico. Tutti i pazienti coinvolti nello studio sono stati rivalutati nel *follow-up* tre volte nel primo anno dopo l'intervento e due ne-**

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tumours were recognized in 27.3% of the tumours of the sigmoideal-rectal junction, in 28.6% of neoplasms of the upper rectum, in 24% of tumours of the medium rectum and 26.6% of cancers of the lower rectum. Overall local recurrence rate was 13.8%, 62.5% of which were identified as pN+. There was significant difference in disease-free survival rate between cN_0 *vs* cN+ (p=0.005), pN_0 *vs* pN+ (p<0.02), intraperitoneal *vs* extraperitoneal tumour pN_0 (p=0.04) and intraperitoneal *vs* extraperitoneal tumour pN+ (p=0.05). Significant difference in overall survival rate was found between intraperitoneal *vs* extraperitoneal tumour pN_0 (p=0.05) and intraperitoneal *vs* extraperitoneal tumour pN+ (p=0.05). *Conclusion*. Lymph node status and the relationship with the peritoneum layer are two important factors that influence the prognosis of rectal cancer treated by neoadjuvant chemoradiotherapy and surgery. Eur. J. Oncol., 11 (1), 41-50, 2006

Key words: **lymph node status, rectal cancer, chemo-radiotherapy, neoadjuvant therapy**

gli anni successivi. La durata media del *follow-up* è stata di 55,25±28,06 mesi (media±DS; range 5-108 mesi). *Risultati*. All'esame istologico il 65,5% dei pazienti è risultato N_0. Le neoplasie pN+ sono state diagnosticate nel 27,3% dei tumori della giunzione sigmoido-rettale, nel 28,6% dei tumori del retto superiore, nel 24% di quelli del retto medio e nel 26,6% di quelli del retto inferiore. L'incidenza complessiva della recidiva locale è stata del 13,8%, il 62,5% della quale è stata registrata nelle neoplasie pN+. Abbiamo registrato una differenza statisticamente significativa nella sopravvivenza libera da malattia tra cN_0 *vs* cN+ (p=0,005), pN_0 *vs* pN+ (p<0,02), neoplasie intraperitoneali *vs* extraperitoneali e pN_0 (p=0,04) e neoplasie intraperitoneali *vs* extraperitoneali e pN+ (p=0,05). Per la sopravvivenza complessiva la differenza significativa è stata calcolata per neoplasie intraperitoneali *vs* extraperitoneali e pN_0 (p=0,05) e neoplasie intraperitoneali *vs* extraperitoneali e pN+ (p=0,05). *Conclusioni*. La diffusione linfonodale e la localizzazione della neoplasia rispetto al limite peritoneale sono due fattori importanti che influiscono sulla prognosi del cancro del retto sottoposto a radiochemioterapia neoadiuvante e chirurgia. Eur. J. Oncol., 11 (1), 41-50, 2006

Parole chiave: **stato linfonodale, cancro del retto, radiochemioterapia, terapia neoadiuvante**

Introduction

The main lymphatic drainage of the rectum is to nodes in the mesorectum, along lymph node chains paired to haemorroideal vessels, according to the rectum regions, and to the uppermost nodes along the inferior mesenteric arteries. Another lymph node pathway is defined as lateral lymphatic drainage, that runs along the internal iliac vessels; the lower and middle third rectum are drained by inguinal lymph nodes and internal iliac chain, respectively[1].

Many studies report lymph node involvement as the most important predictor for local recurrences and survival, even in patients submitted to neoadjuvant chemoradiation[2-4]. Other predictive factors are: tumour localization, histological response to neoadjuvant treatment, tumour regression grade, pTNM stage and the development of distant metastases[5-8].

The reported range of local recurrence rates following surgical treatment for rectal cancer varies from 20% to 45%[1,2]. The incidence of lateral lymph node metastases in patients with low and middle rectal cancer is 15-20%, in accordance with rates reported in the literature[9,10].

A total mesorectal excision (TME) provides adequate dissection of the lymph node pathways of the rectum, and the reported result is good local control with low rates of local recurrence, even in cases of node-positive disease[6,11,12].

A preoperative chemo-radiotherapy (CRT) protocol has been reported to reduce the local recurrence rate both after conventional surgery and after standard TME[3,4,13].

As recently reported in the literature, lateral pelvic lymph node dissection (LPLD) is not necessary for patients who undergo preoperative radiation therapy[14]. Although TME does not involve the removal of the lateral lymph nodes, the local recurrence rate is comparable to that after LPLD, which is not recommended as routine practice: lateral lymph node involvement is relevant only as an indicator of prognosis[15]. The aim of this study is to evaluate the prognostic implications of lymph node status in rectal cancer treated by neoadjuvant CRT followed by surgery.

Methods

Patients

From 1994 to 2003, 58 patients with a primary diagnosis of rectal cancer were studied at our department and enrolled in a neoadjuvant CRT protocol followed by surgery.

The protocol is a single centre, not randomized study based on 5 weeks of daily sessions of radiotherapy (total dose 46 Gy) associated with a 30-day 5-FU continuous infusion (300 mg/m^2 per day), followed by surgical resection.

The study population included 35 males (60.4%) and 23 females (39.6%); the median age was 59.98±10.76 years (range 25-77); 57 patients were affected by rectal adenocarcinoma and one by neuroendocrine rectal cancer. The diagnosis was achieved by clinical evaluation (digital rectal examination), proctoscopy and biopsy of the lesion and the stage was defined on the basis of abdominal-pelvic CT and endorectal ultrasonography (ERUS).

Protocol schedule

All patients were treated by a 30-day CRT, at the end of which every patient underwent clinical examination, including digital rectal examination, proctoscopy and abdominal-pelvic CT, to define the clinical response to CRT.

Surgical resection was performed in all patients three weeks after the end of CRT.

Pathology

Histological analysis was performed on all resected specimens. Lymph nodes were dissected and embedded according to the TNM lymph node groups. The minimum requirement for tumour diagnosis was the presence of vital tumour cells or cell groups. The histological sections were reviewed by two pathologists (CA, DI) and the regression grade was quantified. Regression grading was performed according to the grading system for tumour regression[7], as follows: grade 0: no regression; grade 1: dominant tumour mass with obvious fibrosis and/or vasculopathy; grade 2: dominantly fibrotic changes with few tumour cells or groups (easy to find); grade 3: very few (difficult to find microscopically) tumour cells in fibrotic tissue with or without mucous substance; grade 4: no tumour cells, only fibrotic mass (total regression or response).

The evaluation of lateral spreading was performed as follows: gross rectal specimens, once fixed and ink marked on the resection margin, were transversely sliced at 3-4 mm intervals to select blocks from areas with suspect, clearly neoplastic or fibrotic appearance, in the deepest part, macroscopically closest to the margins. The thickness of the neoplastic area from luminal surface and the distance of the deepest infiltration point from the inked margin were measured on a histological section.

All patients enrolled in the study were followed up three times during the first year after resection and twice in the following years. The follow-up consisted of clinical examination, blood tests, serologic liver function test, αFP and CEA serum levels and CT and/or US examination of the abdomen and pelvis. Mean follow-up was 55.25±28.06 months (range 5-108 months).

Statistical analysis

Data are expressed as mean ± standard deviation; survival was calculated using the Kaplan-Meier method; the difference was estimated by Log-rank test. Multivariate analysis MANOVA was applied to assess independent variables. Significant difference was defined as $p< 0.05$. Data processing was performed using Statistica for Windows®.

Results

The tumour was localized in the sigmoideal-rectal junction in 18.9% of patients, in the upper rectum in 12.1% of cases, in the middle rectum in 43.1% and in the inferior rectum in 25.9%, therefore the intraperitoneal tumours were 31% of cases and extraperitoneal ones 69%. The surgical procedures performed were: 43 (74.1%) anterior resections, 1 (1.7%) Hartmann procedure, 9 (15.5%) abdomino-perineal resections according to Miles and 5 (8.6%) anterior resections plus stoma. The pTNM classification and the pathologic staging are reported in Table 1.

Pathologic response

Pathologic response according to multivariate analysis depends on: regression grading (p=0.006), lateral spread (p=0.04) and lymph node status (p=0.01).

Downstaging

The downstaging obtained by the CRT neoadjuvant protocol defined at histological examination, performed on all resected specimens, is reported in Table 2. pN_0 was

Table 1 - pTNM and pathologic stage

pTNM		%[a]
T	0	12.1
	1	3.5
	2	25.8
	3	50.0
	4	0.0
N	0	65.5
	1	17.2
	2	5.2
	3	3.5
M	0	84.5
	+	6.9
pStage		
0		8.6
I		24.1
II		31.1
III		20.7
IV		6.9

[a] The sum of the percentages is 91.4% because the rest (8.6%) correspond to non-RO cases (with residual disease)

Table 2 - Downstaging

Clinical stage		Pathologic stage			Localization			
					Intraperitoneal		Extraperitoneal	
			No.	%	No.	%	No.	%
N 0 (37)		N_0	31	83.8	10	27	21	56.7
		N_1	3	8.1	2	5.4	1	2.7
1 (16)		N_0	6	37.5	0	-	6	37.5
		N_1	4	37.5	1	6.25	5	31.25
		N_2	1	6.25	0	-	1	6.25
		N_3	1	6.25	1	6.25	0	-
2 (4)		N_0	1	25	0	-	1	25
		N_1	1	25	0	-	1	25
		N_2	2	50	0	-	2	50
3 (1)		N_3	1	100	1	100	0	-

obtained by 37.5% of cN_1 and 25% of cN_2, while 83.8% of cN_0 had no progression during CRT. 15.5% of overall patients recorded no progression of lymph nodal disease while 8.6% had nodal disease progression during CRT. Pathologic response to neoadjuvant treatment was shown to be the only independent variable for regression grading according to multivariate analysis (p=0.04). Independent variables for pT resulted as: distal margin (p=0.00001), grading (p=0.01), lateral spreading (p=0.0005), regression grading (p=0.0003) and pN status (p=0.01) (Table 3).

Table 3 - Multivariate analysis

Factor	Variables	p
Overall survival	Tumour site	0.003
	Distal margin	0.04
	Grading	0.04
Disease-free survival	Tumour site	0.005
	pN	0.006
	Liver metastases	0.007
	Other metastases	0.0003
Regression grading	Pathologic response	0.04
pT	Distal margin	0.00001
	Grading	0.01
	Lateral spreading	0.0005
	Regression grade	0.0003
pN	Margin	0.000002
	Grading	0.0001
	Lateral spreading	0.0000001
	Regression grade	0.00004
	Pathologic response	0.0031
Pathologic staging	Distal margin	0.008
	Grading	0.001
	Lateral spreading	0.002
	Regression grade	0.000001
	Lymph node status	0.000001
	Pathologic response	0.000001
Pathologic response	Lateral spreading	0.04
	Regression grade	0.006
	Lymph node status	0.015
Local recurrence	Lymph node status	0.002
	Regression grading	0.003
Lung metastases	Tumour site	0.04
Liver metastases	Distal margin	0.02
Other metastases	Distal margin	0.01

Nodal disease

The mean number of lymph nodes was 8 per surgical specimen. At clinical staging, before the neoadjuvant CRT, 63.8% of patients were classified N_0, 27.6% N_1, 6.9% N_2 and 3.5% N_3. At pathologic staging, after surgery, 65.5% of patients were classified N_0, 17.2% N_1, 5.2% N_2 and 3.5% N_3. According to the site of the tumour, pN_0 were found in 63.6% of sigmoideal-rectal junction tumours, 42.8% of cancers of the upper rectum (55.5% of which intraperitoneal tumours), 76% of tumours of the medium rectum and 60% of cancers of the lower rectum

Table 4 - Local recurrence and metastases according to pN

pN	Local recurrence		Liver metastases		Lung metastases		Other metastases	
	No.	%	No.	%	No.	%	No.	%
0	3	37.5	5	55.5	2	33.3	2	40
1-2	4	50	4	45.5	3	50	3	60
3	1	12.5	0	-	1	16.7	0	-
N_0	3	37.5	5	55.5	2	33.3	2	40
N+	5	62.5	4	45.5	4	66.7	3	60
Total	8 (13.8%)		9 (15.5%)		6 (10.3%)		5 (8.6%)	

(70% of which extraperitoneal tumours). pN+ tumours were recognized in 27.3% of tumours of the sigmoideal-rectal junction, 28.6% of neoplasms of the upper rectum (27.7% of intraperitoneal tumours), 24% of tumours of the medium rectum and 26.6% of cancers of the lower rectum (25% of extraperitoneal tumours). Out of 7 patients with no evidence of residual tumour in the bowel wall, 6 (85.7%) had no nodal disease, while only one (14.3%) was staged pN_2. In the 2 pT_1 cases, nodal involvement was not observed. Of the 15 pT_2 cases, the rate of pN+ was 20%, and 28% of the 39 pT_3 were pN+. Prognostic factors for pN+ are: grading (p=0.0001), regression grading (p=0.00004), lateral sprading (p=0.0000001), distal margin (p=0.000002), pathologic response (p=0.003) (Table 3).

Pathologic staging

Pathologic staging depends on: distal margin (p=0.008), lateral spreading (p=0.002), regression grading (p=0.000001), grading (p=0.001), pathologic response (p=0.000001) and lymph node status (p=0.000001) (Table 3).

Local recurrence and metastases

As reported in Table 4 the overall local recurrence rate was 13.8%, with 62.5% identified in pN_+ (50% N_{1-2} and 12.5% N_3). Liver metastases were diagnosed in 15.5% of patients, 45.5% of which were associated with pN+ (only N_{1-2}); lung metastases were recognized in 10.3% of patients, 66.7% of which were identified in pN+ (50% N_{1-2} and 16.7% N_3). "Other site" metastases were found in 8.6% of patients, 60% in pN_{1-2} (Table 4). Prognostic factors for local recurrence are: regression grade (p=0.003) and lymph node status (p=0.002). Prognostic factors for distal metastases are: site of the tumour for lung metastases (p=0.04), distal margin for liver metastases (p=0.02), and distal margin for other metastases (p=0.01) (Table 3).

Disease-free survival and overall survival rate

Postoperative mortality rate was 0%. With a median follow-up of 55.25±28.06 months (range 5-108), 5-year disease-free survival rate and overall survival rate are 69.9% and 71.1% respectively, while 9-year disease-free survival rate and overall survival rate are 69.9% and 68.3%. There was significant difference in disease-free survival rate between cN_0 *vs* cN+ (5-year disease-free survival rate was 83.2% *vs* 51.7%; p=0.005), pN_0 *vs* pN+ (5-year disease-free survival rate was 82.3% *vs* 49.8%, p<0.02) (Table 5), both intraperitoneal *vs* extraperitoneal tumour N_0 (5-year disease-free survival rate was 100% *vs* 66.9%; p=0.04) and intraperitoneal *vs* extraperitoneal tumour N+ (5-year disease-free survival rate was 80% *vs* 17.1%; p<0.05) (fig. 1 and 2). Significant difference in overall survival rate was found between both intraperitoneal *vs* extraperitoneal tumour N_0 (5-year overall survival rate was 100% *vs* 69.8%; p<0.05) and intraperitoneal *vs* extraperitoneal tumour N+ (5-year overall survival rate was 80% *vs* 50.9%; p<0.05) (Table 5, fig. 3 and 4). Independent variables associated to disease-free survival resulted as: site of tumour (p=0.005), pN status (p=0.006), liver metastases (p=0.007) and other metastases (p=0.0003); independent variables associated to overall survival resulted as: site of tumour (p=0.003), distal margin (p=0.04), and grading (p=0.04) (Table 3).

Discussion

Preoperative neoadjuvant CRT promises to improve survival by reducing local recurrences in patients undergoing resection for rectal cancer[13,16]. This effect was also shown in patients undergoing TME: significantly reduced local recurrence rates were recorded in patients undergoing TME surgery after neoadjuvant CRT in comparison to patients undergoing TME surgery alone[4,13].

The aim of this study was to evaluate the prognostic

Table 5 - Disease-free survival and overall survival rate: prognostic factors

Factors	Disease free survival			
	5-year (%)	7-year (%)	9-year (%)	p
Lymph node status				
Clinical				0.005
N_0	83.2	83.2	83.2	
N+	51.7	34.5	34.5	
Pathological				<0.02
N_0	82.3	82.3	82.3	
N+	49.8	49.8	39.9	
Localization				
N_0				0.04
Intraperitoneal	100	100	100	
Extraperitoneal	66.9	66.9	0	
N+				<0.05
Intraperitoneal	80	80	80	
Extraperitoneal	17.1	17.1	0	
	Overall survival			
Localization				
N_0				<0.05
Intraperitoneal	100	100	100	
Extraperitoneal	69.8	69.8	0	
N+				<0.05
Intraperitoneal	80	80	80	
Extraperitoneal	50.9	25.5	0	

implications of lymph node status in rectal cancer treated by neoadjuvant CRT followed by surgery.

The CRT contributed in downstaging (to pN_0) the nodal dissemination in 12.1% patients, all affected by extraperitoneal tumour; furthermore 53.4% of overall patients, who were cN_0 had no progression during neoadjuvant therapy. Combined 5-FU infusion and radiotherapy protocol increases the response of the primary tumour to the radiation and probably eliminates systemic micrometastases, with improved rates of both recurrence-free and overall survival rate, in accordance with rates reported in the literature[2,11].

A complete mesorectal dissection provides adequate excision of the lymph node pathways of the rectum: the achieved result is good local control with low rates of local recurrence even in cases of node-positive disease[11,12].

We reported a relatively low mean number of retrieved lymph nodes (nr. 8) compared with that always recommended in surgical guidelines (nr. 12)[17], but we do not believe this is a weak point of the study because, as reported in the literature, preoperative CRT for advanced rectal cancer results in a significant decrease of lymph nodes detected within the tumour-bearing specimen[18-21].

Because the number of lymph nodes identified within the excised specimen, in patients undergoing resection of a rectal cancer, positively correlates with the size of the tumour, the downsizing-downstaging obtained by CRT could lower the number of lymph nodes identified by pathologist[19].

Although neoadjuvant CRT downstages rectal cancer, it results in a significantly low yield of lymph nodes, which are also significantly smaller than those in non-irradiated controls[20]. As reported by Wijesuriya *et al*[20], the median nodal harvest was 4 (range 0-12) in the neoadjuvant CRT group *vs* 9 (range 1-19) in the control (p=0.001); the median size of the largest lymph node was 5 mm (range 2-12 mm) in the neoadjuvant CRT group *vs* 9 mm (range 4-15 mm) in the control group (p= 0.004)[20]. Finally, two previous reports on preoperatively treated patients showed a median of 16 and a mean number of 9.3 nodes[7,22], which confirms the variability of collected nodes within homogeneously examined surgical specimens. There is no

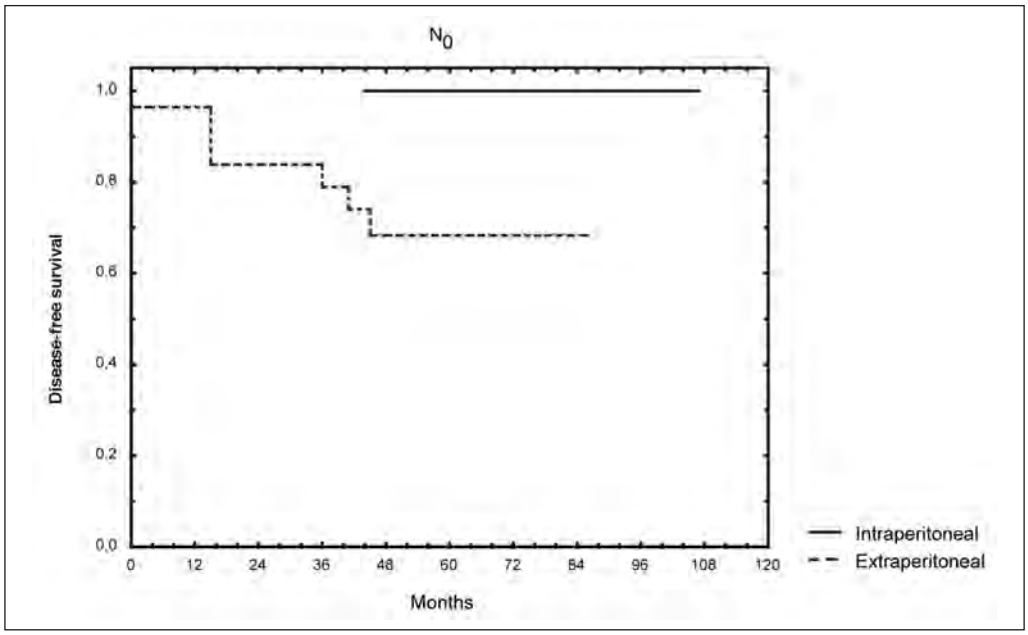

Fig. 1. Disease-free survival: intraperitoneal *vs* extraperitoneal tumour pN_0 (p=0.04)

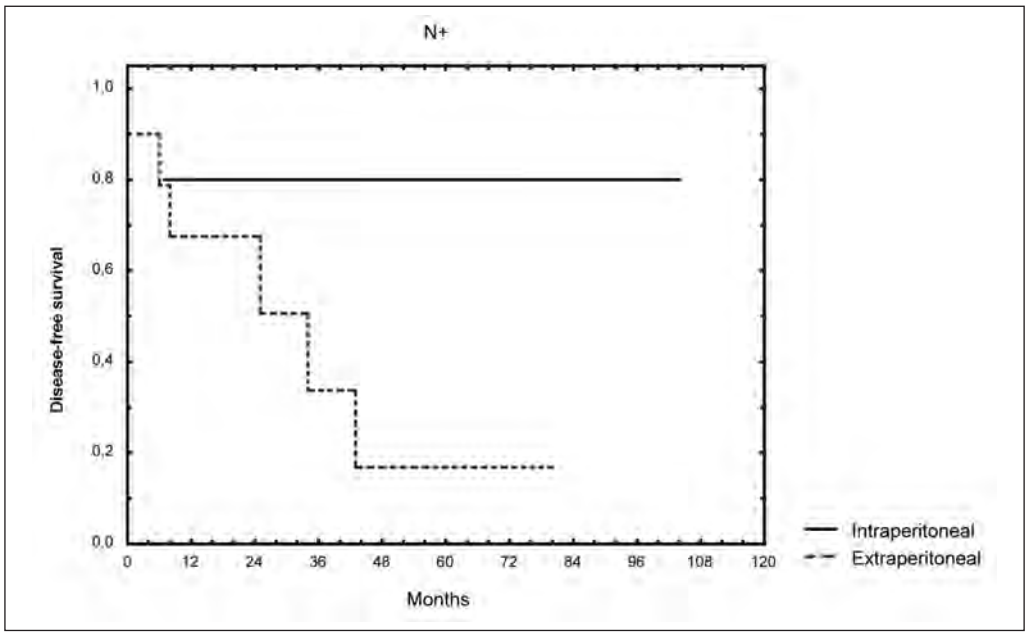

Fig. 2. Disease-free survival: intraperitoneal *vs* extraperitoneal tumour pN_+ (p<0.05)

general and constant agreement on the minimum number of nodes to be examined in this setting. This has been a matter of debate over the last decade. Nevertheless, a report from Goldstein[23] suggests that "*there is no minimum number that reliably or accurately stages all patients*". All palpable nodes should be retrieved, irrespective of their dimension and total number.

Lymph node status, after surgery, was 65.5% N_0, 17.2% N_1, 5.2% N_2 and 3.5% N_3, better than other surgical series, where reported N_0 rates are 50-53%, 29.6% N_1 and 26.1% N_2[16, 24, 25].

Some authors found that the sole independent covariate for local recurrence and survival was the presence of lymph node metastases[2,3]. The overall local recurrence rate is similar to other data recently published by centres routinely performing only TME (8.6%-13%)[26-29].

Liver metastases were diagnosed in 15.5% of patients, 45.5% associated with pN+ (only N_{1-2}), all of them derived from extraperitoneal tumours; in surgical series alone the rate of liver metastases is 26.7%[24]; 80% of lung metastases and all "other site" metastases also arose from extraperitoneal tumours. This event could not be merely explained by either an incomplete lymphatic resection or inappropriate application of CRT protocol[30]. The difference in the development of distant metastases could be explained by the pT stage: as reported by some authors, pT_{3-4} is associated with a higher incidence of distant metastases than pT_{0-2}[31, 32].

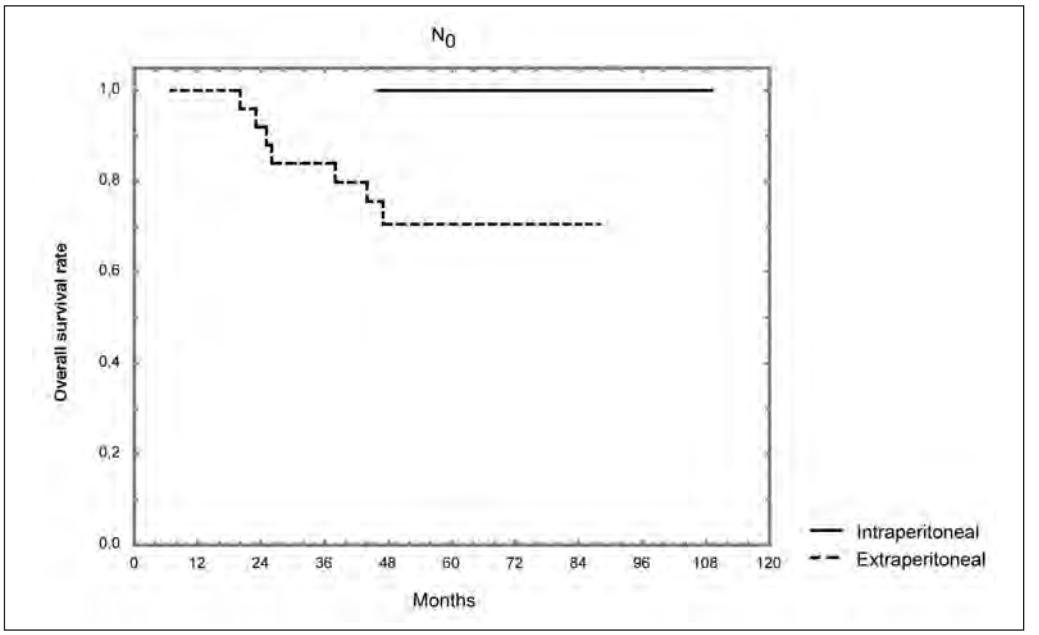

Fig. 3. Overall survival rate: intraperitoneal *vs* extraperitoneal tumour pN_0 ($p<0.05$)

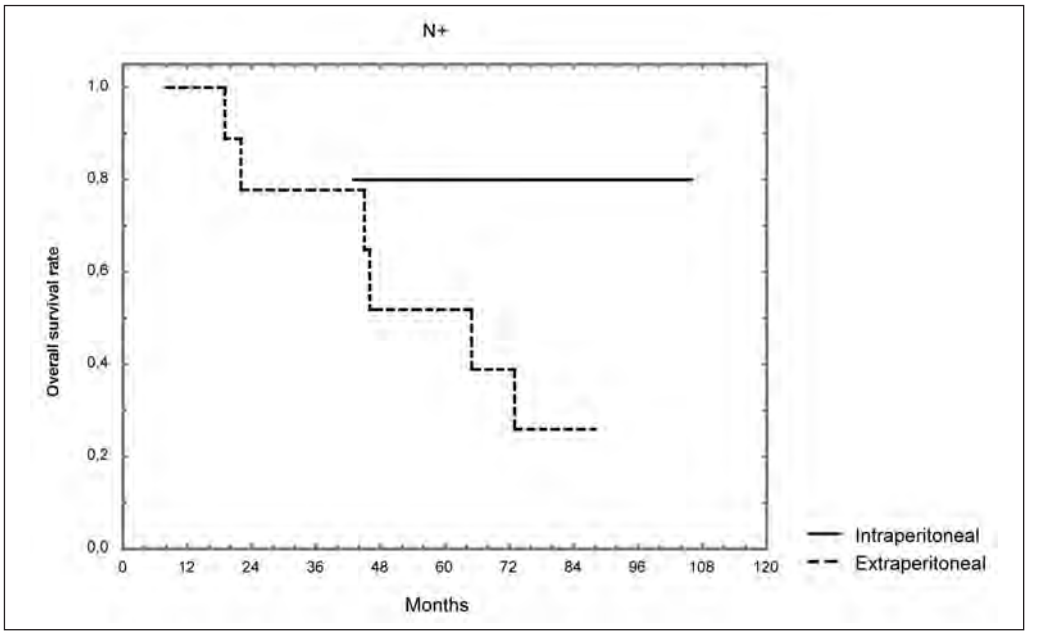

Fig. 4. Overall survival rate: intraperitoneal *vs* extraperitoneal tumour pN_+ ($p<0.05$)

In our study pT_3 extraperitoneal tumours (EPt) developed twice as many distant metastases as $T_{0\text{-}2}$, and the only intraperitoneal tumour (IPt) that developed a lung metastasis was staged as pT_3; in addition, the number of patients with EPt staged pT_3 is significantly larger than IPt (57.5% EPt *vs* 33.3% IPt; p=0.04). EPts might be more aggressive than IPt ones, spreading more precociously, and/or being less responsive to the neoadjuvant CRT on the site of lymph node disease, because of their localization rather than because of differences in biological characteristics[30].

We recorded a 5-year overall survival rate of 71.1%, and a 9-year overall survival rate of 68.3%; the 5-year overall survival rate reported in the literature is about 50-52.8%, and the 10-year overall survival rate is 41.1%[24, 33].

Conclusion

Neoadjuvant CRT and surgery, in comparison with surgery alone, allows the surgeon to obtain optimal results with lower risks[34]. The object of neoadjuvant regimens is a downstaging or downsizing of advanced tumours in order to increase the rate of curative resection and to reduce locoregional failure: 2-3% in irradiated patients *vs* 10-12% in non-irradiated patients[13, 16, 35, 36].

Some authors have reported, as independent prognostic factors that reduce overall survival, not only local recurrence, but also the absence of neoadjuvant treatment[5]. On the grounds of lymph node status of our protocol we recorded better results than the surgical series.

The high rates of liver, lung and other metastases associated with pN+ status, in our series, strengthen the hypothesis reported by some authors that pelvic nodal metastases may indicate haematogenous spread or may spread to distant sites themselves[2]. In summary, neoadjuvant CRT and surgery represent the best choice for the treatment of rectal cancer. The CRT could play a rôle by preventing the development of node metastases in patients with preoperative N_0, as well as downstaging the nodal dissemination in patients with preoperative N+. Neoadjuvant CRT achieves a satisfactory downstaging both in tumour mass and in peritumoural tissue, reducing lymph node disease. The relevant prognostic factors, whose rôle in the prognosis of rectal cancer treated by neoadjuvant protocols is reinforced by data herein reported, are: pN and the associated local recurrence, pathologic staging, pathologic response and pT[3, 22, 37-39].

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Proposta di una tecnica innovativa per la preservazione del complesso areola-capezzolo e radicalità oncologica: la "*delayed nipple-sparing mastectomy*"

Proposal of an innovative technique for the preservation of the nipple-areola complex and oncological radicality: delayed nipple-sparing mastectomy

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Riassunto

La conservazione del complesso areola–capezzolo durante l'intervento di mastectomia per cancro della mammella rappresenta una soluzione tecnica problematica. In effetti, al fine di ottenere una completa rimozione della ghiandola mammaria, nessun residuo tissutale deve essere lasciato dietro al capezzolo, compresi i dotti galattofori maggiori. D'altro canto la vitalità del complesso areola-capezzolo, privato *tout court* della vascolarizzazione ghiandolare, risulta fortemente compromessa e occorre un tempo di adattamento dei circoli collaterali suppletivi dermici per garantire l'integrità di questo apparato escretore. Gli Autori suggeriscono pertanto una procedura chirurgica che si attua in due fasi. La prima, ambulatoriale, in anestesia locale tumescente con tecnica mini-invasiva di "dissezione-coagulazione": tale procedimento è indirizzato a rendere autonomo il supporto vascolare del complesso areola-capezzolo e si effettua staccando il peduncolo dei galattofori dal peduncolo mammario e coagulando il plesso vascolare profondo. La seconda fase, in anestesia generale con tecnica tumescente, consiste nel rimuovere radicalmente la mammella all'interno della sua capsula, con un adeguato controllo dei possibili residui ghiandolari ed un'adeguata via d'accesso per la regione ascellare. La procedura descritta viene completata con l'inserimento di una protesi mammaria

Summary

The problem of nipple-areola-complex (NAC) preservation during mastectomy for breast cancer is a complicated technical question. In fact, in order to achieve a radical excision of the mammary gland, no residual tissue, including the main galactophorous ducts, has to be left behind the nipple; on the other hand, without vascular support from the gland, the NAC viability is greatly impaired, and it takes time for the surrounding vascular dermal network to adapt in order to ensure the integrity of this excretory organ. The authors therefore suggest a two-step surgical procedure: the first one, on an outpatient basis under local tumescent anaesthesia, is a mini-invasive cutting-coagulating procedure; this is intended to autonomize the vascular supply to the nipple-areola complex, by detaching the galactophorous stalk from the nipple and coagulating the deep vascular plexus. The second step, under general anaesthesia, with tumescent technique, consists of radical excision of the breast within its capsule and allows careful control of any gland remnant and adequate approach to the axilla. The procedure is completed by the application of a subpectoralis prosthesis. This technique could be electively suitable for prophylactic mastectomy, but also for *in situ* ductal carcinoma, as well as I and II grade breast cancer, peripheral with respect to the nipple-areola complex.

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sub-pettorale. Questa tecnica si può prospettare in elezione come una mastectomia profilattica, ma anche terapeutica nel cancro duttale *in situ* della mammella e in quello di grado I e II, periferici al complesso areola-capezzolo. È inoltre di esecuzione semplice ed accurata, conservativa nell'apparenza, radicale nella sostanza. Eur. J. Oncol., 11 (1), 51-56, 2006

***Parole chiave:* complesso areola-capezzolo, mastectomia sottocutanea "*delayed nipple-sparing subcutaneous mastectomy*" (DNSSM), cancro della mammella**

The technique is simple and accurate, conservative as regards appearance, but radical in its results. Eur. J. Oncol., 11 (1), 51-56, 2006

***Key words:* nipple-areola complex, delayed nipple-sparing subcutaneous mastectomy (DNSSM), breast cancer**

Introduzione

La predisposizione ereditaria al cancro della mammella (come dimostrato recentemente dagli studi sulla mutazione dei geni BRCA 1 e 2), l'iperplasia lobulare, l'iperplasia duttale atipica ed il carcinoma lobulare *in situ* vengono attualmente identificati e trattati ricorrendo ad un attento *screening* mammografico ed al *follow-up* delle pazienti, con chemioprevenzione ed eventualmente mastectomia profilattica[1-4]. Secondo Crane[5] la semplice mastectomia, la mastectomia sottocutanea e la rimozione del complesso areola-capezzolo assieme alla ghiandola mammaria, sarebbero insufficienti ai fini della radicalizzazione oncologica. La mastectomia totale rimarrebbe dunque l'unica misura atta a contrastare l'alto rischio di cancro mostrato da alcune donne. Tra i vari interventi di mastectomia totale, effettuati in donne con storia familiare di cancro al seno a partire dal 1995, è stato notato un incremento (pari a circa il 13%) di questo tipo di operazione, cui ha fatto seguito un elevato numero di ricostruzioni della mammella. Secondo Metcalfe *et al*[6] la quota di ricostruzioni mammarie in queste pazienti era pari al 60%, comparata al 6-13% delle mastectomie non profilattiche.

La *"delayed nipple-sparing subcutaneous mastectomy"* (DNSSM), da noi messa a punto, rappresenta una tecnica elettiva di ablazione completa della ghiandola mammaria, quando si agisca in via profilattica in donne ad alto rischio di cancro al seno, o in via terapeutica in donne che presentano tumori allo stadio I e II.

Questa tecnica si basa sul principio di rendere autonomo, in un tempo operatorio preliminare ad esso dedicato, il complesso areola-capezzolo, praticando ambulatoriamente l'intervento in anestesia locale tumescente.

Dopo un'attesa di due-quattro settimane, affinché il circolo collaterale dermico assuma il controllo vascolare del complesso areola-capezzolo, si interviene con il completo svuotamento della mammella e con la sua ricostruzione. Noi riteniamo che questa procedura sia sicura ed efficace, verificabile in tempo reale per ciò che riguarda la radicalità profilattica ed oncologica e, soprattutto, confortevole in quanto conferisce integrità al complesso areola-capezzolo. Per tale ragione è prevedibile che questa tecnica operatoria si possa applicare in futuro anche nel cancro duttale *in situ* (DCIS) e negli stadi di cancro I e II, e possa avere sempre maggiore diffusione.

Pazienti e metodi

La casistica comprende 15 donne operate nel periodo aprile 1999–novembre 2005, le cui patologie sono presentate nella Tabella 1.

Tutte le pazienti hanno firmato il consenso informato dopo aver ricevuto dettagliate informazioni sulle fasi e le caratteristiche della procedura. Questa è stata realizzata in due tempi distinti a distanza di 2 settimane uno dall'altro:

– autonomizzazione della rete vascolare del complesso areola-capezzolo in anestesia locale tumescente con strumentazione laparoscopica. È stata inoltre effettuata una biopsia preliminare;
– mastectomia radicale sottocutanea "*nipple-sparing*" in anestesia generale con tecnica tumescente.

Protocollo di autonomizzazione del complesso areola-capezzolo

A. Controllo della temperatura del capezzolo, con termometro a cristalli liquidi (facoltativo).
B. Esame Doppler (Mini-Doppler oppure Laser-Doppler Echoflow) (facoltativo).
C. Anestesia intradermica e sottocutanea per tumescenza, intra-sub-areolare, con soluzione anestetica contenente adrenalina 1x1000 e con gentamicina 80 mg.

Tabella 1 - Casistica

Paziente	Età (anni)	Patologia
1. C.M.	34	Carcinoma lobulare nella mammella contro-laterale operata precedentemente per cancro duttale infiltrante
2. S.C.	42	Cancro della mammella sn in stadio II e mastopatia fibrocistica bilaterale. La paziente rifiutò la mastectomia totale
3. D.D.	37	Mastopatia fibrocistica e cancro familiare della mammella
4. P.C.	39	Quadrantectomia bilaterale per DCIS e terapia radiante monolaterale
5. S.N.	62	Displasia mammaria sn e precedente mastectomia controlaterale
6. D.D.	42	Frequenti ricadute di mastite bilaterale
7. Z.G.	54	Carcinoma lobulare *in situ* mammella dx
8. G.G.	56	Carcinoma duttale infiltrante periferico grado II con alta incidenza familiare
9. S.M.	34	Carcinoma lobulare bilaterale grado I
10. B.G.	61	Carcinoma duttale infiltrante a dx e mastopatia fibrocistica con metaplasia apocrina a sn
11. B.E.	44	Mastiti recidivanti ed alta incidenza familiare di carcinoma mammario
12. A.A.	47	Displasia grave multifocale a sn, fibroadenoma QSE dx
13. N.R.	52	Carcinoma duttale infiltrante a sn e cancro *in situ* a dx
14. C.P.	36	Mastopatia fibrocistica prevalentemente dx e positività per BRCA 1 e 2; alta familiarità per carcinoma mammario e ovarico
15. M.L.	41	Positività BRCA 1 e 2 ed alta incidenza familiare di carcinoma mammario

D. Ottenuta la pelle a "buccia d'arancia" si incide con minibisturi inferiormente o lateralmente al capezzolo a distanza di 15 cm circa; si inserisce una forbice laparoscopica elettrificata in modo tale da non indebolire il circolo collaterale epidermico-dermico; si inizia lo scollamento sotto il capezzolo mantenendo fredda la superficie cutanea con pezze imbevute di soluzione fisiologica sterile ghiacciata e monitorando la temperatura cutanea. La procedura più sicura consiste nell'elettrificare, con la forbice a branche aperte, l'area da sezionare per 5-10 secondi, indi sezionarla a piccoli colpi taglienti.

E. Una volta deafferentato il capezzolo si controlla l'emostasi utilizzando striscioline di garza asciutte.

F. Si inseriscono quindi, attraverso il pertugio praticato, strisce di collageno e spongostan, addizionate con polvere antibiotica (cloramfenicolo).

G. Per isolare in modo più completo le superfici cauterizzate, vengono introdotti anche foglietti di silicone sterile in strisce (sifravit, Fresenius-Kabi) per isolare l'area sottocutanea dal letto vascolare di pertinenza della ghiandola mammaria.

H. Sutura della cute con singolo punto di sutura in Ethylon 3/O.

I. Fasciatura elastica moderatamente compressiva. La paziente viene dimessa immediatamente con prescrizione analgesica da effettuarsi per tre giorni. Dopo sei giorni viene medicata e le viene rimosso il punto di sutura.

Da due a quattro settimane più tardi viene praticato l'intervento finale di mastectomia (o mastectomia più linfonodo sentinella e/o linfoadenectomia e protesi).

Protocollo del secondo intervento

A. In regime di ricovero o *day hospital*, in anestesia generale o locale più sedazione, si pratica tumescenza dell'intera ghiandola infiltrandola di quadrante in quadrante con un volume totale di 250-500 ml di soluzione fisiologica più adrenalina per ogni lato; deve essere indotto l'effetto a buccia d'arancia su tutta la regione mammaria.

B. Si accede attraverso il solco inframammario (con una incisione orizzontale moderata sul quadrante laterale di 1,5 cm circa) oppure utilizzando precedenti cicatrici. Una volta prescelta la via d'accesso si incide l'adipe sottocutaneo fino a raggiungere la capsula mammaria, che risalta nel tessuto adiposo per il suo colore nettamente bianco.

C. Si procede quindi ad uno scollamento, in parte per via smussa, in parte con elettrobisturi (il quale viene utilizzato quasi esclusivamente per i piani profondi, per il timore di ustionare la cute), staccando l'intera ghiandola mammaria a partire dalla superficie pre-pettorale ed isolando completamente dall'atmosfera adiposa il connettivo capsulare.

D. Una volta raggiunta la regione sotto-areolare si scolla, per via smussa, la parte già in precedenza deafferentata dei dotti galattofori; tale manovra risulta molto agevole, ed è completata da rasatura con bisturi (lama N. 16) del capezzolo, asportandone un frammento cilindrico-discoide che viene inviato al laboratorio di anatomia patologica per l'esame estemporaneo. Eventuali residui di collagene, applicato nell'intervento precedente, vengono raccolti ed inviati per una valutazione estemporanea cito-patologica "*imprint*".

E. Nell'area cruentata sub-areolare si tampona con una garza intrisa di soluzione antisettica. Al termi-

ne di queste fasi l'intera ghiandola risulta scollata *in toto*, avvolta interamente dalla sua capsula.

F. Attenzione particolare viene rivolta ad eventuali prolungamenti ascellari o porzioni di ghiandola che fossero sfuggiti all'asportazione *en bloc*. La presenza di noduli biancastri e/o ghiandole mammarie accessorie è facilmente evidenziata grazie all'ingrandimento della telecamera laparoscopica con cui si effettua sistematicamente la revisione della cavità residua alla mastectomia. In caso di chirurgia oncologica vera e propria si eseguono da 4 a 16 biopsie chirurgiche *at random* del tessuto adiposo nei quadranti mammari, per escludere la presenza di residui epitelio-ghiandolari.

G. La ghiandola viene quindi asportata ed inviata al laboratorio di anatomia patologica, mentre il chirurgo cura minuziosamente l'emostasi, considerando un'eventuale ulteriore asportazione di tessuto adiposo peri-ghiandolare sottocutaneo, qualora sussista il rischio di disseminazione tumorale.

H. Se si ritiene necessario da un punto di vista oncologico, viene asportato un altro disco dalla faccia profonda del capezzolo; anch'esso viene inviato al laboratorio per un'ulteriore valutazione estemporanea della radicalità chirurgica.

I. Si osserva la cute, per trans-illuminazione, in modo tale da poter rilevare immediatamente qualsiasi possibile lobulo ghiandolare residuato. Questa fase può essere ulteriormente integrata ed ingrandita dalla ripresa con telecamera laparoscopica onde ottenere un adeguato ingrandimento.

J. Sempre utilizzando transilluminazione o ingrandimento con telecamera laparoscopica è possibile visualizzare il linfonodo sentinella, avendo iniettato il colorante o il tracciante nel sottocute profondo attorno al nodulo.

K. Si effettua ora lo scollamento del muscolo grande pettorale, il quale viene sollevato "a cortina", previo disancoraggio delle inserzioni distali, con accurata emostasi delle arterie profonde. Le fibre muscolari vanno allungate il più possibile con una moderata trazione meccanica, al fine di ottenere un ampio rivestimento della superficie anteriore della protesi, a tutela di una eventuale sua estrusione in caso di sofferenza cutanea.

L. La protesi scelta viene applicata sotto il muscolo, proprio per isolare da una eccessiva pressione la cute scheletrizzata, il capezzolo e l'area sottostante: quest'ultima soprattutto potrebbe venire contaminata. E' preferibile evitare l'uso di protesi eccessivamente voluminose e curare molto la simmetria dei due seni.

M. A questo punto si procede all'applicazione di un drenaggio sottoprotesico ed alla sutura a strati della cute.

Le Tabelle 2-4 sintetizzano le caratteristiche tecniche, l'impiego e le indicazioni per la DNSSM.

*Variante per l'intervento di "*nipple-sparing*" con* lifting *del complesso areola-capezzolo*

Talora è possibile mobilizzare cranialmente il complesso areola-capezzolo come nella mastoplastica riduttiva con mastopessi, avendo cura di effettuare un primo tempo chirurgico che consenta la formazione di un peduncolo adiposo-vascolare di sostegno. Nel secondo tempo, demolitivo-ricostruttivo, è possibile risollevare il complesso areola-capezzolo, utilizzando il peduncolo dermico a base superiore o supero-inferiore. Questa manovra è molto delicata ed occorre tenere presente che il

Tabella 2 - Caratteristiche tecniche della DNSSM

– Tumescenza anestesiologica nella I e II fase: è possibile condurre l'intervento in anestesia locale e facoltativamente in sedazione
– Elettrobisturi con manico lungo oppure forbice elettrificata laparoscopica (fase 1)
– Telecamera d'ingrandimento laparoscopico per l'ispezione del campo operatorio dopo mastectomia
– Aggiunta facoltativa di collagene eterologo liofilizzato a *strips* di silicone sterile in modo da isolare il complesso areola-capezzolo e la mammella la cui vascolarizzazione deriva solo dalla cute circostante
– Uso di protesi mammaria sottomuscolare a fini ricostruttivi

Tabella 3 - Criteri di radicalità oncologica

– Verificare, nel primo e nel secondo tempo chirurgico, con biopsia discoide del capezzolo, l'assenza di tumore o ghiandola residua sia dietro al capezzolo che in altri quadranti mammari
– Effettuare "*envelope mastectomy*"[7] e resezione dell'atmosfera adiposa mammaria. La tecnica consente inoltre accesso a linfonodi sentinella ed ascellari
– Effettuare biopsie *at random* o mirate da telecamera laparoscopica dell'adipe sottocutaneo per verifica di radicalità

Tabella 4 - Indicazioni per la realizzazione della DNSSM

– Mastiti recidivanti
– Mastectomia profilattica in soggetti geneticamente predisposti o in mammelle ad alto rischio sulla base dell'*imaging* diagnostico e dell'agoaspirato
– Cancro mammario di I e II grado con implicazioni bilaterali sincrone o metacrone
– Simmetrizzazione controlaterale dopo mastectomia

supporto al complesso areola-capezzolo dopo la deafferentazione è prevalentemente dermico.

Risultati

Dall'analisi dei risultati e del *follow-up* delle pazienti operate, si evince che non si sono osservati casi di necrosi del complesso areola-capezzolo, se si esclude il primo caso, dove si è avuta una lesione cutanea causata dal surriscaldamento durante la procedura di autonomizzazione. In questo caso il secondo intervento chirurgico è stato posticipato di sei settimane, al fine di ottenere una buona cicatrizzazione oltre alla rigenerazione della rete vascolare dermica. Il risultato finale è stato soddisfacente ed il complesso areola-capezzolo non mostrava segni di depigmentazione né danni di altro tipo. La sensibilità risultò intatta come dimostrato dall'erezione del capezzolo dopo stimolazione.

In due casi, durante la fase di ricostruzione, si è potuto sollevare il complesso areola-capezzolo al fine di ottenere un gradevole effetto estetico, ripristinandone la simmetria. Per questa procedura si è fatto ricorso ad una tecnica bi-peduncolata tipo McKissock in un caso e ad un peduncolo superiore nel secondo.

In un caso non abbiamo inserito la protesi poiché questa paziente doveva essere sottoposta a chemio-radioterapia; il complesso non risultava danneggiato e la paziente è in attesa per l'intervento di ricostruzione.

In altri due casi è stato effettuato l'intervento di mastectomia "*nipple-sparing*" unilateralmente ed è stata inserita una protesi; non è stata invece eseguita mastopessi controlaterale.

A distanza di uno-quattro anni non sono state identificate metastasi o recidive. La soddisfazione delle pazienti è stata invariabilmente elevata. Parziale ripresa della sensibilità del complesso areola-capezzolo è stata osservata in sei casi su otto a distanza di sei-nove mesi.

Discussione

In questo studio preliminare viene proposta una soluzione tecnica innovativa per la conservazione del complesso areola-capezzolo in corso di intervento di mastectomia. In base ai risultati conseguiti abbiamo dimostrato che la nostra procedura in due tempi per la mastectomia radicale sottocutanea "*nipple-sparing*" è una tecnica semplice, sicura ed efficace che consente di asportare completamente la ghiandola mammaria oltre alla possibilità di poter valutare, in sede intraoperatoria, ogni possibile residuo.

L'autonomizzazione del complesso areola-capezzolo, in accordo con gli studi anatomici di Nakajima *et al* [8], è ottenuta per dissezione "a cieco" con strumentazione per laparoscopia. Questa delicata rete vascolare è sufficiente a garantire l'integrità degli annessi cutanei della mammella (tenendo conto che la loro resezione deve esser eseguita molto vicino al derma), come pure a mobilizzare il complesso areola-capezzolo per poter effettuare l'intervento di mastopessi, durante la seconda operazione.

Tre sono i punti cardine che garantiscono un'efficiente circolazione collaterale e l'autonomizzazione della cute sovrapposta:

1) l'emostasi ed il distacco dei dotti galattofori;
2) l'irrigazione con antibiotici dell'area scheletrizzata (per prevenire infezioni dermo-epidermiche che precluderebbero la sicurezza delle protesi impiantate e potrebbero ricreare circoli collaterali di compenso tra ghiandola e capezzolo);
3) l'isolamento della superficie profonda del capezzolo con collagene e membrane di silicone, onde favorire una neoangiogenesi dermo-epidermica escludendo completamente il supporto vascolare della mammella.

La riconnessione vascolare del complesso areola-capezzolo con la cute viene ripristinata nelle due-quattro settimane successive all'intervento.

Inserendo la protesi in sede sottomuscolare dopo mastectomia, si riducono le probabilità di un'infezione dovuta a batteri presenti a livello intraduttale.

La possibilità di effettuare, durante la seconda fase dell'intervento, esami cito-istologici anche per apposizione delle sezioni criostatate fornisce ulteriori prove di radicalità oncologica ed è per questo motivo che la tecnica chirurgica viene consigliata, con intento curativo, nei primi stadi del cancro della mammella, purché la lesione sia distante 1-1,5 cm dall'areola, e non superi 1,5 cm di diametro.

Il punto d'accesso per la mastectomia radicale dovrebbe essere sufficientemente ampio per consentire una buona esposizione di tutti i segmenti della ghiandola mammaria e, eventualmente, del cavo ascellare; questo obiettivo è ottenuto attraverso l'uso del trans-illuminatore o della telecamera e nel caso della dissezione ascellare da un accesso laterale, orizzontale, al confine tra il quadrante superiore ed inferiore.

Conclusioni

Questo intervento, pienamente rispettoso dell'integrità anatomo-funzionale del complesso areola-capezzolo, risulta gradito alle pazienti e costituisce un approccio preventivo del cancro mammario, dotato di notevole effica-

cia dal punto di vista estetico. Nel caso che tale tecnica venga adottata per la rimozione di carcinomi di grado iniziale, la sua dimostrata radicalità istologica potrebbe consentire di escludere il ricorso alla radioterapia, la quale però non è comunque controindicata, pur con tutte le ben note limitazioni inerenti il danno attinico alla cute e al materiale protesico. Una casistica multicentrica ben selezionata in quanto a protocollo oncologico ed arruolamento di casi potrà meglio definire se tale metodica potrà confrontarsi quanto ad affidabilità prognostica, in casi selezionati, all'attuale tecnica di mastectomia sottocutanea e irradiazione intraoperatoria (ELIOT) messa a punto dal gruppo di Veronesi e Petit[9].

Non bisogna però dimenticare, soprattutto per la paziente, anche il risvolto ricostruttivo ed estetico: per questo motivo si preferisce incidere su una preesistente cicatrice, se questa è presente.

Crediamo quindi che la nostra tecnica possa essere utile nel preservare l'integrità della cute dopo interventi radicali di mastectomia. Questo approccio viene consigliato soprattutto per le donne di giovane età, con un alto rischio di familiarità per il cancro della mammella, ma anche in donne in età peri-menopausale affette da mastopatia fibrocistica istologicamente accertata o con displasie a rischio di cancro, in quanto consente da un lato la completa rimozione della ghiandola e dall'altro la successiva ricostruzione cosmetica in modo molto semplice.

Il ricorso a due fasi, la prima delle quali ambulatoriale, rende semplice e sicura la conservazione del complesso areola-capezzolo. L'intervallo temporale tra la prima e la seconda operazione (tre-quattro settimane) è sufficiente per consentire alla rete vascolare collaterale, aperta nella prima fase, di completare il processo di neo-angiogenesi da essa innescato.

Infine vorremmo enfatizzare il ruolo svolto dall'anestesia in tumescenza, sia a livello locale nella prima fase del metodo, che a livello generale durante la fase di *clearance* del tessuto e della capsula mammaria, durante la successiva mastectomia.

Il metodo che proponiamo si prefigge l'obiettivo di adeguata autonomizzazione vascolare del complesso areola-capezzolo, conseguendo nel contempo anche una adeguata radicalità oncologica. Stiamo ora perfezionando la possibilità tecnica di eseguire una pessi dell'areola e del capezzolo, laddove nella simmetrizzazione di un intervento monolaterale cio' si renda necessario; le premesse tecniche e i riscontri anatomo-patologici, sia macroscopici che microscopici, fanno prospettare un suo più ampio utilizzo, soprattutto nel campo della chirurgia profilattica del cancro della mammella.

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Treatment of neuroendocrine gastroenteropancreatic tumours with somatostatin analogues: a personal case series and review of the literature

Trattamento dei tumori neuroendocrini gastroenteropancreatici con analoghi della somatostatina: casistica personale e revisione della letteratura

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Summary

Neuroendocrine tumours (NETs) are rare neoplasms characterized by low clinical incidence (approximately 1 case/100,000/year). The most frequent sites are in the digestive tract (70%). The aim of this retrospective study is to assess objective and symptomatic responses (limited to refractory chronic diarrhoea) after somatostatin analogue treatment and to compare our case history data with that available in the literature. The Rare Hormonal Tumour Group of Cremona has, since 1990, observed 165 patients with digestive NETs. Of these, 57 (34.5%) were treated with somatostatin analogues, of whom 20 were considered eligible for this study. The patients were divided into two groups: the first group included all 20 patients, while the second group included 9 tumour cases in 7 patients already studied in the first group who, during the follow-up, received a modified dose or different molecule type or a diverse formulation of the analogue. In this study, the high rate of disease stabilization in both groups (60% and 66.6%, respectively) confirmed that somatostatin analogues guarantee better responses in NETs with low grades of malignancy. Control of chronic refractory diarrhoea also demonstrated an overall partial response (PR) in 90.9% of the cases, which corresponds with improvement in the patient's

Riassunto

I tumori neuroendocrini (NETs) sono neoplasie rare caratterizzate da una bassa incidenza (circa 1 caso/100.000/anno). La sede più frequentemente colpita è il tratto digestivo (70%). L'obiettivo di questo studio retrospettivo è di valutare la risposta obiettiva e sintomatica (limitatamente alla diarrea cronica refrattaria) dopo trattamento con analoghi della somatostatina e di confrontare i dati della nostra casistica con quelli disponibili in letteratura. Il Polo Tumori Ormonali Rari di Cremona dal 1990 ha osservato 165 pazienti affetti da NETs del tratto digestivo. Di questi, 57 (34,5%) sono stati trattati con analoghi della somatostatina di cui 20 sono stati considerati eligibili per questo studio. I pazienti sono stati suddivisi in due gruppi: nel primo gruppo sono rappresentati tutti i 20 pazienti, nel secondo gruppo sono invece inseriti 9 casi di tumore relativi a 7 pazienti già studiati nel primo gruppo ai quali, nel corso del *follow-up*, è stata modificata o la dose del farmaco, o il tipo di molecola, o la formulazione dell'analogo. In questo studio l'elevata percentuale di stabilizzazione della malattia nei due gruppi (60% e 66,6% rispettivamente) conferma il dato che gli analoghi della somatostatina garantiscono migliori risposte nei NETs a basso grado di malignità. Anche il controllo sulla diarrea cronica refrattaria ha mostrato una ri-

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quality of life. In conclusion, somatostatin analogues can play a rôle in the treatment of digestive NETs with low grades of malignancy, with good differentiation, a low cellular proliferation index and a high specific receptorial density *in vivo*. An increased dosage of the analogue appears to determine better control of the disease and of the chronic refractory diarrhoea. New opportunities may arise from the synthesis of new, more powerful and selective analogues. Eur. J. Oncol., 11 (1), 57-64, 2006

Key words: **neuroendocrine tumours, somatostatin analogues, chronic diarrhoea, quality of life**

sposta parziale complessiva (PR) nel 90,9% dei casi che coincide con un miglioramento della qualità di vita del paziente. In conclusione, gli analoghi della somatostatina possono trovare un loro posto nel trattamento dei NETs digestivi a basso grado di malignità, con buona differenziazione, un basso indice di proliferazione cellulare e un'alta specifica densità recettoriale *in vivo*. L'aumento della dose dell'analogo sembra determinare un miglior controllo della malattia e della diarrea cronica refrattaria. Nuove opportunità potranno arrivare dalla sintesi di nuovi analoghi sempre più potenti e selettivi. Eur. J. Oncol., 11 (1), 57-64, 2006

Parole chiave: **tumori neuroendocrini, analoghi della somatostatina, diarrea cronica, qualità di vita**

Introduction

Neuroendocrine tumours (NETs) are rare neoplasms with a frequent hormonal component, characterized by a low clinical incidence (almost 1 case/100,000/year)[1]. The more frequent sites are the in digestive tract (70%), and the respiratory tract (25%)[2]; followed in 5% of cases by the skin, the thyroid and the adrenal glands.

The most recent WHO NETs classification[3] totally revised the previous one, finally providing a *dynamic* classification which considers not only the classic anatomo-pathological evaluation parameters (histotype, dimensions, site of the tumour, and grading) but also introduces new immuno-histochemical parameters (cellular proliferation index, number of mitosis, angio-invasiveness) and clinical parameters (hormonal hyperfunction symptoms, presence of metastases), distinguishing well differentiated tumours and carcinomas (with low grades of malignancy) and with good prognosis, from poorly differentiated carcinomas (with high grade of malignancy) characterized by an elevated cellular proliferation index and a bad prognosis.

The increased number of cases observed in recent years justifies the growing attention given by clinicians and pathologists to the improved imaging and laboratory methods, and to the use of new molecules, also with a radio-metabolic approach, with positive benefits for survival and quality of life.

The aim of this retrospective study is to assess objective and symptomatic responses (limited to refractory chronic diarrhoea) after somatostatin analogue treatment and to compare our case history data with that available in the literature.

Patients and methods

Eligibility criteria

Patients were required to have histologically confirmed metastatic NETs. Additional eligibility requirements were: bidimensionally measurable diameters, survival expectancy more than 12 weeks, Eastern Cooperative Oncology Group performance status of less than 2, adequate bone marrow function, and hepatic and renal function. Prior pretreatments with surgery, α-interferon, chemotherapy, and chemo-embolization were allowed.

A period ranging from one week to one month was required in the case of prior treatment with α-interferon, chemotherapy, and chemo-embolization. For surgical treatment, we considered complete enucleation of the primitive lesion or palliative care treatment (biliary and/or digestive by-pass); for the metastatic sites we considered only incomplete debulking.

Pretreatment and on-treatment evaluations

Pretreatment evaluation included complete medical history, physical examination, vital signs, assessment of symptoms, and complete biochemical profile.

All patients had a baseline chest radiograph, a computed tomography scan (CT) of pertinent lesions, and a scintigraphy with ^{111}In-DTPA-D-Phe^{1}-octreotide (OctreoScan®).

Clinical examinations were scheduled at start and after 6 months and included complete physical examination, vital signs, and assessment of symptoms.

At the end of the sixth month period of somatostatin analogue treatment, the patients underwent a CT of pertinent indicator metastatic lesions, in order to highlight the objective and symptomatic responses, where present; our attention was focussed on the refractory chronic diarrhoea as, in our experience, this is the most common symptom (11%) of those characterizing the functioning forms of NETs[4]. The patient indicated a score from 0 to 10 at the beginning and after 6 months' somatostatin analogue treatment.

Treatment

The patients enrolled in this retrospective study were treated with somatostatin analogues in different therapeutic lines.

The molecules used were as follows:
– octreotide subcutis (sc) (range: 1500-2000 μg/*die*);
– octreotide LAR 20 mg (every 14 days);
– octreotide LAR 30 mg (range: from 30 mg/every 14 days, to 30 mg/every 28 days);
– lanreotide PR 60 mg (range: from 60 mg/every 14 days, to 60 mg/every 28 days);
– lanreotide Autogel 120 mg/every 28 days;
– association of octreotide LAR 30 mg/every 14 days with lanreotide PR 60 mg/every 14 days.

Response

Two response categories were assessed: objective and symptomatic. Objective responses were defined according to International Union Against Cancer criteria, and were the following: complete response (CR): complete disappearance of all known disease for a minimum of 1 month; partial response (PR): more than 50% decrease in the product of perpendicular diameters of measurable tumour lesion for at least 1 month; stable disease (SD): tumour size decrease by less than 25% or increased by less than 25%; progressive disease (PD): tumour size increased by more than 25%. Measurements were taken on the largest lesion.

Symptomatic response (limited to refractory chronic diarrhoea) was defined as follows: CR, complete relief of symptoms, and PR, a reduction of at least 50% in both the frequency and intensity of symptoms.

The response was calculated from the beginning of therapy up to 6 months.

Patient characteristics

The Rare Hormonal Tumours Group of the Azienda Ospedaliera Istituti Ospitalieri at Cremona, has studied NETs since 1990. To date it has observed 165 patients with gastroenteropancreatic NETs, 18 with carcinoid tumours, 11 with Merkel cell carcinoma, 6 with pheochromocytoma, 3 with medullary thyroid carcinoma, and over 30 cases of carcinomas with neuroendocrine differentiation of different sites (such as breast, prostate, paraganglions, soft tissue, and paranasal sinus).

Of all the digestive NETs, 57 (34.5%) were treated with somatostatin analogues, and 20 of them were considered eligible for this study. Overall patients characteristics are given in Table 1.

Thirteen patients were male and 7 female, with a mean age of 59 years (range: 20-74 years).

All patients included in the study exhibited advanced disease, and most patients showed progressive disease. Seven patients had endocrine pancreatic tumours (EPT), 5 mid-gut carcinoids, 3 primitive unknown site, and 1 gastric, colonic, rectal, duodenal and mesenteric neuroendocrine carcinoma.

In 19/20 patients the tumour was well differentiated (G_2). In 9/20 patients the cell proliferating index (ki67) was studied and in only one case (1/9) it was found to be

Table 1 - Patient characteristics

Patients enrolled	20
Female/male	7/13
Mean age/ (range)	59 (20-74)
Type of tumour	
• EPT[a]	7
• Mid-gut carcinoid	5
• Primitive unknown site	3
• Gastric carcinoid	1
• Colonic carcinoid	1
• Rectal carcinoid	1
• Duodenal net	1
• Mesenteric net	1
Well differentiated tumour	19/20
Sites of metastases	
• Liver	17
• Lymph-nodes	9
• Lung	2
Previous treatment	
• Surgery (explorative)	16 (6/16)
• Chemotherapy	9
• α-interferon	3
• Chemoembolization	1
• No treatment	1
OctreoScan® positive	16/17
Functioning disease	9/20

[a] Endocrine pancreatic tumour

more than 60%; in the other cases the value of ki67 varied in a range from less than 1% to 5%. All patients had measurable disease.

The sites of metastases were as follows: liver 17, lymph nodes 9, lung 2.

Sixteen patients had received primary surgery (6 only explorative), 9 were pretreated with chemotherapy, 3 with α-interferon, 1 with chemo-embolization, and 1 had no previous treatment. Nine out of 20 patients (45%) were symptomatic (refractory chronic diarrhoea).

Octreotide scintigraphy was positive in 16 out of 17 cases (94%, 3 patients not available).

The patients in the study were divided into two groups: the first group included all 20 patients, while the second group included 9 tumour cases in 7 patients already studied in the first group who, during the follow up, received a modified dose or different molecule type or a diverse formulation of the analogue.

The patients in the second group were as follows: 3 EPTs, 1 mid-gut carcinoid, 1 primitive unknown site, 1 duodenal, and 1 mesenteric neuroendocrine tumour. All tumours were well differentiated with ki67 below 3%. The metastases sites were: 7 liver, 2 lung, and 2 lymph nodes. Octreotide scintigraphy was positive in 6/6 patients (100%, 1 patient not available). Five patients received primary surgery (2 explorative), 4 chemotherapy, 1 α-interferon, and 1 no previous treatment. Two out of 7 patients were symptomatic (28.5%). The median age was 62 years.

Therefore, the full study considered a total of 29 cases of patients with digestive NETs treated with different somatostatin analogue molecules, with diverse formulations and dosage.

Results

In the first group we separated patients treated with octreotide sc at the dose of 1500 μg/*die* (13 patients), from patients treated with long acting analogues: octreotide LAR 30 mg/every 28 days (5 patients); lanreotide PR 60 mg/every 14 days (1 patient), and octreotide LAR 30 mg in association with lanreotide PR 60 mg/every 14 days (1 patient).

In the second group, 9 tumour cases (7 patients) were distributed in the following way: 1 patient treated with lanreotide PR 60 mg/every 28 days, 1 patient treated with octreotide LAR 20/every 14 days, 1 patient treated with octreotide sc with 2000 μg/*die*, 1 patient treated with octreotide LAR 30 mg/every 14 days, 1 patients treated with lanreotide Autogel 120 mg/every 28 days, 1 patients treated with the association of octreotide LAR 30 mg and lanreotide PR 60 mg every 14 days, and 3 patients treated with octreotide LAR 30 mg/every 28 days. These were patients for whom we adopted an increased dose of the current analogue therapy (3), or a shift from the sc formulation to LAR (4), or a different analogue type (1), or an association of the two different analogues (1).

While recognizing a different pharmacokinetic profile in the two analogues used[5] and different clearance[6], we decided to consider the second group as a whole in order to avoid impediment of recognition of statistically significant data due to data dispersion.

In both groups of patients we measured the product of perpendicular diameters of the major metastatic lesion at the start of the treatment and after 6 months. In the same way, we scored the different number of stools at the start and after 6 months of treatment with somatostatin analogues.

First group

In the first group, in 13 patients with measurable disease and treated with octreotide sc 1500 μg/*die*, 2/13 (15.3%) achieved a PR, 8/13 (61.5%) had no change (SD), and 3/13 (23%) had PD. Of 5 patients treated with octreotide LAR 30 mg/every 28 days, 4/5 (80%) had no change (SD), 1/5 (20%) had PD. In particular, one patient treated with lanreotide PR 60 mg/every 14 days had PR, while the only one treated with the association of analogues achieved CR. Overall objective response was CR 5% (1/20), PR 15% (3/20), SD 60% (12/20), and PD 20% (4/20).

In relation to control of symptoms, 8/20 (40%) had functioning tumours with refractory chronic diarrhoea. Of the 8 patients with a measurable number of stools, 5 were treated with octreotide sc, and 3 with octreotide LAR 30 mg: 4/5 (80%) treated with octreotide sc achieved a PR, and 1/5 (20%) had no change (SD), whereas 3/3 patients (100%) treated with octreotide LAR 30 mg had CR. In one patient with one carcinoid of the right colon, treated with octreotide sc, the progression of disease coincided with the appearance of diarrhoea not present at the start of the therapy. Applying the Student test a difference is encountered, albeit of low significance, in refractory chronic diarrhoea control with octreotide LAR 30 mg *vs* octreotide sc ($p=0.087$ *vs* $p=0.163$).

Second group

In the second group, among 9 cases with measurable disease, 6/9 (66.6%) had no change (SD), and 3/9 (33.3%) had PD. Regarding the control of refractory chronic diarrhoea, only 2 patients had a functioning tumour with a 100% response (Table 2).

Table 2 - Evaluation of response

	Objective		Symptomatic response	
	N.	%	N.	%
First group				
Assessable	20		8	
CR[a]	1	5	3	37.5
PR[b]	3	15	4	5.0
SD[c]	12	60	1	12.5
PD[d]	4	20		
Second group				
Assessable	9		2	
CR	-		2	100.0
PR	-		-	
SD	6	66.6	-	
PD	3	33.3	-	

[a] Complete response
[b] Partial response
[c] Stable disease
[d] Progressive disease

Discussion

Somatostatin analogues have, without doubt, written an important chapter in the treatment of neuroendocrine tumours. Not only have they stimulated clinical interest in a little known neoplastic pathology, they have also permitted, in many cases, a change in the approach to the disease, often delivering, in the case of advanced forms, an equilibrium between the interlocutory position of oncologists and the aggressive position of surgeons, producing improved patient quality of life.

Somatostatin analogues are highly effective in the control of clinical symptoms in patients with functioning NETs. Inhibition of tumour growth or even decrease in tumour size have also been reported[7-15]. With the use of octreotide, partial responses are not encouraging (0-31%), yet the observation of a high percentage of stabilization of the disease (15-63%) authorizes us to think that octreotide may modify cellular growth progress and may thus be indicated in metastatic patients with neuroendocrine tumours with a low grade of malignancy [2, 16-19].

It is also interesting to note that the best partial response rate (31%) is referable to high dose octreotide treament (1,500-6,000 μg/*die*) (Table 3).

The heterogeneity of the cases recruited in the multi-centre studies (mid-gut carcinoid, endocrine tumour of the pancreas, Merkel cell carcinoma, medullary thyroid carcinoma, pheochromocytoma), the different hormonal state (functioning/non functioning tumours), the often unknown receptorial state *in vivo*, the ample variability of the octreotide dosages (50-6,000 μg/*die*) and, finally, its use in different lines can put the objectivity of the data collated in doubt.

Lanreotide activity has been evaluated in numerous studies. With daily lanreotide doses ranging from 2,250 to 15,000 μg, and monthly lanreotide PR from 10 to 30 mg, a total of 431 patients have been treated[8, 20-33] (Table 4).

We currently observe a certain superimposition of lanreotide and octreotide, especially in symptom control and in the biological response. Significant objective

Table 3 - Antiproliferative activity of octreotide (50-6000 μg/*die*) in the scientific literature

Author	Year	Patients	PR (%)[a]	SD (%)[b]	PD (%)[c]
Gorden[7]	1989	94	13	63	24
Kvols *et al*[14]	1989	66	17	-	-
Eriksson *et al*[15]	1990	14	28.5 (16-21 months)	-	-
Öberg *et al*[13]	1991	22	9	-	-
Arnold *et al*[12]	1992	68	4.4	50	45
Anthony *et al*[8]	1993	13	31	15	54
Saltz *et al*[9]	1993	34	-	50 (0-27 months)	-
Arnold *et al*[10]	1994	47	-	40	-
Di Bartolomeo *et al*[11]	1996	58	3	43 (> 6 months)	-
Total		416	11.7	43.5	41

[a] Partial response
[b] Stable disease
[c] Progressive disease

Table 4 - Somatostatin analogues: objective responses (882 patients)

	Octreotide	Lanreotide	Lanreotide 10	Lanreotide 30	Lanreotide 30	RC-160
Dose	50-6000 μg/*die*	2250-15000 μg/*die*	10 mg x 3/month	30 mg x 2/month	30 mg x 3/month	1.5 mg (continuous infusion)
Patients	416	62	10	341	18	35
PR[a]	0-31%	14.1%	-	6.9%	-	-
SD[b]	40-63%	47%	90%	45%	77.7%	68%
PD[c]	24-54%	52.4%	-	39.1%	-	24%

[a] Partial response
[b] Stable disease
[c] Progressive disease

responses were observed with high doses of lanreotide[30] with incremented apoptosis associated with stabilization of the disease[27,34-35]. As in the case of octreotide, lanreotide treatment determines a global improvement in the quality of life, influenced by optimal diarrhoea control and a secondary gain in night time rest[25,26].

The introduction of lanreotide 60 to therapy has delivered an unquestionable benefit to patients in terms of compliance, with data relative to symptom control and to the disease in comparison to earlier formulations[36].

More recently, the synthesis of the Autogel® 120 preparation created a basis for a new formulation of the drug, more potent and with different pharmacokinetic characteristics[37,38].

In our survey, the PR percentage in the first group (15%) and the SD percentages in both the first (60%) and second groups (66.6%) confirm the data in the literature. Of the 3 cases in the first group with PR, 2 were observed after 6 months' treatment with octreotide sc (1 mid-gut carcinoid tumour with low grade of malignancy treated with surgery and chemo-embolization, and 1 duodenal metastatic low grade gastrinoma treated with surgery); the response was complete (CR) after therapy with octreotide LAR 30 mg/every 28 days for both patients (the first, after 3 years of treatment, and the second after 4 years). The third PR in the first group was observed after 6 months of treatment with lanreotide PR 60 mg/every 14 days (a low grade metastatic EPT not previously treated). The only CR in this study refers to a case of metastatic mid-gut carcinoid tumour treated surgically and previously with chemotherapy, α-interferon, and association of the two analogues for 6 months. The latter two cases confirm the data in the literature where the best objective responses were observed with increased doses of somatostatin analogues, suggesting a dose-dependent function for somatostatin analogues in the treatment of NETs.

In the second group too, despite the absence of PR, we emphasise that in 3 cases SD was achieved with increased analogue dosage: 1 metastatic EPT treated with octreotide LAR 20 mg/every 14 days, 1 primitive unknown metastatic tumour treated with octreotide LAR 30 mg/every 14 days, and 1 metastatic pancreatic GRFoma treated with octreotide 2000 μg sc/*die*. In the latter case, at a dosage of 1500 μg sc/*die* an important objective control of the disease after 6 months of treatment was observed, with reduction in the diameters of the primitive lesion from 7x3 to 2.7 cm, and after 6 months treatment with 2000 μg sc/*die* from 5x3.6 to 4x3 cm. The patient continued to refuse surgical treatment, and after 8 years of chronic hormonal treatment the disease spontaneously progressed[39].

By contrast, the only patient treated with the association of analogues progressed after 6 months. But it is very important to note that this patient had been in chronic treatment with analogues since 1993: he had SD with octreotide sc (first group), and with octreotide LAR (second group) before shifting to PD, despite supplementing with other therapies, such as chemotherapy and immunotherapy.

The disease control observed in both groups confirms the rôle of OctreoScan® as an effective prognostic indicator, as our survey indicates that benefits from somatostatin analogue treatment are delivered principally in metastatic NETs with a low grade of malignancy and with elevated receptorial density *in vivo*, as also reported in the literature[2,17]. Some new elements are provided by a recent consensus by Öberg *et al*[40], which confirms the unquestionable rôle of OctreoScan® as an indispensable method in the selection of patients for somatostatin analogue treatment. The treatment of patients with non functioning disease (also metastatic) remains controversial, as does adjuvant or precautionary therapy after surgical debulking, radio-frequency or embolization, all the more in the absence of residual disease[40].

In our study also, the elevated SD percentage in the two groups (60% and 66.6%) confirms that somatostatin

analogues deliver better responses in tumours with low grades of malignancy, represented in 95% of cases in the first group (19/20) and 100% in the second group. In our study, OctreoScan® presented a very low density of receptors in the only case with scarce cell differentiation (G_3), and with ki67 more than 60%; the patient went at once to PD before the end of the study.

In relation to symptom control, somatostatin analogue treatment showed a good control of diarrhoea as confirmed in the literature[41, 42]. Diarrhoea control thus assumes a determinant importance as it improves patients' quality of life, considered as improvement of fatigue, sleeping problems, and general health[25].

Conclusions

In conclusion, somatostatin analogues play a rôle in the treatment of NETs with a low grade of malignancy, with good differentiation, a low cellular proliferation index and OctreoScan® with high receptorial density *in vivo*.

An increased analogue dose appears to determine better control of the disease and of the chronic diarrhoea.

Hormonal symptom control, and, in particular, that of chronic diarrhoea, delivers improved quality of life in the patients studied. New opportunities may derive from SOM 230 experimentation[43], from the synthesis of new selective BIMs and of other peptidomimetic analogues still under evaluation by researchers[44]. Finally, studies in receptorial homo/heterodimerization[45] and in the synthesis of molecular hybrids[46] may produce new opportunities for better hormonal regulation and better receptorial affinity with favourable effects on treatment of NETs.

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World Cancer Day - Saturday, 4 February 2006
Giornata Mondiale sul Cancro - Sabato, 4 Febbraio 2006

New report on childhood cancer shows early detection can save thousands of children's lives

The rôle of the International Union Against Cancer

The International Union Against Cancer (UICC), a Geneva-based NGO, launched a new report, *Childhood Cancer: Rising to the Challenge*, on World Cancer Day. Findings from the report show that childhood cancer is the second highest cause of death in children and more than 160,000 children are newly diagnosed with cancer each year[1,a]. In developing countries roughly 60% of children with cancer still die of their disease, as opposed to 25% in the developed world. But significant advances in diagnosis and therapy during the past four decades mean that childhood cancer can largely be cured if detected early. That is why UICC and its cancer-fighting organisations have dedicated this year's **World Cancer Day** to childhood cancer and the importance of early detection and equal access to treatment.

The importance of early detection

"Too many children are unnecessarily dying each year, since they are never diagnosed or diagnosed too late. Knowing the common signs and symptoms of childhood cancer is one of the most important steps in fighting this disease and saving thousands of children's lives each year", says Isabel Mortara, Executive Director, UICC.

The prevalence and types of childhood cancer differ between populations and ages, however in around 85 per cent of all cases, one or more of the following symptoms are usually present[2]:

- Continued, unexplained weight loss and fever
- Pallor
- Headaches, often with early-morning vomiting
- Unusual swelling or abdominal mass
- Swollen head
- Development of excessive bruising or bleeding
- Sudden changes in balance or behaviour
- White glow in the eye

"Most of the symptoms of cancer can be interpreted as common childhood ailments", says Dr John Seffrin, President, UICC. "It is therefore extremely important for parents to take their child to a physician for further investigation, if any of these symptoms appear. Parents must insist that, where possible, physicians carry out tests to rule out cancer".

UICC and its members around the world ran World Cancer Day under the slogan "My Child Matters". Members are organising a wide range of activities and fundraising events to educate parents about early detection and to join together to celebrate the lives of children who are fighting against the disease.

On World Cancer Day, UICC launched 14 projects in 10 low- and middle-income countries to help improve

[a] The exact number of new cases is not known because in many countries not all children with cancer are registered.

early detection, treatment, care and support of children with cancer. The chosen countries are Bangladesh, Egypt, Honduras, Morocco, Philippines, Senegal, Tanzania, Ukraine, Venezuela and Vietnam. "Across the world, we need to ensure that the survival rates of childhood cancer are increased. In developing countries, where over 80 per cent of children with cancer live and survival rates are lowest, governments have limited funding for health projects. This is why UICC is leading this initiative to help save children's lives. These projects will help communicate the message that childhood cancer can be treated and is often curable", says Dr Franco Cavalli, Chair of the UICC Childhood Cancer Campaign Advisory Committee[b].

Cancer in children

- 'Childhood cancer' refers to all cancers in children aged 14 and under.
- It is estimated that more than 160,000 children around the world are diagnosed with cancer each year and this number may be considerably higher. Information on the occurrence of childhood cancer in developing countries is largely inadequate. More population-based cancer registries are needed to measure the real number of children with cancer.
- Eighty per cent of all children with cancer live in developing countries, and this proportion will grow as infectious diseases are eliminated.
- In developed countries, three in four children with cancer survive at least five year after diagnosis. In developing countries, more than one in two children diagnosed with cancer is likely to die; late diagnosis and limited access to effective cancer therapy result in only a small percentage of patients receiving the life-saving medical treatment they need.

Common childhood cancers[3]

Leukaemia

– Leukaemia is a disease of the blood cells and represents almost a third of all childhood cancers in Europe, America and East Asia.
– The predominant type occurring in children is acute lymphoblastic leukaemia that affects the lymphocyte producing cells in the bone marrow. This is the single most common cancer in children in Caucasian populations.

Lymphomas

– Lymphomas are tumours of the lymphatic tissues and are the third most common cancer of children in developed countries, and in some children in Africa and Oceania.
– Hodgkin's lymphoma is more common in developed countries whereas Burkitt's lymphoma, associated with malaria and infection from the Epstein Barr virus, accounts for half of all childhood lymphomas in African countries.

Central nervous system tumours

– These tumours occur in the brain and spinal cord and are the second most common form of childhood cancer in developed countries. They are much less often diagnosed in many developing countries, partly due to lack of advanced diagnostic techniques.

Neuroblastoma

– Neuroblastoma is a malignant disease of the sympathetic nervous tissue, originating in adrenal medulla or other sites.
– This form of cancer occurs frequently in infants and very young children in developed countries.

Retinoblastoma

– Retinoblastoma is cancer of the retina, the nervous tissue of the eye. About half of all cases are inherited.
– Its incidence peaks in the first year of life; it is extremely uncommon in children aged ten and over.
– In Europe, North America and Australia, retinoblastoma accounts for up to four per cent of all tumours in children. In African populations, the malignancy may represent 10 to 15 per cent.

Renal cancer

– The most common renal cancer in childhood is Wilms' tumour (95 per cent), occurring mostly in children under five years of age.
– In most populations in Europe, Australia and Caucasian Americans, Wilms' tumour represents up to six per cent of all cancers diagnosed in children. In contrast, among black populations in North America and in Africa, its proportion is around 10 per cent.

Bone tumours

– Bone tumours arise in various types of cells of bone tissue and include osteosarcoma (50%), chondrosarcoma, Ewing's sarcoma (35%) and others. They represent about three to five per cent of childhood cancers.

[b] Funding of these projects is made possible by sanofi-aventis, with additional support from the National Cancer Institute, USA.

– The occurrence of osteosarcoma and Ewing's sarcoma differ between ethnic groups.

Soft tissue sarcomas

– Rhabdomyosarcoma is a cancer arising in cells that normally develop into skeletal muscles of the body. It is a typical soft tissue sarcoma of childhood, with two thirds of cases developing before the age of ten
– Kaposi's sarcoma is caused by the herpes virus, in which cancerous cells and abnormally grown blood vessels form solid lesions in connective tissue. Since the early 1980s, an aggressive form of Kaposi's sarcoma has risen dramatically in the African countries affected by the HIV/AIDS epidemic. Kaposi's sarcoma may represent half of all cancers in children in these countries with around 70 new cases per million children per year. This tumour is rare in other populations.

For further information

Childhood Cancer: Rising to the challenge is published by the UICC in the framework of the World Cancer Campaign. For more information on the campaign, visit our website (www.uicc.org) or contact Jose Julio Divino, World Cancer Campaign Coordinator, at wcc@uicc.org

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EAPCCT

European Association of Poisons Centres and Clinical Toxicologists

XXVI International Congress

Wednesday 19 to Saturday 22 April 2006
Prague, Czech Republic

Co-sponsored by the American Academy of Clinical Toxicology

A view of Prague Castle and the Vltava River

Venue and date
The XXVI Congress of the European Association of Poisons Centres and Clinical Toxicologists will be held at the Hilton Hotel from Wednesday 19th April to Saturday 22nd April 2006.

Information: EAPCCT website - www.eapcct.org

The EAPCCT XXVI International Congress will be held in the picturesque and historic city of Prague, the capital of the Czech Republic. The Congress will take place at the Hilton Hotel, which offers meeting facilities of the quality that our delegates expect. The Hilton is a magnificent, modern glass building located close to the Vltava river, and is about 15 minutes walking distance from Prague's Old Town, Wenceslas Square and major attractions. There is a metro station, Florenc (lines B and C), close by for those wishing to use public transport to reach the centre. Prague is very accessible by air, with most carriers flying to Ruzyne Airport, which is some 20 km northwest of the city. The taxi journey to the hotel takes about 20-25 minutes. There are also buses that link the airport to the city centre. Prague has excellent road connections.

This will be the sixth Congress organised both academically and administratively by the EAPCCT Scientific and Meetings Committee. This will ensure that delegates benefit maximally from the Congress. As in past years the Congress will be co-sponsored by the American Academy of Clinical Toxicology, which will also accredit the Congress for continuing medical education. Selected abstracts from this meeting will be published in future issues of the Journal of Toxicology – Clinical Toxicology, the official Journal of both the American Academy of Clinical Toxicology and the European Association of Poisons Centres and Clinical Toxicologists. As in past years EAPCCT members have chosen the principal themes of the academic programme. The Congress main theme is *Evidence-based Toxicology,* and part of the programme will include symposia on Controversies in the management of poisoning, Poisoning in special patient groups, Education in Clinical Toxicology and Forensic, medico-legal and ethical aspects of poisoning.

At the XXVI International Congress in Prague we hope to offer you an outstanding academic programme, balanced with the usual social events in a beautiful and vibrant city. Of course your participation will guarantee the quality of our programme and in return you will be stimulated by four days of the finest continuing education in clinical toxicology. So, we look forward to seeing you in Prague, and hope that you will take the opportunity to visit the Czech Republic and its capital, and sample their many delights in the days either side of the meeting.

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