EUROPEAN JOURNAL OF ONCOLOGY
GIORNALE EUROPEO DI ONCOLOGIA

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

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

Associato all'Unione Stampa Periodica Italiana

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

Publisher
Mattioli 1885 - Spa
Casa Editrice

Autorizzazione del Tribunale di Parma n. 14/97 del 11/6/1997 - ISSN 1128-6598

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

EUROPEAN JOURNAL OF ONCOLOGY
GIORNALE EUROPEO DI ONCOLOGIA

CONTENTS/INDICE

VOLUME 11 - N. 2 - 2006

Eur. J. Oncol., vol. 11, n. 2, pp. 85-88, 2006

Editorial/Editoriale

Asbestos is not banned in North America

L'amianto non è stato messo al bando nel Nord America

Barry I. Castleman
Environmental Consultant, Garrett Park, MD, USA

Summary

Around the world, local asbestos industries oppose asbestos bans by pointing out that asbestos is not banned in the US. The US Environmental Protection Agency ban rule was overturned in a court challenge in 1991, and legislation to ban asbestos in the US has been proposed but not yet been enacted. US asbestos use has nonetheless continued to plummet by over 90% in the years since the ban was overturned, driven by public concerns, liability considerations, and government regulations. The US continues to import asbestos products that have not been manufactured in the US for many years. Canada exports almost all of the asbestos from its mines, almost all of it to poorer countries, as Canadian scientists travel the world promoting asbestos and the Canadian government sponsors propaganda seminars in market countries. Only 11 small countries that are not asbestos-exporting countries and those countries where asbestos has been banned have ratified the International Labour Organization Asbestos Convention providing for minimal basic safeguards in asbestos use. Public health workers seeking to widen the number of national asbestos bans (from about 40 countries so far) consider Canada and the US to be high priorities, despite the minimal market for asbestos products in these countries. Eur. J. Oncol., 11 (2), 85-88, 2006

Key words: **asbestos, ban, US, Canada**

Riassunto

In tutto il mondo le industrie locali dell'amianto si oppongono alla sua messa al bando, facendo notare che l'amianto non è stato messo al bando negli USA. La regolamentazione dell'Environmental Protection Agency degli USA, che lo metteva al bando, è stata abrogata a seguito di una contestazione legale nel 1991, e la legislazione per mettere al bando l'amianto negli USA è stata proposta ma non ancora promulgata. Tuttavia l'uso dell'amianto negli USA ha continuato a ridursi drasticamente di oltre il 90% dagli anni in cui il bando è stato contestato, sulla spinta della preoccupazione pubblica, di valutazioni di responsabilità civili e penali e di regolamenti governativi. Gli USA continuano ad importare prodotti contenenti amianto che non sono più stati fabbricati negli USA da molti anni. Il Canada esporta quasi tutto l'amianto delle sue miniere, e quasi tutto nei paesi più poveri, dato che gli scienziati canadesi girano il mondo per pubblicizzare l'amianto ed il governo canadese sponsorizza seminari di propaganda nei paesi in cui è venduto. Solo 11 piccoli Paesi che non sono esportatori di amianto ed i Paesi in cui l'amianto è stato messo al bando hanno ratificato la Convenzione sull'Amianto dell'International Labour Organization che fornisce criteri minimi di sicurezza nell'uso dell'amianto. Gli operatori di sanità pubblica, che cercano di allargare il numero delle messe al bando dell'amianto a livello nazionale (che finora riguardano circa 40 Paesi), considerano il Canada e gli USA delle priorità assolute, nonostante il ridotto mercato dei prodotti contenenti amianto in questi Paesi. Eur. J. Oncol., 11 (2), 85-88, 2006

Parole chiave: **amianto, messa al bando, USA, Canada**

Received/Pervenuto 7.3.2006 - Accepted/Accettato 16.3.2006
Address/Indirizzo: Dr. Barry I. Castleman, PO Box 188, Garrett Park, MD 20896, USA - E-mail: bcastleman@earthlink.net

Around the world, when public health workers call for national bans on asbestos, one of the things they hear from the local asbestos industry is that the US has not banned asbestos. The US Environmental Protection Agency (EPA) issued regulations to phase out the use of almost all asbestos products in 1989, and these rules were overturned in a court challenge in 1991. Industry spokesmen accordingly emphasize that the sale of almost all asbestos products is still allowed in the US and point to the existence of a 1986 International Labour Organization (ILO) Convention on Asbestos to assert that there are international standards in effect for "controlled use" of asbestos[1].

The decision in 1991 of the US Court of Appeals in New Orleans criticized the EPA for not identifying all the substitute products that could replace the asbestos products and for not evaluating their toxicity, in order to justify the ban[2]. The EPA wanted to appeal against this ruling to the Supreme Court and asked the US Department of Justice to take on the appeal. After the Justice Department refused, the EPA asked Justice to reconsider and was turned down again. The EPA had to settle for issuing a statement criticizing the court for "*significant legal errors*" in interpreting the law and substituting its judgement for that of the EPA in balancing the costs and benefits of asbestos products banned under the rule[3].

When the EPA issued its asbestos ban rule, the companies that had constituted the US asbestos industry were beset by many thousands of personal injury lawsuits, based on their long-term failure to warn product users that there were lethal, non-obvious hazards from the dust created when these products were used. The US industry was acutely aware that if substitute products were anywhere near as dangerous as asbestos, the manufacturers would end up facing new liabilities, dealing with adverse media reports and facing government regulations. But the court – by setting the requirement that EPA, in effect, look into a crystal ball and predict the future breakdown of substitute usage that would follow an asbestos ban, and then do a risk analysis on all of these substitute products – set an impossible burden for the EPA in banning asbestos products. EPA has not banned any substance for any use since 1991 under the provision of the law used for the asbestos ban.

Sweden and other European countries led in forcing technological advance in the replacement of asbestos in vehicle brakes, the last major application in which it could be claimed that asbestos use was essential. The EPA tried in 1992 to get car manufacturers to voluntarily agree to stop using asbestos in vehicle brakes, gaskets, etc, and seemed to be successful at first. General Motors, for example, wrote that it would honour the deadlines for the elimination of asbestos in various products that were contained in the overturned EPA regulation: that would have ended the sale of asbestos in some vehicle friction products and gaskets in 1994 and the rest in 1997. The asbestos industry then charged that the proposed agreement of the automobile manufacturers would be illegal and, as a consequence, the EPA's effort to get auto makers' voluntary agreement collapsed. In 1998, General Motors was still selling asbestos brakes on two models of new cars in the US, even though all its sales of new cars and replacement parts in major European countries were by then required to be asbestos-free[4].

At that time, I realized that the US classification of imported "asbestos" products allowed non-asbestos products to be counted in the same categories. My request to the US International Trade Commission[a] to separate these commodity classifications into asbestos and non-asbestos product categories was turned down, and to this day the extent to which the US imports most asbestos products is not clearly evident from import statistics. Trends of importation from asbestos-using countries are nonetheless apparent.

Worldwide imports of brake linings and pads for cars and trucks composed "*of asbestos and other minerals*" went from US $59 million in 1996 to US $110 million in 2005. Importation of these products from Brazil, China, Colombia, and Mexico went up in value from US $23 million to US $76 million between 2000 and 2005.

The US imported 60 million kg of "*asbestos and cellulose-based cement sheet, panel*" from Mexico in 2005, triple the quantity in 2000, accounting for about two-thirds of worldwide imports of this commodity. Mexico also supplied all US imports of "*asbestos yarn and thread*", over 99,000 kg, in 2005 (about doubled since these imports began in 2002). If such imports are allowed to continue, the US government should examine Customs information on importers and exporters to identify the importation of particular asbestos products and see how they are used in the US. These asbestos products have not been made in the US for many years.

Consumption of asbestos fibres for manufacturing in the US has gone steadily down. Worker and public concern, insurers' aversion, and costs imposed by EPA and Occupational Safety and Health Administration (OSHA) regulations for asbestos have combined to all but end the use of asbestos in manufacturing in the US.

[a] US import statistics from US International Trade Commission are available at: http://dataweb.usitc.gov

The country's use of asbestos, mainly in asphalt roofing shingles, was 2,500 metric tons (m.t.) in 2005, down from 803,000 m.t. in the peak year of 1973 and 35,000 m.t. in 1991. It is ridiculous for the US to continue to allow the importation of asbestos products no longer even made in the US.

The current toll from historic asbestos use in the US is estimated at 10,000 deaths per year[5]. Legislation debated in the US Senate, to close the courts to asbestos victims in exchange for a $140 billion, industry-financed, government-run trust fund, failed to be adopted in February 2006. A major concern was that the trust fund would run short and become a burden on the taxpayers. Asbestos litigation had cost US manufacturers and insurers US $70 billion by the end of 2002.

With such experience, you might think the US would be ready to join such countries as Argentina, Chile, Gabon, Honduras, Japan, Kuwait, Saudi Arabia, and Uruguay, and all 25 countries of the European Union that have banned asbestos.

In 2002, Senator Patty Murray and others introduced the "Ban Asbestos in America Act". This would accomplish what EPA was unable to, and it would initiate additional efforts to examine the usage of other minerals that may be contaminated with asbestos (e.g., talc, vermiculite, and stone used in construction). Unfortunately, this legislation has not been brought to a vote in the Senate.

Canada, like the US, uses very little asbestos in domestic manufacturing. Canada's asbestos mines export virtually all of their output to poorer countries. Many of the perennial defenders of chrysotile asbestos on the global scene today are Canadian scientists, who carry on the tradition started in the 1960s by spokesmen for multinational asbestos corporations. But they would be less effective as globe-trotting asbestos industry propagandists, featured in news reports with titles like "*Asbestos cement products are absolutely safe*"[6], if Canada banned asbestos. Canada's continuing efforts to promote asbestos included a seminar this January, co-sponsored by the Canadian Embassy in Jakarta and the Fibre Cement Manufacturers Association of Indonesia. At this event, despite his expressed willingness to participate, the world-renowned authority on asbestos, Dr. Douglas Henderson of Australia, was excluded from the programme.

When Canada unsuccessfully challenged France's asbestos ban at the World Trade Organization in 1999, the former country was the world's largest exporter of asbestos and the second largest producer[7]. By 2003, Canada was no longer among the three largest asbestos producing countries. Canada's asbestos mines now employ only hundreds of workers, yielding an annual output of over 200 m.t. for each miner. It has been estimated that, for every 170 m.t. of asbestos mined and consumed in the world, one person has contracted mesothelioma[8]. Noting the proportionality between asbestos-caused mesotheliomas and lung cancers in the epidemiology studies, the mesothelioma mortality can be used to project the added cases of lung cancer resulting from the same usage of asbestos.

Even taking the conservative estimate that there is one death from lung cancer for every death from mesothelioma caused by asbestos, the toll *for every year* that a Canadian asbestos miner mines asbestos is at least two lives in the asbestos-importing countries (and Canada).

The remaining markets in Asia, Africa, and Latin America are rapidly being taken over by competitors in Russia, Kazakhstan, Zimbabwe, and Brazil. The competition will close the Quebec mines before long, if Canada does not ban asbestos and pension off the miners first (as recommended by Irving J. Selikoff over 25 years ago). Though most business lost by Canada may be picked up by others, Canada's withdrawal as an advocate for asbestos use on the world stage would make a major difference[9]. In recent years, there have been organized efforts to get asbestos banned in Canada by unionists, environmentalists, asbestos victims, public health scientists, and doctors.

If Canada limited its exports to countries that have ratified ILO Convention 162 on safeguards for the use of asbestos[b], the asbestos mines would probably have to close. Only 11 countries that still permit the use of asbestos and are not asbestos-exporting countries themselves have ratified this convention; and none of these mostly small countries are in Asia. Actual conformity with the terms of the ILO convention to protect workers would make the prices of asbestos products far less competitive, and such measures are probably not achieved even in most of the asbestos-consuming countries that have ratified the ILO convention. The profitability of the asbestos business depends on avoiding the costs of prevention and compensation of occupational disease.

Bans of asbestos in the US and Canada would have great symbolic, political, and public health value outside North America, even though the market for asbestos products in each of these countries is only a minuscule part of the global asbestos trade.

[b] Information on ILO's Asbestos Convention #162 can be found at http://www.ilo.org/ilolex/english/convdisp2.htm

References

1. Range J. Globally asbestos-free automobiles. Int J Occup Environ Health 1999; 5: 242.
2. Leary WE. Appeals court strikes down major parts of federal asbestos ban. New York Times, October 22, 1991.
3. Fisher L. US EPA, letter to Z. Anavi, Israel Ministry of Environment, August 17, 1992.
4. Castleman BI. Globally asbestos-free automobiles. Int J Occup Environ Health 1998; 4: 277-8.
5. Environmental Working Group. Asbestos: Think Again 2004. www.ewg.org/reports/asbestos
6. Surjit E. Asbestos cement products are absolutely safe. Times of India, September 13, 2004.
7. Castleman BI. WTO confidential: the case of asbestos. Int J Health Serv 2002; 32: 489-501.
8. Tossavainen A. Global use of asbestos and the incidence of mesothelioma. Int J Occup Environ Health 2004; 10: 22-5. http://www.ijoeh.com/pfds/1001_Tossavainen.pdf
9. Castleman BI. Controversies at international organizations over asbestos industry influence. Int J Health Serv 2001; 31: 193-201.

Ecuadorian Project
International Conference
Occupational and Environmental Health: Emergencies in Developing Countries

Quito Declaration
(March, 2006)

We, as participants in the International Conference "Occupational and Environmental Health: Emergencies in Developing Countries", held between the 6th and 10th of March, 2006, in Quito, under the aegis of the Collegium Ramazzini, an international Academy of experts in the field of occupational and environmental medicine, and the IFA Corporation, a non Governmental Organization from Ecuador, after learning of the advances attained and the topics requiring attention related to Occupational and Environmental Health Sciences and their consequences on health, and after the deliberations of sectorial working groups on Occupational and Environmental Epidemiology, Ethics and the Precautionary Principle, Reproductive Health, Chronic Effects of Pesticides, Occupational and Environmental Cancer, Children's Health and their Environment, the Construction Industry and Health, Heavy Metals and Industrial Toxicology; and, determined to improve health and life conditions of the Ecuadorian people.

Declare, on behalf of all the Latin American Nations present:

1) Our interest and willingness to work together in order to get "Occupational and Environmental Health" to be considered a priority in the National Public Agenda, to reach equity in health for the general population and particularly for workers, looking for a better social and economical development in the country.

2) Our endorsement of the conclusions and agreements reached in this Conference, for their establishment as a State Policy to guarantee its application and validity.

3) Our exhortation and request to the Authorities of the National Government to support and update the existing legislation in Occupational and Environmental Health.

4) Our demand for an adequate budget assignation for public institutions responsible for these issues (such as the Ministries of Public Health, Labour, Environment, Agriculture and Livestock, and the Municipalities) to be able to work on Occupational and Environmental Health matters, and, in this way, fulfil the pertinent topics in the Millennium Objectives signed by 189 Chiefs of State, of which Ecuador is also signatory.

5) Our support for the participation of sectional organisms in the Area of Occupational and Environmental Health, under the supervision of pertinent National Authorities.

6) Our exhortation to competent Governmental Institutions, to develop actions that strengthen Occupational and Environmental Health and their participation in different instances of management and social control.

7) Our demand for social solidarity from the private sector through financial support to improve occupational and environmental health.

8) Our demand for the participation of collective communication media in the diffusion, on the basis of the Right to Information, of National Policies of Occupational and Environmental Health, with the objective of generating a culture for health and life.

9) Our interest to generate alternative systems directed to a good and immediate utilization by policy makers and social actors (employers and workers together with the State) of the scientific discoveries that are made in the area of Occupational and Environmental Health.

10) Our request that the Human Resources training entities give the necessary support to guarantee academic excellence in education in the Occupational and Environmental Health area.

11) Our request for the application of the international agreements in occupational and environmental health.

12) Our demand to the authorities and institutions for the monitoring of the principal pollutants and its official publication.

We confirm with this statement our willingness and commitment to encourage the consensus obtained in the International Conference "Occupational and Environmental Health: Emergencies in Developing Countries" as a primary condition for reaching human development in the country, and hope that this becomes a daily guideline for policies, that will become possible through the active and wide participation of the citizenship and the commitment of all the institutions.

Quito, March 10th, 2006

The Quito Declaration is underwritten by 210 signatures of participants from Ecuador, Nicaragua, El Salvador, Panama, Costa Rica, Venezuela, Colombia, Peru, Bolivia, Chile, Brazil and Argentine and by Fellows of the Collegium Ramazzini from the USA, Italy, Sweden, Denmark, Belgium, Germany, Brazil, Argentina and Ecuador.

For further information on the Quito Declaration, please contact:
Dr. Raul E. Harari Arjona, IFA Corporación para el Medio Ambiente Laboral, Domingo de Brieva N. 38-107 y Villalengua, Urbanizacion Granda Centeno, Casilla de Correo 17-08-8386, Quito, Ecuador
E-mail: ifa@ifa.org.ec

Progetto Ecuadoregno
Conferenza Internazionale
Salute Occupazionale ed Ambientale: le Emergenze nei Paesi in via di Sviluppo

Dichiarazione di Quito
(Marzo 2006)

Noi, partecipanti alla conferenza internazionale "Salute Occupazionale ed Ambientale: Emergenze nei Paesi in via di Sviluppo", tenuta il 6-10 Marzo 2006 a Quito, promossa dal Collegium Ramazzini, un'accademia internazionale di esperti nel campo della medicina del lavoro e ambientale, e dall'IFA Corporación, un'Organizzazione non Governativa ecuadoregna, dopo aver constatato i progressi ottenuti e gli argomenti ancora in discussione relativi alla Salute Occupazionale ed Ambientale e le loro conseguenze per la salute, e dopo le delibere di gruppi di lavoro settoriali su Epidemiologia Occupazionale ed Ambientale, Etica e Principio di Precauzione, Medicina dell'Età Riproduttiva, Effetti Cronici dei Pesticidi, Oncologia Occupazionale ed Ambientale, Salute dei Bambini e loro Ambiente, Industria Edilizia e Salute, Metalli Pesanti e Tossicologia Industriale; e determinati a migliorare le condizioni di salute e di vita della popolazione ecuadoregna.

Dichiariamo da parte di tutte le Nazioni Latino-Americane presenti:

1) Il nostro interesse e volontà a lavorare insieme per far sì che la "Salute Occupazionale ed Ambientale" sia considerata una priorità nelle Agende Pubbliche Nazionali, per raggiungere un'equità in fatto di salute per la popolazione generale ed in particolare per i lavoratori, cercando un migliore sviluppo sociale ed economico nel paese.

2) La nostra approvazione per le conclusioni e gli accordi raggiunti in questa conferenza, per la costituzione di una politica statale che garantisca la sua applicazione e legittimazione.

3) La nostra raccomandazione e richiesta alle Autorità del Governo Nazionale a sostenere e aggiornare la legislazione esistente in tema di Salute Professionale ed Ambientale.

4) La nostra richiesta di assegnazione di finanziamenti adeguati da parte delle istituzioni pubbliche responsabili di questi problemi (come i Ministeri della Sanità, del Lavoro, dell'Ambiente, dell'Agricoltura e Allevamento ed i Comuni) per poter operare su temi occupazionali ed ambientali, ed in questo modo affrontare gli argomenti elencati negli Obiettivi del Millennio firmati da 189 Capi di Stato, di cui anche l'Ecuador è firmatario.

5) Il nostro sostegno alla partecipazione di organismi di parte nell'Area della Salute Occupazionale ed Ambientale, sotto la supervisione delle Autorità Nazionali competenti.

6) La nostra raccomandazione alle Istituzioni Governative competenti a sviluppare azioni che rafforzino la Salute Occupazionale ed Ambientale e la loro partecipazione nei vari contesti di amministrazione e controllo sociale.

7) La nostra richiesta di solidarietà sociale da parte del settore privato mediante sostegno finanziario per migliorare le condizioni di salute occupazionale ed ambientale.

8) La nostra richiesta di partecipazione dei mezzi di comunicazione di massa nella diffusione, sulla base del Diritto all'Informazione, di Politiche Nazionali di Salute Occupazionale ed Ambientale allo scopo di generare una cultura per la salute e per la vita.

9) Il nostro interesse a produrre sistemi alternativi orientati ad un utilizzo corretto ed adeguato da parte di chi prende decisioni politiche e degli operatori sociali (datori di lavoro e lavoratori insieme allo Stato) delle scoperte scientifiche riguardanti il settore della Salute Occupazionale ed Ambientale.

10) La nostra richiesta del necessario supporto, da parte degli organismi che formano le Risorse Umane, per garantire un alto livello accademico nell'istruzione nel settore della Salute Occupazionale ed Ambientale.

11) La nostra richiesta di applicazione degli accordi internazionali in tema di salute occupazionale ed ambientale.

12) La nostra richiesta alle autorità ed istituzioni di monitoraggio dei principali inquinanti e la sua pubblicazione ufficiale.

Riaffermiamo con questa dichiarazione la volontà e l'impegno ad incoraggiare il consenso ottenuto nella Conferenza Internazionale "Salute Occupazionale ed Ambientale: Emergenze nei Paesi in via di Sviluppo" come condizione primaria per realizzare lo sviluppo umano nel Paese e nella speranza che questo diventi un orientamento politico quotidiano, che sarà possibile attraverso l'attiva ed ampia partecipazione dei cittadini e l'impegno di tutte le istituzioni.

Quito, 10 marzo 2006

Seguono 210 firme di partecipanti da Ecuador, Nicaragua, El Salvador, Panama, Costarica, Venezuela, Colombia, Perù, Bolivia, Cile, Brasile ed Argentina, e Soci del Collegium Ramazzini da USA, Italia, Svezia, Danimarca, Belgio, Germania, Brasile, Argentina ed Ecuador.

Per ulteriori informazioni sulla Dichiarazione di Quito, contattare:
Dr. Raul E. Harari Arjona, IFA Corporación para el Medio Ambiente Laboral, Domingo de Brieva N. 38-107 y Villalengua, Urbanizacion Granda Centeno, Casilla de Correo 17-08-8386, Quito, Ecuador
E-mail: ifa@ifa.org.ec

Proyecto Ecuatoriano
Conferencia Internacional
Salud Ocupacional y Ambiental: Emergencias en los Países en Desarrollo

Declaración de Quito
(Marzo, 2006)

Nosotros, como participantes en la Conferencia Internacional "Salud Ocupacional y Ambiental: Emergencias en los Países en Desarrollo", que se llevó a cabo entre el 6 y el 10 de marzo de 2006, en Quito, organizada por el Collegium Ramazzini una academia internacional de expertos en el campo de la medicina ocupacional y medioambiental y Corporación IFA de Ecuador, y después de conocer los avances logrados y los temas pendientes con respecto a la Salud Ocupacional y Ambiental y sus consecuencias en la salud, y luego de las deliberaciones de los diferentes grupos sectoriales sobre Epidemiología Ocupacional y Ambiental, Ética y Principio de Precaución, Salud Reproductiva, Efectos crónicos de los Plaguicidas, Cáncer de Origen Ocupacional y Ambiental, Salud Infantil y Ambiente, Industria de la Construcción y Salud, Metales Pesados, Toxicología Industrial; y determinados en mejorar la salud y las condiciones de vida de la población ecuatoriana,

Declaramos como parte de todas las Naciones de Latinoamérica presentes:

1) Nuestro interés y voluntad de trabajar juntos para lograr que la "Salud Ocupacional y Ambiental" sea considerada una prioridad en la Agenda Pública Nacional, para lograr la equidad en salud para la población en general y particularmente para los trabajadores, en busca de un mejor desarrollo social y económico en el país.

2) Nuestro respaldo a las conclusiones y los acuerdos alcanzados en esta Conferencia, para su establecimiento como una Política de Estado que garantice su aplicación y validez.

3) Nuestra exhortación y demanda a las Autoridades del Gobierno Nacional y de los diferentes países para apoyar y actualizar la legislación existente en Salud Ocupacional y Ambiental.

4) Nuestra demanda por la asignación de presupuestos adecuados para las entidades públicas responsables del tema (Ministerios de Salud Pública, de Trabajo, Ambiente, Energía y Minas, Agricultura y Ganadería, Municipios, entre otros) para poder trabajar en los temas de Salud Ocupacional y Ambiental, y de esta forma cumplir los temas pertinentes en los Objetivos del Milenio firmado por 189 Jefes de Estado, del cual Ecuador es también signatario.

5) Nuestro apoyo a la participación de organismos sectoriales y gobiernos locales en el Área de Salud Ocupacional y Ambiental, bajo la supervisión de las Autoridades Nacionales pertinentes.

6) Nuestra exhortación a las Instituciones Gubernamentales Competentes, a impulsar el desarrollo de acciones que fortalezcan la Salud Ocupacional y Ambiental y su participación en las diferentes instancias de gestión y control social.

7) Nuestra demanda de solidaridad social por parte de las empresas privadas mediante la entrega de recursos financieros para el desarrollo y manejo del tema de salud ocupacional y medio ambiente.

8) Nuestra exigencia de la participación de los medios de comunicación colectivos en la difusión, en base al Derecho a la Información, de las Políticas Nacionales de Salud Ocupacional y Ambiental con el objetivo de generar una cultura para la salud y la vida.

9) Nuestro pedido de generar sistemas alternativos dirigidos hacia una buena e inmediata utilización por parte de los tomadores de decisiones políticas y actores sociales (empleadores y trabajadores) conjuntamente con el Estado, de los descubrimientos científicos que se sucedan en materia de Salud Ocupacional y Ambiental.

10) Nuestra solicitud a las instituciones de formación y entrenamiento de profesionales, del compromiso necesario para garantizar la excelencia académica en la educación y el desarrollo de la investigación

científica y tecnológica en el área de la Salud Ocupacional y Ambiental.

11) Nuestra exigencia del cumplimiento de los acuerdos internacionales oficiales en los temas de control de riesgos laborales y ambientales.

12) Nuestra demanda a las autoridades y organismos de control del monitoreo de los principales agentes contaminantes y la publicación oficial de informes de los seguimientos realizados.

Aseguramos con esta declaración, la voluntad y el compromiso para incentivar el consenso obtenido en la Conferencia Internacional "Salud Ocupacional y Ambiental: Emergencias en los Países en Desarrollo" como una condición primaria para alcanzar el desarrollo humano en el país, y esperamos que se convierta en una guía diaria de políticas, que será posible a través de la activa y amplia participación ciudadana y el compromiso de todas las instituciones.

Quito, 10 de Marzo de 2006

Siguen 210 firmas de asistentes de Ecuador, Nicaragua, El Salvador, Costa Rica, Panamá, Venezuela, Colombia, Perú, Bolivia, Chile, Brasil y Argentina y Miembros del Collegium Ramazzini de USA, Italia, Bélgica, Dinamarca, Suecia, Alemania, Brasil, Argentina y Ecuador.

Para ulterior información sobre la Declaración de Quito, ponerse en contacto con:
Dr. Raul E. Harari Arjona, IFA Corporación para el Medio Ambiente Laboral, Domingo de Brieva N. 38-107 y Villalengua, Urbanizacion Granda Centeno, Casilla de Correo 17-08-8386, Quito, Ecuador
E-mail: ifa@ifa.org.ec

Eur. J. Oncol., vol. 11, n. 2, pp. 95-102, 2006

Aspects of the fatal malignant disease among the Tasmanian devil population (*Sarcophilus laniarius*)

Aspetti della malattia maligna mortale tra la popolazione dei diavoli di Tasmania (Sarcophilus laniarius)

Neil D. McGlashan*, David L. Obendorf**, John S. Harington***
* School of Geography and Environmental Studies, University of Tasmania, Hobart, Tasmania, Australia
** Wildlife Veterinary Consultant, Hobart, Tasmania, Australia
*** School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa

Summary

The world's largest remaining marsupial carnivore, the Tasmanian devil (*Sarcophilus laniarius*, formerly *harrisii*) has over the last ten years been found to be suffering from a previously unknown and invariably fatal malignancy, now known as Devil Facial Tumour (DFT), which results in disfiguring and debilitating tumours of the facial skin and within the oral cavity. The disease has caused high mortality by apparently infectious spread and, with its high prevalence, seriously affects the continuing viability of the species. In the face of this epizootic, the devil has now been listed on Tasmania's and on Australia's threatened species registers as "vulnerable", below "endangered", "rare" and only one more step before "extinct". This paper describes the research challenges and outlines some approaches to the investigation of DFT pathobiology and aetiology. The environment of Tasmania is widely contaminated by human activities, whose residual health effects on native wildlife are unknown. The Tasmanian devil is the major carnivore at the head of a diverse native animal food chain of grazing herbivorous marsupials. The rôle of bioaccumulated persistent organic pollutants and possibly genotoxic chemicals requires investigation as do conventional infectious pathogens such as exogenous and endoge-

Riassunto

Negli ultimi dieci anni si è scoperto che il più grande marsupiale carnivoro rimasto al mondo, il Diavolo di Tasmania (*Sarcophilus laniarius*, in passato *harrisii*) è colpito da un tumore maligno precedentemente sconosciuto ed invariabilmente fatale, oggi conosciuto come Tumore Facciale del Diavolo (*Devil Facial Tumour* = DFT), che comporta tumori sfiguranti e debilitanti localizzati nella cute del muso e all'interno del cavo orale. La patologia causa alta mortalità con andamento apparentemente infettivo e, con la sua alta prevalenza, mette seriamente in pericolo la sopravvivenza della specie. Di fronte a questa epizoozia, il diavolo è stato ora inserito nel registro delle specie minacciate in Tasmania e in Australia come "vulnerabile", sotto a "in pericolo", "raro" e solo un posto prima di "estinto". Questo articolo descrive le problematiche della ricerca e indica alcuni approcci di indagine sulla patobiologia ed eziologia del DFT. L'ambiente della Tasmania è fortemente inquinato dalle attività umane, i cui effetti sanitari sulla fauna selvatica indigena sono sconosciuti. Il diavolo di Tasmania è il principale carnivoro a capo di una catena alimentare indigena diversificata di marsupiali erbivori che brucano. Il ruolo di agenti inquinanti organici, bioaccumulati persistenti e forse anche di agenti chimici genotossici richiede un

Received/Pervenuto 15.3.2006 - Accepted/Accettato 3.4.2006
Address/Indirizzo: Dr. Neil D. McGlashan, 2 Protea Place, Kingston, Tasmania 7050, Australia - E-mail: jill@keypoint.com.au

nous viruses (or their genomes). Several potentially analogous conditions of known viral origin are described and recently the likelihood of infective cellular transmission from devil to devil has been reported. The risks inherent in any disease of unknown origin with potential to spread to other species cannot be overstressed. Eur. J. Oncol., 11 (2), 95-102, 2006

Key words: **Tasmanian devil facial tumour, environmental toxins, endogenous and exogenous viruses**

approfondimento, così come i patogeni infettivi convenzionali quali virus esogeni ed endogeni (o i loro genomi). Vengono descritte molte condizioni potenzialmente analoghe di origine virale conosciuta e recentemente è stata riferita la possibilità di una trasmissione di cellule infette da diavolo a diavolo. I rischi legati a qualsiasi patologia di origine sconosciuta con la possibilità di diffondersi ad altre specie non possono essere sottovalutati. Eur. J. Oncol., 11 (2), 95-102, 2006

Parole chiave: **tumore facciale del diavolo di Tasmania, tossine ambientali, virus endogeni ed esogeni**

Introducing *Sarcophilus laniarius*

Since the opening of the Bass Strait between Tasmania and Australia by rising sea levels as a consequence of climatic change some 12,000-10,000 years ago, the world's only two large marsupial carnivores have found safe haven in the island state. The last known specimen of the thylacine (*Thylacinus cynocephalus*), colloquially known as the Tasmanian Tiger, died in a Hobart zoo in 1936. Despite extensive searches, the species is now widely thought to be extinct, bounty-hunted out of existence because of its believed predatory attacks upon livestock, particularly sheep and chickens[1].

Meanwhile, although the Tasmanian devil became extinct on the Australian mainland perhaps 450 years ago[2], it has survived, successfully adapting with the environment in Tasmania (fig. 1) and achieving large numbers - perhaps 150,000 in 1990[3] - and has reached an almost iconic status locally, widely contrasting with its Disney-esque characterisation abroad.

The devil is a powerfully built carnivore of up to 13 kg (males average 12 kg, females 7-8 kg) that is secretive and usually shy of people[4]. Fertility commences between one and two years of age and a female will usually rear only one litter of up to four cubs per year. Fertility decreases from the age of five and senescence and death occur by six years[5]. In any year and over her lifetime, a female will have had several sexual partners. Young are pouch-fed and incubated for about 25 weeks and become independent at about 40 weeks old. Mortality is high in the first year of independent life[5]. The devil in its environment performs services similar to those of the hyaena[6]. In one recent case in South Africa's Kruger National Park, a hyaena has been found with a spontaneous, highly anaplastic fibrosarcoma originating from the buccal mucosa (Bengis R., personal communication). The

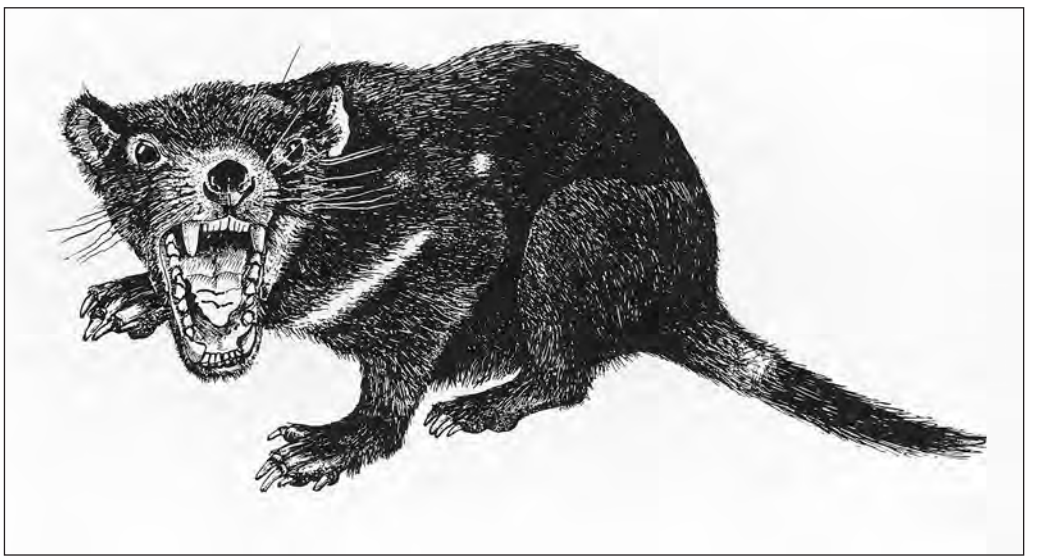

Fig. 1. *Sarcophilus laniarius,* acknowledgement Larry A. Belbin, 1983

Tasmanian devil and the African hyaena (*Crocuta crocuta*) are also both known to prey upon young and weak animals of their own species as well as carrying out useful and hygienic work by cleaning their habitats of road-kill, carcasses, carrion and the like. As a consequence of these cannibalistic and scavenging habits, devil carcasses are not generally found in the wild.

Like other dasyurid marsupials, the Tasmanian devil has six pairs of autosomal chromosomes and two sex chromosomes (XX/YY), with the Y chromosome being minute[7]. Very low genetic diversity, taken together with the known long-term history of major falls in population numbers, followed each time by recovery[8], supports the theory of a nucleus of population founders with a small genetic pool typified by low heterozygosity and low allelic diversity. These features may reflect island effects and repeated periods of fluctuations in the devil population numbers[9, 10]. Some additional alleles, not represented in the dense eastern devil populations, occur in the lower-density western ones and this allelic variation may have important implications for the long-term survival of the species[9].

The Devil Facial Tumour (DFT)

Several types of neoplasia are known in the larger dasyurids, particularly among captive Tasmanian devil populations; skin and mammary adenomas or adenocarcinomas and squamous cell carcinomas with metastatic spread to the lungs are common[11-13]. In recent years the devil has fallen victim to a mysterious and fatal disease, previously unknown (figs. 2, 3), and first observed in 1996 among the high-density, contiguous populations of the far north-east of Tasmania[14]. DFT was described in February 2005, as "a unique disease with no diagnostic test or vaccine and no understanding of its cause, transmission, its lag time or potential to affect other species"[15]. At that date only adults had been found with the disease: by March 2006 juveniles (under two years old) were also affected[16].

The disease is characterised by disfiguring and eventually debilitating tumours involving the subcutaneous tissues of the face, muzzle and oral cavity[17]. The clinical signs and histopathology of DFT are now clear. Large, rapidly increasing tumours are erosive, fungating and ulcerating lesions with metastases to cervical and submandibular lymph nodes. Multi-focal secondary tumours occur; commonly in the lungs and, less commonly, in the spleen, kidney, heart, mammary gland and brain[16]. Tumours are almost certainly transmissible between devils[7] and are currently described as anaplastic, round cell sarcomas of neuroendocrine origin[17]. Such tumours are rarely seen in humans[18]. To date no basic morphological and pathological definition has been published, although the cancer shows constant gross anatomical, histological and structural properties[7]. In addition, the karyotype of neoplastic cells from eleven devils shows a consistent chromosomal anomaly including the presence of three marker chromosomes whether these cells are derived from different tumours in the same animal or between different animals[7]. The disease is progressive and invariably fatal, with no evidence of spontaneous remission or regression[14]. Animals usually die within a few months of the lesions appearing. Particularly in high-density populations, numbers may have been reduced by an estimated 50% in as little as three years[15]. By contrast, no incidence of DFT in the devil populations of the State's wildlife parks has been reported[15]. This may relate to absence of potential infectivity, differing environmental risks, social contacts among captive devils[19], or by possessing an immune system not compromised by toxic chemicals in their protected habitat.

The geographic distribution of DFT

As a means of determining the spatial distribution of DFT, field monitoring of the geographical spread is difficult and possibly unreliable. Mapping has had to proceed on only manifestational bases of where diseased animals have been captured. At present, diagnosis can only be based on the presence or absence of observed tumours on the skin of trapped devils whereas histology would provide a more specific test. A screening test for devils incubating DFT either by marker protein, antibody presence or genomic fingerprint is an urgent need. It is therefore not at present possible to ascertain where or when any population is totally clear of disease. Pre-clinical stages of DFT will not normally enter the record, although in one case a devil was found asymptomatic but believed to be positive for DFT[20]. Capture-release-recapture surveys prove that devils without visual DFT lesions may subsequently develop grossly obvious cancers in a matter of months[20]. Prevalence-by-area calculations are further hampered by loss of animals due to possible local variations in other causes of devil mortality, especially old age deaths and road-kills.

Accepting these difficulties, the Tasmanian Department of Primary Industries, Water and Environment (DPIWE) reports that DFT is widespread across some two-thirds of Tasmania's total area of 26,383 sq ml (68,331 sq km), predominantly in the east, centre and north-east[20]. Early results from trapping had quickly

Fig. 2. Healthy young adult male

Fig. 3. Diseased juvenile devil with tumour, aged approximately 13 months

distinguished between areas containing only healthy devils in the west from those with confirmed cases of DFT, broadly in the east (fig. 4).

A presumed spread of the pattern from an initial index region of occurrence in the far north-east of Tasmania, where devils were previously at high density, may be occurring into the west and north-west of the state where diseased animals have previously not been recognised[21]. New cases of DFT are being detected along the extreme western boundary of the current range. This has led to speculation that natural boundaries, such as major river courses or other environmentally adverse conditions for devils may delay or limit spread[21] (fig. 4). Lower densities of devil populations in western Tasmanian habitats may reduce direct animal to animal contact and hence slow the progress of cancer in these areas.

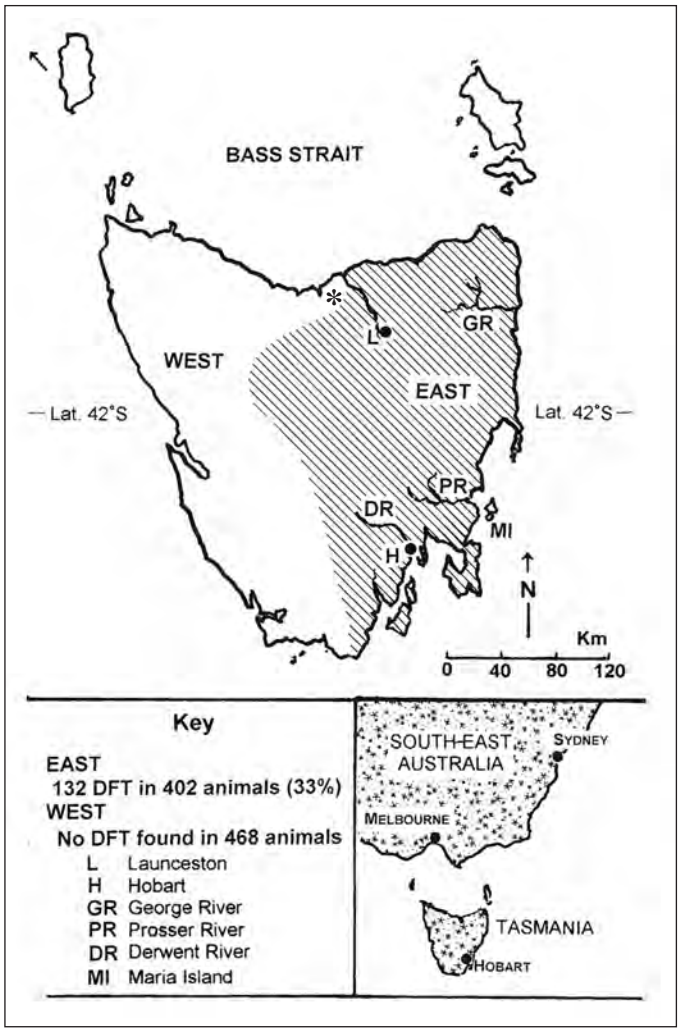

Fig. 4. Occurrence of DFT in Tasmania up to 31 December 2004 (From DPIWE[20])
* A diseased devil was found in January 2006 as a road-kill north-west of Launceston in a previously disease-free area

Ensuring species survival?

The serious threat of extinction posed by DFT, with an estimated 75,000 dead animals in eight years, has caused concern regarding how best to protect and save the species. The task of researching and protecting the Tasmanian devil has been largely controlled by the DPIWE, in conjunction with the University of Tasmania's Zoology Department and other authorities[20]. Major effort by the DPIWE has gone into monitoring the progress of the disease in wild populations of devils and in developing a more detailed understanding of the natural demographics of these animals[5].

One response by the DPIWE has been to isolate "insurance" populations of DFT-free healthy devils (based on visual assessment of trapped animals) from their wild kin[19]. Captive holding pens at an urban quarantine facility and on an offshore island (Maria Island, see fig. 4) are currently being utilised. From a total of 25 apparently unaffected post-weaning juveniles derived from a population considered DFT-free, two female devils selected as healthy in one such "Noah's Ark" population had developed facial tumours within three and ten months of capture. In the absence of a pre-clinical diagnostic test or any firm understanding of routes of transmission, the disease status and infectivity of progeny reared from these females would be questionable. The selection of animals for insurance purposes requires isolation facilities for each animal. Risk is also sharply increased if such animals are selected from devil populations known to be affected by DFT. Facilities in other Australian states are loath to receive Tasmanian devils without lengthy quarantine because, as this scenario has demonstrated, "… rapid development of lesions can occur in previously visually-free animals"[19]. Any captive insurance population is subject to the additional long-term burden that "… the genetic diversity of devils is not large enough to maintain an isolated population for longer than about five years"[19].

A second response centres on the multiple peninsula character of Tasmania's coastal outline. The objective has been to create peninsular zones artificially freed of infection by re-doubling culling efforts locally in the Forestier and Tasman peninsulas until no infective animals can be found to remain. A single road bridge connects these areas to mainland Tasmania and diseased animals trapped beyond it are being euthanased (Hamilton J., personal communication) although viral-contaminated faeces would remain. The trial will assess the effect of culling diseased devils from defined peninsular areas and preventing the ingress of devils from the mainland of Tasmania. If this method is successful, other areas may be selected for similar treatment but, again, the lack of a pre-symptomatic diagnostic test will become a significant limitation on assessing the effectiveness of this approach.

Unlike other neoplasms of Tasmanian devils[11-13], the spontaneous appearance and high prevalence of DFT suggests an unusual aetiology which remains only partially elucidated[7]. With limited scientific literature on DFT to date, defining the pathobiology and aetiology has been protracted and has focussed on collecting field data in free-ranging populations and on laboratory studies of histopathology and cytology. Two main hypotheses are now suggested here[22]: an initiating environmental trigger for the presence of viral infections, or activated oncoviral gene sequences, or both.

Environmental hypotheses

One aetiological contender, either as a primary, triggering or contributory factor, is pollution in Tasmania, where a wide variety of potentially noxious and cumulative chemicals has been used as herbicides, insecticides and animal poisons[23]. The highly toxic sodium monofluoroacetate (commonly known as 1080) has been used for some 50 years and is now in widespread use by forestry companies and agriculturalists to protect young plantation trees and farmlands from herbivorous wildlife. Widespread use of poisons has particular implications for top-order scavenging species such as devils, and poisoned native herbivores have ensured a constant supply of carcasses to support artificially high devil populations as well as introducing highly toxic agents into their habitat. While dogs and foxes are known to be very susceptible to secondary 1080 poisoning (indeed, they can die outright[24]), it is not known whether this poison causes any sub-lethal effect on devils, such as lowering their immunity to infection.

Meanwhile, due to their perceived habit of preying on weak livestock, devils themselves have been targeted for poisoning. Mevinphos (Phosdrin®), which causes high mortality, especially among dispersing juveniles in early summer[25] is suspected of being genotoxic[26] and was in wide use in north-east Tasmania in the early to mid-1990s, specifically to kill devils (Cronin S. personal communication), before being withdrawn from commercial sale in 1997. This period and location would therefore cover the first recognised sighting of a devil with DFT in 1996. Moreover, while scavenging, the devil's facial tissues would be the first point of contact with toxins in carrion. The actual mechanism whereby devils survive acute exposure to an index event and then go on to develop neoplastic cells is unclear. Pollutants and other compounds that become long-lived environmental contaminants are known to affect the normal function of mammalian genes, even at extremely low exposure levels, and can also lead to the bioaccumulation of lipophilic organic pesticides. The toxicological effects of both 1080 and mevinphos as well as some eight other agricultural chemicals on the devils' health and procreation are not clear. All these need unequivocal definition.

Infective transmission of DFT: cellular and viral

Two actions are compatible with the presence of primary tumours on the head, either or both of which suggest direct transmission of infectious material by facial biting[7,14]. The first could be the direct transfer of infected malignant cells and the second could be viral transmission, with neither of these being necessarily incompatible with the other.

Endogenous cellular transmission

Pearse and Swift[7] have recently shown that the chromosomes in the tumours have undergone a rearrangement identical in each animal studied (n=11). In the light of this and of the known fighting behaviour of the devils[25], these authors propose that the disease is transmitted by allograft, whereby infectious cells are directly passed between the animals by facial biting.

The only known analogue with this process is canine transmissible venereal tumour or sarcoma (CTVT)[27] (which also occurs in other *Canidae* such as foxes, coyotes and wolves). The cells are easily transplanted and, moreover, are known to respond well to chemotherapy. Both naturally-occurring and experimental transplants of CTVT show an initial stage of rapid tumour growth followed by spontaneous regression and remission[28], whereas no such regression is seen in DFT.

The hypothesis therefore states that, in devils, the direct transfer of viable neoplastic cells from facial sarcomas could implant as infective cells or allografts between affected and unaffected animals[7]. This could account for the apparently contagious or infectious pattern of spread observed, but would necessarily assume that these xenograft cells have the capacity to evade the recipient's major histocompatibility complex (MHC) and are not recognised as "non-self".

In vitro cultures of cells from DFT cases showing consistent chromosomal alteration[7] imply that this unique cancer epizootic was derived from a single source origin of a particular tumour cell clone with viable cells transformed from infected (and infectious) devils to others through direct bite inoculation. Confirmation of this direct transmission of DFT cells to "uninfected" devils will lie in successful laboratory or field transplantation of infected cells from donor to recipient devils.

Evidence that these xenograft cells evade immunosurveillance by cell-mediated and humoral immune function in their new hosts would be a further significant discovery. Such a theory might be supported by the generally low heterozygosity recorded in Tasmanian devils across their range and by the ability of cancer cells to evade the MHC within the whole population. If neoplastic cells, experimentally inoculated, are to establish and proliferate in recipient disease-free devils – as in natural DFT cases – then proof of this means of transmission would assist substantially in understanding DFT in devil populations.

Conventional exogenous oncoviruses

A second causative hypothesis suggests that transmissible oncogenic viruses could well be the primary infective agents of DFT; transmission of CTVT with cell-free filtrates was mentioned as long ago as 1960[28] and C-type particles were reported in 1970 to be associated with CTVT[29], implying that the agent of DFT may also be a C-type retrovirus. Clearly, a fresh look at this possibility produced by old evidence is indicated. Similarly, DFT might represent the resurgence of an ancient virus such as that responsible for the transmissible infection of seven species of marine turtle[30, 31], including some in Australia[32].

The transfer of an oncogenic virus, perhaps encoding an active oncogene infection, should not be ruled out. There are now numerous examples of viruses successfully crossing from one species to another[13]. Human-induced changes to natural ecologies, new intensive animal husbandry systems or dietary regimes, global movements of live or processed animal products, unusual wild animal migrations or overpopulations are among the recognised catalysts for the emergence of new pathogens or disease syndromes which may lead to serious epizootics or epidemics.

By analogy with other cryptic virus with ability to cross species boundaries[33, 34], even a mutation from a known virus or viral gene may yet be found to be the underlying cause of DFT. At least two retroviruses known to occur in the domestic cat (*Felis catus*) population[35], and presumably also in feral cats common in the Tasmanian bush, offer another cross-species retroviral possibility. The danger in this case would extend to other Tasmanian dasyurids, such as the Spotted-tail and Eastern Quoll (*Dasyurus maculatus* and *D. viverrinus*) populations, or even to other mammalian species.

If DFT were due to a conventional exogenous oncovirus, this could produce cryptic oncogenic insertions into the host genome. Many known oncogenes have now been isolated from RNA viruses and would be comparable with gamma retroviruses such as Feline Leukaemia Virus (FeLV) and Koala (*Phascolarctos cinereus*) Retrovirus (KoRV), both of which lead to immunosuppression and high prevalences of lymphoreticular tumours. KoRV is unusual in that it is a truly endogenous, yet highly active, retrovirus that is transmitted vertically from parents to offspring *via* the gametes. KoRV is present in virtually all koala populations in Australia[36] and is closely related to the exogenous, horizontally transmitted Gibbon Ape Leukaemia virus. In some host species, for example cats, FeLV disease results from recombination of exogenous with endogenous strains[35] and immunosuppression by chemical pollutants, or Kaposi sarcoma-type tumours, as in AIDS in which KSHV (human herpesvirus 8) is the causative agent[22].

Infection, possibly by haematogenous blood-feeding insects, with a fibropapillomavirus (PV) or with Epstein-Barr virus (as in Burkitt's sarcoma in which mosquitoes are a co-factor), should also be considered. PV infection is ubiquitous, difficult to miss and has been recognised in humans, non-human primates, cattle, and other species[37]. In such cases, electron microscopy has proved equivocal[38]. Lesions similar to PV-induced ones have been reported in over a score of animals including in Tasmanian devils[11, 39]. Electron microscopy has revealed no virus particles in any tissues from devils affected with DFT but a cryptic viral instigator for the condition may yet exist. Indeed, all or part of the viral genome may persist in the transformed cell and there is often no production of infectious progeny virus. In addition, animals may inherit viral genes.

The research effort to date has been largely conducted within the Tasmanian Government's veterinary laboratory in Launceston. The need now is for these questions to be opened up far more broadly to wider avenues of multidisciplinary enquiry. Research institutions with expertise in molecular technology, gene probes and retroviral sequencing should be given access to a range of devil tissues.

Acknowledgement

One of the authors, Dr. J.S. Harington, has long received support from the University of the Witwatersrand and this is gratefully acknowledged here.

References

1. Paddle RN. The last Tasmanian tiger: the history and extinction of the thylacine. Cambridge: Cambridge University Press, 2000.
2. Archer M, Baynes A. Prehistoric mammal faunas from two small caves in the extreme south-west of Eastern Australia. J R Soc Wn Aust 1972; 55: 80-9.
3. Mooney N. The devil's new hell. Nature Australia, Summer 2004-5; 34-41.
4. Guiler ER. Tasmanian devils - Australia's ugliest marsupials. Aust Nat Hist 1983; 21: 117-9.
5. Jones ME, Paetkau D, Geffen E, *et al*. Genetic diversity and population structure of Tasmanian devils. Mol Ecol 2004; 13: 2197-209.
6. Estes RD. The behavior guide to African mammals. Berkeley: University of California Press, 1991: 20: 323-44.
7. Pearse A-M, Swift K. Transmission of devil facial-tumour disease. Nature 2006; 439: 549.
8. Green RH. Notes on the devil and quoll in northeastern Tasmania. Rec Q Vic Mus 1967; 27: 1-13.

9. Jones ME. Disease decimates the devil. Wilderness News, Summer 2003, 14-5.
10. Bradshaw CJA, Brook BW. Disease and the devil: density-dependent epidemiological processes explain historical population fluctuations in the Tasmanian devil. Ecography 2005; 28: 181-90.
11. Effron M, Griner L, Benirschke K. Nature and rate of neoplasia found in captive wild mammals, birds and reptiles at necropsy. J Natl Cancer Inst 1977; 59: 185-94.
12. Twin JE, Pearse A-M. Diseases in wild Tasmanian dasyurids. Wildlife Disease Association, Australasia Section Meeting, 1985 (abstract).
13. Obendorf DL. Diseases of dasyurid marsupials. Biology and management of Australian carnivorous marsupials. In Biology and Management of the American Association of Zoos, Parks and Aquariums, 1993; 3, 39-46: 158.
14. Pyecroft S. In Young E. Tasmanian devil's bite is the kiss of death. New Scientist 2005; 9; 14.
15. Department of Primary Industries, Water and Environment. (DPIWE) DFTD: Disease management strategy, 2005a.
16. Department of Primary Industries, Water and Environment. Devil Facial Tumour Disease Newsletter, March 2006.
17. Loh R, Hayes D, Mahjoor A, *et al*. Devil facial tumour disease in Tasmanian devils. Proceedings of the Wildlife Disease Association International Conference, Cairns (Australia) 2005; 266.
18. Cirillo F. The epidemiology of neuroendocrine tumours. Eur J Oncol 2005; 10: 55-62.
19. Andrewartha R. Animal health issues. In Department of Primary Industries, Water and Environment, Disease Management Strategy 2005; 3.
20. Department of Primary Industries, Water and Environment. Research into DFTD: Progress Report, 2005b.
21. Hawkins CE, Jones ME, Mooney N, *et al*. Epidemiological features of a new disease in the Tasmanian devil. In Proceedings of the Wildlife Disease Association International Conference, Cairns, Australia, 2005; 16: 58.
22. Kaleeba JAR, Berger EA. Kaposi's sarcoma-associated herpesvirus fusion-entry receptor. Science 2006; 311: 1921-4.
23. Scammell M. Environmental problems in Georges Bay, Tasmania. Report to Tasmanian Fisheries Industries Council, 2004; 7.
24. McIlroy RC. Susceptibility of target and non-target animals to 1080. Proc R Soc NZ Series, 1993, 28.
25. Guiler ER. Observations on the Tasmanian devil. Aust J Zoology 1970; 18: 49-70.
26. Australian Pesticides and Veterinary Medicines Authority 1997; Mevinphos toxicological assessments.
27. Das U, Das AK. Review of canine transmissible venereal sarcoma. Vet Res Comm 2000; 24: 545-56.
28. Ajello P. Trasmissione del tumore di Sticker con materiale acellulare. La Nuova Veterinaria 1960; 36: 179-83.
29. Sapp WJ, Adams EW. C-type viral particles in canine venereal tumor cell cultures. Am J Vet Res 1970; 31: 1321-3.
30. Herbst LH. Fibropapillomatosis of marine turtles. Ann Rev Fish Dis 1994; 4: 389-425.
31. Herbst LH, Ene A, Su M, *et al*. Tumor outbreaks in marine turtles are not due to recent herpesvirus mutations. Curr Biol 2004; 14: R697-9.
32. Limpus CJ, Miller JD. The occurrence of cutaneous fibropapillomas in marine turtles in Queensland. In James R (ed). Proceedings Australian Marine Turtle Conservation Workshop, Gold Coast, Australia, 1990; 186-8.
33. Mayer JD. The geographical understanding of HIV/AIDS. Norsk Geografisk Tidsskrift 2005; 59: 6-13.
34. Webster R, Hulse D. Controlling avian flu at the source. Nature 2005; 435: 415-6.
35. Hardy WD, Hess PW, Essex M, *et al*. Horizontal transmission of feline leukaemia virus. Nature 1973; 244: 266-9.
36. Tarlinton R, Young P, Meers J, *et al*. Koala Retrovirus (KoRV): the link to disease and its place in Koala ecology. Proceedings of the Wildlife Disease Association. International Conference, Cairns (Australia) 2005; 42: 101.
37. Canfield PJ, Sabine JM, Love JM. Virus particles associated with leukaemia in a koala. Aust Vet J 1998; 65: 327-8.
38. Shah KV, Howley PM. Papillomaviruses. In Fields BN. (ed). Virology, vol. 2, chapter 66. Berlin: Springer-Verlag, 1996, 2007-109.
39. Sundberg JP. Papillomavirus infections in animals. In Syrjänen K, Gissmann L, Koss LG (ed). Papillomaviruses and human disease. Berlin: Springer-Verlag, 1987; 40-103.

Eur. J. Oncol., vol. 11, n. 2, pp. 103-111, 2006

The prognostic rôle of the Fas/APO-1 receptor, related to immune response, in colorectal cancer

Il ruolo prognostico del recettore Fas/APO-1, correlato con la risposta immunitaria, nel carcinoma del colon-retto

Giovanni Teodossiu*, Edoardo Triggiani**, Nicola Filiotis**, Rita Lazzaro*, Alessandra Provenzano*, Panaghiotis Ginopoulos***

* Clinical Oncology Unit, Division of General Surgery De Blasi, University Hospital, Bari, Italy
** Division of General Surgery Oliva, University Hospital, Bari, Italy
*** Division of Oncology, San Andreas Hospital, Patras, Greece

Summary

***Aim*. The aim of this work is to estimate serum values of FasR and FasL in patients with colorectal cancer and to compare them with normal values in order to establish a relationship between serum values and prognosis of colorectal cancer. *Patients and methods*. The FasR and FasL serum values of 45 patients were analyzed for two years (from March 2003 to April 2005), during which period the patients were enrolled and treated with chemotherapy. The ELISA technique was used to analyse FasR and FasL serum values. *Results*. The serum values of FasR and FasL increased in neoplastic patients starting from the baseline. These values remained high in non-responders, whereas they were reduced in responders. Furthermore, there was a correlation between the cancer stages and serum values of FasR and FasL. *Conclusions*. Prognosis can be related with FasR and FasL serum values. Eur. J. Oncol., 11 (2), 103-111, 2006**

***Key words:* Fas/APO-1, colorectal cancer, FasR, FasL**

Riassunto

***Finalità*. Lo scopo del lavoro è quello di valutare i valori sierici di FasR e di FasL nei pazienti con cancro del colon-retto e paragonarli con quelli normali al fine di trovare una correlazione tra valori sierici e prognosi del carcinoma del colon-retto. *Pazienti e metodi*. Sono stati analizzati i valori sierici di FasR e di FasL di 45 pazienti per due anni (da marzo 2003 ad aprile 2005), periodo in cui i pazienti sono stati arruolati e trattati con chemioterapia. Per analizzare i valori sierici di FasR e di FasL è stata utilizzata la tecnica ELISA. *Risultati*. I valori sierici di FasR e di FasL erano aumentati nei pazienti neoplastici fin dal *baseline*. Tali valori erano ancora elevati nei pazienti non-responsivi, mentre erano diminuiti nei pazienti responsivi. Inoltre, c'era una correlazione tra lo stadio della neoplasia ed i valori sierici di FasR e di FasL. *Conclusioni*. La prognosi può essere correlata con i valori sierici di FasR e di FasL. Eur. J. Oncol., 11 (2), 103-111, 2006**

***Parole chiave:* Fas/APO-1, carcinoma del colon-retto, FasR, FasL**

Received/Pervenuto 10.3.2006 - Accepted/Accettato 27.3.2006
Address/Indirizzo: Prof. Giovanni Teodossiu, Ambulatorio di Oncologia Clinica, Clinica Chirurgica De Blasi, Policlinico, Piazza G. Cesare 11, 70124 Bari, Italia - E-mail: g.teodossiu@cimedoc.uniba.it

Introduction

Many experimental and clinical studies support the hypothesis that patients with neoplastic disease have a lower immune cellular response and that this could be related to the stage and grade of cancer. The most common mechanisms that play a rôle in the limitation and the prevention of tumoural growth are: specific cytotoxicity mediated by T lymphocytes (CTL) against tumours with high antibody production; cell-mediated immunity mainly modulated by natural killer (NK) cells and activated macrophages, which lead to "apoptosis".

Apoptosis is a programmed cellular death. It is very important in many physiological processes, such as embryogenesis, immune response regulation, menstruations, etc. In normal cells apoptosis can also be induced by the binding between the Fas Ligand (FasL) and its receptor, Fas Receptor (FasR). FasL is a protein, similar to the tumour necrosis factor (TNF)[1, 2] and it is always expressed on T lymphocytes and NK cells[3]. FasR is a monomeric protein expressed on different types of cells in each organ; it is also called Fas/APO-1 or CD95[4].

There is much evidence that apoptosis, a very important type of cell cycle regulation, is damaged or even missing in tumoural cells. Furthermore, patients with neoplastic diseases have lower lymphocytic cell values than healthy people.

Colorectal cancer is the second most frequent cancer in the USA and Europe. It seems to be clear that its origin is related to alimentary habits, but overweight plays an important rôle in colorectal cancer predisposition.

The aim of this study was to evaluate the changes in the immune system in patients operated for colorectal cancer before and after the treatment with chemotherapy.

Moreover, we considered possible correlations between the percentage of CD95 positive (+) cells, serum FasR (sFasR) and FasL (sFasL) values and clinical-anamnestic data (stage of cancer, grade of malignancy), which could be very important for the prognosis when evaluating both survival and response to chemotherapy.

Patients and methods

Patient selection

Forty-five patients with colorectal cancer were selected and were followed at the day-hospital of the Clinical Oncology Unit of the University Hospital of Bari. These patients were enrolled over two years, from March 2003 to April 2005.

About 30% of the 45 patients were withdrawn from the study because they continued therapy elsewhere or because they died. Thirty-two patients completed the study. They were aged 55-75 years, 18 men and 14 women. At the time of selection none of the patients had received any type of treatment (radiotherapy or chemotherapy). The patients were moreover diagnosed with different stages of cancer (18 were in the B2 stage, 13 in C1, 10 in C2, and 4 were in the D stage, according to Dukes classification).

Thirty-two healthy subjects were selected as control cases: they were paired by sex and age and they had been free from infectious or auto-immune diseases for at least six months.

At the first examination (baseline) the patients and the controls had peripheral blood samples taken, each of 10 ml: one to check lymphocytic populations (including CD 95) and for the haemochromocytometric examination; the other to study the serum concentration of FasR and FasL. The first blood samples were taken 40 days after the operation to resect the initial neoplasia. The second blood test was made two months after the end of chemotherapy and the same two types of samples were taken.

All 45 patients were treated with FOLFOX (oxaliplatin associated with 5-fluorouracil and folinic acid = 5-FU/FA) in order to estimate: 1) its efficacy in the increase of complete and partial responses; 2) its efficacy in improving the disease-free survival rate, and to evaluate whether a better prognosis is related to a faster reinstatement of immunological functions.

The first blood samples were used to analyze immunologic populations with a fluorescence-activated cell sorter (FACS) scan (we checked with the following antibodies: anti-CD3, anti-CD4, anti-CD8, anti-CD20, anti-CD16/56, anti-CD95 for FasR positive cells). In particular, for the study of CD95+ lymphocytes, a double labelling system was used, made with two monoclonal antibodies: anti-CD3 labelled Fluorescein (FITC) and anti-CD95 labelled fluorescent phycoerythrin (PE).

The second blood samples were used to analyze the FasR and FasL serum concentration with ELISA (Enzyme Linked Immuno-Sorbent Assay).

Statistical analysis

The main aim of the study was to show whether there is a correlation between the FasR and FasL serum values and the prognosis of colorectal cancer. Assuming that the values found in the 32 healthy subjects are representative of the general population and considering an α error of 0.05 (so that the confidence interval is 0.95), we compared each value between healthy subjects and

patients and, for every patient, we checked before and after the chemotherapy. Values were also compared between responding and non-responding patients. A Z test was used in order to evaluate whether a difference was significant.

Results

The differences in lymphocytic values between patients and healthy subjects were analyzed (Table 1). Their values were different before treatment.

At baseline, the results on the lymphocytic population showed that:

1) the average percentage value of total lymphocytic population (CD3+) in patients with colorectal cancer before chemotherapy was lower than that in control subjects;
2) the average value of lymphocytic sub-population helper/inducer (CD4+) was significantly reduced in neoplastic patients (with Z calculated = -13.01 < Z tabulated = -1.96);
3) also cytotoxic/suppressor lymphocytes (CD8+) were significantly increased (with Z calculated = 3 > Z tabulated=1.96);
4) the average value of NK lymphocytic type (CD16+/56+ CD3-) was significantly increased in neoplastic patients (with Z calculated = 9.9 > Z tabulated = 1.96);
5) on the contrary, there was no significant difference in the average percentage value of B lymphocytes (CD20+) between cancer patients at baseline and normal subjects (with Z calculated = -1.9 < Z tabulated = -1.96);
6) the changes in lymphocytic subpopulation distribution involved a progressive reduction in CD4/CD8 ratio;
7) already at baseline, cancer patients showed a considerable increase both in CD95+CD3+ cell percentage (FasR lymphocytes) (with Z calcu-

Table 1 - Average percentage values of lymphocytograms performed on neoplastic patients, at baseline and at the end of chemotherapy, and on healthy subjects

Lymphocytic subset	Control group (32 patients)	Patients before chemotherapy (baseline) (45 patients)	Patients after chemotherapy (follow-up)	
			Responders (23 patients)	Non-responders (9 patients)
CD3 Total T lymphocytes	71.3 ± 7.5	64.2 ± 7.8	68.8 ± 6.0	56.5 ± 4.0
CD4 Helper/Inducer T lymphocytes	45.5 ± 5.6	34.7 ± 4.9	42.9 ± 3.0	25.9 ± 2.9
CD8 Cytotoxic/suppressor T lymphocytes	26.0 ± 4.8	28.1 ± 5.0	26.4 ± 4.1	31.7 ± 3.2
CD20 B lymphocytes	10.4 ± 4.2	9.2 ± 3.1	11.3 ± 3.8	9.1 ± 2.0
CD16/56+CD3- Natural killers	16.1 ± 6.8	26.1 ± 6.9	18.2 ± 4.0	29.6 ± 3.7
CD95 Cell FasR+	51.3 ± 10.1	70.2 ± 9.0	54.6 ± 9.1	73.0 ± 8.9
CD95+CD3+ FasR+ T lymphocytes	38.9 ± 8.8	52.8 ± 8.7	40.8 ± 7.8	53.0 ± 8.6
CD95+CD3-	13.7 ± 8.0	18.5 ± 9.1	14.1 ± 5.5	19.8 ± 7.9
CD4/CD8	1.75 ± 2.41	1.23 ± 2.58	1.62 ± 2.05	0.82 ± 1.01

lated=10.1 > Z tabulated=1.96) and in CD95+CD3- cells (FasR cells) (with Z calculated=12.6 > Z tabulated=1.96).

Follow-up of patients with colorectal cancer after chemotherapy showed:

1) the average value of T lymphocytes (CD3+) in non-responders after treatment was significantly different from that found in the controls;
2) the average value of CD4+ lymphocytes was significantly reduced in non-responders (with Z calculated = -10.48 < Z tabulated = -1.96), whereas responder values returned almost to normal (with Z calculated = -2.2 < Z tabulated = -1.96);
3) all the patients, before and during chemotherapy, had an increase in CD8+ lymphocytes values; but these values became normal again in responders (with Z calculated = 0.4 < Z tabulated = 1.96), while they were still high in non-responders (with Z calculated = 3.56 > Z tabulated = 1.96);
4) B lymphocytes (CD20+) percentage did not change significantly in any patient group;
5) NK lymphocytes (CD16/56+ CD3-) were constantly increased only in non-responders (with Z calculated = 5.9 > Z tabulated = 1.96), while they returned to normal in responders (with Z calculated = 1.47 < Z tabulated = 1.96);
6) the average percentage value of FasR cells (CD95+) was still high in non-responders (with Z calculated = 6.44 > Z tabulated = 1.96) and this became lower in responders (with Z calculated = 1.57 < Z tabulated = 1.96);
7) in responders there was a normalization of the CD4/CD8 ratio, which was reduced at baseline because of the changed lymphocytic subpopulation distribution.

Z must be included in the interval (-1.96 – +1.96), considering an α error of 0.05 and therefore a confidence interval of (1-α) of 0.95.

Table 2 - Measurement (by ELISA) of FasR serum values in healthy subjects and in patients with colorectal cancer at baseline

Subjects from whom blood samples were taken	sFasR values (ng/ml) (mean ± SD[a])
Healthy subjects (n = 32)	2.8 ± 1.9
Patients with colorectal cancer (n = 45)	9.1 ± 3.9

[a] SD = standard deviation

The sFasR and sFasL values were also considered.

The sFasR concentration in men (3.35 ± 0.5 ng/ml, n = 30) was significantly higher than in women (2.9 ± 0.39 ng/ml, n = 30) (considering an age between 60 and 69). Moreover sFasR was shown to be related to age; it increased with ageing. In fact, in older people, cells quickly release FasR, although these cells express less FasR.

The sFasL concentration is not usually measurable, but it increases in a quite remarkable way in patients with different diseases.

The sFasR and sFasL values were measured with the ELISA technique.

The first blood test, to analyze sFasR and sFasL, was made on 45 patients with colorectal cancer 40 days after the operation to resect the neoplasia (baseline). Moreover, sFasR and sFasL values were measured in 32 healthy subjects (controls). The second blood test was made on the 32 patients, who remained in the study, two months after the end of chemotherapy (follow-up).

As shown in Table 2 and in fig. 1, sFasR values in healthy people were 2.8 ng/ml, while in neoplastic patients they were 9.1 ng/ml at baseline: thus showing how sFasR values were four times higher in patients than in healthy subjects. Furthermore, sFasR had a progressive tendency to rise according to the worsening of the grade of neoplasia (Dukes' classification) (Table 3, fig. 2).

At the follow-up, two months after the end of chemotherapy, there was a diminution in sFasR values in responders (3.03 ng/ml), while they were still high in non-responders (9.53 ng/ml) (Tables 4, 5; figs. 3, 4).

The sFasL values in healthy subjects were 0.06 ng/ml, while in neoplastic patients, at baseline, they were 4.9 ng/ml (Table 6, fig. 5). Analyzing the values according to Dukes' classification, there was a non-statistically significant trend of sFasL to increase with the progression of cancer (Table 7, fig. 6).

At the follow-up, two months after the end of chemotherapy, sFasL values were therefore reduced only in responders (although they were still measurable, with values

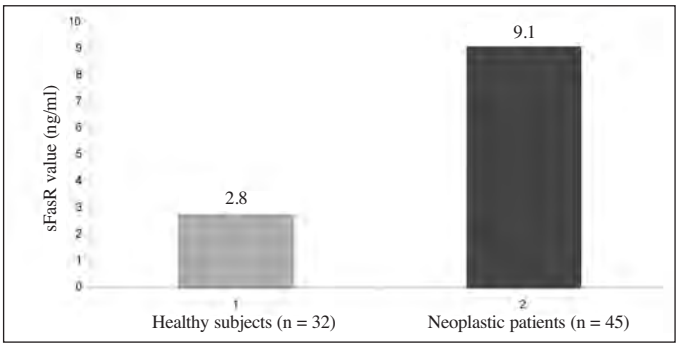

Fig. 1. Measurement (by ELISA) of FasR serum values in healthy subjects and in patients with colorectal cancer

Table 3 - Measurement (by ELISA) of FasR serum values in patients with colorectal cancer at baseline according to Dukes' Classification

Dukes' Classification	sFasR values (ng/ml) (mean ± SD[a])
Colorectal cancer (B2) (n = 18)	8.1 ± 3.0
Colorectal cancer (C1) (n = 13)	8.8 ± 3.7
Colorectal cancer (C2) (n = 10)	9.4 ± 4.2
Colorectal cancer (D) (n = 4)	10.1 ± 4.6

[a] SD = standard deviation

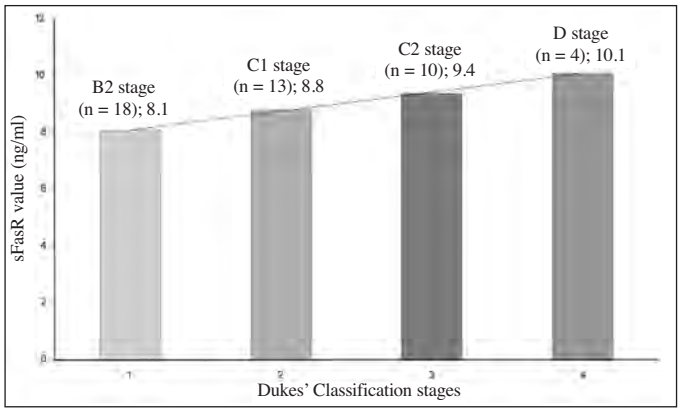

Fig. 2. Measurement (by ELISA) of FasR serum values in patients with colorectal cancer at the baseline according to Dukes' Classification

Table 4 - Measurement (by ELISA) of FasR serum values in patients with colorectal cancer before and after chemotherapy

N. of patients	sFasR values (ng/ml)		
	Baseline (mean ± SD[a])	Follow-up	
		Responders (mean ± SD)	Non-responders (mean ± SD)
45	9.1 ± 3.9		
23		3.03 ± 1.3	
9			9.53 ± 4.5

[a] SD = standard deviation

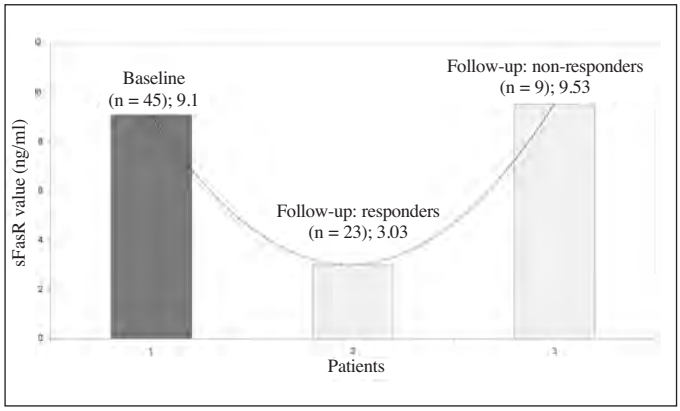

Fig. 3. Measurement (by ELISA) of FasR serum values in patients with colorectal cancer before and after chemotherapy

Table 5 - Measurement (by ELISA) of FasR serum values in healthy subjects and in patients with colorectal cancer after chemotherapy

N. of patients	sFasR values (ng/ml)		
	Controls (mean ± SD[a])	Follow-up	
		Responders (mean ± SD)	Non-responders (mean ± SD)
32	2.8 ± 1.9		
23		3.03 ± 1.3	
9			9.53 ± 4.5

[a] SD = standard deviation

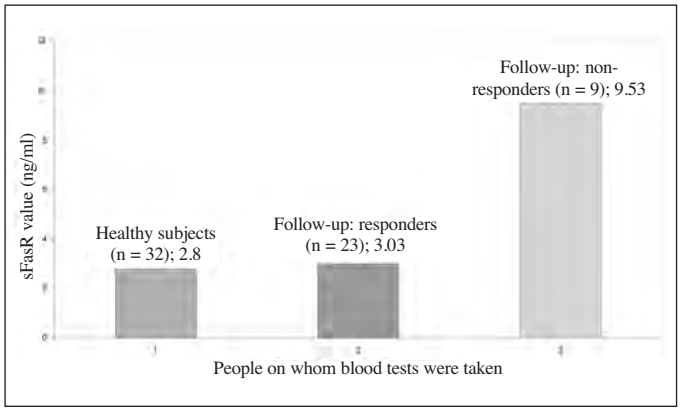

Fig. 4. Measurement (by ELISA) of FasR serum values in healthy subjects and in patients with colorectal cancer after chemotherapy

Table 6 - Measurement (by ELISA) of FasL serum values in healthy subjects and in patients with colorectal cancer at baseline

Subjects from whom blood samples were taken	sFasL values (ng/ml) (mean ± SD[a])
Healthy subjects (n = 32)	0.06 ± 0.07
Patients with colorectal cancer (n = 45)	4.9 ± 2.3

[a] SD = standard deviation

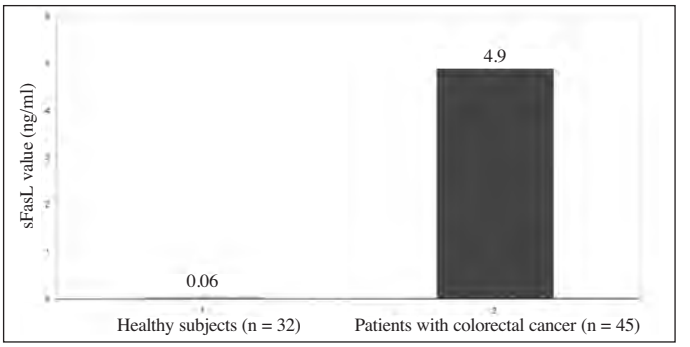

Fig. 5. Measurement (by ELISA) of FasL serum values in healthy subjects and in patients with colorectal cancer

Table 7 - Measurement (by ELISA) of FasL serum values in patients with colorectal cancer at baseline according to Dukes' Classification

Dukes' Classification	sFasL values (ng/ml) (mean ± SD[a])
Colorectal cancer (B2) (n = 18)	3.8 ± 2.2
Colorectal cancer (C1) (n = 13)	4.3 ± 2.3
Colorectal cancer (C2) (n = 10)	5.3 ± 2.6
Colorectal cancer (D) (n = 4)	6.2 ± 2.7

[a] SD = standard deviation

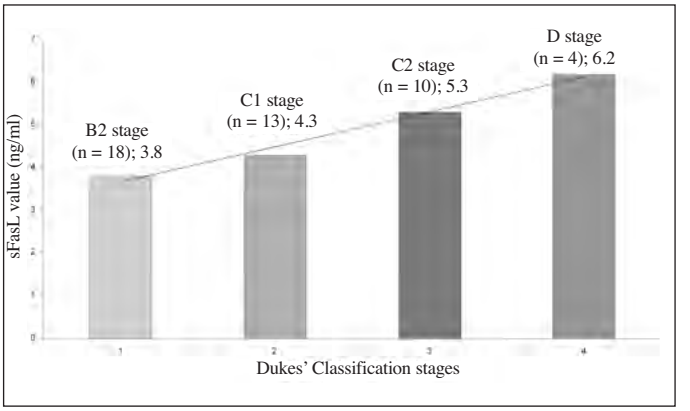

Fig. 6. Measurement (by ELISA) of FasL serum values in patients with colorectal cancer at the baseline according to Dukes' Classification

Table 8 - Measurement (by ELISA) of FasL serum values in patients with colorectal cancer before and after chemotherapy

N. of patients	sFasL values (ng/ml)		
	Baseline (mean ± SD[a])	Follow-up	
		Responders (mean ± SD)	Non-responders (mean ± SD)
45	4.9 ± 2.3		
23		0.6 ± 1.3	
9			5.6 ± 2.2

[a] SD = standard deviation

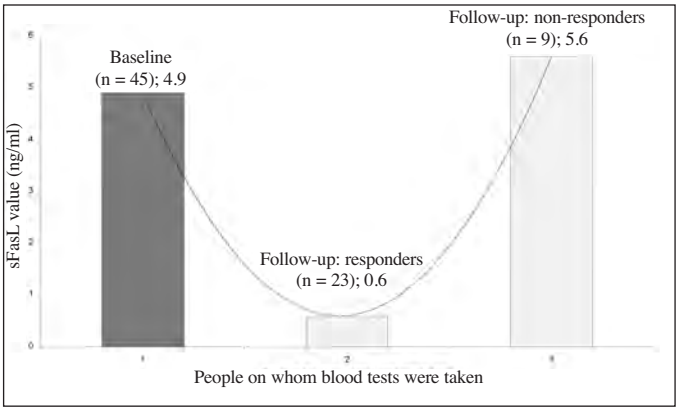

Fig. 7. Measurement (by ELISA) of FasL serum values in patients with colorectal cancer before and after chemotherapy

of 0.6 ng/ml), while they were still high in non-responders (with values of 5.6 ng/ml) (Tables 8, 9; figs. 7, 8).

Discussion

Different experimental studies showed a probable rôle played by several immune-regulating factors in the progression of cancer.

Lymphocytic subpopulations had changed noticeably in neoplastic patients already at baseline and they returned to normal only in responders patients[5].

During the follow-up, cytotoxic/suppressor lymphocytes (CD8+), NK cells (CD16/56+) and FasR cells remained high only in non-responders, indicating that this was an attempt of the body to contrast the disease. The inversion of the CD4/CD8 ratio seems therefore to be related with an insufficient response to the treatment and with a faster evolution of cancer[5,6].

Other immuno-histopathologic studies showed that

Table 9 - Measurement (by ELISA) of FasL serum values in healthy subjects and in patients with colorectal cancer after chemotherapy

N. of patients	sFasL values (ng/ml)		
	Controls (mean ± SD[a])	Follow-up	
		Responders (mean ± SD)	Non-responders (mean ± SD)
45	0.06 ± 0.07		
23		0.6 ± 1.3	
9			5.6 ± 2.2

[a] SD = standard deviation

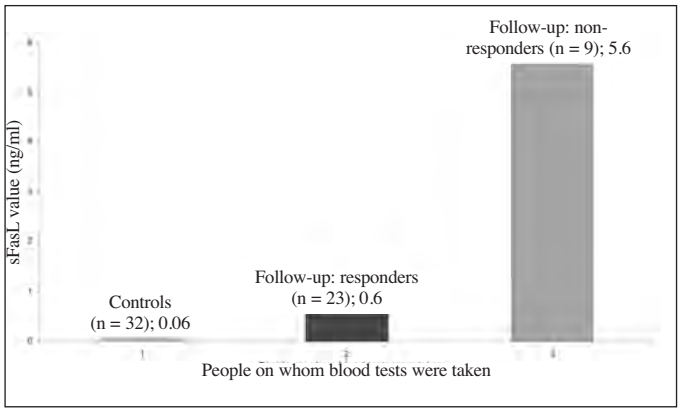

Fig. 8. Measurement (by ELISA) of FasL serum values in healthy subjects and in patients with colorectal cancer after chemotherapy

most cells in colorectal cancers had low Fas/APO-1 (CD95) values or, although, they did not express this at all. Moreover, these cells were *per se* resistant to apoptosis mediated by CD95[7,8]. The causes of their resistance are not completely clear, however this could not be related to the diminished cell FasR expression[9].

These studies demonstrated that cells, in colorectal cancer and during their malignant transformation, down-regulated or lost Fas/APO-1 receptor (CD95) and/or acquired increased resistance in the link with CD95.

On the contrary, FasL was expressed in 100% of colorectal tumours, showing that the Fas ligand could be a very important factor allowing the tumour to escape the defence of the immune system. The tumour was therefore shown to have developed many mechanisms to survive immune system attack, one of which is FasL[10].

FasL expression could eventually facilitate the establishment of the neoplasia or of metastases in sites where cells expressed FasR and could not therefore be submitted to the FasL cytotoxicity produced by the tumour[11-13]. Furthermore, it could be suggested that FasL, used like a weapon for a counter-attack, could induce apoptosis in activated cytotoxic lymphocytes with increased sensitivity, which expressed FasR, through death pathway signal FasR/FasL: this hypothesis is supported by *in vitro* data which show that different FasL tumoural cells could kill immune cells during co-culture experiments. It might therefore be possible that a similar mechanism could facilitate tumour progression *in vivo* and its elusion of the immune system. This is demonstrated by the fact that CD4+ and CD8+ lymphocytes are differently killed by tumoural cells[13]. This also happens for T lymphocytes infiltrating tumour (TIL) and it is probable that a higher percentage of T cells, which express FasR on their surface, could be more sensitive to an attack by the tumour[14].

The above hypothesis could therefore explain how, in responding patients, the return to normal FasR cell (CD95) values could be related to therapeutic response: a reduced FasR lymphocyte number could be a sign of a more active immune system, less sensitive to apoptosis mediated by FasR/FasL.

Furthermore, the FasL action of cytotoxic lymphocytes is neutralized by tumoural sFasR that, linking itself with the FasL, stops its action. This could explain why non-responders had a higher sFasR concentration, given that it was released by tumoural cells (sFasR production is widely increased in neoplastic cells).

In this study, the sFasR and sFasL values of patients with colorectal cancer were not only shown to be higher than those in healthy subjects, but their increase was directly proportional to the stage of the neoplasm: this seems to suggest that the serum values of these molecules could be prognostic indices for non-haemopoietic neoplasias.

Patients, who showed sFasR and sFasL reduction during follow-up after chemotherapy had a greater life expectancy and a longer disease-free interval than non-responding patients, in whom these values remained high.

There are other studies that show how sFasR and sFasL values are high in diseases such as Systemic Lupus Erythematosus (SLE)[15], but also in B and T lymphatic leukaemias, and in other types of cancer, such as oesophageal, uterine and lung cancer (Table 10).

Therefore, high sFasR and sFasL values can be seen to be associated with a worse prognosis than in cases with lower values.

sFasR and sFasL values in patients with colorectal cancer could indicate a prognostic significance determining the relationship between serum values of these proteins and correspondent cytotoxic activity by lymphocytes of peripheral blood (PBL) against autologue tumoural cells[28].

Table 10 - Some studies on FasR and FasL in tumours

Authors	Research	Types of tumour
Younes *et al*[16]	FasL expression	Oesophageal carcinomas
Hughes *et al*[17]	Fas/APO-1 (CD95)	Oesophageal adenocarcinoma
Shibakita *et al*[18]	FasR and FasL	Oesophageal cancer
Younes *et al*[19]	Fas/APO-1 (CD95)	Metaplasia in Barrett's oesophagus
Mitani *et al*[20]	sFasR and FasR	Malignant pleural effusion, lung cancer
Yasuda *et al*[21]	Fas/APO-1 (CD95)	Lung cancer
Kim *et al*[22]	Fas/APO-1 (CD95)	Lung cancer
Yu *et al*[23]	FasL expression	Brain tumour endothelium
Riffkin *et al*[24]	FasR and FasL	Brain tumours
Imai *et al*[25]	FasR and FasL	Endometrial cancer
Kondera-Anasz *et al*[26]	sFasR and sFasL	Uterine tumour
Gordon *et al*[27]	FasL	Osteosarcoma (lung metastasis)

Conclusions

For many years prognosis and the efficacy of chemotherapy were related to the clinical stages of cancer diffusion.

Now other parameters are considered very important: histologic grading, nuclear indices, and also proto-oncogenes.

Results obtained for lymphocytic sub-populations and for sFasR and sFasL, are impaired at follow-up, by a very high α error (false positives) because of the low number of patients in the study, due to the loss of subjects who continued chemotherapy elsewhere or who died during chemotherapy (withdrawal of 30% of patients).

The data, however, show the important rôle played by the immune system, by FasR/FasL link and by sFasR and sFasL, and how important they are for the prognosis of patients with colorectal cancer.

References

1. Nagata S. Apoptosis by death factor. Cell 1997; 88: 355-65.
2. Nagata S, Volgestein P. The Fas death factor. Science 1995; 267: 1449-56.
3. Suda T, Takahashi, Golstein P, *et al*. Molecular cloning and expression of the Fas ligand: a novel member of the tumour necrosis factor family. Cell 1993; 75: 1169-78.
4. Cheng J, Liu C, Koopman WS, *et al*. Characterization of human Fas gene. J Immunol 1995; 154: 1239-45.
5. Hamaoka T, Fujiwara H. Phenotipically and functionally distinct T-cell subset in anti-tumour response. Immunol Today 1987; 8: 267-70.
6. Chirigos MA. Immunomodulators: current and future development and implications. Thimus 1992; 19: 7-14.
7. Moller P, Korentz K, *et al*. Expression of APO-1 (CD95), a member of the NGF/TNF receptor superfamily, in normal and neoplastic colon epithelium. Int J Cancer 1994; 57: 371-7.
8. Von Reyher U, Strater J, Kittstein W, *et al*. Colon carcinoma cells use different mechanisms to escape CD95-mediated apoptosis. Cancer Research 1998; 58: 526-34.
9. O'Connell J, O'Sullivan GC, Collins JK, *et al*. The Fas counterattack: Fas-mediated t cell killing by colon cancer cells expressing fas ligand. J Exp Med 1996; 184: 1075-82.
10. O'Connell J, Bennett MW, O'Sullivan GC, *et al*. The Fas counterattack: cancer as a site of immune privilege. Immunol Today 1999; 20: 46-53.
11. Hahne M, Rimoldi D, Schroter M, *et al*. Melanoma cell expression of Fas(Apo-1/Cd95) ligand: implication for tumour immune escape. Science 1996; 274: 1363-6.
12. Mann B, Gratchev A, Bohm C, *et al*. FasL more frequently expressed in liver metastases of colorectal cancer than in matched primary carcinomas. Br J Cancer 1999; 79: 1262-9.
13. Walker PR, Sasa P, Dietrich P. Role of Fas ligand (CD95L) in immune escape (the tumour cell strikes back). J Immunol 1997; 158: 4521-4.
14. Kayagaki N, Kawakami A, Ebata T, *et al*. Metallo-proteinase-mediated release of human Fas ligand. J Exp Med 1995; 182: 1777-83.
15. Suzuki N, Ichino M, Michore S, *et al*. Inhibition of Fas/Fas ligand-mediated apoptotic cell death of lymphocytes in vitro by circulating anti-fas ligand autoantibodies in patients with systemic lupus erythematosus. Arthritis Rheum 1998, 41: 344-53.
16. Younes M, Schwartz MR, Ertan A, *et al*. Fas ligand expression in esophageal carcinomas and their lymphnode metastases. Cancer 2000; 88(3): 524-8.
17. Huges SJ, Nambu Y, Soldes OS, *et al*. Fas/APO-1 (CD95) is not translocated to the cell membrane in esophageal adenocarcinoma. Cancer Res 1997; 57(24): 5571-8.
18. Shibakita M, Tachibana M, Dhar DK. Prognostic significance of Fas and Fas ligand expression in human esophageal cancer. Clin Cancer Res 1999; 5(9): 2464-9.
19. Younes M, Lechago J, Ertan A, *et al*. Decreased expression of Fas (CD95/APO1) associated with goblet cell metaplasia in Barrett's esophagus. Hum Pathol 2000; 31(4): 434-8.

20. Mitani K, Nishioka Y, Yamabe K, *et al*. Soluble Fas in malignant pleural effusion and its expression in lung cancer cells. Cancer Sci 2003; 94(3): 302-7.
21. Yasuda M, Tanaka Y, Fujii K, *et al*. CD44 stimulation downregulates Fas expression and Fas-mediated apoptosis of lung cancer cells. Int Immunol 2001; 13(10): 1309-19.
22. Kim PK, Park SY, Koty PP, *et al*. Fas-associating death domain protein overexpression induces apoptosis in lung cancer cells. J Thorac Cardiovasc Surg 2003; 125(6): 1336-42.
23. Yu JS, Lee PK, Ehtesham M, *et al*. Intratumoral T cell subset ratios and Fas ligand expression on brain tumour endothelium. J Neurooncol 2003; 64(1-2): 55-61.
24. Riffkin CD, Gray AZ, Hawkins CJ, *et al*. Ex vivo pediatric brain tumors express Fas (CD95) and FasL (CD95L) and are resistant to apoptosis induction. Neuro-oncol 2001; 3(4): 229-40.
25. Imai A, Takagi A, Horibe S, *et al*. Fas and Fas ligand system may mediate antiproliferative activity of gonadotropin-releasing hormone receptor in endometrial cancer cells. Int J Oncol 1998; 13(1): 97-100.
26. Kondera-Anasz Z, Mielczarek-Palacz A, Sikora J. Solubile Fas receptor and solubile Fas ligand in the serum of women with uterine tumors. Apoptosis 2005; 10(5): 1143-9.
27. Gordon N, Arndt CA, Hawkins DS, *et al*. Fas expression in lung metastasis from osteosarcoma patients. J Pediatr Hematol Oncol 2005; 27(11): 611-5.
28. Mizutani Y, Yoshida O, Bonavida B. Prognostic significance of soluble in the serum of patients with bladder cancer. J Urology 1998, 160: 571-6.

Eur. J. Oncol., vol. 11, n. 2, pp. 113-120, 2006

Clinical, histopathological and immunohistochemical features in familial and sporadic breast cancers: differences in the progesterone receptor contents with stratification by patient age

Caratterisiche cliniche, istopatologiche ed immunoistochimiche nelle forme familiari e sporadiche del cancro mammario: differenze nella espressione del recettore del progesterone stratificando le pazienti in rapporto all'età

Giovanni D'Eredità*, Carmela Giardina**, Giuseppe Ingravallo**, Marcella Carella*, Tommaso Berardi*
* Department of General and Special Surgery, University of Bari, Bari, Italy
** Department of Anatomical Pathology, University of Bari, Bari, Italy

Summary

***Aim*. Several researchers have indicated that hormone receptor genetic polymorphism or hormone receptor status could be linked with familial breast cancer (FBC) development. In the present study we have investigated the clinical, histopathological and immunohistochemical features in FBCs and compared them with these findings in cases of sporadic breast cancer (SBC); in particular we evaluated hormone receptor status in patients stratified by age. *Patients and methods*. 392 patients treated for breast cancer were included in the study. The patients were stratified into two groups: FBC 67 patients (17%), and SBC 325 patients (83%). Variables evaluated for the analysis were: age, menopausal status, tumour type (palpable or not), multifocality/multicentricity, mammographic appearance, type of surgery, tumour size, histological type, grading, lymphadenectomy, axillary lymph node status, ER and PR content, MIB- 1, c-ErbB2, p53 and bcl 2 expression. *Results*. The following variables showed a statistically significant difference in the two groups: type of tumour (p=0.003), mammographic appearance (p=0.002),**

Riassunto

***Finalità*. Numerose ricerche hanno evidenziato la possibilità che il polimorfismo genetico dei recettori ormonali o il loro stato sia connesso con lo sviluppo di forme familiari di cancro della mammella. Nel nostro studio abbiamo analizzato le caratteristiche cliniche, istopatologiche ed immunoistochimiche dei cancri familiari confrontandole con le corrispondenti nei carcinomi sporadici. In particolare per lo stato dei recettori ormonali lo studio è stato approfondito stratificando le pazienti in fasce d'età. *Pazienti e metodi*. Sono state studiate 392 pazienti con cancro mammario. Le pazienti sono state stratificate in due gruppi: 67 (17%) avevano una forma familiare e 325 (83%) un cancro sporadico. Sono state valutate le seguenti variabili: età, stato menopausale, tipo di tumore (palpabile o no), multicentricità/multifocalità, aspetto mammografico, tipo di intervento, dimensioni del tumore, istotipo, *grading*, linfoadenectomia, stato dei linfonodi ascellari, contenuto degli ER e PR, espressione del MIB-1, c-ErbB2, p53 e bcl 2. *Risultati*. Le seguenti variabili hanno dimostrato una differenza statisticamente significativa nei due gruppi: tipo di tumore**

Received/Pervenuto 5.1.2006 - Accepted/Accettato 9.3.2006
Address/Indirizzo: Prof. Giovanni D'Eredità, Via S. Hahnemann, 2, 70126 Bari, Italia - Tel. +39/080/5482107 - Fax: +39/080/ 5478887
E-mail: gderedita@chirges.uniba.it

tumour size (p=0.04), histological type (p=0.009). Having divided the patients into subgroups (≤ 60 years and > 60 years), it was found that the PR content was clearly lower in FBCs (p=0.02) in patients over 60 years of age. *Conclusions*. PR gene polymorphism leads to an increased risk of breast cancer, since it determines inadequate control of ER-driven proliferative function. Moreover, PR gene polymorphism is associated with a varying effect based on a family history of breast cancer. In some breast cancers there is a possibility that a rich PR expression may thus be lost during cancer development. It is possible that the ER and/or PR gene instability are linked with some late-onset FBCs. Eur. J. Oncol., 11 (2), 113-120, 2006

Key words: **breast cancer, oestrogen receptor, progesterone receptor, family history, pathological characterization**

(p=0,003), aspetto mammografico (p=0,002), dimensioni del tumore (p=0,04), istotipo (p=0,009). Suddividendo le pazienti in sottogruppi (≤ 60 anni e > 60 anni), si è osservato che nelle pazienti con età maggiore di 60 anni il contenuto di PR era notevolmente più basso nelle forme familiari (p=0,02). *Conclusioni*. Il polimorfismo genetico dei PR comporta un aumentato rischio di cancro mammario poiché determina un inadeguato controllo della funzionalità degli ER. Inoltre il polimorfismo genetico dei PR è associato ad un effetto variabile correlato ad una storia familiare di carcinoma mammario. In alcuni cancri mammari è possibile che una elevata espressione di PR possa essere persa durante lo sviluppo tumorale. È infine ipotizzabile che l'instabilità genetica degli ER e/o PR sia connessa ad alcune forme di cancro familiare ad insorgenza tardiva. Eur. J. Oncol., 11 (2), 113-120, 2006

Parole chiave: **cancro mammario, recettori estrogenici, recettori progestinici, storia familiare, caratterizzazione patologica**

Introduction

Clinical evidence and large epidemiological studies have shown that some women have a familial predisposition to breast cancer, although the impact of risk factors for breast cancer correlated to family history has not been well defined. Family history can be regarded as a representation of genetic and environmental factors that work together to cause cancer[1]. Such studies have also shown that there are families in which breast cancer risk is inherited in an autosomal-dominant fashion (hereditary breast cancer).

The recent demonstration and chromosomal localization of susceptibility genes associated with breast cancer such as BRCA1[2] and BRCA2[3] can provide a useful tool for recognizing this important subset of hereditary breast cancer. Although BRCA1 and BRCA2 may cause as much as 90% of breast cancer and ovarian cancer in some families, no more than 5-10% of all breast cancer in the United States is probably attributable to these two *loci*[4]. Before the cloning of the BRCA1 and BRCA2 genes, a number of studies reported an association of histopathological type with a family history, but these studies had been difficult to interpret due to the small number of samples and to differing criteria for "positive family history" and pathological typing. After the identification of BRCA1 and BRCA2, many studies examined the histological features of familial breast cancer (FBC) attributable to mutations in these genes[5].

A number of genes have been identified during the past 10 years which, when inherited in a mutant form, confer a high lifetime risk for breast cancer. Several researchers have indicated that hormone receptor genetic polymorphism or hormone receptor status could be linked with FBC development[6-9].

In the present study we have investigated the clinical, pathological and immunohistochemical features in FBC cases and compared them with these findings in cases of sporadic breast cancer (SBC); in particular we evaluated hormone receptor status in patients stratified by age.

Patients and methods

From January 1999 to August 2005, 392 patients, 389 women and 3 men with breast cancer were treated. The average age was 57.48 years (range: 27-90).

The patients were stratified into two groups: those with a family history of breast cancer: 67 patients (17%); and those with no family history of the disease, whose breast cancer was sporadic: 322 patients (83%).

According to Lynch *et al*[10, 11], hereditary breast cancer is diagnosed when a family group exhibits a high

frequency of mammary carcinomas that are distributed in different generations in a pattern consistent with Mendelian (autosomal dominant) transmission. Lynch *et al*[10] stated that at least three family members with breast cancer were required, i.e. the proband plus two other first or second degree relatives, in order to make the diagnosis.

The following criteria were used to define FBC[10]:

– three or more first-degree relatives had been affected by breast cancer;
– two first-degree relatives had been affected by breast cancer, and either of them were under 40 years old and/or had had bilateral breast cancers.

In the absence of the above criteria, the cases were considered sporadic and were included in the second (control) group.

Clinical status variables evaluated for the analysis were: age, menopausal status, tumour type (palpable or not), multifocality/multicentricity, mammographic appearance, type of surgery, lymphadenectomy, and axillary lymph node status.

Pathological variables considered were: tumour size, histological type and tumour grading. Histological typing and histological grading were evaluated according to the WHO classification.

Hormonal variables evaluated were: oestrogen receptor (ER) and progesterone receptor (PR) content in all the patients, moreover we considered these variables dividing the patients in subgroups stratified by patients' age (≤ 60 and > 60 years). Hormonal receptor status in the two groups were then compared.

Genetic variables evaluated were: MIB-1, ErbB2, p53 and bcl 2 expression.

Methodological considerations

The surgical specimen was fixed overnight in 10% neutral-buffered formalin, sampled and embedded in paraffin blocks, sectioned at a thickness of 4 μm and stained with haematoxylin-eosin (HE).

Furthermore, additional sections, collected on poly-L-lysine coated slides, were used for the immunohistochemical staining with the avidin-biotin-based detection method. The sections were incubated, overnight at 4°C, with the primary antibodies listed in Table 1. Appropriate negative controls, obtained by substituting the primary antibodies with pre-immune serum, and positive controls, indicated in Table 1, were included in the procedure.

The immunohistochemical staining was semi-quantitatively evaluated by two pathologists.

We scored ER/PR levels as negative (–) if positive tumoral cells were ≤ 10%, positive (+) if tumoural cells were 11-25%, (++) if positive tumoural cells were 26-50%, (+++) if tumoural cells were more than 50%. Only nuclear staining was evaluated.

ErbB2 status was scored on the following basis: 0 (no detectable staining); + (<10% positive cells with partial membranous staining); ++ (>10% positive cells with concentric and weak membranous staining); +++ (>10% positive cells with concentric and strong intense membranous staining).

Bcl 2 and p53 staining was scored in a three-way categorization: negative (no detectable staining); <50% positive cells; >50% positive cells. Only nuclear staining was evaluated.

The presence of steroid hormone receptors (ER and PR), ErbB2, p53 and bcl 2 was not evaluated on all the specimens because of technical problems. In particular: ER and PR were evaluated on 321/322 cases (99.6%) in SBC patients and 64/67 (95.5%) in FBC patients; MIB1 on 312/322 (96.9%) in SBCs and 63/67 (94%) in FBCs; ErbB2 on 272/322 (84.5%) in SBCs and 55/67 (82.1%) in FBCs; p53 on 121/322 (37.6%) in SBCs and 27/67 (40.3%) in FBCs; bcl 2 on 112/322 (34.7%) in SBCs and 26/67 (38.8%) in FBCs.

Statistical analysis

The data were analyzed with SPSS for Windows (release 10; SPSS Inc., Chicago, IL). Comparison of group averages was determined by *t*-testing, and comparison of data with the multiplication tables was determined by a Pearson χ^2 test. p values <0.05 were considered significant.

Table 1 - Pertinent data of the monoclonal antibodies used to immuno-characterize carcinoma of the breast

Antigen	Clone	Dilution	Source	Positive control
Oestrogen receptor	1D5	1/150	Zymed - San Francisco, CA, USA	Normal breast
Progesterone receptor	PR88	1/60	BioGenex - San Ramon, CA, USA	Normal breast
bcl 2	100	1/800	BioGenex - San Ramon, CA, USA	Tonsils
ErbB2	CB11	1/20	BioGenex - San Ramon, CA, USA	*In situ* breast carcinoma
Ki 67	MIB-1	1/100	Dako - Glostrup, Denmark	Undifferentiated carcinoma
p53	Do-7	1/50	Dako - Glostrup, Denmark	Colonic carcinoma

Results

Table 2 shows the results of our study on the physical characteristics of the patients. The age distribution of FBCs and SBCs was comparable (p=0.6) as well as menopausal status (p=0.24).

Considering the tumour type, a higher frequency of palpable lesions was recorded in SBCs (78,5%) as compared with FBCs (61.2%), with a high statistical significance (p=0.003). There was a higher percentage of multifocal and/or multicentic tumours in FBCs as compared to SBCs, in accordance with other authors[12], but this result did not reach statistical significance (p=0.33).

When comparing the mammographic appearance of the tumours, FBCs appeared more often as microcalcifications (22.4% *vs* 9.3%), whereas SBCs appeared frequently as stellate lesions (72.8% *vs* 52.2%), with high statistical significance (p=0.002).

In our study FBCs occurred more often as nonpalpable or smaller lesions, and therefore they more frequently underwent a limited surgery (quadrantectomy) (50.7% *vs* 48.3%), even though the variation in the type of surgery did not reach statistical significance.

For the above reason, axillary lymphadenectomy, was more frequently not performed in FBCs as compared with SBCs (20.9% *vs* 17.9%) (p=0.56). Axillary lymph node

Table 2 - Patient[a] characteristics (clinical status)

Variable	SBC[b]		FBC[c]		χ^2	df[d]	p value
Age median ± SD	56.81±13.3		58.16±10.9		-0.523[e]	139	0.60
	N.	%	N.	%			
Menopausal status							
Premenopausal	110	34.2	18	26.9			
Postmenopausal	212	65.8	49	73.1	1.337	1	0.24
Type of tumour							
Not palpable	70	21.5	26	38.8			
Palpable	255	78.5	41	61.2	8.957	1	**0.003**
Multifocality							
No	239	73.5	42	62.7			
Multifocal	40	12.3	12	17.9			
Multicentric	29	9.0	9	13.4			
Multifocal and multicentric	17	5.2	4	6.0	3.394	3	0.33
Mammographic appearance							
Stellate lesions	237	72.9	35	52.2			
Microcalcifications	30	9.2	15	22.4			
Microcalcific. + stellate lesion	53	16.4	17	25.4			
Nipple discharge	5	1.5	0	-	15.269	3	**0.002**
Type of surgery							
Quadrantectomy	157	48.3	34	50.7			
Modified radical mastectomy	153	47.1	29	43.3			
Total mastectomy	15	4.6	4	6.0	0.45	2	0.79
Lymphadenectomy							
No	58	17.9	14	20.9			
Yes	267	82.1	53	79.1	0.331	1	0.56
Axillary lymph node status							
Negative	162	60.9	34	64.2			
Positive	104	39.1	19	35.8	0.197	1	0.65

[a] 389 female and 3 male
[b] Sporadic breast cancer
[c] Familial breast cancer
[d] Degrees of freedom
[e] *t-testing*

status did not show any significant difference between the two groups (p=0.65).

Table 3 shows the results on histopathological status. There was a marked difference in tumour size between the two groups (p=0.04), since in FBCs there were generally smaller tumours (T1a).

Histological type showed a higher rate of carcinoma *in situ* in FBCs (17.9% *vs* 9.5%), often combined with invasive cancers (40.3% *vs* 36%) (p=0.009). We did not find a higher rate of lobular or medullary histological type in FBCs. As regards the grading, although there was no statistically significant difference (p=0.76), we noted a higher number of well differentiated tumours (G1) in FBCs (24.6%) as compared with SBCs (20.9%).

Table 4 shows the results according to hormonal status: the ER and PR content was similar in the two groups in all patient ages, even if the difference regarding PR content had borderline significance (p=0.05)

We divided the patients into two subgroups (≤60 and >60 years). The results of the analysis were very interesting. With regard to ER expression, we did not find any difference in patients under 60 years of age. In patients over 60, on the contrary, we noted a non significant trend towards higher ER++ content in FBCs (16.7%) than in SBCs (6.8%) (p=0.54).

Similarly the PR content, in patients under 60 years of age, did not show any differences, whereas in patients over 60 the PR content was clearly lower in FBCs (58.3% *vs* 23.7%) with a statistically significant difference (p=0.02).

Table 5 gives the results on genetic status: the expression of MIB-1, c-ErbB2, p53 and bcl 2 did not reach statistically significant differences.

Discussion

Several studies have compared the characteristics of FBCs and those of sporadic controls. Clinical, histopathological and prognostic differences between FBCs and SBCs have been found[12-15].

In comparison with controls, FBCs have been shown to have the following characteristics: early age at onset, and medullary and lobular histotype. Lobular tumours were

Table 3 - Patient characteristics (histopathological status)

Variable	SBC[a]		FBC[b]		χ^2	df[c]	p value
	N.	%	N.	%			
Tumour size							
Tis	30	9.2	12	17.9			
T1a	15	4.6	7	10.4			
T1b	51	15.7	11	16.4			
T1c	101	31.1	22	32.8			
T2	103	31.7	14	20.9			
T3	11	3.4	1	1.5			
T4	14	4.3	0	-	13.051	6	**0.04**
Histological type							
Dis	31	9.5	10	14.9			
Lis	0		2	3.0			
Ductal invasive	139	42.8	21	31.3			
Lobular invasive	15	4.6	7	10.4			
Medullary	2	0.6	0	-			
Tubular, mucinous	8	2.5	0	-			
Papillary	7	2.2	0	-			
Ductal invas. + Dis	108	33.2	26	38.8			
Lobular invas. + Lis	9	2.8	1	1.5			
Other	6	1.8	0	-	21.988	9	**0.009**
Grading							
G1	67	20.6	16	23.9			
G2	141	43.4	29	43.3			
G3	117	36.0	22	32.8	0.541	2	0.76

[a] Sporadic breast cancer
[b] Familial breast cancer
[c] Degrees of freedom

Table 4 - Patient characteristics (hormonal status)[a]

Variable	SBC[b]		FBC[c]		χ^2	df[d]	p value
	N.	%	N.	%			
ER							
Negative	74	23.1	16	25.0			
+	24	7.5	4	6.3			
++	42	13.1	11	17.2			
+++	181	56.4	33	51.6	1.073	3	0.78
ER (age ≤ 60 years)							
Negative	54	28.7	15	28.8			
+	20	10.6	4	7.7			
++	33	17.6	9	17.3			
+++	81	43.1	24	46.2	0.443	3	0.93
ER (age > 60 years)							
Negative	20	15.0	1	8.3			
+	4	3.0	0	-			
++	9	6.8	2	16.7			
+++	100	75.2	9	75.0	2.125	3	0.54
PR							
Negative	110	34.2	31	48.4			
+	45	14.0	10	15.6			
++	71	22.1	14	21.9			
+++	95	29.6	9	14.1	7.757	3	0.05
PR (age ≤ 60 years)							
Negative	79	41.8	24	46.2			
+	29	15.3	8	15.4			
++	34	18.0	11	21.2			
+++	47	24.9	9	17.3	1.483	3	0.68
PR (age > 60 years)							
Negative	31	23.5	7	58.3			
+	16	12.1	2	16.7			
++	37	28.0	3	25.0			
+++	48	36.4	0	-	9.567	3	**0.02**

[a] The presence of hormone receptors (ER and PR) was not evaluated on all the specimens (321/322 SBC patients and 64/67 FBC patients) because of technical problems
[b] Sporadic breast cancer
[c] Familial breast cancer
[d] Degrees of freedom

more likely to be bilateral than other types of breast tumours[13, 14]. FBC tumours were of significantly lower grading and showed lower scores for pleomorphism and tubule formation[15]. When the onset of breast cancer in relatives occurred at or before the age of 45 years, increased risks were evident for ER– PR+ and ER– PR– tumours[7].

In our study we pointed out the clinical, histopathological and immunohistochemical differences between patients with FBC and patients with SBC. We noticed that FBCs were more frequently non-palpable tumours, of smaller size (diameter <1 cm), and with mammographic appearance as microcalcifications and a lower grading as compared with SBCs. For the above reasons FBC patients more often underwent conservative surgery, without axillary lymphadenectomy when sentinel lymph node biopsy was performed and proved negative.

Moreover, in our study, we demonstrated an increased frequency of carcinoma *in situ* even associated with invasive cancers. These lesions have been found increasingly in recent years, as a result of mammographic screening.

In view of our results, it seems logical to suppose that women with a family history of breast cancer should take

Table 5 - Patient characteristics (genetic status)[a]

Variable	SBC[b]		FBC[c]		χ^2	df[d]	p value
	N.	%	N.	%			
MIB 1							
Negative	23	7.3	1	1.6			
+	126	40.4	32	50.8			
++	115	36.9	20	31.7			
+++	48	15.4	10	15.9	4.477	3	0.21
ErbB2							
Negative	234	86.0	48	87.3			
+	8	2.9	3	5.4			
++	8	2.9	2	3.6			
+++	22	8.0	2	3.6	2.175	3	0.73
p53							
Negative	88	72.7	19	70.4			
<50%	13	10.7	4	14.8			
>50%	20	16.5	4	14.8	0.375	2	0.82
bcl 2							
Negative	26	23.2	4	15.4			
<50%	19	17.0	8	30.8			
>50%	67	59.8	14	53.8	2.779	2	0.24

[a] The genetic status was not evaluated on all the specimens because of technical problems:
- MIB1: 312/322 SBC pts; 63/67 FBC pts;
- ErbB2: 272/322 SBC pts; 55/67 FBC pts;
- p53: 121/322 SBC pts; 27/67 FBC pts;
- bcl 2: 112/322 SBC pts; 26/67 FBC pts

[b] Sporadic breast cancer
[c] Familial breast cancer
[d] Degrees of freedom

greater care of their health, with particular regard to the early detection of breast cancer.

However, in accordance with Lakhani *et al* [15], these findings need to be interpreted with caution and may be partially or completely attributable to an important bias: breast cancers arising within families with multiple cases may have been detected earlier in their natural history as a result of mammographic screening of unaffected family members and hence may present features indicative of lower grading.

In the present study the PR content is lower in FBCs than in SBCs in patients of all ages, but especially in patients over 60 years of age, where it reaches statistical significance (p=0.02), whereas the ER content is similar in the two groups. Okamura *et al* [16] reported the positivity rate of ER to be 70% in Japanese, late-onset FBCs whereas that of PR was only 50%. In addition, a high frequency of ER+ PR– tumours was observed in FBC patients over 60 years of age, although the difference did not reach statistical significance.

Fukutomi *et al* [17] reported that in FBCs, in all age groups, the PR contents were significantly lower than those in SBCs, particularly in patients over 60 years old. In addition there was a non significant trend towards a high frequency of ER+ PR– tumours in FBC patients aged 60 years and over.

More recently, Eerola *et al* [18] demonstrated a significantly higher content of PR– in breast cancer of non-BRCA1/2 families *vs* SBCs and even more significantly *vs* BRCA1 breast cancers. Moreover a PR gene polymorphism leads to an increased risk of breast cancer, since it causes inadequate control of ER–driven proliferation. The PR-A form of PR has been shown to repress ER activation and contribute to oestrogen-related tumour promotion in the mammary gland of premenopausal women[19]. Wang-Gohrke *et al* [19] reported that PR gene polymorphism is associated with a varying effect based on a family history of breast cancer.

It is possible that the ER and/or PR gene functions are linked with some late-onset FBCs. In some breast cancers there is a possibility that a rich PR expression may thus be lost during cancer development[17].

In conclusion, these immunohistochemical and pathological characteristics of the tumours should be of value in evaluating the possibility of mutation and in targeting mutation screening in such families, especially when considering a family history of cancer and the characteristics of the tumours[18]. Finally, it is well-known that breast cancers with lower expression of ER and PR as well as ER+ and PR– have a worse prognosis. As a clinical consequence, late-onset FBCs, since they are frequently PR–, need more intensive therapy and a more careful follow-up.

References

1. Hirose K, Tajima K, Hamajima N, *et al*. Association of family history and other risk factors with breast cancer risk among Japanese premenopausal and postmenopausal women. Cancer Causes Control 2001; 12: 349-58.
2. Hall JM, Lee MK, Newman B, *et al*. Linkage of early-onset familial breast cancer to chromosome 17q21**.** Science 1990; 250: 1684-89.
3. Wooster R, Bignell G, Lancaster J, *et al*. Identification of breast cancer susceptibility gene BRCA2. Nature 1995; 8: 789-92.
4. Castilla LH, Couch FJ, Erdos MR, *et al*. Mutations in the BRCA1 gene in families with early-onset breast and ovarian cancer. Nat Genet 1994; 8: 387-91.
5. Lakhani SR, van de Vijver MJ, Jacquemier J, *et al*. The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, Her-2, and p53 in patients with mutations in BRCA1 and BRCA2. J Clin Oncol 2002; 20: 2310-18.
6. Lehrer S, Levine E, Savoretti P, *et al*. Estrogen and progesterone receptor dissociation and family history of breast cancer. Lancet 1992; 339: 1108-9.
7. Tutera AM, Sellers TA, Potter JD, *et al*. Association between family history of cancer and breast cancer defined by estrogen and progesterone receptor status. Genet Epidemiol 1996; 13: 207-21.
8. Roodi N, Bailey LR, Kao WY, *et al*. Estrogen receptor gene analysis in estrogen receptor-positive and receptor-negative primary breast cancer. J Natl Cancer Inst 1995; 87: 446-51.
9. Zuppan P, Hall JM, Lee MK, *et al*. Possible linkage of the estrogen receptor gene to breast cancer in a family with late-onset disease. Am J Hum Genet 1991; 48: 1065-8.
10. Lynch HT, Watson P, Conway TA, *et al*. Clinical genetic features in hereditary breast cancer. Breast Cancer Res Treat 1990; 15: 63-71.
11. Lynch HT, Watson P, Conway TA, *et al*. Breast cancer family history as a risk factor for early onset breast cancer. Breast Cancer Res Treat 1988; 11: 263-7.
12. Fukutomi T, Akashi-Tanaka S, Nanasawa T, *et al*. Multicentricity and histopathological background features of familial breast cancers stratified by menopausal status. Int J Clin Oncol 2001; 6: 80-3.
13. Rosen PP, Lesser ML, Senie RT, *et al*. Relationship of family history to tumor type. Cancer 1982; 50: 171-9.
14. Claus EB, Risch N, Thompson WD, *et al*. Relationship between breast histopathology and family history of breast cancer. Cancer 1993; 71: 147-53.
15. Lakhani SR, Gusterson BA, Jacquemier J, *et al*. The pathology of familial breast cancers: histological features of cancers in families not attributable to mutations in BRCA1 or BRCA2. Clin Cancer Res 2000; 6: 782-9.
16. Okamura T, Fukutomi T, Tsuda H, *et al*. Japanese late-onset breast cancer families: their clinicopathological characteristics and absence of BRCA1 and BRCA2 germline mutations. Breast Cancer Res 1998; 5: 251-4.
17. Fukutomi T, Akashi-Tanaka S. Differences in the progesterone receptor contents between familial breast cancers and sporadic breast cancers stratified by patient age. Surg Today 2001; 31: 963-7.
18. Eerola H, Heikkila P, Tamminen A, *et al*. Histopathological features of breast tumours in BRCA1, BRCA2 and mutation-negative breast cancer families. Breast Cancer Res 2005; 7: 93-100.
19. Wang-Gohrke S, Chang-Claude J, Becker H, *et al*. Progesterone receptor gene polymorphism is associated with decreased risk for breast cancer by age 50. Cancer Res 2000; 60: 2348-50.

Eur. J. Oncol., vol. 11, n. 2, pp. 121-132, 2006

Identificazione di carcinoma occulto della mammella in pazienti con linfonodi ascellari metastatici (*CUP syndrome*): confronto preliminare tra procedure diagnostiche convenzionali e non

Detection of occult breast cancer in patients with axillary lymph node metastases (CUP syndrome): preliminary comparison of conventional diagnostic procedures and other imaging techniques

Stefano Panareo*, Stefano Corcione**, Luciano Maria Feggi*
* Struttura Complessa di Medicina Nucleare, Azienda Ospedaliero-Universitaria di Ferrara, Ferrara, Italia
** Centro di Senologia, Azienda Ospedaliero-Universitaria di Ferrara, Ferrara, Italia

Riassunto

***Finalità*. Lo scopo dello studio è di valutare l'utilità dell'integrazione di procedure diagnostiche convenzionali, quali l'Ecografia mammaria (US) e la Mammografia (MM), e di tecniche non convenzionali, come la Risonanza Magnetica mammaria (RM), la Scintimammografia con 99mTc-MIBI (SM), e la Tomografia ad Emissione di Positroni con 18F-Fluoro-Desossi-Glucosio (PET) per la ricerca e la stadiazione di carcinoma occulto (*cancer unknown primary* = *CUP*), di possibile origine mammaria, in pazienti affette da metastasi linfonodali ascellari. *Materiali e metodi*. Sono state studiate sei pazienti giunte alla nostra osservazione per linfonodi ascellari metastatici con diagnosi bioptica di adenocarcinoma di probabile origine mammaria. Tutte le pazienti, senza storia nota di patologia oncologica e con esame obiettivo generale negativo, sono state sottoposte ad una serie di indagini strumentali convenzionali e non, secondo procedure standardizzate, mirate alla ricerca della neoplasia primitiva: US, MM, RM, SM e PET. I risultati di queste indagini sono stati confrontati e correlati con l'esame istopatologico conseguente ad exeresi chirurgica della neoplasia primitiva e dei linfonodi ascellari metastatici. *Risultati*. In tutti i**

Summary

***Objectives*. The aim of the study is to evaluate the utility of conventional integrated diagnostic procedures, such as breast Ultrasonography (US), Mammography (MX) and non conventional procedures, such as breast Magnetic Resonance Imaging (MRI), Scintimammography with 99mTc-MIBI (SM), and 18F-FDG-Positron Emission Tomography (PET) for the detection and staging of possible occult breast cancer (cancer unknown primary = CUP) in patients with axillary lymph node metastases. *Materials and methods*. Six women with biopsy-proven metastatic adenocarcinoma in the axillary lymph nodes and probable occult breast lesion, were studied. All patients, without a history of known cancer and with a negative physical examination, underwent a series of conventional and non conventional instrumental diagnostic examinations, following standard procedures, aimed at the detection of the primary tumour: US, MX, MRI, SM and PET. The results of these examinations were compared and correlated with the histopathologic findings subsequent to breast MRI-guided biopsy, the exeresis of the primary neoplasia and metastatic axillary lymph node clearance. *Results*.**

Received/Pervenuto 14.3.2006 - Accepted/Accettato 31.3.2006
Address/Indirizzo: Dr. Stefano Panareo, Struttura Complessa di Medicina Nucleare, Azienda Ospedaliero-Universitaria di Ferrara, Corso Giovecca 203, 44100 Ferrara, Italia - Tel. +39/0532/236359 - 237458 - Fax +39/0532/237553 - E-mail: s.panareo@ospfe.it

casi sia l'esame clinico che l'US e la MM sono risultati negativi. La RM, invece, ha rilevato il tumore primitivo come un'area ad elevato *contrast-enhancement* monofocale in 5 casi e come un'area di *contrast-enhancement* quadri-focale in 1 caso. In tutte le pazienti la SM ha confermato la presenza della lesione mammaria vista alla RM come un'area di aumentato *uptake* del tracciante metabolico, tranne in un caso dove ha evidenziato solo la lesione di maggiori dimensioni e non quelle concomitanti di diametro inferiore al centimetro. Alla SM la captazione dei linfonodi ascellari è stata rilevata in tutti i casi: in 5 delle 6 pazienti omolateralmente alla lesione biopsiata, in 1 caso controlateralmente. La PET ha evidenziato la localizzazione mammaria in 5 pazienti, mentre in 1 caso è risultata negativa. D'altra parte la PET ha individuato le metastasi ai linfonodi ascellari in tutte e 6 le pazienti. Inoltre la PET ha evidenziato una metastasi epatica in un caso. Questo ultimo reperto è stato confermato con l'US e con la Tomografia Computerizzata (TC) addominale. *Conclusioni*. PET, SM e RM rappresentano un utile strumento in caso di *CUP syndrome*. In casi selezionati queste indagini hanno un valore superiore rispetto alle procedure di *imaging* convenzionale. La PET è particolarmente utile per la valutazione della estensione a distanza della malattia. Il caso di lesione mammaria osservata alla SM e alla RM, non evidenziata dalla PET, è probabilmente correlato al basso metabolismo glucidico del tumore. È dimostrato inoltre che l'accuratezza diagnostica della SM nelle pazienti con neoplasia mammaria è comparabile a quella della RM. Comunque la RM è più sensibile nella identificazione di lesioni multiple (multicentriche e/o multifocali), soprattutto quando di dimensioni inferiori al centimetro. D'altra parte la SM può identificare le metastasi ascellari. Il caso di captazione linfonodale ascellare controlaterale alla sede del tumore, osservata alla SM, potrebbe essere legata ad una reazione di tipo infiammatorio. Nelle pazienti con MM e US non diagnostiche, l'utilizzo complementare di SM, RM e PET aumenta la sensibilità dell'*imaging* della mammella. Essendo la nostra casistica limitata, non è possibile alcuna inferenza sul valore clinico delle metodiche ivi confrontate. Eur. J. Oncol., 11 (2), 121-132, 2006

***Parole chiave: CUP syndrome*, metastasi linfonodali ascellari, scintimammografia, risonanza magnetica mammaria, 18F-FDG PET**

The clinical examination, the US and the MX resulted negative in all cases. Instead the MRI revealed the primary cancer as an area of high monofocal contrast-enhancement in 5 cases and an enhancing quadrifocal lesion in 1 case. In all patients the SM confirmed the presence of a primary breast lesion identified on the MRI as an area of increased uptake of the metabolic tracer with the exception of one case, where it showed only the largest lesion and not those with a diameter of less than 1 cm. With the SM, axillary uptake was detected in all cases: in 5 out of 6 patients homolaterally to the biopsied lesion, in 1 case controlaterally. The PET showed the breast localization in 5 patients, whereas it proved negative in 1 case. On the other hand, the PET found the axillary lymph node metastases in all the patients. Moreover the PET discovered a liver metastasis in one case, which was confirmed by abdominal US and Computed Tomography (CT). *Conclusions*. PET, SM and MRI are useful tools for identifying the primary tumour site in patients with axillary metastases from occult breast cancer. In selected cases they are superior to conventional imaging diagnostic procedures. Moreover PET is useful for evaluating the distant extension of the disease. One case of breast lesion observed with SM and MRI, but not found by PET, is probably related to low glucose metabolism of the tumour. It is furthermore demonstrated that the diagnostic accuracy of SM in patients with breast cancer is comparable to that of MRI. However MRI is more sensitive in detecting multiple site lesions (multicentric and/or multifocal) expecially those of less than one centimeter. On the other hand SM was shown to identify axillary metastases. The axillary lymph node uptake controlateral with respect to the site of tumour, observed with SM, may be related to an inflammatory reaction. In patients with non diagnostic MX and US, the complementary use of SM, MRI and PET increases the sensitivity of breast imaging. The present case series is too limited in number to be able to draw any firm conclusions on the clinical value of each of the diagnostic methods considered. Eur. J. Oncol., 11 (2), 121-132, 2006

***Key words:* CUP syndrome, lymph node axillary metastases, scintimammography, breast magnetic resonance imaging, 18F-FDG PET**

Introduzione

Si definisce *CUP* (*Cancer Unknown Primary*) *syndrome* una condizione di malattia metastatica per la quale non è nota la sede primitiva tumorale che la determina. Rappresenta circa il 5-10% di tutti i tumori maligni ed è uno dei 10 più frequenti tumori diagnosticati nell'uomo. È comunemente accettato che la *CUP syndrome* rappresenta un gruppo eterogeneo di patologie maligne che condivide un comportamento clinico unico, caratterizzato da rapida progressione e da localizzazioni metastatiche atipiche e, presumibilmente, una unica biologia[1].

Ci sono diverse entità clinico-patologiche riconosciute che rientrano nel gruppo CUP: 1) CUP primariamente metastatico soprattutto al fegato o in sedi multiple, 2) CUP metastatico ai linfonodi compresi quelli mediastinico - retroperitoneali, ascellari, cervicali o inguinali, 3) CUP metastatico della cavità peritoneale compresa la carcinomatosi sierosa papillare peritoneale femminile e la carcinomatosi non papillare peritoneale maschile o femminile, 4) CUP metastatico ai polmoni caratterizzato da metastasi parenchimali o diffusione pleurica maligna isolata, 5) CUP metastatico scheletrico, 6) CUP metastatico cerebrale, 7) carcinoma metastatico di tipo neuroendocrino e 8) melanoma metastatico di origine sconosciuta.

Le sedi neoplastiche primitive più frequentemente rilevate e causa di *CUP syndrome* sono il carcinoma occulto del polmone o del pancreas. Sulla base della sensibilità-responsività alla chemioterapia sistemica e/o al trattamento locoregionale, sono stati distinti diversi sottogruppi di CUP: quelli, per esempio, responsivi alla chemioterapia con cisplatino sono i carcinomi scarsamente differenziati che interessano i linfonodi mediastinico-retroperitoneali, l'adenocarcinoma sieroso papillare peritoneale femminile ed i carcinomi scarsamente differenziati di tipo neuroendocrino. Altri tumori controllati con successo dal trattamento locoregionale con la chirurgia e/o l'irradiazione sono l'adenocarcinoma metastatico dei linfonodi ascellari isolati, il carcinoma squamoso metastatico dei linfonodi cervicali o qualunque altro singolo sito metastatico[2].

Recentemente la diagnosi di CUP è stata migliorata dall'introduzione di tecniche istopatologiche (immunoistochimica, microscopia elettronica, biologia molecolare) e di *imaging*, quali la Tomografia Computerizzata (TC), la Risonanza Magnetica (RM), e la Tomografia ad Emissione di Positroni con 18F-Fluoro-Desossi-Glucosio (PET) nonostante la sede primaria rimanga sconosciuta, nella maggior parte dei pazienti, anche in sede autoptica. Per quanto concerne le indagini istopatologiche, il *sampling*, per esempio, degli indicatori istochimici come le citocheratine 7 e 20 appare utile per la diagnosi differenziale in pazienti che manifestano un adenocarcinoma. In riferimento all'utilità delle tecniche di *imaging*, la TC è stata introdotta primariamente per la ricerca del tumore primitivo sconosciuto, anche se i risultati non sono così confortanti[3]. Dall'altra parte, la RM (per es. mammaria) è in grado di identificare un tumore primitivo occulto nel 75% delle pazienti che presentano adenocarcinoma linfonodale ascellare, influenzandone, così, il decorso chirurgico. La PET può essere utilizzata per la diagnosi di CUP, anche se il suo valore è controverso[4].

Entrando nello specifico del tumore mammario primitivo, esso può essere diagnosticato in diversi modi. La mammografia (MM) è la tecnica più usata per le sue eccellenti *performances*, per la *compliance* del paziente e per il buon rapporto costo-beneficio. Altre tecniche radiologiche, come l'ecografia (US) sono indicate in particolari circostanze, mentre altre, come la Mammografia Digitale (MMD) e la RM sembrano veramente promettenti anche se in fase di valutazione[5].

I recenti progressi in Medicina Nucleare hanno consentito di introdurre due procedure diagnostiche validate da estese esperienze scientifiche internazionali: la Scintimammografia con catione lipofilico marcato con 99mTc (MIBI o Tetrofosmin) (SM) e la PET. Il 99mTc-MIBI è un radiofarmaco che penetra all'interno delle cellule neoplastiche attraverso un meccanismo attivo e viene immagazzinato nei mitocondri: in competizione con tale meccanismo di *uptake* endocellulare agisce un altro meccanismo di efflusso, espressione di "*multidrug resistance*" (MDR-1), condizionante la resistenza di vari tipi di tumore al trattamento chemioterapico. Tale MDR-1 sembra essere in particolare correlata al contenuto cellulare della glicoproteina P (gP). La concentrazione significativamente maggiore di mitocondri e quindi di MIBI nelle cellule metabolicamente attive della neoplasia rispetto a quelle circostanti del tessuto normale consente l'individuazione del tumore, ove il catione si concentra e viene rilevato, previa opportuna marcatura con 99mTc[6]. Attualmente la SM ha una limitata applicazione diagnostica dovuta alla bassa risoluzione spaziale delle gamma-camere tradizionali in commercio, la quale non va al di sotto di 8-10 mm. Tale *gap* sembra poter essere risolto dalla introduzione di una gamma-camera dedicata allo studio della mammella (Lumagem®), in fase di sperimentazione, che migliora la risoluzione spaziale (1,5 mm) e quindi la sensibilità della SM. La SM è indicata per lo studio della lesione mammaria in pazienti nelle quali MM e US sono non diagnostiche o difficili da interpretare. È anche utile per valutare e perfino predire la risposta alla chemioterapia primaria[7].

È lecito chiedersi se la Tomografia ad Emissione di Singolo Fotone (Single Photon Emission Computed Tomography = SPECT) possa offrire maggiori informazioni sulla patologia mammaria; di certo la SPECT è più preci-

sa nella rilevazione dei linfonodi ascellari. Senza dubbio il vantaggio generale della Medicina Nucleare è che il radiofarmaco (detto anche "indicatore positivo" o "oncotropo") si accumula nella cellula neoplastica esprimendo, quindi, un segnale di attività-vitalità neoplastica. Per contro le tecniche radiologiche convenzionali (MM, US, TC) forniscono informazioni di tipo morfologico, non funzionale[8].

Le tecniche più interessanti offerte, oggi, dalla Medicina Nucleare sono la PET e la Linfoscintigrafia per la rilevazione intraoperatoria di lesione occulta mammaria e di linfonodo sentinella (Sentinel Node and Occult Lesion Localisation = SNOLL). La tecnica del linfonodo sentinella sta acquisendo sempre più consenso nella pratica clinica, per la sua affidabilità e riproducibilità, migliorando l'*outcome* della paziente[9]. Nello stesso tempo molti Autori riconoscono il valore della PET nella diagnosi differenziale delle lesioni mammarie e nello *staging* locoregionale, poiché il cancro della mammella è, solitamente, molto avido di glucosio[10, 11]. La PET è sempre più utilizzata in oncologia ed è particolarmente utile nel cancro mammario, perché sembra fornire informazioni più esatte rispetto alla SM nella valutazione di pazienti con MM ambigua e nel discriminare fra il possibile tumore, la cicatrice fibrosa o la necrosi post-chirurgica, chemio e/o radioterapica[12, 13]. La captazione del fluoro-desossi-glucosio (FDG) da parte del tumore è correlata con il *grading* e la potenziale aggressività della neoplasia, che può avere implicazioni prognostiche. In aggiunta alla sua utilità nello studio delle lesioni mammarie, la PET mostra grande efficacia nel riscontro di linfonodi ascellari e non interessati da malattia, prima della chirurgia, anche se non può avere una risoluzione spaziale sufficiente per diagnosticare la micrometastasi linfonodale[14]. La PET totale corporea fornisce, inoltre, informazioni sui tessuti molli e sull'osso attraverso una singola scansione "*whole body*", svolgendo, pertanto, un ruolo importante nella ricerca di metastasi locoregionali o a distanza. La PET totale corporea può, quindi, sostituire altre indagini strumentali per la ricerca di potenziali diffusioni, a distanza, del tumore[15].

In fase di sperimentazione c'è anche la Mammografia ad Emissione di Positroni (PEM) (fig. 1). Si tratta di un tomografo dedicato per lo studio della mammella che rileva la radioattività emessa dal tumore in seguito alla somministrazione ev di 18F-FDG[16]. Altre tecniche innovative, come la Tomografia Ottica, contano, invece, sul rilievo della neoangiogenesi tumorale[17]. Le metodiche di ultimissima generazione potrebbero contribuire a migliorare sia la sensibilità che la specificità nella diagnosi di cancro, migliorando, da un lato, l'*outcome* di pazienti oncologiche, dall'altro, evitando procedure inutili nelle pazienti esenti da patologia neoplastica.

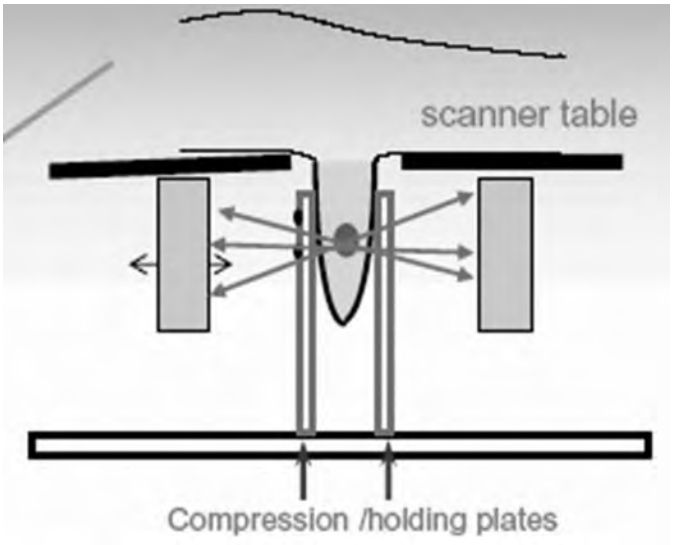

Fig. 1. PEM (*Positron Emission Mammography*). Per concessione del Jefferson Lab Detector and Imaging Group, Newport, VA, USA

Gli esami che correntemente vengono eseguiti per la stadiazione pre- e post- chirurgica della neoplasia mammaria sono: RX torace, US addominale, MM, Scintigrafia ossea con 99mTc-difosfonato e test ematochimici (*markers* tumorali). La TC e la RM possono essere utilizzate per risolvere problemi diagnostici particolari. L'applicazione corrente di alcune di queste modalità dipende dal rischio del singolo paziente di sviluppare diffusione metastatica che è legata a diversi parametri come aggressività tumorale e, naturalmente, stadio clinico della malattia. La scintigrafia ossea e la PET possono essere utili nel controllo della risposta alla chemio-radioterapia e alla individuazione di recidive tumorali. Sulla base delle suddette considerazioni, le tecniche di *imaging* medico nucleare, integrate da quelle radiologiche convenzionali, offrono una opportunità interessante di accrescere il valore dell'*imaging* senologico integrato nella diagnosi e *staging* della neoplasia mammaria, con conseguente miglioramento della gestione clinica del paziente in termini sia di sopravvivenza che di qualità della vita[4].

Lo scopo di questo studio è di valutare l'utilità dell'integrazione di procedure diagnostiche convenzionali, quali l'US mammaria e la MM, e tecniche non convenzionali, come la RM mammaria, la SM con 99mTc-MIBI, e la 18F-FDG-PET, per la ricerca e la stadiazione di carcinoma occulto, di possibile origine mammaria, in pazienti affette da metastasi linfonodali ascellari.

Materiali e metodi

Sono state studiate 6 pazienti giunte, in tempi diversi, alla nostra osservazione per linfoadenomegalia ascellare.

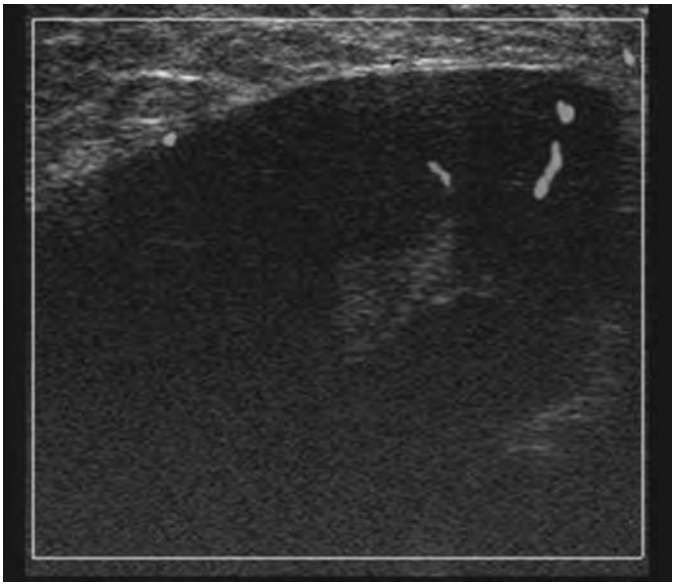

Fig. 2. US ascellare: linfonodo metastatico (caso I)

Le pazienti sono state sottoposte ad esame obiettivo del cavo ascellare e delle mammelle che ha evidenziato, in tutti i casi, una tumefazione ascellare non dolente, aderente ai piani profondi. Assenti tumefazioni palpabili o visivamente apprezzabili alle mammelle. L'US del cavo ascellare ha evidenziato, in tutti i casi, aspetto irregolarmente ovalare, ipoecogeno e con *pattern* vascolare sovvertito del linfonodo palpabile, orientativo quindi di localizzazione metastatica (fig. 2). Tale diagnosi è stata confermata dalla biopsia linfonodale US-guidata. Tutte le pazienti, senza storia nota di patologia oncologica e con esame obiettivo generale negativo, sono state sottoposte ad una serie di indagini strumentali convenzionali e non, secondo procedure standardizzate, mirate alla ricerca della neoplasia primitiva, partendo dallo studio delle mammelle. Sono state eseguite: US mammaria, MM, RM mammaria, SM e PET.

L'US è stata eseguita in tempi diversi, da due differenti operatori, con sonda ecografia da 10 Mhz, osservando tutti e quattro i quadranti di entrambe le mammelle. La MM è stata condotta secondo tecnica standardizzata che prevede la ricostruzione 3D, secondo i piani coronale, assiale e sagittale, di entrambe le mammelle. La RM mammaria è stata eseguita utilizzando un apparecchio di ultima generazione ad alto campo (1T) con gradienti intensi (25 mT/m) e bobine dedicate bilaterali multicanale. Previo posizionamento di un'ago-cannula in una vena periferica, ogni paziente è stata fatta coricare prona, con i seni alloggiati in apposite coppe (bobine). Alla fase di centratura è seguita una prima acquisizione, senza mezzo di contrasto, per un totale di 60 immagini. Si è proceduto quindi con iniezione rapida per via e.v. di Gadolinio DTPA in dose di 0,2 ml/kg e sono state eseguite, in rapida successione, altre 5 acquisizioni identiche alla prima. Alla fine sono state ottenute così 360 immagini per un esame della durata di circa 8 minuti. Alla fase di acquisizione è seguita, poi, una fase di elaborazione che ha previsto:

- sottrazione: dalla prima serie di immagini acquisite dopo iniezione di mezzo di contrasto si è sottratta quella acquisita prima dell'iniezione per facilitare l'identificazione delle aree di *enhancement*;
- ricostruzione multiplanare *(Multiplanar reconstruction projection* - MRP*)*: ha consentito di visualizzare le lesioni secondo i differenti piani dello spazio;
- MIP = *Maximum intensity projection* (somma delle immagini sottratte in un'unica immagine tridimensionale): ha fornito una rappresentazione d'insieme di entrambe le mammelle e delle eventuali lesioni;
- elaborazione semi quantitativa: mediante un idoneo *software* si è selezionata un'area di interesse (*Region of interest* = ROI) nel contesto della lesione che ha permesso di quantificare l'entità e la velocità di *enhancemen*t elaborando una curva intensità di segnale/tempo, utile per la caratterizzazione di lesione (fig. 3).

La SM è stata effettuata somministrando per via ev 99mTc-MIBI. La quantità di radioattività somministrata per via ev, nel braccio controlaterale a quello della nodularità ascellare presente, è dell'ordine di 740-1000 MBq (circa 25 mCi). Per l'esame, che inizia 10 minuti dopo la somministrazione del radiofarmaco, è stata utilizzata una gamma-camera (Siemens - ECAT o Philips ADAC VERTEX) con collimatore planare ad alta risoluzione. Di significativo aiuto è stato un lettino, appositamente costruito per agevolare il mantenimento della posizione prona con mammella pendula, necessario per l'acquisizione che si protrae per circa 30 minuti. L'esame prevede l'osservazione di entrambe le mammelle nelle proiezioni laterali, obliqua posteriore di circa 10° a paziente prona e l'acquisizione anteriore a paziente supina comprendente nel campo di osservazione anche i cavi ascellari. Con le immagini ottenute si è proceduto alla definizione di una ROI sia della lesione che di un'area normale della mammella controlaterale. Delle ROI descritte è stata successivamente calcolata e comparata la statistica di conteggio ottenuta.

La PET è stata eseguita a digiuno, previa idratazione delle pazienti, dopo circa 60 minuti dall'iniezione ev del tracciante metabolico (370 MBq di 18F-FDG), nel braccio controlaterale a quello della nodularità ascellare, con osservazione totale corporea e con studio particolare delle mammelle, utilizzando un tomografo dedicato (Siemens-ECAT-ACCEL).

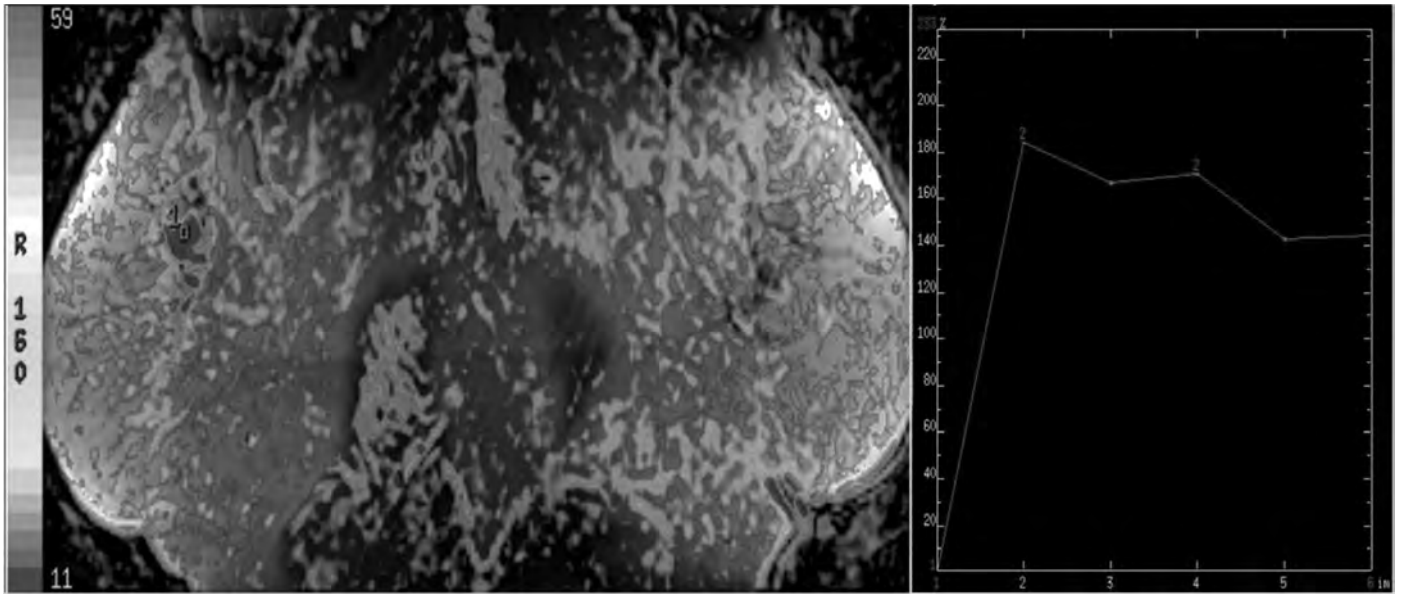

Fig. 3. Esempio di curva intensità segnale/tempo RM

I risultati di queste indagini strumentali sono stati confrontati e correlati con il risultato istopatologico definitivo, ottenuto in un caso anche con biopsia mammaria RM-guidata, e in tutti i casi con exeresi chirurgica della neoplasia primitiva e dei linfonodi ascellari metastatici.

Risultati

I risultati dello studio sono schematicamente visibili nella Tabella 1. In tutte le pazienti sia l'US mammaria, che la MM sono risultate negative (figg. 4-6). La RM,

Tabella 1 - Risultati schematici dello studio

	Caso I	Caso II	Caso III	Caso IV	Caso V	Caso VI
US (mammella)	-[a]	-	-	-	-	-
MM	-	-	-	-	-	-
RM	****[b]	*[c]	*	*	*	*
SM (mammella)	+[d]	+	+	+	+	+
SM (cavo ascellare)	+	+	+	+c[e]	+	+
PET (mammella)	+	+	-	+	+	+
PET (cavo ascellare)	+	+	+	+	+	+
PET (totale corporea)	e[f]	-	-	-	-	-
US/TC addome	e	No[g]	No	No	No	No
Istologia definitiva lesione mammaria	Ca duttale inf + ca *in situ* comedo	Ca duttale inf + ca *in situ*	Ca lobulare inf	Ca duttale inf + ca *in situ* comedo	Ca duttale inf + ca *in situ* solido e comedo	Ca duttale inf + ca *in situ* di tipo papillare
Istologia definitiva linfonodi ascellari	N+[h]	N+	N+	N+	N+	N+

[a] - = indagine negativa
[b] **** = area di *contrast enhancement* quadri-focale
[c] * = area di *contrast enhancement* mono-focale
[d] + = indagine positiva
[e] +c = area di aumentato *uptake* nell'ascella controlaterale alla sede tumorale
[f] e = metastasi epatica
[g] No = indagine non eseguita
[h] N+ = metastasi da adenocarcinoma

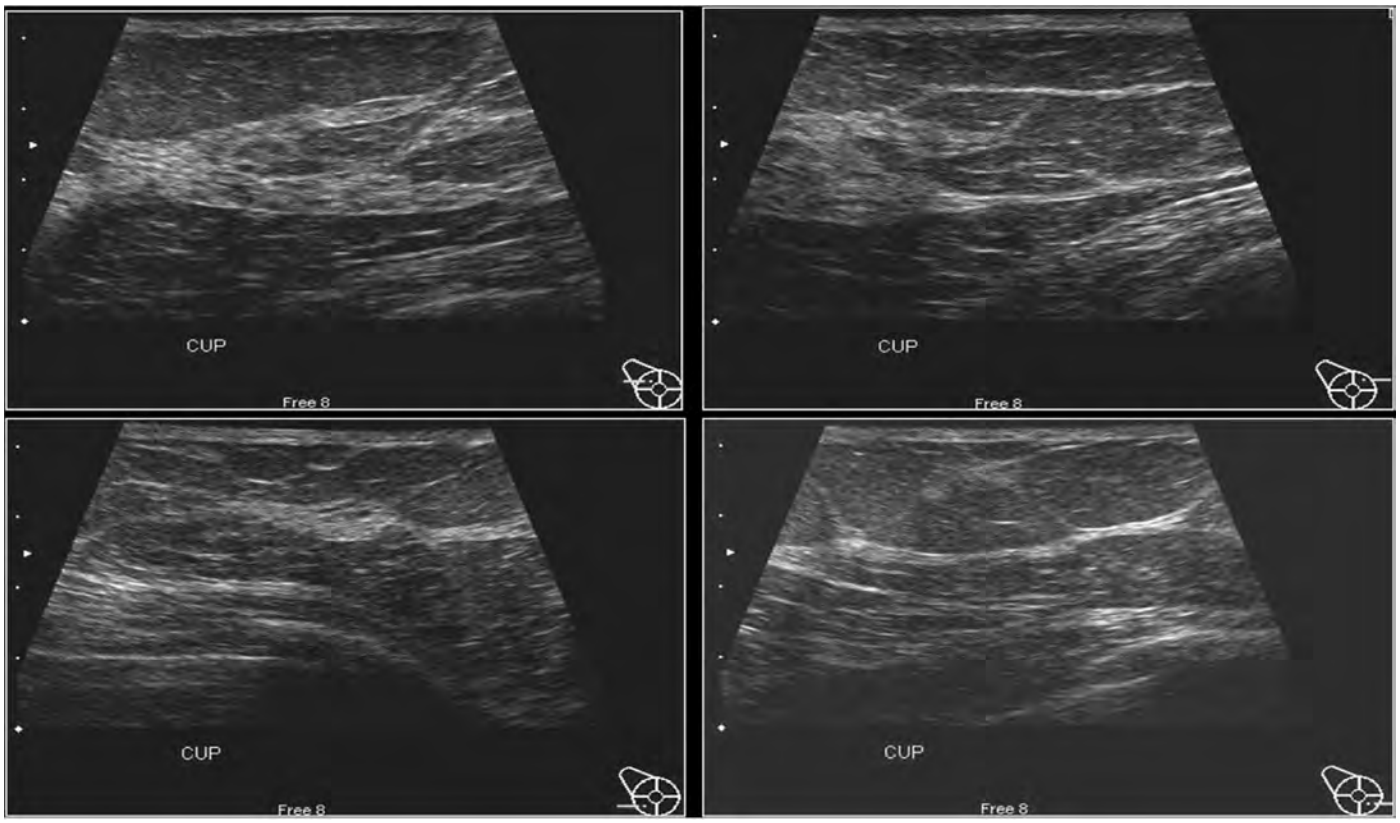

Fig. 4. US mammaria di tutti e quattro i quadranti: negativa (caso I)

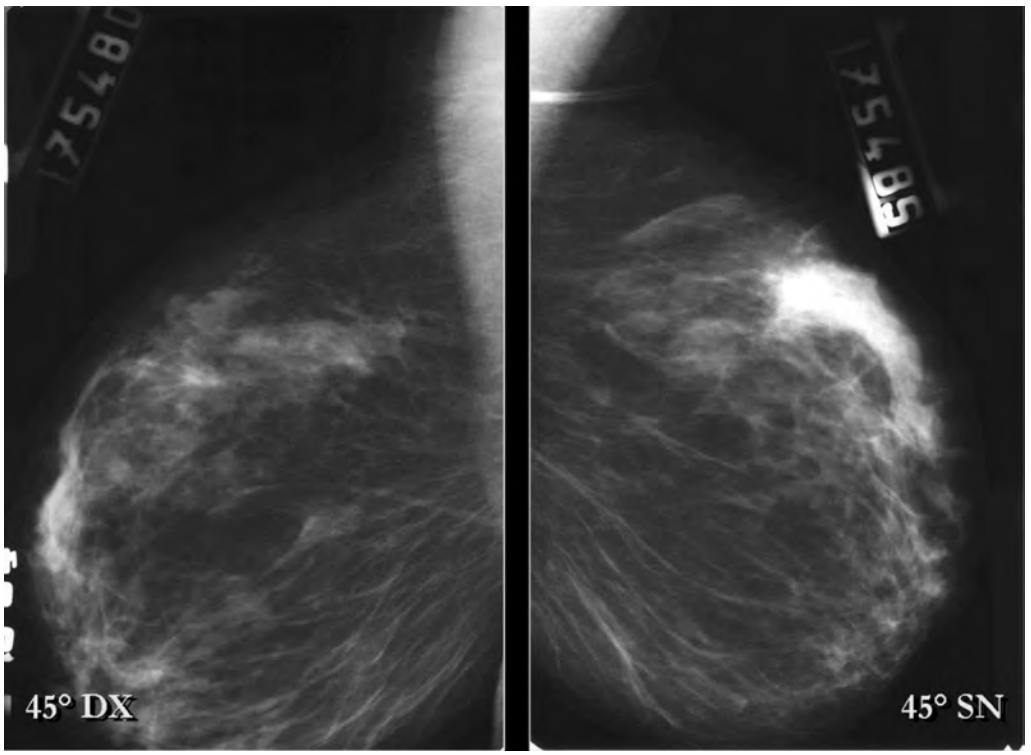

Fig. 5. MM (proiezione obliqua 45°) di ambedue le mammelle: negativa (caso I)

invece, ha rilevato il tumore primitivo come un'area ad elevato *contrast-enhancement* monofocale in 5 casi e come un'area di *contrast-enhancement* quadri-focale in 1 caso (figg. 7, 8). In tutte le pazienti la SM ha confermato la presenza delle lesioni mammarie mono-multifocali viste alla RM come aree di aumentato *uptake* del tracciante metabolico tranne un caso nel quale si è evidenziata un'unica area di ipercaptazione, quella di maggiori dimensioni (fig. 8). In tutte le pazienti, sempre alla SM, è stata osservata un'aumentata captazione del tracciante

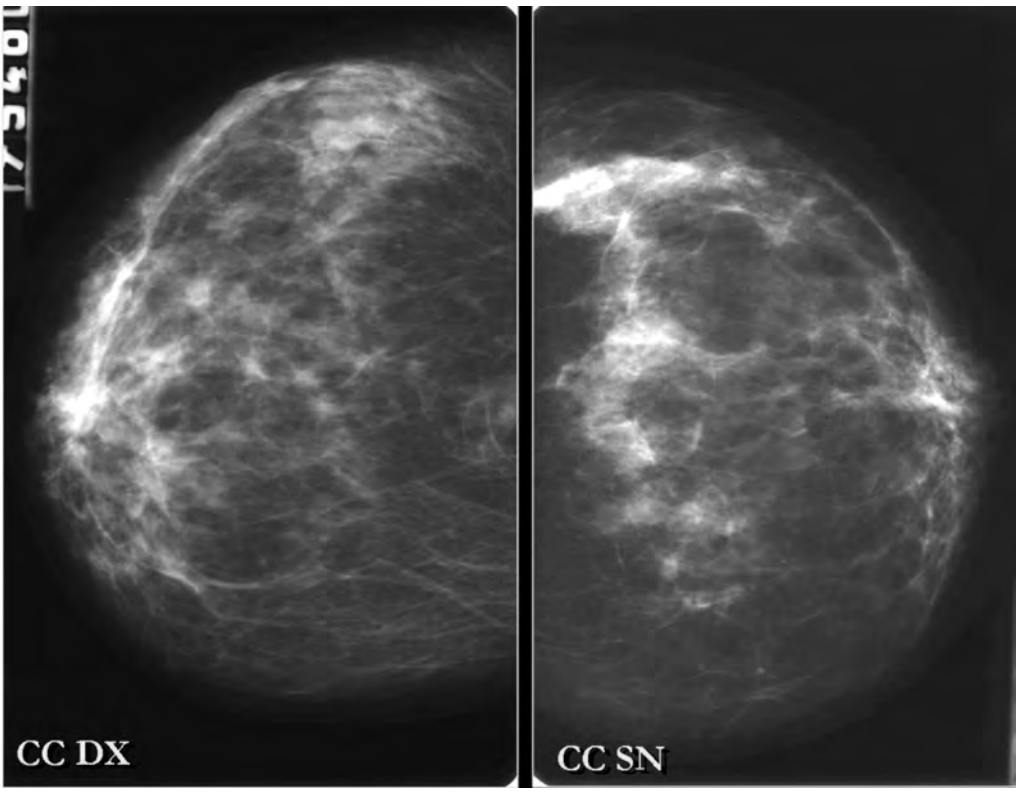

Fig. 6. MM (proiezione craniocaudale) di ambedue le mammelle: negativa (caso I)

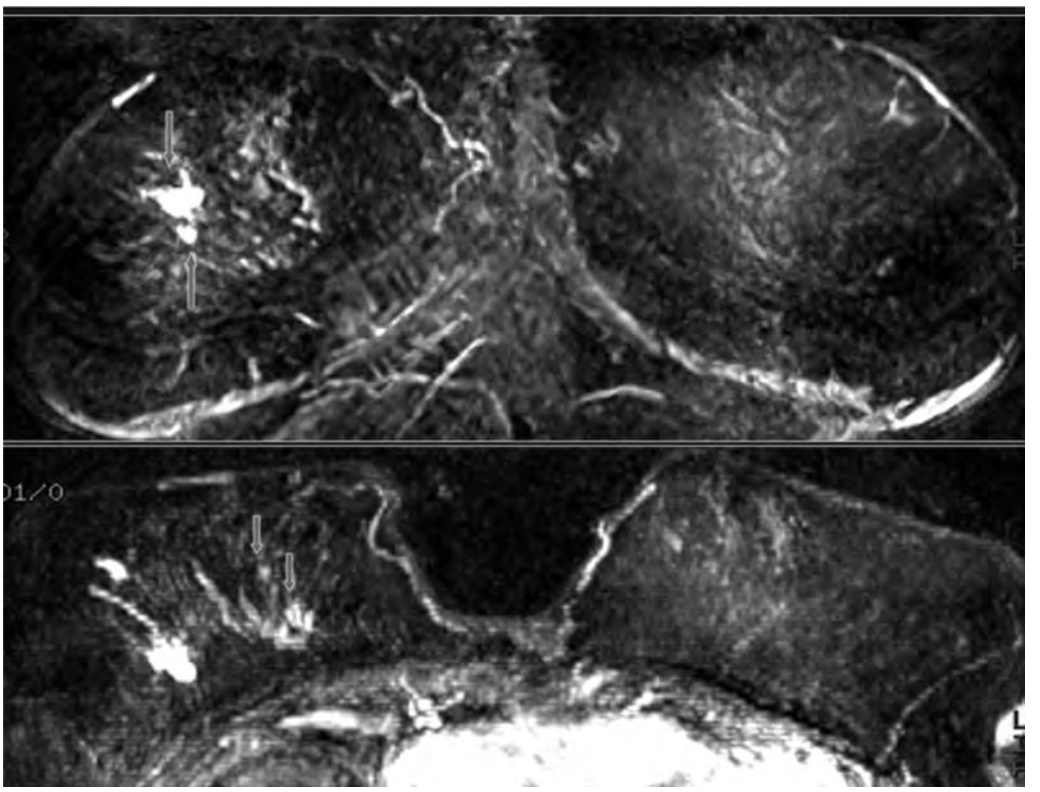

Fig. 7. RM mammelle (sezioni coronale e assiale): aree multifocali ad elevato *contrast enhancement* (caso I)

metabolico a carico dei linfonodi ascellari: in 5 dei 6 casi nella sede della localizzazione metastatica linfonodale biopsiata, in 1 caso nella regione ascellare controlaterale alla sede di metastasi.

L'indagine PET ha evidenziato la localizzazione mammaria neoplastica primitiva in 5 pazienti, mentre in 1 caso è risultata negativa. Per contro, in tutte e 6 le pazienti, la PET ha evidenziato un aumento di fissazione del tracciante nella sede di metastatizzazione linfonodale ascellare. Inoltre la PET ha permesso di individuare, in virtù della possibilità di eseguire una scansione *total body*, una localizzazione metastatica epatica in una paziente (fig. 9).

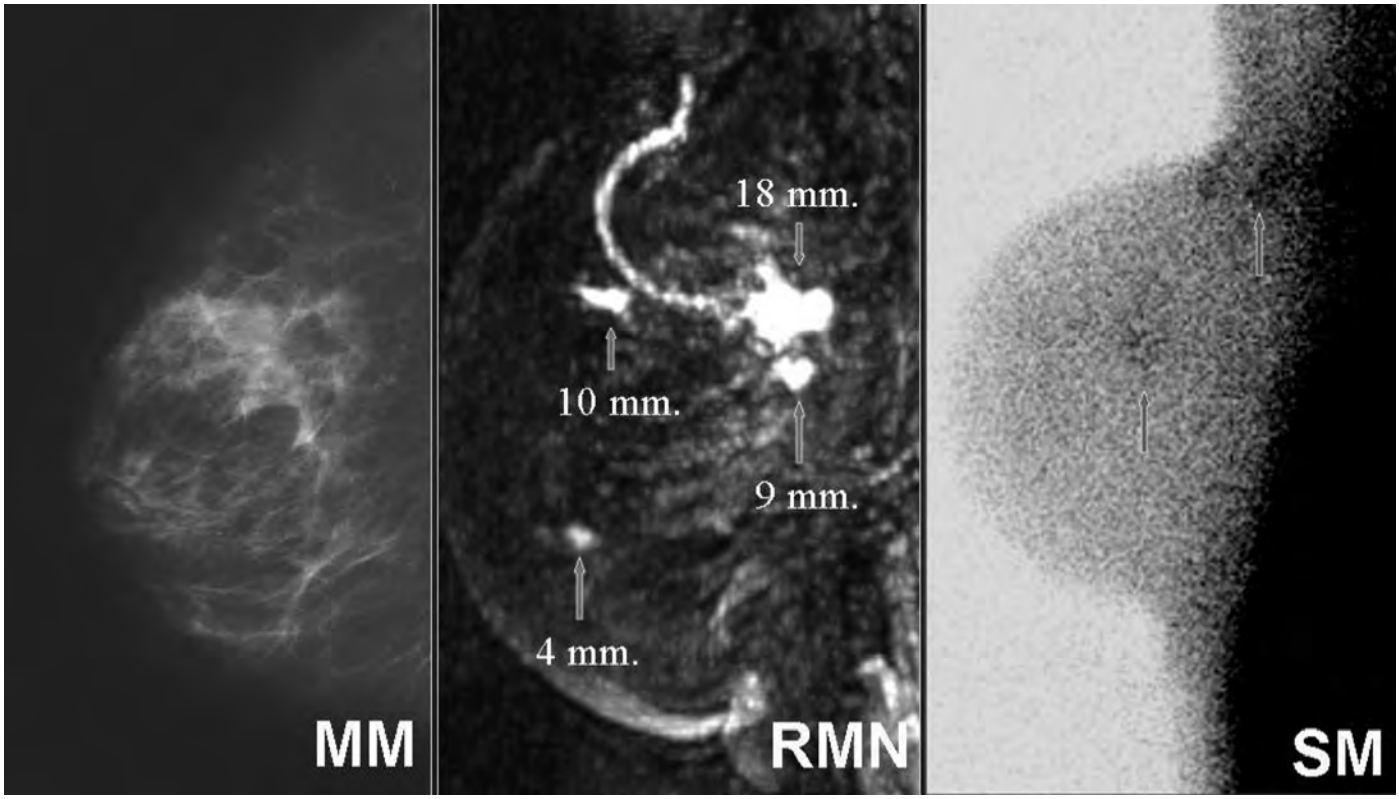

Fig. 8. Confronto tra MM, RM e 99mTc-MIBI (SM) della mammella destra: MM negativa, RM con aree multifocali ad elevato *contrast enhancement*, SM con area di aumentato *uptake* sia mammario che ascellare (caso I)

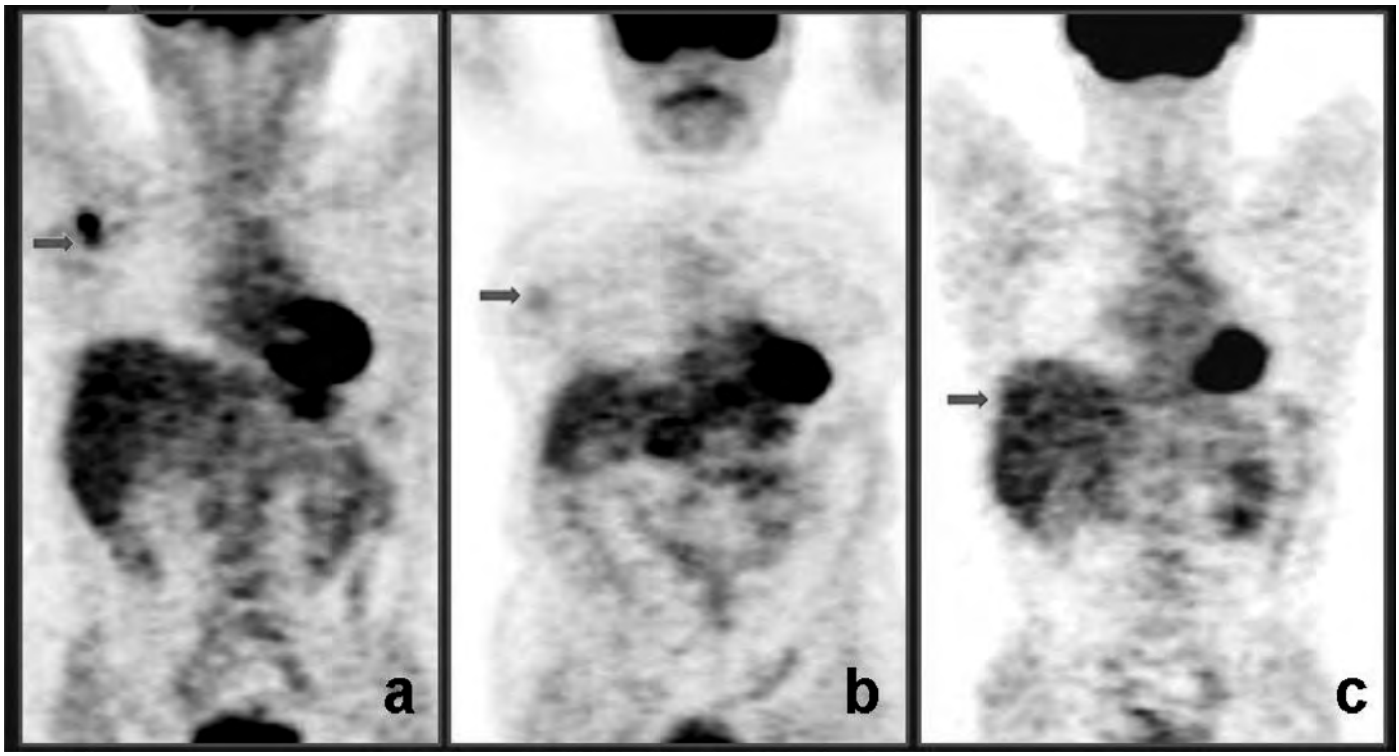

Fig. 9. 18F-FDG PET (sezioni coronali): iperaccumulo del tracciante metabolico in sede ascellare destra (a); tenue iperfissazione mammaria destra (b) e localizzazione sostitutiva epatica (c) (caso I)

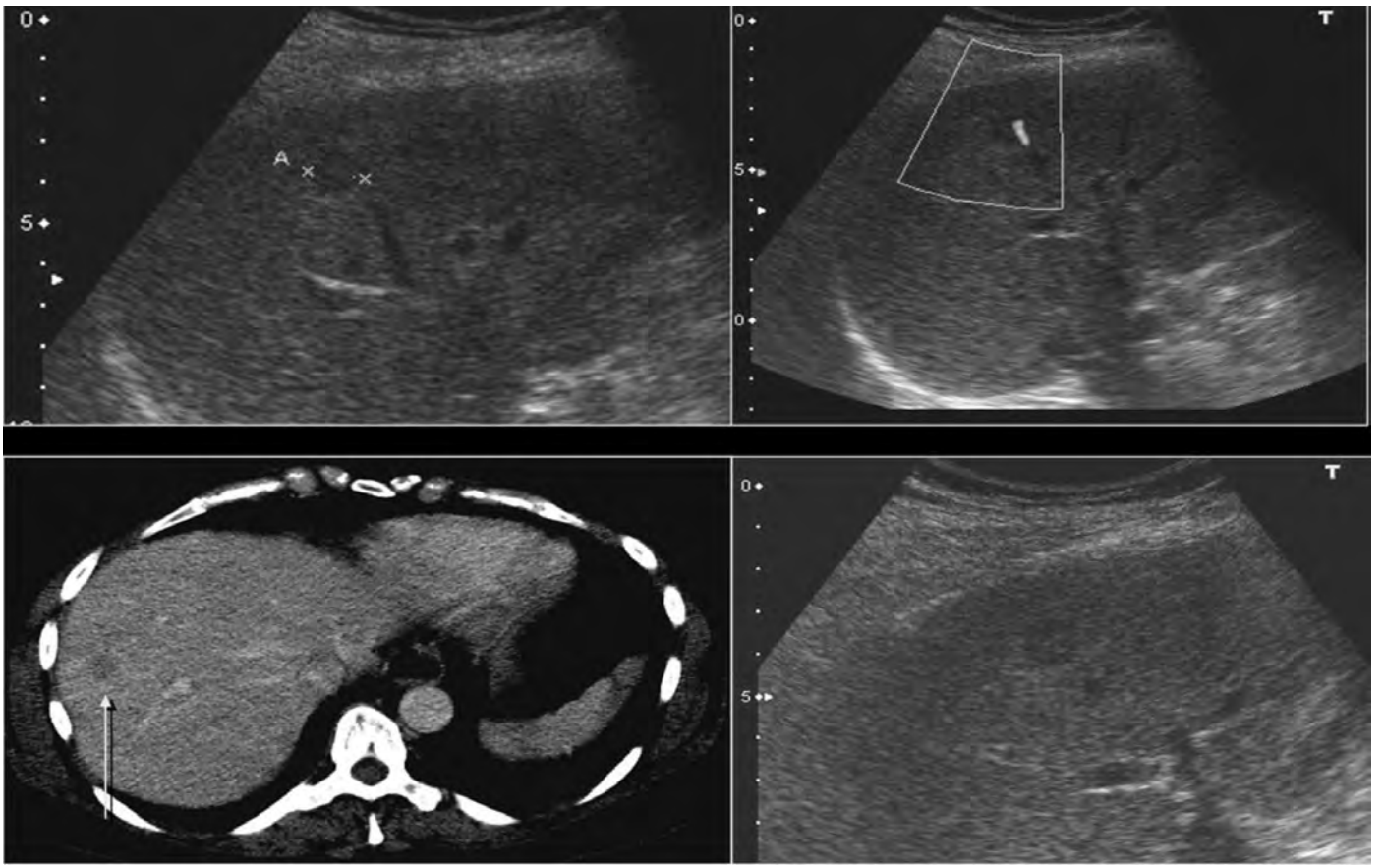

Fig. 10. US e TC addominale: metastasi epatica (caso I)

Questa diffusione a distanza della malattia (M+) è stata confermata dalla US e dalla TC addominale (fig. 10). Gli esami istologici definitivi, conseguenti all'exeresi chirurgica della lesione mammaria e alla linfoadenectomia ascellare omolaterale, hanno confermato la diagnosi, rispettivamente, di carcinoma mammario e di adenocarcinoma metastatico linfonodale (Tabella 1). Tutte le pazienti sono attualmente in *follow-up*.

Discussione

L'*imaging* senologico integrato rappresenta un utile mezzo, spesso indispensabile, per individuare e per stadiare una neoplasia occulta mammaria soprattutto quando le singole indagini strumentali risultano negative.

Nel presente studio, la PET, la SM e la RM, che possono essere definite indagini strumentali non convenzionali, rappresentano un utile mezzo per la ricerca di localizzazioni neoplastiche mammarie e sembrano avere un valore diagnostico superiore all'*imaging* convenzionale (US e MM). La RM ha permesso di individuare più foci neoplastici come aree ad elevato *contrast-enhancement* mono o multifocale (indicative quindi di neoangiogenesi tumorale mono e multifocale) con una sensibilità che appare superiore rispetto alle altre tecniche non convenzionali (PET, SM). Questo perché sia la SM che la PET hanno individuato, in un caso, un'unica area di aumentato *uptake* del tracciante (99mTc-MIBI o 18F-FDG) quando, invece, la RM ha rilevato un *enhancement* quadri-focale. La PET, inoltre, è risultata essere negativa per localizzazione mammaria in una paziente e ciò è probabilmente da correlare al basso metabolismo glucidico del tumore, come osservabile, per esempio, nel carcinoma lobulare infiltrante: tale è stato il risultato all'esame istologico definitivo della lesione in quella paziente (caso III).

In virtù della possibilità di eseguire una scansione di tutto il corpo, la PET è anche utile per valutare l'eventuale diffusione a distanza della malattia primitiva neoplastica. Infatti la PET ha permesso di individuare, in una paziente, una metastasi epatica (confermata all'US/TC).

Nello studio emerge che l'accuratezza diagnostica della SM nella neoplasia mammaria è quasi comparabile a quella della RM, ove si escluda il limite rappresentato dalla risoluzione spaziale che per la SM tradizionale è ≥ a 10 mm. Con l'introduzione di gamma-camere dedica-

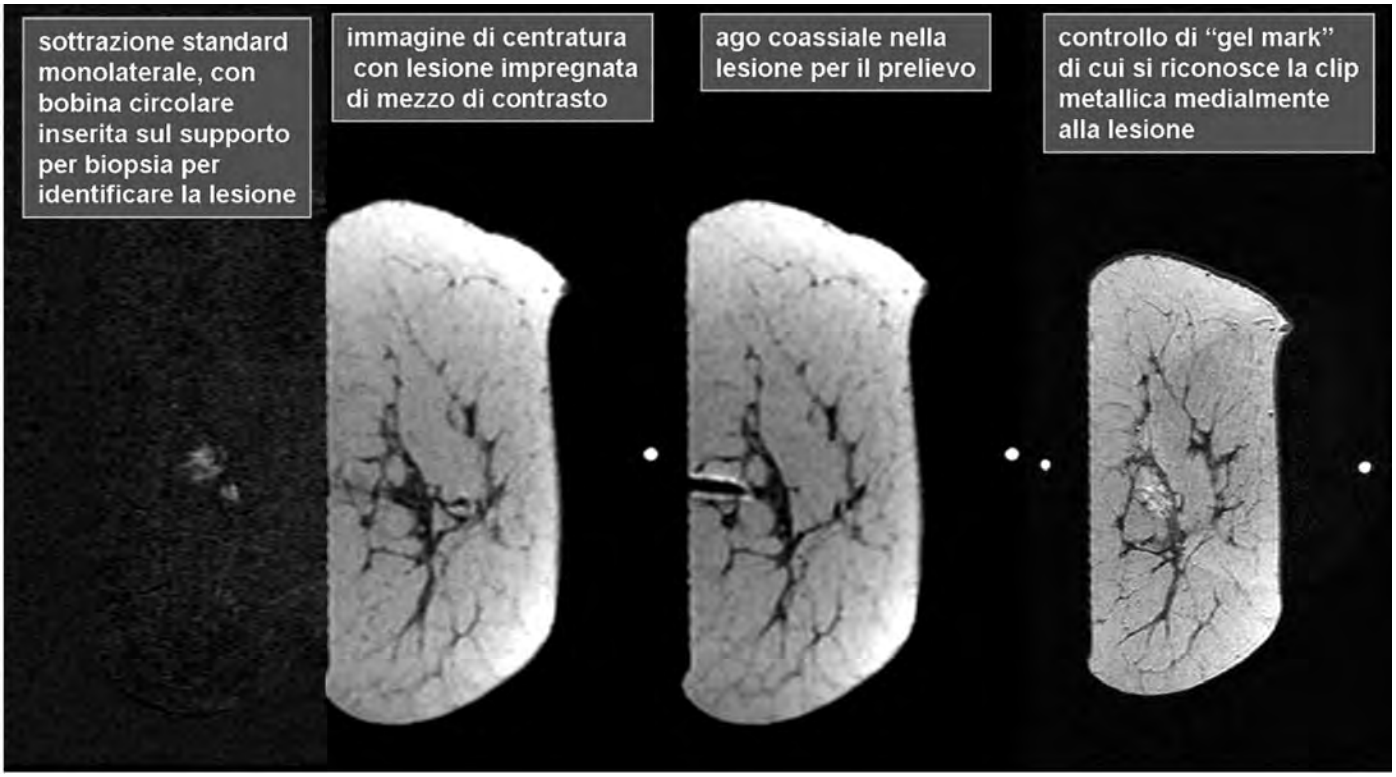

Fig. 11. Biopsia mammaria RM-guidata (con la collaborazione del Dott. Panizza, Ospedale San Raffaele, Milano)

te per l'esecuzione della SM (Lumagem®)[18], caratterizzate da un potere risolutivo che può raggiungere anche 1,5 mm, il problema della risoluzione spaziale potrà essere decisamente ridotto o eliminato. Comunque la RM è più sensibile della SM, sia convenzionale che dedicata, nell'identificazione di lesioni multiple (multicentriche e/o multifocali), come osservato in uno dei nostri casi. La captazione del tracciante metabolico, sia esso 18F-FDG che 99mTc-MIBI, evidenziato a livello linfonodale ascellare, ha generalmente un significato aspecifico perché potrebbe rappresentare o un esito post-bioptico con flogosi attiva, o la persistenza di malattia neoplastica. Nel nostro caso la diagnosi istologica conseguente alla linfoadenectomia ascellare suggerisce che la positività PET dovrebbe essere conseguente all'N+. L'aumentata captazione in regione ascellare controlaterale alla sede di metastatizzazione linfonodale, nonché alla sede tumorale mammaria, osservata in un caso, potrebbe essere legata ad una reazione flogistica locoregionale (linfoadenite).

Vista la non routinaria applicazione diagnostica, merita una breve considerazione la biopsia RM-guidata[19] di una lesione tumorale mammaria occulta: qualora l'US e la MM non siano in grado di identificare una lesione mammaria, non dimentichiamo che la RM mammaria può essere utilizzata sia per localizzare la lesione sia per caratterizzarla da un punto di vista istologico mediante l'agobiopsia RM-guidata (fig. 11).

Conclusioni

Alla luce dei risultati ottenuti, in caso di MM e US non diagnostiche, l'utilizzo di SM, RM e PET ha consentito di aumentare decisamente la sensibilità dell'*imaging* senologico integrato. Questo risultato avvalora quindi la necessità di un apporto interdisciplinare tra senologi, chirurghi, radiologi e medici nucleari nella diagnosi e stadiazione del tumore mammario primitivo per rendere la diagnostica senologica più efficace e tale da aumentare la sopravvivenza e migliorare la prognosi delle pazienti affette da malattia tumorale. Occorre, infine, sottolineare che, essendo la nostra casistica limitata, non è possibile alcuna inferenza di questo studio sul valore clinico delle tecniche diagnostiche, convenzionali e non, messe a confronto.

Bibliografia

1. Nissenblatt MJ. Carcinoma with unknown primary tumour (CUP syndrome). South Med J 1981; 12: 1479-502.
2. Jentsch-Ullrich K, Kalinski T, Roessner A, *et al*. Long-term remission in a patient with carcinoma of unknown primary site. Chemotherapy 2006; 52 (1): 12-5.
3. Pavlidis N, Briasoulis E, Hainsworth J, *et al*. Diagnostic and therapeutic management of cancer of an unknown primary. Eur J Cancer 2004; 14: 1990-2005.

4. Varadhachary GR, Abbruzzese JL, Lenzi R. Diagnostic strategies for unknown primary cancer. Cancer 2004; 100 (9): 1776-85.
5. Berg WA, Gutierrez L, Nessaiver MS, *et al*. Diagnostic accuracy of mammography, clinical examination, US and MR imaging in preoperative assessment of breast cancer. Radiology 2004; 233 (3): 830-49.
6. Marian T, Szabo G, Goda K, *et al*. In vivo and in vitro multitracer analyses of P-glycoprotein expression-related multidrug resistance. Eur J Nucl Med Mol Imaging 2003; 30 (8): 1147-54.
7. Buscombe JR, Cwikla JB, Thakrar DS, *et al*. Scintimammography: a review. Nucl Med Rev Cent East Eur 1999; 2 (1): 36-41.
8. Buscombe JR, Holloway B, Roche N, *et al*. Position of nuclear medicine modalities in the diagnostic work-up of breast cancer. Q J Nucl Med Mol Imaging 2004; 48 (2): 109-18.
9. Chao C, McMasters KM. Sentinel lymph node biopsy in breast cancer. Methods Mol Med 2006; 120: 91-111.
10. Bombardieri E, Crippa F, Baio SM, *et al*. Nuclear medicine advances in breast cancer imaging. Tumori 2001; 87 (5): 277-87.
11. Demir H, Berk F, Raderer M, *et al*. The role of nuclear medicine in the diagnosis of cancer of unknown origin. Q J Nucl Med Mol Imaging 2004; 48 (2): 164-73.
12. Crippa F, Gerali A, Alessi A, *et al*. FDG-PET for axillary lymph node staging in primary breast cancer. Eur J Nucl Med Mol Imaging 2004; 31 suppl 1: S97-102.
13. Gallowitsch HJ, Kresnik E, Gasser J, *et al*. F-18 fluorodeoxyglucose positron-emission tomography in the diagnosis of tumor recurrence and metastases in the follow-up of patients with breast carcinoma: a comparison to conventional imaging. Invest Radiol 2003; 38 (5): 250-6.
14. Lind P, Igerc I, Beyer T, *et al*. Advantages and limitations of FDG PET in the follow-up of breast cancer. Eur J Nucl Med Mol Imaging 2004; 31 suppl 1: S125-34.
15. Wu D, Gambhir SS. Positron emission tomography in diagnosis and management of invasive breast cancer: current status and future perspectives. Clin Breast Cancer 2003; 4 suppl 1: S55-63.
16. Abreu MC, Almeida P, Balau F, *et al*. Clear-PEM: a dedicated PET camera for improved breast cancer detection. Radiat Prot Dosimetry 2005; 116: 208-10.
17. Agnese DM. Advances in breast imaging. Surg Technol Int 2005; 14: 51-6.
18. Coover LR, Caravaglia G, Kuhn P. Scintimammography with Dedicated Breast Camera Detects and localizes Occult Carcinoma. J Nucl Med 2004; 45: 5533-58.
19. Perlet C, Heywang-Kobrunner SH, Heinig A, *et al*. Magnetic resonance-guided, vacuum-assisted breast biopsy: results from a European multicenter study of 538 lesions. Cancer 2006; 106 (5): 982-90.

Eur. J. Oncol., vol. 11, n. 2, pp. 133-135, 2006

Testicular metastasis from colon carcinoma

Metastasi testicolare da carcinoma del colon

Stefano Creti*, Daniele Mannini*, Michele Malizia*, Cesare Calandri**, Cristina Baldoni***, Diego Ettore Cuzzocrea*
* Department of Urology, Maggiore Hospital, Bologna, Italy
** Department of Oncology, Bellaria-Maggiore Hospital, Bologna, Italy
*** Department of Pathology, Maggiore Hospital, Bologna, Italy

Summary

Metastatic involvement of the testis is a rare event and is most often found incidentally at autopsy or after orchiectomy for prostatic carcinoma. The authors report a case of testicular metastasis from colonic adenocarcinoma. Although the onset of a nonlymphomatous cancer presenting as an intrascrotal mass is extremely rare, seldom detected clinically and almost never as the first sign of disease, this possibility should be considered, particularly in the young adult presenting with a mass involving the testicle or epididymis. Eur. J. Oncol., 11 (2), 133-135, 2006

Key words: **testis, metastasis, colon carcinoma**

Riassunto

La metastasi testicolare da carcinoma è un'evenienza rara, e viene diagnosticata per lo più casualmente all'autopsia o dopo orchiectomia per carcinoma prostatico. Gli Autori riferiscono un caso di metastasi testicolare da adenocarcinoma del colon. Sebbene la presentazione di tumori non linfomatosi come massa intrascrotale sia estremamente rara, raramente diagnosticata clinicamente e quasi mai come primo segno di malattia, tale evenienza dovrebbe essere presa in considerazione, soprattutto nei giovani adulti con una massa che interessi il testicolo e l'epididimo. Eur. J. Oncol., 11 (2), 133-135, 2006

Parole chiave: **testicolo, metastasi, carcinoma del colon**

Introduction

Metastatic tumours involving the testis or its tunic are rare, with an incidence of only 0.02 to 0.06% in large autopsy series. According to McWilliam *et al* [1] and Meacham *et al* [2] a total of only 218 cases have been reported in the literature worldwide, 11 cases of which were primary colon carcinomas[2,3].

Colon cancer usually metastasizes to the regional lymph nodes, liver, lung, bone and brain, but rarely to the spermatic cord and/or intrascrotal organs. The latter diagnosis is often incidental and associated with advanced cancer. The identification and confirmation of the primary tumour is important in choosing the correct treatment; this can, however, prove difficult, given the unusual site of the metastasis.

Case report

A 50-year-old male presented with an enlarged, non-tender left testis, associated with mild discomfort but no significant urinary

Received/Pervenuto 22.3.2006 - Accepted/Accettato 29.3.2006
Address/Indirizzo: Dr. Stefano Creti, Dipartimento di Urologia, Ospedale Maggiore, Largo Nigrisoli 2, 40100 Bologna, Italia - Tel. +39/051/6478609
Fax +39/051/6478509 - E-mail: screti@katamail.com

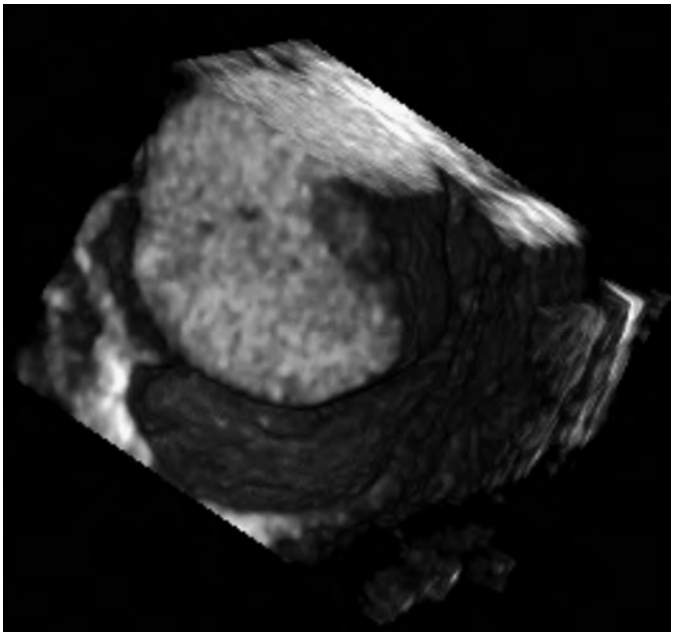

Fig. 1. Ultrasonography: hypoecoic area (measuring 1.2x1.0 cm) of the lower pole of the testis

symptoms. Colon cancer had been diagnosed about one year before and had been treated with surgical resection. After surgery, the patient did reasonably well for about six months. Then, a computed tomography (CT) revealed liver and lymph node metastases, and the patient received chemotherapy (FOLFOX). An abdominal CT six months after chemotherapy showed a complete regression of the secondary lesions.

The patient's urogenital symptoms started one month before being admitted to hospital, with mild pain, increase of size of left hemiscrotum, without any fever. Physical examination revealed a hard left testicle. Testicular 3D-ultrasound demonstrated an hypoecoic area, measuring 1.2x1 cm, in the lower pole of the testis (fig. 1). Left orchiectomy was then performed through an inguinal incision. The tumour measured 1.5 x1.3 cm but did not involve the epididymis or spermatic cord (fig. 2). Histopathological examination showed a mucus-secreting adenocarcinoma of colonic origin (fig. 3).

The patient was discharged two days after surgery.

Discussion

It is well known that distant metastases from colorectal carcinoma most often occur in the liver and lung; however, metastases to the bone, adrenal glands, lymph nodes, brain and skin have also been reported. Metastases involving the intrascrotal organs are generally found in conjunction with widespread advanced disease, and are extremely rare. Only 9.5% of metastases appear as the first sign of an occult neoplasm, and in 47.6% of these cases, the metastases and the primary tumour are diagnosed simultaneously[4].

The most frequent cancers of the digestive organs which metastasize to the testis or peritestis are: colon (28.9%), stomach (26.3%), pancreas (15.8%), bowel (13.2%), rectum (7.9%) and appendix, bile duct and jejunum (2.6%)[5].

Although metastases to the testicle are uncommon, it is even more unusual for such metastases to be the first manifestation of the primary tumour. In a recent review Haupt *et al*[5] reported a total of 9 cases. Various modalities of metastatic spread to the testicle have been hypothesized. The mechanisms vary with the type and location of the primary tumour and more than one route of metastatization is possible. Metastatic routes include arterial embolization, retrograde venous spread, retrograde

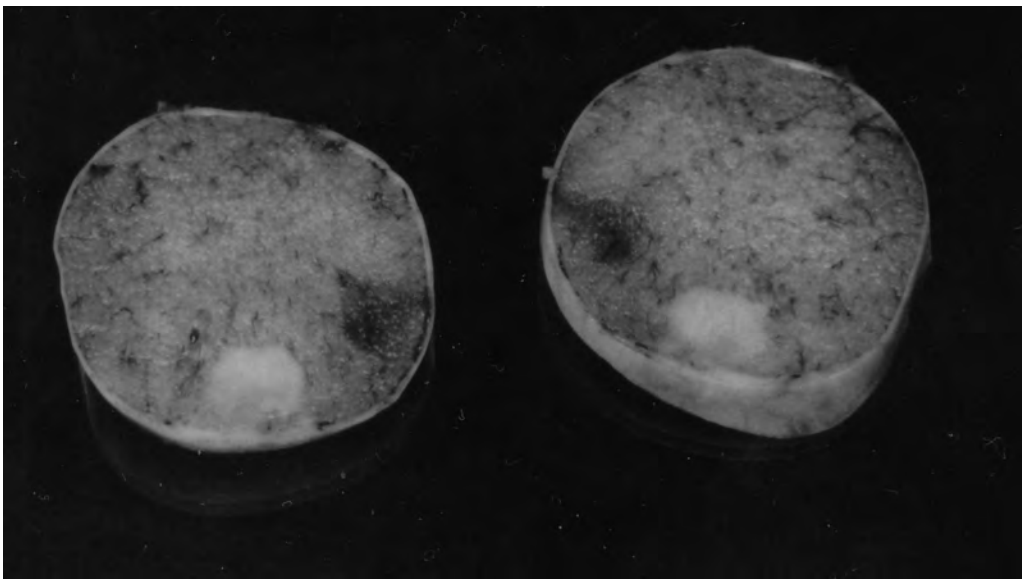

Fig. 2. Overall photograph of metastasis involving testicular parenchyma

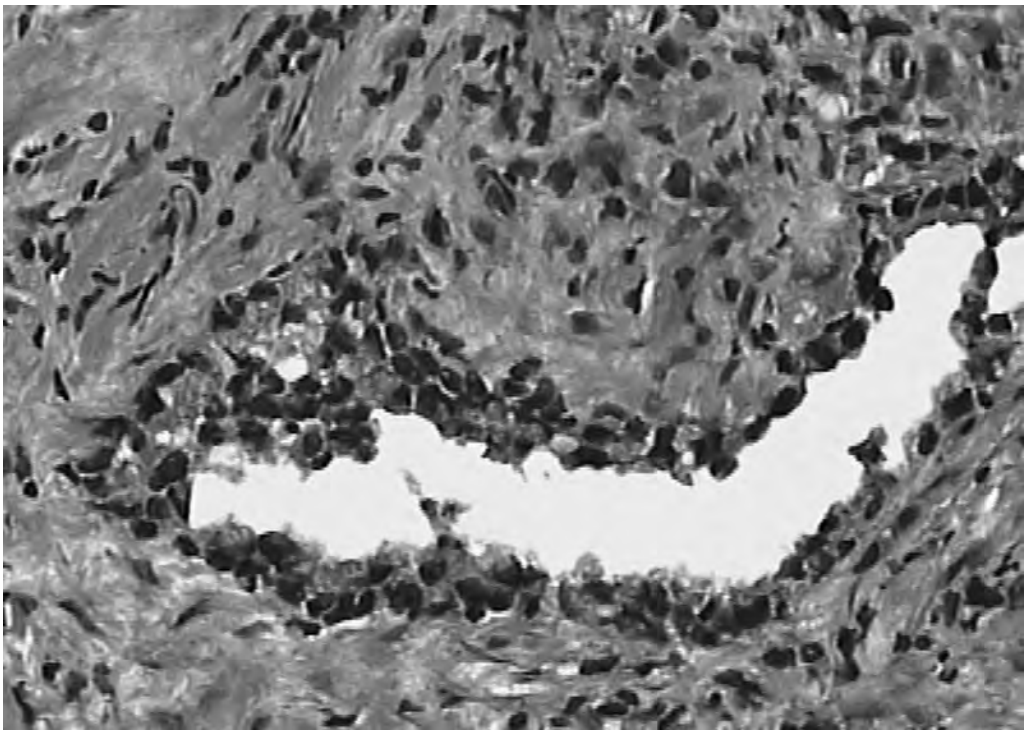

Fig. 3. Microphotograph: metastasis of adenocarcinoma with abundant mucin production adjacent to uninvolved atrophic testicular tubules (haematoxilin and eosin)

lymphatic spread and diffusion along the *vas deferens* to the epididymis and left testis. The prognosis is poor: the average survival for metastatic tumours of the spermatic cord is 9.1 months, with a maximum of 3 years and a minimum of 1 month[5,6].

This case confirms and emphasizes that a clinical diagnosis of metastatic cancer should be considered when a patient, past the usual age for a primary testis tumour, presents with a testicular mass or hydrocele, especially if the clinical findings suggest involvement of other organs and the clinical history is positive for cancer in other sites.

References

1. McWilliam WA, Zein TA, Koppelnick M, *et al*. Testicular metastasis from carcinoma of prostate. Urology 1983; 21: 570-2.
2. Meacham RB, Espada JA, Wheeler TM, *et al*. Testicular metastasis as the manifestation of colon carcinoma. J Urol 1988; 140: 621-2.
3. Almagro UA. Metastatic tumors involving testis. Urology 1988; 32: 357-60.
4. Polychronidis A, Tsolos C, Sivridis E, *et al*. Spermatic cord metastasis as an initial manifestation of sigmoid colon carcinoma : report case. Surg Today 2002; 32: 376-7.
5. Haupt HM, Mann RB, Trump DL, *et al*. Metastatic carcinoma involving the testis. Clinical and pathologic distinction from primary testicular neoplasm. Cancer 1984; 54: 709-14.
6. Algaba F, Santualaria JM, Villavicencio H. Metastatic tumour of the epididymis and spermatic cord. Eur Urol 1983; 9: 56-9.

Eur. J. Oncol., vol. 11, n. 2, pp. 137-138, 2006

Doctor Ruth Lilis
(1926-2006)

Dr. Ruth Lilis, a world-renowned occupational medicine physician and Professor Emerita in the Department of Community and Preventive Medicine of the Mount Sinai School of Medicine, died on March 17, 2006. She was 80.

Dr. Lilis, who was born in Romania, received her medical education at the Institute of Medicine and Public Health at Bucharest University. In 1960, the Institute awarded her the highest level of certification in the specialty of Occupational Medicine. Due to harsh work conditions in Romania, Dr. Lilis gained unique clinical experience in her early years with overwhelmingly severe cases of disease resulting from occupational exposures to lead, silica, asbestos and carbon disulphide. In 1972, Dr. Lilis and her family came to New York and she was a member of the faculty at Mount Sinai from 1972 until her death.

Throughout her tenure at Mount Sinai, Dr. Lilis was known for her deep knowledge of occupational medicine, her dedication to the learning process and her accomplishments in clinical research. She was an exemplary teacher who brought the highest professional standards to her work. She was an exceptionally knowledgeable clinician with vast first hand experience in the diagnosis and treatment of occupational diseases.

In 1976, Dr. Lilis established the Clinical Center for Occupational Medicine at Mount Sinai. This centre began as a part-time specialized clinic but grew under her able guidance into the Irving J. Selikoff Center for Occupational and Environmental Medicine, the flagship of the statewide network of occupational medicine clinics in New York State. This internationally respected Center is a diagnostic and treatment centre for persons with environmentally mediated disease or with toxic occupational exposures.

Dr. Lilis was revered for her originality in the development of innovative research perspectives and her incredible productivity. She authored 107 research publications addressing the clinical expression of the seminal occupational diseases of the past 50 years.

Dr. Lilis' research findings extended the knowledge of lead-induced disease to the broader range of environmental and occupational circumstances currently encountered. In the late 1970s, Dr. Lilis' group, in collaboration with Bell laboratory physical chemists, was the first to use the zinc protoporphyrin (ZPP) test as an indicator of lead's effect on haeme synthesis. Her research demonstrated a dose-response relationship between ZPP, lead, and lead-induced symptoms, and kidney and brain dysfunction. This research led to the understanding that lead toxicity occurs at levels much lower than previously understood. This work formed a major part of the basis for current standards and regulations designed to control occupational and environmental lead exposure.

Dr. Lilis' asbestos-related research was similarly groundbreaking. For two decades, Dr. Lilis was the chief clinical colleague at Mount Sinai of Dr. Irving J. Selikoff, the noted asbestos research physician. Together, they studied insulators, shipyard workers, other construction workers and factory workers, documenting the severity and breadth of asbestos-induced diseases. This work not only increased medical understanding of asbestos-related cancer and chronic lung disease, but had critical importance in changing public awareness and policy to promote the control of asbestos use and exposure in the United States in the 1970s and 1980s.

The clinical knowledge and research interests of Dr. Lilis were encyclopaedic. In addition to lead and asbestos, she studied the harm caused by occupational exposures to carbon disulphide, styrene, arsenic, mercury, vinyl chloride, cadmium, solvents, and polychlorinated biphenyls.

At the Mount Sinai School of Medicine, Dr. Lilis served as Director of the Occupational Medicine Outpatient Clinic, Director of Clinical Studies within the Division of Environmental Medicine, Associate Director of the Environmental Sciences Laboratory for clinical studies, as well as serving on the Residency Committees of the Department and the Division.

Dr. Lilis had served on the Working Group for the

International Classification of Radiographs of Pneumoconiosis and the International Committee of Lead Standards, of the International Labour Office. She was a member of the International Scientific Committee for Seveso of the Italian government and had served as a consultant to the International Agency for Research on Cancer, the National Institute of Environmental Health Sciences and the National Institute for Occupational Safety and Health.

She is survived by her husband, Dr. Michael Lilis, daughter, Elena and son-in-law, Louis Werner, two grandchildren, Genevieve and Kennett, her sister, Dr. Judith Lowe and nephew, Dr. Andrew Lowe.

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