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The efficacy of long-term bioassays in predicting human risks: mesotheliomas induced by fluoro-edenitic fibres present in lava stone from the Etna volcano in Biancavilla, Italy

L'efficacia dei saggi a lungo termine nel predire i rischi per l'uomo: mesoteliomi indotti dalle fibre di fluoroedenite presenti nella roccia lavica dell'Etna, Biancavilla, Italia

Fiorella Belpoggi, Eva Tibaldi, Michelina Lauriola, Luciano Bua, Laura Falcioni, Daniela Chiozzotto, Fabiana Manservisi, Marco Manservigi, Morando Soffritti

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Summary

Subsequent to an increased mortality for malignant pleural neoplasms in Biancavilla near the Etna volcano (Sicily, Italy), 17 cases of pleural mesothelioma were ascertained from 1980 to 1997. An occupational exposure to asbestos was considered possible in 7 cases and an environmental exposure for the other 10 cases. The epidemiological study was carried out by the Italian Superior Institute of Health (Rome, Italy). On the basis of this finding, the Earth Sciences Institute of "La Sapienza" University (Rome, Italy) carried out a careful mineralogical and environmental study in the area of Biancavilla, where incohesive volcanic materials had been widely used by the building industry and in road paving, particularly in the 1960-70s. This research brought to the identification of fluoro-edenitic prismatic crystals composed of calcium

Riassunto

In conseguenza ad un incremento della mortalità per neoplasie maligne della pleura a Biancavilla nei pressi del vulcano Etna (Sicilia, Italia), sono stati accertati 17 casi di mesotelioma maligno della pleura tra il 1980 ed il 1997. In 7 casi è stata considerata possibile un'esposizione professionale ad asbesto, mentre negli altri 10 casi l'esposizione è stata considerata di tipo ambientale. Gli studi epidemiologici sono stati condotti dall'Istituto Superiore di Sanità di Roma (Italia). Sulla base di questi risultati, l'Istituto di Scienze della Terra dell'Università "La Sapienza" di Roma ha eseguito un accurato studio mineralogico ed ambientale nell'area di Biancavilla, dove materiali vulcanici non coesivi sono stati ampiamente utilizzati dall'industria edilizia e nella pavimentazione delle strade, in particolare negli anni 1960-70. Questa ricerca ha portato all'identifica-

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amphiboles with microgranular feldspars, haematite and pyroxenes. In order to acquire further information on the relationship between the exposure to these fibres and the onset of mesotheliomas, a long-term bioassay was performed on Sprague-Dawley rats at the Cesare Maltoni Cancer Research Center of the Ramazzini Institute (CMCRC/RI). The preliminary results of this study, published in this Journal in 2004, showed that fluoro-edenitic fibres induce peritoneal and pleural mesotheliomas, with an incidence comparable to asbestos fibres. In this article we reported the final results of this study and the characterization of the types of the observed mesothelioma. Once again the CMCRC/RI experimental animal model demonstrates to be an effective tool to predict or confirm human risks. Eur. J. Oncol., 16 (4), 185-195, 2011

Key words: **asbestiform amphiboles, fluoro-edenite, lava stone, long-term bioassays, mesothelioma, rat**

zione di cristalli di calcio con feldspati microgranulari, ematite e piroxeni. Per acquisire ulteriori informazioni sulla relazione tra l'esposizione a queste fibre e l'insorgenza di mesoteliomi, è stato condotto un saggio a lungo termine su ratti Sprague-Dawley presso il Centro di Ricerca sul Cancro Cesare Maltoni dell'Istituto Ramazzini (CRCCM/IR). I risultati dello studio, pubblicati come preliminari in questa rivista nel 2004, hanno dimostrato che le fibre di fluoroedenite inducono mesoteliomi peritoneali e pleurici, con un'incidenza paragonabile alle altre fibre asbestiformi. In questo articolo vengono riportati i risultati finali dello studio e la caratterizzazione dei tipi di mesotelioma osservati. Ancora una volta il modello sperimentale animale utilizzato dal CRCCM/IR dimostra essere un efficace strumento nel predire o confermare il rischio per l'uomo. Eur. J. Oncol., 16 (4), 185-195, 2011

Parole chiave: **anfiboli asbestiformi, fluoro-edenite, roccia lavica, saggi a lungo termine, mesotelioma, ratto**

Introduction

In the framework of a national survey on mortality from pleural mesothelioma in Italy, a cluster of cases was detected in Biancavilla (Catania Province, Sicily), a town of about 23,000 inhabitants situated on the south-western slope of the Etna volcano (fig. 1), where 4 cases of pleural mesothelioma were observed in the years 1988-1992 (1, 2). According to statistical data calculated on the basis of Sicily's population, in the same period the expected cases in Biancavilla were 0.9 (SMR=417; 95% CI: 142-954) (3). Following this preliminary finding, an epidemiological study was started, leading to the identification of a total of 17 cases (10 men and 7 women) of pleural mesothelioma, 16 of them with histological/cytological confirmed diagnosis, among the inhabitants of the town in the years 1980-1997 (2, 4, 5). For these cases an asbestos exposure was hypothesized: in 9 cases no evidence of occupational exposure was present, in 2 cases the exposure was possible (1 building worker and 1 foundry worker), and in the last 5 cases the occupational exposure was not demonstrated. In a pilot study (6), performed on hospitalized people from Biancavilla, suffering from exacerbation of chronic obstructive pulmonary disease symptoms, 6 out of 12 patients (2 men and 4 women) showed fluoro-edenite fibers in the sputum. Because the data on professional exposure did not indicate that the cluster of mesotheliomas should have been correlated with the exposure to asbestos, it was thought that the source might have been in the general environment.

More recent epidemiological studies have shown that, in the period 1998-2004, 19 more cases of mesothelioma in Biancavilla (9 men and 10 women) were observed. In this period the incidence rate of mesothelioma in Biancavilla was 10 times higher than the one of Sicily and was comparable to those regions which had industrial activities related to the use of asbestos. The age at diagnosis (less than 66 years) and the high number of women suffering from

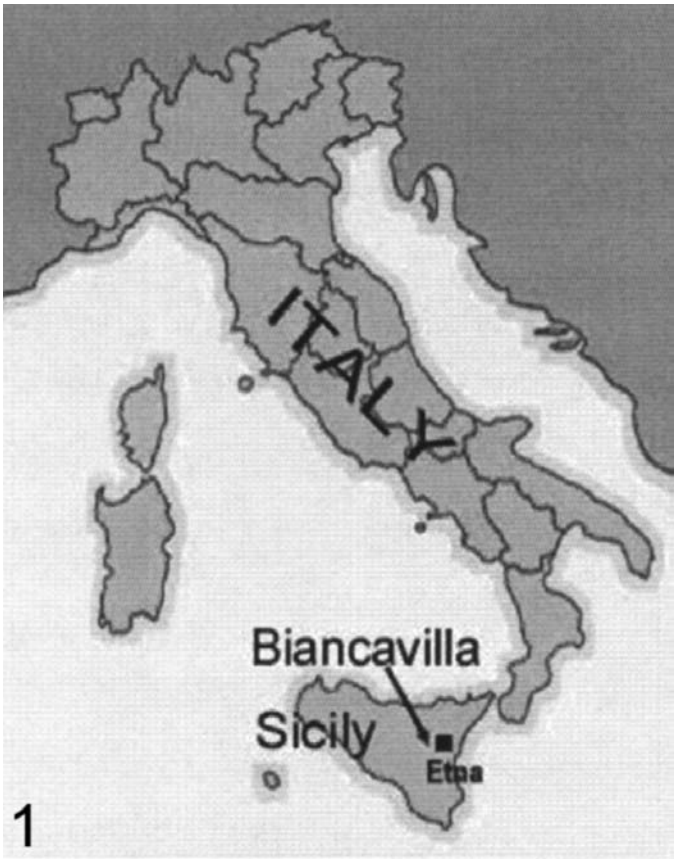


Fig. 1. Biancavilla is located on the south-western slope of the Etna volcano (Sicily)

mesothelioma supported the environmental origin of the exposure (7).

A careful mineralogical investigation was duly started in the area of Biancavilla, where incohesive volcanic materials have been widely used for the building industry and for road paving, particularly in the 1960s and 1970s. The studies carried out detected prismatic, acicular and fibrous amphiboles in the quarry material (8). The prismatic crystals were later identified as fluoro-edenite [$NaCa_2Mg_5Si_7AlO_{22}(OH,F)_2$], a new end-member of the calcium amphibole group, whose mineralogical and crystal-chemical data were reported by Gianfagna and Oberti. The fluoro-edenite of Biancavilla is a calcium amphibole with a high fluorine content (4% by weight), of transparent and intense yellow colour; it has been found both as prismatic or acicular crystals and as asbestiform fibres (9). Gianfagna *et al.* (2003) reported mineralogical data (based on optical, chemical and X ray investigations) obtained from asbestiform amphiboles from Biancavilla and showed them to be closely related to the associated prismatic and acicular fluoro-edenite (10).

In the framework of the Ramazzini Institute (RI) cancer research programme, which is aimed at identifying carcinogenic agents of industrial and environmental origin, a large and integrated project of long-term bioassays on fibrous materials was started in our laboratory at the end of 1970s. Among the studied materials, asbestos of many types and origins, various types of natural (sedimentary and hydrothermal) zeolites including erionite, several synthetic zeolites, and other organic, natural and man-made solid compounds, such as silica, alumina, talc, kaolin, man-made mineral fibres, propylene fibres, etc. are present (Table 1). The aim of the project was not only to verify the carcinogenicity of the compounds, but also to provide information regarding the relative carcinogenic potency. For this purpose all the compounds were tested under the same experimental conditions.

In order to acquire more detailed information on the relationship between exposure to fluoro-edenite compounds present in the area of Biancavilla and the onset of mesothelioma, a long-term bioassay started at the Cesare Maltoni Cancer Research Center (CMCRC) of the RI in 2002.

After 109 weeks of biophase, preliminary results were published in 2004 in order to make the population and authorities aware of the probable risk Biancavilla inhabitants were exposed to, and to promote urgent measures of environmental and health controls. The preliminary results showed indeed a higher incidence of mesothelioma in treated animals (11). The paper herein outlines the final results.

Materials and methods

Two samples of fluoro-edenite, fibrous (FFE) and prismatic (PFE), were supplied by Dr. Gianfagna (Hearth Sciences Institute, "La Sapienza" University, Rome; Italy). The FFE material contains 30-35% fibrous fluoro-edenite, feldspars, hematite and pyroxenes.

Male and female Sprague-Dawley rats from the colony of the CMCRC/RI were used. These rats have been employed in our laboratory for the last 30 years. Data are available on more than 17,000 histor-

Table 1 - Long-term bioassays on natural and man-made mineral fibres, performed at the CMCRC/RI

No.	Compounds/agents	No. of bioassays	Animals		Route of exposure[a]
			Species	No.	
	Natural and man-made mineral materials				
	Asbestos				
1	- Crocidolite	5	Rat	860	Ing,Int,Ip,Ipl,Sc
2	- Chrysotile (Canada)	5	Rat, mouse	1,260	Int,Ip,Ipl,Sc
3	- Chrysotile (Rhodesia)	1	Rat	80	Ip
4	- Chrysotile (California)	1	Rat	80	Ip
5	- Amosite	1	Rat	80	Ip
6	- Anthophyllite	1	Rat	80	Ip
7	- Asbestos-cement	1	Rat	240	Ip,Ipl,Sc
	Modified asbestos				
8	- Compound 1	1	Rat	160	Ip, Ipl
9	- Compound 2	1	Rat	160	Ip, Ipl
10	- Compound 3	1	Rat	160	Ip, Ipl
11	- Compound 4	1	Rat	160	Ip, Ipl
12	- Compound 5	3	Rat, mouse	860	Ip, Ipl
13	- Compound 6	3	Rat, mouse	860	Ip, Ipl
14	- Compound 8	3	Rat, mouse	860	Ip, Ipl
15	Wollastonite	1	Rat	240	Ip,Ipl,Sc
16	Rock wool	3	Rat	640	Int,Ip,Ipl
17	Ceramic fibres	1	Rat	440	Ip,Ipl
18	Glass fibres	1	Rat	200	Int,Ip
19	Crystalline silica	1	Rat	160	Ip,Sc
20	Amorphous silica	1	Rat	160	Ip,Sc
21	Alumina	1	Rat	160	Ip,Sc
22	Talc (pure)	1	Rat	160	Ip,Sc
23	Talc (industrial)	1	Rat	240	Ip,Ipl,Sc
24	Kaolin	1	Rat	160	Ip,Sc
25	Bentonite	1	Rat	240	Ip,Ipl
26	Erionite	2	Rat, mouse	400	Ip,Ipl,Sc
27	Fluoro-edenite	1	Rat	270	Ip,Ipl
	Other natural zeolites				
28	- Mordenite (sed)	1	Rat	240	Ip,Ipl,Sc
29	- Phillipsite	1	Rat	240	Ip,Ipl,Sc
30	- Clinoptilolite	1	Rat	160	Ip,Sc
31	- Cabasite	1	Rat	160	Ip,Sc
32	- Ferrierite	1	Rat	160	Ip,Sc
33	- Mordenite (crystalline)	1	Rat	240	Ip,Ipl,Sc
34	- Heulandite	1	Rat	160	Ip,Sc
35	- Mesolite	1	Rat	160	Ip,Sc
36	- Natrolite	1	Rat	160	Ip,Sc
37	- Solecite	1	Rat	160	Ip,Sc
38	- Stilbite	1	Rat	160	Ip,Sc
39	- Thomsonite	1	Rat	160	Ip,Sc
	Man-made zeolites and precursors				
40	- TE-16460	1	Rat	360	Ip,Ipl,Sc
41	- TE-16461	1	Rat	360	Ip,Ipl,Sc
42	- TE-16462	1	Rat	360	Ip,Ipl,Sc
43	- WGB 1189-2-1 (catalyst)	1	Rat	360	Ip,Ipl,Sc

(continued)

Table 1 (continued) - Long-term bioassays on natural and man-made mineral fibres, performed at the CMCRC/RI

No.	Compounds/agents	No. of bioassays	Animals		Route of exposure[a]
			Species	No.	
	Man-made zeolites and precursors				
44	- WGB 1189-2-1 (elutriated)	1	Rat	360	Ip,Ipl,Sc
45	- WGB 1190-2-1 (catalyst)	1	Rat	360	Ip,Ipl,Sc
46	- WGB 1190-2-1 (elutriated)	1	Rat	360	Ip,Ipl,Sc
47	- WGB 1191-2-1 (catalyst)	1	Rat	360	Ip,Ipl,Sc
44	- WGB 1189-2-1 (elutriated)	1	Rat	360	Ip,Ipl,Sc
45	- WGB 1190-2-1 (catalyst)	1	Rat	360	Ip,Ipl,Sc
46	- WGB 1190-2-1 (elutriated)	1	Rat	360	Ip,Ipl,Sc
47	- WGB 1191-2-1 (catalyst)	1	Rat	360	Ip,Ipl,Sc
48	- WGB 1191-2-1 (elutriated)	1	Rat	360	Ip,Ipl,Sc
49	- WGB 1192-1-1	1	Rat	360	Ip,Ipl,Sc
50	- WGB 1193-1-1	1	Rat	360	Ip,Ipl,Sc
51	- WGB 1194-1-1	1	Rat	360	Ip,Ipl,Sc
52	- Paulsboro (catalyst)	1	Rat	360	Ip,Ipl,Sc
53	- Paulsboro (catalyst fines)	1	Rat	360	Ip,Ipl,Sc
54	- Kaiser alumina	1	Rat	360	Ip,Ipl,Sc
55	- Mobil Joliet	1	Rat	360	Ip,Ipl,Sc
56	- Joliet fresh	1	Rat	360	Ip,Ipl,Sc
57	- MS 4A	1	Rat	160	Ip,Ipl,Sc
58	- MS 5A	1	Rat	160	Ip,Ipl,Sc
59	- MS 13X	1	Rat	160	Ip,Ipl,Sc
60	Carbon fibers (disks)	1	Rat	140	Imp

[a] Imp=subcutaneous implantation; Ing=ingestion; Int=intratracheal instillation; Ip=intraperitoneal injection; Ipl=intrapleural injection; Sc=subcutaneous injection

ical controls, kept under observation for their life span and submitted to systematic necropsy and standardised histopathological examination. Data are therefore available on the expected incidence of the different types of tumours in control animals and on its fluctuations: in particular, mesotheliomas are extremely rare (mean <1% range 0-2%) among the untreated rats of this colony. After weaning, at 4-5 weeks of age, the experimental animals were identified by ear punch, randomised in order to have no more than one male and one female of each litter in the same group and housed 5 per cage/sex. The animals were 8 weeks old at the start of the experiment. The fibrous fluoro-edenite was administered to groups of 80 rats (40 males and 40 females) through intraperitoneal or intrapleural injection, *una tantum*, at the dose of 25 mg suspended in 1 cc of water. The prismatic fluoro-edenite was administered to 30 rats (15 males and 15 females) only through intraperitoneal injection, *una tantum*, at the dose of 25 mg suspended in 1 cc of water. A group of 80 rats (40 males and 40 females), injected with water alone, served as control. The animals were kept under observation until spontaneous death. During the experiment, the animals were clinically examined every 2 weeks in order to detect and record all gross changes, and weighed every 4 weeks. Animal status and behaviour were examined 3 times daily. A systematic necropsy was performed on each animal. Histopathological examination was carried out on all experimental animals and regarded the tissue at the site of injection, and brain, thymus, lung, heart, liver, spleen, pancreas, kidneys, adrenal glands, stomach, uterus, gonads, subcutaneous, mediastinal and mesenteric lymph nodes, and any other organs with pathological changes. All organs and tissues are preserved in 70% ethyl alcohol. The specimens were trimmed and then processed as paraffin blocks; 3-5 μm sections of every specimen are obtained and routinely stained with haematoxylin-eosin. The experiment was performed following the principle of Good Laboratory Practice

(GLP), with the Standard Operating Procedures of the CMCRC/RI, in accordance with Italian governmental Guiding Principles for the use of experimental animals (12).

Results

In this study, survival was deeply affected by the treatment with fibrous fluoro-edenite administered with intraperitoneal injection, both in males and females (figs. 2A and 2B respectively). This early mortality was greatly due to the onset of mesotheliomas. The fluoro-edenite showed a mesotheliomatogenic effect (Table 2). Among 80 animals treated with intraperitoneal injection, 66 cases (37 in males and 29 in females) of mesothelioma have been observed, with an average latency time of 63.7 weeks. The first case was observed in a male rat only after 36 weeks from treatment. Among 80 animals treated through intrapleural injection, 13 cases (6 in males and 7 in females) of pleural mesothelioma have been observed with an average latency time of 79.7 weeks. The first case was observed in a male rat after 51 weeks from treatment. This study confirms that the peritoneum is more responsive than the pleura, as usually observed in our experimental model.

No cases of mesothelioma have been observed among the 30 rats treated with prismatic fluoro-edenite, and one case was observed in a female of the control group with longer latency time (122 weeks). Macroscopically, the peritoneal tumours involved the whole abdominal cavity, with whitish and yellowish tissue on the surface of all the organs. In some cases, a serosal effusion was present. The pleural tumours involved the visceral and/or parietal pleura. In 8 of the 13 pleural mesothelioma the diaphragm was largely involved, with subsequent extension of the tumour into the peritoneal cavity. Microscopically, malignant mesothelioma in rats, as in humans, are classified based on three types of cells: epithelioid, sarcomatoid (or fibrous) and mixed/biphasic. The mesotheliomas observed in this study were polymorphic, with a predominance of epithelial-solid and fibrous spindle cell patterns: the different types of peritoneal and pleural mesothelioma observed in this study are reported in Table 3.

In animals treated with FFE through peritoneal injection the prevalent histotype was sarcomatous, both in males and females. The pleural injection prevalentely induced epithelial mesothelioma in males; the mixed type was prevalent in females, where no tumour of the epithelial type alone was observed.

The epithelioid type lesion was composed of papillary and/or tubular structures (figs. 3A and 3B), similar to an adenocarcinoma; areas with a solid pattern were more frequently present (fig. 3C). Solid areas were composed of epithelial-like cells with vacuolated to foamy cytoplasm and large nuclei. Local invasion into underlying tissues (figs. 3D and 3E) and metastases were observed.

In the sarcomatoid or fibrous type, the following mesenchymal components were observed: fibrosarcomatoid, rhabdosarcomatoid, chondrosarcomatoid and osteosarcomatoid. Secondary organs and tissues were affected. Fibrous pattern consisted in bundles of uniform spindle-shaped cells separated by thick collagen fibres (fig. 4A). Metastases were sometimes observed (fig. 4B).

Mixed type was observed both in pleural and peritoneal mesothelioma. Epithelial and mesenchimal components were present: pseudoglandular structures embedded in sarcomatoid spindle-shaped cells were found (figs. 5A and 5B). Distant metastases of the peritoneal tumours were detected in the mediastinal lymph nodes, mediastinum (fig. 5C), lung, pleura and pericardium. The presence of the test fibres has been detected in some neoplasms, surrounded by inflammatory cells (fig. 6).

Conclusions

FFE showed to be mesotheliomatogenic in our experimental model. The effect was strong on the peritoneum and to a lesser extent on the pleura. Peritoneal and pleural mesotheliomas increased in incidence and the exposure to FFE anticipated the latency time. These results are comparable with those obtained in our laboratory, under the same experimental conditions on various types of fibrous materials (11, 13) (Tables 4 and 5).

The lack of any mesotheliomatogenic effect by prismatic fluoro-edenite is not surprising if compared to the results of previous experiments

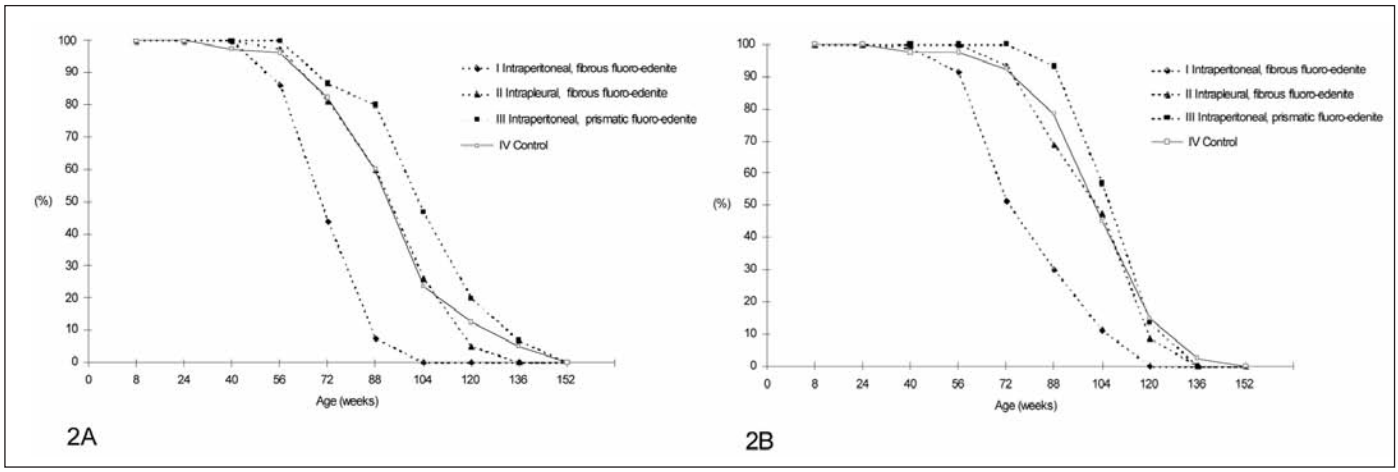


Fig. 2. Survival in Sprague-Dawley rats: (A) males; (B) females

Table 2 - Long-term carcinogenicity bioassay on FFE and PFE administered *una tantum* to 8-week-old male (M) and female (F) Sprague-Dawley rats. Incidence and latency time of mesotheliomas at the site of injection.

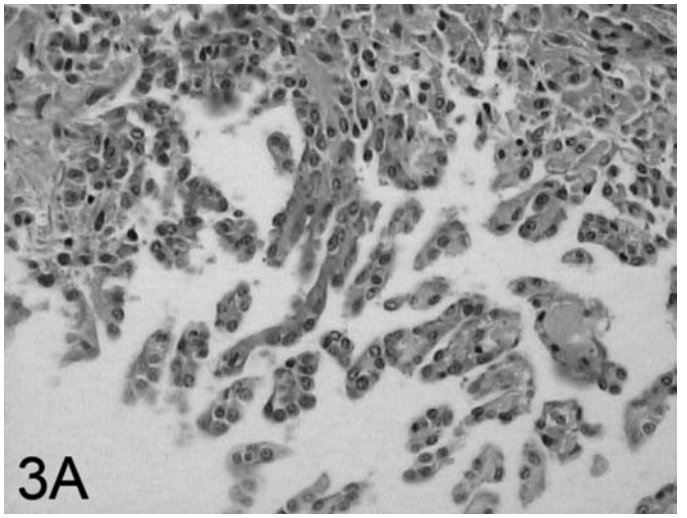
Group	Treatment/dose (mg/1 cc H_2O)	Animals examined		Animals bearing mesotheliomas[a]		
		Sex	No.	No.	%	Average latency time (weeks)
I	FFE 25 mg/1 cc H_2O	M	40	37	92.5	61.6
	Intraperitoneal injection	F	40	29	72.5	66.4
		M+F	80	66	82.5	63.7
II	FFE 25 mg/1 cc H_2O	M	40	6	15.0	82.3
	Intrapleural injection	F	40	7	17.5	77.4
		M+F	80	13	16.3	79.7
III	PFE 25 mg/1 cc H_2O	M	15	0	-	-
	Intraperitoneal injection	F	15	0	-	-
		M+F	30	0	-	-
IV	1 cc H_2O (control)	M	40	0	-	-
	Intraperitoneal injection	F	40	1	2.5	122.0
		M+F	80	1	1.3	122.0

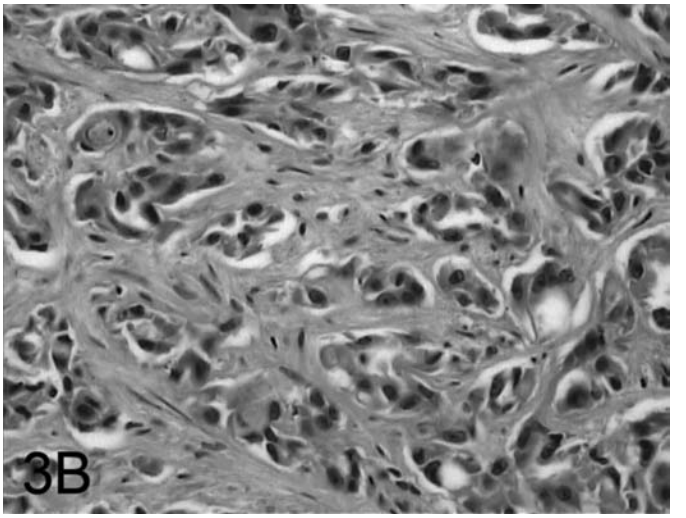
[a] Percentage referred to the examined animals

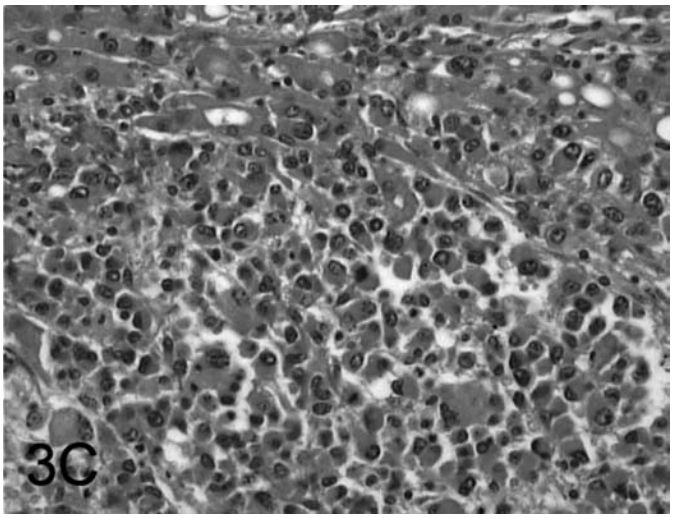
Table 3 - Mesotheliomas in experimental animals treated with FFE distributed by histotype

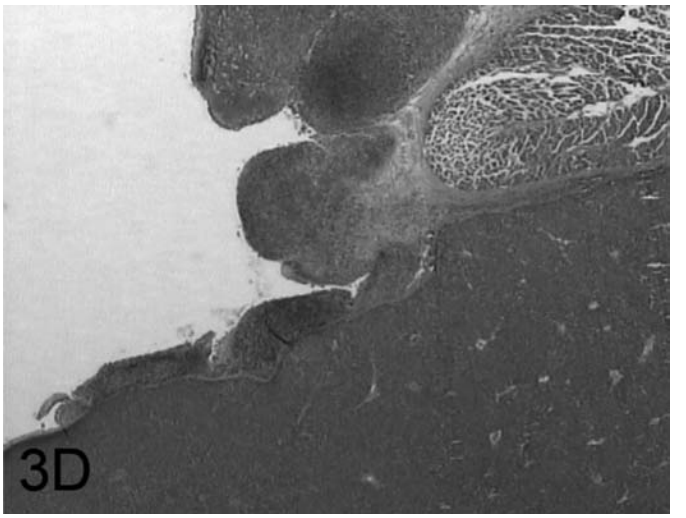
Route of administration (injection)	Animals examined		Mesotheliomas (%)			
	Sex	No.	Total[a]	Epithelial[b]	Mixed[b]	Sarcomatous[b]
Peritoneal	M	40	92.5	2.7	37.8	59.5
	F	40	72.5	3.5	44.8	51.7
Pleural	M	40	15.0	50.0	33.3	16.7
	F	40	17.5	-	57.1	42.9

[a] Percentage referred to the number of animals examined
[b] Percentage referred to the number of total mesotheliomas









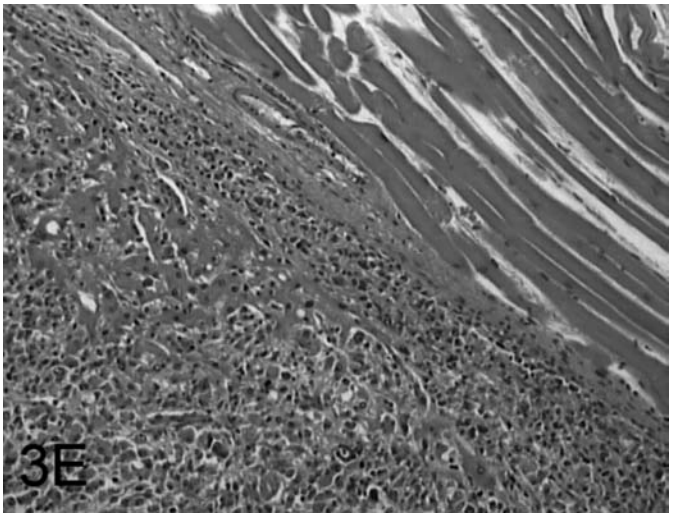


Fig. 3. Different pattern of epithelioid mesothelioma in rats treated with FFE. (A) Pleural epithelioid mesothelioma with papillary pattern, 400x; (B) pleural epithelioid mesothelioma with tubular pattern, 400x; (C) peritoneal epithelioid mesothelioma with solid pattern composed by vacuolated cells, 400x; (D) peritoneal epithelioid mesothelioma invading both the liver and the diaphragm, 25x; (E) same case, 200x

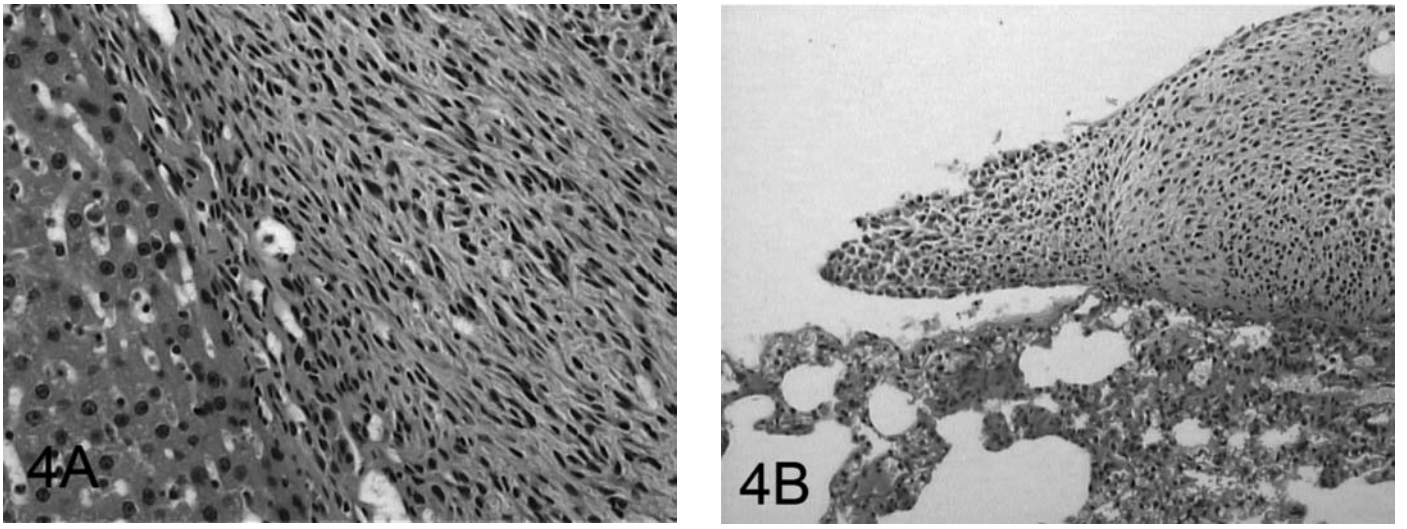


Fig. 4. Sarcomatoid or fibrous type mesothelioma in rats treated with FFE. (A) Peritoneal sarcomatous mesothelioma with bundles of uniform spindle-shaped cells, 200x; (B) same case: metastases in the pleura, 100x

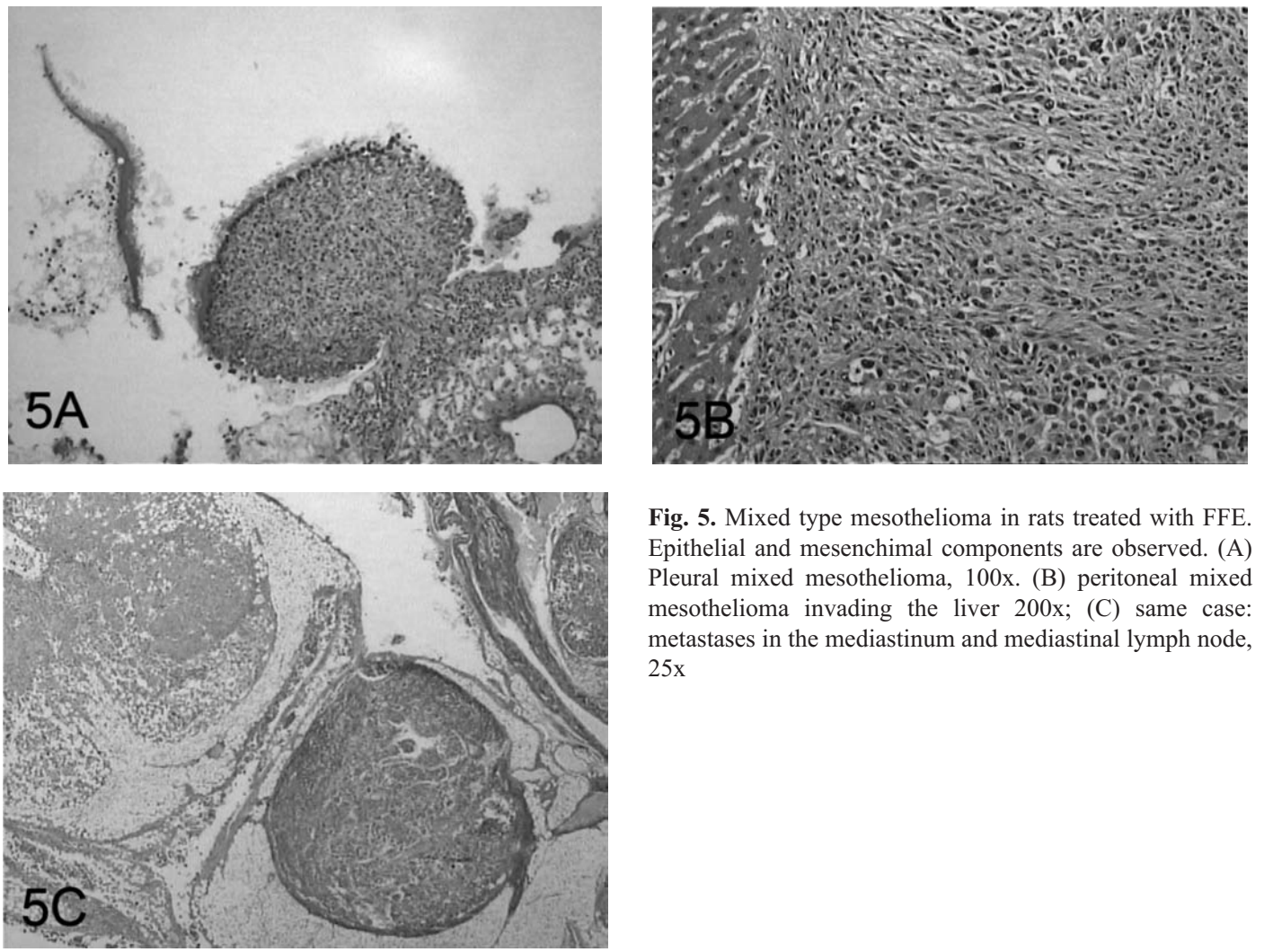


Fig. 5. Mixed type mesothelioma in rats treated with FFE. Epithelial and mesenchimal components are observed. (A) Pleural mixed mesothelioma, 100x. (B) peritoneal mixed mesothelioma invading the liver 200x; (C) same case: metastases in the mediastinum and mediastinal lymph node, 25x

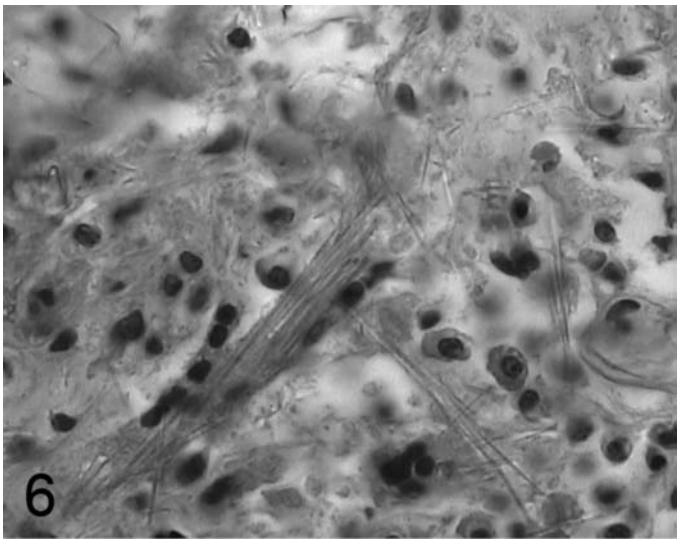


Fig. 6. Deposits of needle shaped fibrous material, surrounded by inflammatory cells, can be observed in a pleural mesothelioma, 1,000x (oil).

Table 4 - Ramazzini Institute Cancer Research program on fibers: comparative incidence and latency time of peritoneal mesotheliomas induced by single injection of different kinds of fibrous material, in male and female Sprague-Dawley rat

Material	Mesotheliomas		
	Incidence (%)	Average latency time (weeks)	Latency time of the first observed tumour (weeks)
Crocidolite	97.5	59.5	29
Amosite	90.0	66.7	44
Anthophyllite	87.5	73.3	18[a]
Chrysotile (Rhodesia)	82.5	89.7	48
Fibrous fluoro-edenite	82.5	63.7	36
Chrysotile (Canada)	80.0	92.2	65
Chrysotile (California)	72.5	85.3	39
Asbestos-cement	52.5	99.7	60
Erionite	50.0	106.1	67
Glass fibres	42.5	90.4	65
Ceramic fibres	32.5	84.9	58
Rock wool	10.0	78.7	69
Kevlar fibres	0	-	-

[a] Microscopic finding in a rat not deceased for peritoneal mesothelioma

performed in our laboratory on a series of non-fibrous materials, such as natural and man-made zeolites, which have proved to have limited or no effect. Our results are important for different reasons: 1) in only 36 weeks our experimental model showed a mesotheliomatogenic effect as observed in human population; 2) the different pattern of mesotheliomas observed are the same as those of humans; 3) this confirms that our model of long-term carcinogenicity bioassays on rodents is a valuable tool to predict human risks. If appropriately planned and carried out, carcinogenesis studies may provide information that allows not only the qualitative identification of carcinogenic risks, but also the quantitative extrapolation of such risks to humans. Furthermore, the observation of the same histotype/pattern of mesothelioma observed in humans, confirms that our model could be used for the assessment of adequate therapy against this tumour, nowadays still far from having success.

Table 5 - Ramazzini Institute Cancer Research program on fibers: comparative incidence and latency time of pleural mesotheliomas after a single injection of different kinds of fibrous material, in male and female Sprague-Dawley rats

Material	Mesotheliomas		
	Incidence (%)	Average latency time (weeks)	Latency time of the first observed tumour (weeks)
Erionite	87.5	64.2	32
Chrysotile (Canada)	65.0	111.1	80
Crocidolite	45.0	104.8	33
Asbestos-cement	35.0	113.1	82
Fibrous fluoro-edenite	16.3	79.7	51

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References

1. Di Paola M, Mastrantonio M, Carboni M, *et al.* La mortalità per tumore maligno della pleura in Italia negli anni 1988-1992. Istituto Superiore di Sanità, Roma: Rapporti ISTISAN 96/40, 1996.

2. Paoletti L, Battisti D, Bruno C, *et al*. Unusually high incidence of malignant pleural mesothelioma in a town of eastern Sicily: an epidemiological and environmental study. Arch Environ Health 2000; 55: 392-8.
3. Pasetto R, Bruni B, Bruno C, *et al.* Problematiche sanitarie della fibra anfibolica di Biancavilla. Aspetti epidemiologici, clinici e sperimentali. Notiziario Istituto Superiore di Sanità 2004; 17(1): 8-12.
4. Cernigliaro A, Fano V, Addario SP, *et al*. Mortality and hospital discharges in the population of Biancavilla (Sicily) contaminated by natural fibres. Epidemiol Prev 2006; 30(4-5): 227-31.
5. Fazzo L, Comba P. The role of high risk groups in environmental health research. Ann Ist Super Sanità 2004; 40(4): 417-26.
6. Putzu MG, Bruno C, Zona A, *et al*. Fluoro-edenitic fibres in the sputum of subjects from Biancavilla (Sicily): a pilot study. Environ Health 2006; 16 (5): 20.
7. Bruno C, Belli S, Cernigliaro A, *et al*. An estimate of pleural mesothelioma incidence in Biancavilla, Sicily, Italy, 1998-2004. Eur J Oncol 2007; 12(3): 183-7.
8. Gianfagna A, Paoletti L, Ventura P. Segnalazione di fibre di amianto anfibolico nei prodotti lavici metasomatizzati di Monte Calvario, Biancavilla (Sicilia orientale). Plinius, suppl. Eur J Mineral 1997; 18: 117-9.
9. Gianfagna A, Oberti R. Fluoro-edenite from Biancavilla (Catania, Sicily, Italy): crystal chemistry of a new amphibole end-member. Am Mineral 2001; 86: 1489-93.
10. Gianfagna A, Ballirano P, Bellatreccia F, *et al.* Characterization of amphibole fibres linked to mesothelioma in the area of Biancavilla, Eastern Sicily, Italy. Mineral Magazine 2003; 67(6): 1221-9.
11. Soffritti M, Minardi F, Bua L, *et al*. First experimental evidence of peritoneal and pleural mesotheliomas induced by fluoro-edenite fibres present in Etnean volcanic material from Biancavilla (Sicily, Italy). Eur J Oncol 2004; 9: 169-75.
12. Governo Italiano. Decreto Legislativo 116. 1992. Attuazione della direttiva n. 86/609/CEE in materia di protezione degli animali utilizzati a fini sperimentali o ad altri fini scientifici [in Italian]. Suppl Ordinario Gazz Ufficiale 40: 5-25.
13. Maltoni C, Minardi F. Recent results of carcinogenicity bioassays of fibres and other particulate materials. In Bignon J, Peto J, Saracci R, eds. Non-occupational Exposure to Mineral Fibres. Lyon: IARC Scientific Publications No. 90, 1989, 46-53.

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Cancer mortality and ELF-EMFs exposure association among young people: a case-control study

Studio caso-controllo sull'associazione tra mortalità per tumore nei giovani ed esposizione a ELF-EMFs

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Summary

Aim: **To study cancer mortality and extremely low frequency electro-magnet fields (ELF-EMFs) association among young people (under 18 yrs).** ***Methods:*** **A case-control study. Recruited all cancer deaths till 18 years of age in Bologna province in the 1993-2008 period. Three controls were matched for each case by sex, year of birth, and last city of residence. Using Geographical Information System (GIS) the residence distances from ELF-EMFs sources were measured. A conditional logistic regression was used.** ***Results:*** **80 cases were divided into three groups (brain cancer, hematologic neoplasms, other cancers). An inverse association between hematologic neoplasms in young males and Aerial Power Lines (ALs) distances from residences with OR= 0.38 (CI 95%=0.19-0.74) was found.** ***Conclusion:*** **Due to the small numbers of cases, it is only possible to state that male leukemia cases lived closer to aerial transmission lines. No association between brain cancer or other cancers and ELF-**

Riassunto

Finalità: **Studiare l'associazione tra esposizione a campi elettromagnetici a bassa frequenza (ELF-EMFs) e mortalità per tumori in giovani.** ***Metodi:*** **Studio caso-controllo. Vengono reclutati tutti i casi di decessi per tumori in giovani di età fino a 18 anni nel periodo 1993-2008 in provincia di Bologna. Ad ogni caso sono accoppiati tre soggetti di controllo per sesso, anno di nascita e comune di residenza al decesso. Mediante Geographical Information System (GIS) vengono calcolate le distanze delle residenze dalle fonti di ELF-EMFs. Viene effettuata una regressione logistica condizionale.** ***Risultati:*** **80 casi vengono suddivisi in 3 categorie (tumori al cervello, tumori al sistema linfoematopoietico, altri tumori). Risulta significativa solo l'associazione inversa tra tumori al sistema linfoematopoietico e distanza dagli elettrodotti aerei (ALs) con un OR di 0,38 (CI 95%=0,19-0,74) nei maschi.** ***Conclusione:*** **A causa dei pochi casi si può affermare solo che i maschi deceduti per leucemia vivano più vicini agli elettrodotti dei**

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EMFs distances was found. More confounders need to be studied. Eur. J. Oncol., 16 (4), 197-202, 2011

Key words: **hematologic neoplasms, brain cancers, EMF, case-control study, GIS**

rispettivi controlli. Non è stata individuata nessuna associazione tra distanze da ELF-EMFs e decessi per tumori cerebrali o di altro tipo. È necessario studiare meglio ulteriori possibili fattori confondenti. Eur. J. Oncol., 16 (4), 197-202, 2011

Parole chiave: **neoplasie ematologiche, tumori cerebrali, campi elettro-magnetici, studio caso-controllo, GIS**

Introduction

Since the 70s of the last century several individual studies (1-4), reviews and meta-analysis (5, 6) have tried to find an association between Extreme Low Frequency Electromagnetic Fields (ELF-EMFs) and cancer in young people. In 2001 the International Agency for Research on Cancer has included exposure to ELF as "possibly carcinogenic to humans" (Group 2B) (7) on the basis of limited epidemiological evidence and inadequate evidence in experimental animals. With the new studies a possible association between exposure to ELF-EMFs and leukaemia (8) has been found. Recently the World Health Organization declared "new human, animal and in vitro studies, published since the 2002 IARC monograph, do not change the overall classification of ELF magnetic fields as a possible human carcinogen" (9). In addition to leukaemia, also brain cancers in young people were studied to evaluate the association with ELF-EMFs (10). Up to now, partly because of the few cases, this association was not traceable (11). In order to evaluate exposure assessment several methods have been tried (wire-code, spot measures, distance) (12). Some studies used the residential distances from ELF-EMFs as a proxy of exposure level, to find correlations with the development of cancer (13, 14). The precise measure of distance and residential mobility from the different sources is a major problem. The use of Geographical Information System (GIS) could be a solution (15). The aim of this case-control study is to investigate whether there is an increased risk of cancer death associated with the distances from ELF measured by GIS. Although the number of cases is small, lifetime addresses were available, thus allowing to assess risks associated with living close to ELF sources at any time in life.

Materials and methods

Many companies manage electricity transmission lines (voltage from 50 kV to 380 kV). The Regional Prevention and Environment Agency (ARPA) provided the number of Medium-Voltage Stations (MVSs) in the Bologna province area (16); the website of Bologna Provincial Administration provided the geographical maps (in shape format) of aerial electric power lines (ALs), underground lines (ULs) and MVSs (17). For each element, the starting and closing year and operating parameters were recorded.

The Bologna mortality register, held at the Local Health Unit (AUSL), includes all death cases from 1993 to 2008; these deaths are coded according to the rules of International Code Diseases (ICD) IX rev.. We selected all cancer death cases of young people aged till 18 years. The cases were grouped by type of cancer: brain (ICD IX rev.=191-192), lymphatic and hematopoietic tissue (ICD IX rev.=200-208), others (single ICD codes). For each case date of birth and death, cause of death, last residence address were recorded. Moreover, for each case three control subjects were randomly drawn from Bologna AUSL's health register of residents on 30 June 2009. Matching was by gender, year of birth and last residence city. Any subject with even a short period of residence outside the Bologna province was discarded.

Through queries from the municipal registers, for each case and controls, all the residence places from

birth to end of follow-up have been retrieved. The period of follow-up ended for the cases at the time of death and for controls at the time of death of the matched case. All addresses were codified in geographic coordinates and mapped on the territory of the Bologna province. By GIS for each case and controls the minimum distances from each address to each source (AL, UL, MVS) and the days of residence were calculated. In case of multiple addresses for each subject the average distance in meters was calculated. Due to the non-normality distributions of the distances and days of residence (skewness e kurtosis tests $p<0.0001$), a logarithmic transformation was made.

A conditional logistic regression was carried out having as outcome cancer group type and as continue variables the logarithms of distances from the sources (AL, UL, MVS). As adjustment covariates gender was used and, as categorical variables for equal numbers, age years (0-7; 7.1-13.8; 13.9-18) and the logarithmic of exposure days (0-3.33; 3.34-3.67; 3.68-3.84) were chosen. Odds Ratios (OR) and confidence intervals at 95% probability (95% CI) were calculated.

The software used for statistical analysis was STATA v. 10 (18); for distance calculation ESRI's ArcGIS 9 (19) was used.

Results

The distribution in km as a function of voltage, both for aerial and underground lines, is shown in Table 1. Most kilometers (over 813 Km) are represented by aerial lines at 132 kV. There are over 150 Km at 380 kV for a single operator. The underground lines are mostly at 132 kV and do not reach 40 Km. The MV stations, distributed in all municipalities of the province in 2007, are 9.040.

A total of 80 deaths from cancer (49 males and 31 females) were identified: 20 cases of cancers in the nervous system, 33 cases of various types of lymphatic and hematopoietic tissue neoplasms (LHT) (mostly Lymphocytic and Myelocytic leukaemias) and 27 cases of cancers of other types (Table 2). The differences of average distances from residences to each ELF-EMFs source between cases and controls are shown in Table 3.

The results of conditional logistic regression for each cancer type by ELF-EMFs sources are shown in Table 4. An inverse significant association between log(distances) from the aerial electrical power lines (ALs) and LHT neoplasms with Odds Ratios = 0.49 (95% CI: 0.29-0.84) was found; such association was not found between LHT neoplasms and other EMF-ELFs sources (ULs or MVSs). No significant association was found for brain cancers or for other cancers with the log(distances) from any source of electromagnetic field.

Entering covariates in the model and performing the analysis only for aerial power lines (ALs), the results presented in Table 5 were obtained. Once again the LHT neoplasms are significantly associated but only for males with OR = 0.38 (95% CI: 0.19-0.74). The second age category (7.1-13.8) has a OR=0.31 (95% CI:0.09-1.00) at limit of statistical significance. All other cancers do not appear to be significantly associated with any source. No particular trend can be seen in various tests.

Table 1 - Companies, length (Km) and voltage (kV) of aerial and underground electric power lines in Bologna province area

Companies	Aerial (Km) Voltage (kV)				Buried (Km) Voltage (kV)	
	50	132	220	380	50	132
AMI		10.264				
ENEL	2.138	361.044	3.357		7.020	30.551
GRTN		0.072				
RFI		333.495				4.256
TERNA		108.308	135.724	153.542		4.256
Total Km	2.138	813.183	139.081	153.542	7.020	39.063

Table 2 - Numbers and average age (standard deviation) of cases and controls stratified for cancer type with International Code Diseases IX rev., at the end of follow-up

Cancer type	ICD IX rev.	Cases		Controls	
		No.	Av.Age (sd)	No.	Av.Age (sd)
Brain	191	17			
other and unspecified parts of nervous system	192	3			
Total Cancer type 1		20	8.9 (5.1)	60	8.8 (4.9)
Lymphosarcoma and reticulosarcoma	200	3			
Hodgkin's disease	201	2			
non-Hodgkin lymphomas	202	4			
Multiple myeloma	203	1			
Lymphocytic leukaemia	204	13			
Myelocytic leukaemia	205	10			
Total Cancer type 2		33	12.1 (5.5)	99	12.1 (5.5)
Nasopharynx	147	1			
Liver	155	1			
Peritoneum	158	1			
Bone and articular cartilage	170	2			
Connective and other soft tissue	171	4			
Eye	190	1			
Endocrine glands	194	8			
Other and ill-defined sites	195	5			
Hemangioma	228	2			
Uncertain behavior of endocrine glands and nervous system	237	1			
Uncertain behavior of other and unspecified sites and tissues	238	1			
Total Cancer type 3		27	9.4 (5.8)	81	9.5 (5.6)
Total cases and controls		80	10.4 (5.6)	240	10.4 (5.6)

Table 3 - Average distances (standard deviation) and range in meters from residences to ELF-EMFs source for cases and controls

Sources	Cases		Controls	
	Average (sd)	Range	Average (sd)	Range
ALs	1,248 (1133)	42 - 6,281	1,319 (1257)	47 - 8,028
ULs	4,143 (4913)	129 - 19,282	4,068 (4736)	13 - 20,903
MVSs	190 (199)	15 - 1,206	191 (165)	19 - 901

Abbreviation: ALs, Aerial power Lines, ULs, Underground power Lines; MVSs, Medium-Voltage Stations; sd, standard deviation

Conclusions

We investigated the possible association between deaths from LHT neoplasms, brain cancers and other cancers in 80 deaths in the Bologna province, in people aged 0-18 years and exposed to different sources of ELF-EMFs; the exposures were measured by distances as proxy. We followed all residential movements of the subjects and measured the minimal distance from each residence to each source of ELF-EMFs, using GIS.

Through the GIS and conditional logistic regres-

Table 4 - Odds Ratios (and 95 % Confidential Interval) for association among cancer types and distances from residences in log(m) to EMF-ELFs sources

Cancers	ALs OR (95% CI)	ULs OR (95% CI)	MVSs OR (95% CI)
Brain Cancers	1.11 (0.53-2.30)	1.23 (0.66-2.29)	1.38 (0.64-2.97)
LHT neoplasms	0.49 (0.29-0.84)*	1.02 (0.56-1.85)	0.84 (0.42-1.70)
Other Cancers	1.07 (0.59-1.94)	1.06 (0.57-1.99)	0.72 (0.38-1.37)

* $p<0.01$

Abbreviations: ALs, Aerial power Lines; ULs, Underground power Lines; MVSs, Medium-Voltage Stations; LHT, lymphatic and hematopoietic tissue neoplasms; OR, Odds Ratio; CI, Confidence Interval

Table 5 - Numbers of cases and Odds Ratios (with 95% CI) for cancer types and distances in log(m) from residences to Aerial Power Lines (ALs), stratified by gender, age at the end of follow-up and Log(exposure days)

	No.	Brain Cancers OR (95% CI)	No.	LHT neoplasms OR (95% CI)	No.	Other Cancers OR (95% CI)
Gender						
Males	12	1.97 (0.77-5.02)	21	0.38 (0.19-0.74)*	16	1.43 (0.55-3.71)
Females	8	0.59 (0.21-1.69)	12	0.89 (0.32-2.43)	11	0.85 (0.37-1.95)
Age (years)						
0 - 7	9	0.99 (0.36-2.77)	7	0.75 (0.24-2.34)	10	1.07 (0.45-2.51)
7.1 - 13.8	6	2.27 (0.25-2.11)	10	0.31 (0.09-1.00) **	11	0.82 (0.29-2.33)
13.9 - 18	5	0.63 (0.19-2.11)	16	0.60 (0.23-1.60)	6	3.32 (0.27-41.6)
Log (exposure days)						
0 – 3.33	9	1.40 (0.36-5.42)	11	0.67 (0.21-2.15)	11	1.05 (0.45-2.48)
3.34 – 3.67	7	1.58 (0.45-5.51)	8	0.36 (0.09-1.36)	9	0.63 (0.17-2.26)
3.68 – 3.84	4	0.83 (0.17-3.96)	14	0.58 (0.21-1.62)	7	2.48 (0.38-16.3)

* $p<0.005$; ** $p=0.051$

Abbreviations: ALs, Aerial Power Lines; LHT, lymphatic and hematopoietic tissue neoplasms; OR, Odds Ratio; CI, Confidence Interval

sion a reliable inverse association between the distances from the aerial electric power lines and LHT neoplasms mostly in males was found.

Several authors have investigated the possible association between childhood leukaemia and potential environmental and territorial factors, including the electromagnetic fields produced by current carrying lines. A recent meta-analysis on studies completed after 2000 confirmed this association (20), albeit with some methodological reservations. For some time even the hypothesis of a possible viral aetiology as leukaemia origin was considered (21). Recently, using GIS S. Schmiedel *et al.* (22) studied the possible presence of cluster products based on the assumption of the presence of infectious leukaemia in Germany. His conclusion did not provide support for this hypothesis in leukaemia aetiology in childhood.

Regarding the possible association between childhood brain cancers and ELF-MFs, a recent meta-analysis by Kheifets *et al.* (23) has confirmed little or no association, as evidenced also by our results.

The province of Bologna is situated in Northern Italy at the crossing of several power lines and electrical railroads. Many people potentially exposed to ELF live near them. Epidemiological studies have long noted an association between childhood leukemia and residential exposure to magnetic fields (1). The scientific evidence was judged as "limited" by IARC (7) and by WHO (9), as it is not possible to

rule out other explanations for the observed association, such as possible additional confounding factors, bias related to the recruitment of subjects (selection bias), or problems in exposure assessment that often occurred in the years before the study development. In our study, selection bias is almost non-existent, since we recruited all cases and controls identification was randomly performed.

A limit of our study is the small number of cases. Confounding factors could currently be considered: absence of precise measures of exposure at the residences, absence of information on lifestyles and socioeconomic status of parents, previous exposure of the mothers, absence of information on electric power transmission and timing.

Analysis of other factors impacting on subjects' lifestyle could prove further results for the onset of the disease. Therefore, this study should be considered as a preliminary investigation that may provide a small contribution to the solution of a complex issue. More investigations are needed.

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References

1. Wertheimer N, Leeper E. Electrical wiring configurations and childhood cancer. Am J Epidemiol 1979; 109, (3): 273-84.
2. Tomenius L. 50-Hz electromagnetic environment and the incidence of childhood tumors in Stockholm County. Bioelectromagnetics 1986; 7: 191-207.
3. Myers A, *et al*. Childhood cancer and overhead power lines. A case-control study. Br J Cancer 1990; 62: 1008-11.
4. Tynes T, Haldorsen T. Electromagnetic fields and cancer in children residing near Norwegian high-voltage power lines. Am J Epidemiol 1997; 145: 219-26.
5. Meinert R, Michaelis J. Meta-analyses of studies on the association between electromagnetic fields and childhood cancer. Radiation and Environmental Biophysics 1996; 35 (1): 11-8.
6. Ahlbom IC, *et al*. Review of the epidemiologic literature on EMF and Health. Environ Health Perspec 2001; 109 (Suppl 6): 911-33.
7. IARC Working Group on the Evaluation of Carcinogen. Non-Ionizing Radiation, Part 1: Static and Extremely-Low Frequency (ELF) Electric and Magnetic Fields. Monographs on the Evaluation of Carcinogenic Risks to Humans. 2002, Vol. 80.
8. Feychting M, Ahlbom A, Kheifets L. EMF and health. Ann Rev Public Health 2005; 26: 165-89.
9. World Health Organization. Extremely low frequency fields. Environ Health Criteria 2007, 238.
10. Kheifets L. Electric and magnetic field exposure and brain cancer: a review. Bioelectromagnetics 2001; 22 (suppl 5): S120-S131.
11. Mezei G, Gadallah M, Kheifets L. Residential magnetic field exposure and childhood brain cancer: a meta-analysis. Epidemiology 2008; 19 (3): 424-30.
12. Kheifets L, Oksuzyan S. Exposure assessment and other challenges in non-ionizing radiation studies of childhood leukaemia. Radiation Protection Dosimetry 2008; 132: 139-47.
13. Draper G, Vincent T, Kroll ME, Swanson J. Childhood cancer in relation to distance from high voltage power lines in England and Wales: a case-control study. BMJ 2005; 330 (7503): 1290.
14. Lowenthal RM, Tuck DM, Bray IC. Residential exposure to electric power transmission lines and risk of lymphoproliferative and myeloproliferative disorders: a cas-control study. Int Med J 2007; 37: 614-9.
15. Burrough PA. Principles of geographical information systems for land resource assessment. Oxford: Clarendon Press, 1986.
16. ARPA. Elettrodotti 2008. ARPA. [Online] dicembre 31, 2008. [Cited: Settembre 15, 2010.] http://www.arpa.emr.it/pubblicazioni/cem/generale_1169.asp
17. SIT, Provincia di Bologna. Catasto Provinciale delle Linee Elettriche. [Online] [Cited: Settembre 15, 2010.] http://cst.provincia.bologna.it/catalogo/
18. Corporation, Stata. Stata Statistical Software. Release 10, College Station, TX, USA: s.n., 2007.
19. Inc., ESRI. ArcMap. 9.0, Redlands, CA, USA: s.n., 2004.
20. Kheifets L, Ahlbom A, Crespi CM, *et al*. Pooled analysis of recent studies on magnetic fields and childhood leukaemia. Br J Cancer 2010; 103 (7): 1128-35.
21. Duc Dodon M, Mesnard JM, Barbeau B. Adult T-cell leukemia induced by HTLV-1: before and after HBZ. Med Sci 2010; 26 (4): 391-6.
22. Schmiedel S, Blettner M, Kaatsch P, Schüz J. Spatial clustering and space-time clusters of leukemia among children in Germany, 1987-2007. Eur J Epidemiol 2010; 25 (9): 627-33.
23. Kheifets L, Ahlbom A, Crespi CM, *et al*. A pooled analysis of extremely low-frequency magnetic fields and childhood brain tumors. Am J Epidemiol 2010; 172 (7): 752-61.

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Interaction between allelic polymorphisms in the modification of the risk of colorectal cancer in the Hungarian population

Rischio di tumore colon-rettale nella popolazione ungherese in relazione ai polimorfismi allelici

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Summary

Aim: **The effect of p53 Arg72Pro, and X-ray cross complementing 1 Arg399Gln allelic polymorphisms on the risk of colorectal cancer was studied in a case control study. The results were combined with the data from a previous study (polymorphisms of metabolizing enzymes), and a combined analysis for the joint effect of 7 allelic polymorphisms was also performed.** ***Materials and methods:*** **Five hundred colorectal cancer patients and 500 cancer- free controls were genotyped for the p53 Arg72Pro polymorphism by an allele-specific PCR, and by a PCR-RFLP for the XRCC1 polymorphism. Genotype frequencies were compared between cases and controls.** ***Results:*** **The number of p53 codon 72 Pro homozygotes and heterozygotes was higher among colorectal cancer patients than in the control group (53 vs. 28 and 188 vs. 123, respec-**

Riassunto

Finalità**: Lo studio caso-controllo descritto prende in esame la relazione tra i polimorfismi allelici dei geni p53Arg72Pro e X-Ray Cross Complementing 1 (XRCC1)Arg399Gln ed il rischio di cancro colon-rettale. I risultati sono stati associati ai dati ottenuti da un precedente studio (relativo a polimorfismi di enzimi metabolizzanti) ed è stata effettuata un'analisi combinata relativa ad un totale di 7 polimorfismi allelici.** ***Materiali e Metodi*****: 500 casi di pazienti affetti da cancro colon-rettale e 500 pazienti di controllo non affetti da cancro sono stati genotipizzati per il polimorfismo del gene p53Arg72Pro mediante PCR allele-specifica e del gene XRCC1 Arg399Gln mediante PCR-RFLP. Le varianti genotipiche sono state confrontate tra casi e controlli.** ***Risultati*****: Il numero di omozigosi ed eterozigosi del codone 72Pro del gene p53 è risultato più alto tra i pazienti af-**

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tively). Having the Pro homozygous genotype (OR: 2.00, 95% CI: 1.25-3.21) or carrying the Pro allele (OR: 2.15, 95% CI: 1.66-2.79) was associated with an elevated risk for colorectal cancer. The occurrence of XRCC1 heterozygotes (236 vs. 216) and Gln homozygotes (78 vs. 65) was higher among cases than in controls. Gln-carriers occurred more frequently among patients than in cancer-free controls (OR: 1.32, 95% CI: 1.02-1.69). Combined analysis of 7 polymorphisms showed that carrying at least 6 high-risk alleles substantially increases the risk of colorectal cancer (OR: 6.39, 95% CI: 2.73-14.92). ***Conclusions:*** **The p53 Arg72Pro and XRCC1 Arg399Gln polymorphisms affect the risk of colorectal cancer. Accuracy of risk estimation can be increased by a simultaneous analysis of several allelic polymorphisms. Eur. J. Oncol., 16 (4), 203-210, 2011**

Key words: **p53, X-ray cross complementing 1, polymorphism, colorectal cancer**

fetti da cancro colon-rettale rispetto al gruppo di controllo (53 vs 28 e 188 vs 123, rispettivamente). Il genotipo portatore di omozigosi Pro (OR: 2.00, 95% CI: 1.25-3.21) o avente un solo allele Pro (OR: 2.15, 95% CI: 1.66-2.79) è stato associato ad un elevato rischio di cancro colon-rettale. La presenza di eterozigosi e di omozigosi Gln del gene XRCC1 è risultata più alta nei pazienti malati rispetto al gruppo di controllo (236 vs 216 e 78 vs 65, rispettivamente). Nei pazienti con cancro colon-rettale è stata rilevata una maggiore frequenza di variazioni dell'allele Gln rispetto al gruppo di controllo. L'analisi combinata di 7 polimorfismi ha evidenziato che i portatori di almeno 6 polimorfismi allelici ad alto rischio hanno un maggiore rischio di sviluppare il cancro colon-rettale (OR: 6.39, 95% CI: 2.73-14.92). ***Conclusioni*****: I polimorfismi di p53Arg72Pro e XRCC1 Arg399Gln aumentano il rischio di sviluppare cancro colon-rettale. L'analisi combinata di diversi polimorfismi allelici può incrementare l'accuratezza della stima del rischio. Eur. J. Oncol., 16 (4), 203-210, 2011**

Parole chiave: **p53, X-Ray Cross Complementing 1, polimorfismo, tumore colon-rettale**

Introduction

Cancer is the second most important cause of deaths in developed countries (1). Cancer is a multifactorial disease, several environmental and genetic factors play a part in human carcinogenesis. Concerning genetics, a huge variety of different factors may affect the carcinogenetic process and thus possibly influence the risk of tumour formation. The so- called high-penetrance susceptibility factors are responsible for hereditary tumours and cancer syndromes. Since the familial aggregation of these tumours can be easily identified, studies on hereditary cancers had already been carried out several decades ago, and the majority of such genes were identified (2-5). Because of their relatively weak effect, studies on low-penetrance susceptibility factors started later, and today these investigations are in the focus of cancer molecular epidemiology.

The approximately 3-billion-base pairs in the human genome show a very high interindividual similarity, 99-99.9% of our genome has the same sequence, while the only 0.1-1% difference is responsible for the huge diversity of the numerous human populations and individuals (6). The most frequent and thus probably the most important such interindividual genetic differences are caused by single nucleotide polymorphisms (SNPs), which represent the major form of allelic polymorphisms. According to our present knowledge, SNPs are present in the human genome at a density of 1 SNP per 0.3-1 kilobase. SNPs in oncogenes, tumour suppressor genes, metabolizing enzymes, DNA repair enzymes may slightly alter the properties of the encoded proteins and thus have an influence on the risk of malignant transformation and tumour formation. The p53 tumour suppressor gene is known to take part in the regulation of cell cycle and apoptosis (7), and its

allelic polymorphism at codon 72 has been described as a risk modifier in human carcinogenesis (8-10). The X-ray cross complementing 1 gene (XRCC1) is an important component of the DNA repair system, it plays an important part in base excision and single-strand break repair (11, 12). Functional and regulatory SNPs of the XRCC1 gene (e.g. the Arg399Gln polymorphism) have been studied in order to identify their possible cancer risk modifying effect. One goal of the present study was to characterize the effect of p53 Arg72Pro and XRCC1 Arg399Gln polymorphisms on colorectal carcinogenesis in the Hungarian population. Colorectal cancer is the second most common cancer in Hungary, with a mortality rate of 49.4 per 100.000. Baranya County, the location of our study, shows similar colorectal cancer mortality (49.1/100.000). The incidence and mortality figures for other types of cancer are also similar to the overall Hungarian data.

The effect of one SNP alone is generally moderate, but carrying several "high-risk" alleles interacting with each other might cause a more pronounced risk increase. In the present study we also tested the combined effect of allelic polymorphisms on the risk of colorectal cancer, by evaluating the p53 and XRCC1 allelic distributions together with the results of our previous studies on polymorphisms of glutathione-S-transferase (GST) and N-acetyltransferase (NAT) enzymes.

Materials and methods

Patients

Five hundred colorectal cancer patients and five hundred cancer-free controls (non-cancer patients from in- or outpatient wards and volunteers for health status examination) primarily from the area of Baranya County, Hungary, were included in the study. Patients with genetic conditions affecting colorectal cancer risk (familial adenomatous polyposis, hereditary non-polyposis colorectal cancer, ulcerative colitis, *etc.*) were excluded. The cases and controls were matched according to age, sex, smoking habits, and red meat consumption. Participation was voluntary, and a written informed consent was obtained from all the participants.

Molecular investigation

Determination of GST and NAT genotypes was earlier performed, and the methods and results were published previously (13). Genotypings were made from peripheral blood or healthy tissue samples. The DNA isolation was carried out with the phenol-chlorophorm method, and the samples were stored at -70°C.

PCR-ARMS (Polymerase Chain Reaction-Amplification Refractory Mutation System)

The p53 Arg72Pro genotyping was made using an allele-specific amplification. Two simultaneous PCR reactions were performed for each sample. Both tubes contained the same 3' primer (GCAACTGACCGTGCAAGTCA), but different 5' primers. The 5' primers differed only in their last base: ATGCCAGAGGCTGCTCCCCC or ATGCCAGAGGCTGCTCCCCG. The amplification took place only in the tube containing the exactly matching 5' primer. The reaction mix contained 0.1 μg DNA template, 20 mM Tris-HCl, 50 mM KCl, 2.0 mM $MgCl_2$, 0.2 mM each dNTP, 0.3 μM each primer and 0.5 U Taq DNA polymerase (Promega), in 20 μl total volume. The PCR reaction with 30 cycles of 60 s 94°C, 60 s 60°C, 60 s 72°C was performed in a Techne Genius thermal cycler (14).

PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism)

Genotyping of the XRCC1 Arg399Gln polymorphism was performed using a PCR-RFLP method. The reaction mix was the same as described for the p53 polymorphism, but 40 cycles of 94°C for 15 s, 57°C for 45 s, 72°C for 45 s were performed in the thermal cycler. The amplification product was digested overnight with *Msp*I restriction endonuclease at 37°C, and subsequently run in 2% agarose gel. The Arg allele was characterized by 269 and 133 bp fragments, while the undigested 402 bp fragment indicated the presence of the Gln allele. The primer sequences were as follows: TCTCCCTTGGTCTCCAACCT and AGTAGTCTGCTGGCTCTGG (15).

Statistics

Odds ratios and 95% confidence intervals (CIs) were calculated to express the effects of genotypes on cancer susceptibility. Simultaneous occurrence of high-risk genotypes in cases and controls was compared with the same method. A multivariate analysis was also performed by logistic regression analysis, in order to describe the interaction between the polymorphisms studied/SPSS for Windows statistical package (SPSS Inc., Chicago, IL, USA).

Results

Five hundred colorectal patients and 500 controls were genotyped for p53 Arg72Pro and XRCC1 Arg399Gln polymorphisms. The genotype distributions of the cases and controls are shown in Table 1. At the p53 allelic polymorphism, the number of Arg homozygous individuals was higher in the control group than among cases [349 (69.8%) vs. 259 (51.8%)], while there were less heterozygotes [123 (24.6%) vs. 188 (37.6%)] and Pro homozygotes [28 (5.6%) vs. 53 (10.6%)] in this group. Having the Pro homozygous genotype (OR: 2.00, 95% CI: 1.25-3.21) or carrying the Pro allele (Pro homozygotes and heterozygotes, OR: 2.15, 95% CI: 1.66-2.79) was statistically and significantly associated with an elevated risk for colorectal cancer.

The distribution of XRCC1 genotypes also showed a statistically significant difference between cases and controls. Occurrence of heterozygotes [216 (43.2%) vs. 236 (47.2%)] and Gln homozygotes [65 (13.0%) vs. 78 (15.6%)] was lower among controls than in the case group (Table 2). Carrying the Gln allele, either in heterozygous or in homozygous form, was statistically and significantly more frequent among colorectal cancer patients than in cancer free participants (OR: 1.32, 95% CI: 1.02-1.69).

In order to test the interaction between the p53 and the XRCC1 polymorphisms, the number of individuals carrying both high-risk alleles in the patients' group was compared to that in the control group. In the case group 169 (33.8%) patients carried both high-risk alleles (XRCC1 Gln and p53 Pro), while only 72 (14.4%) individuals had this allelic combination among controls (OR: 3.04, 95% CI: 2.20-4.19). These results demonstrate that carrying both the p53 Pro and the XRCC1 Gln alleles causes a more intensive risk increase, compared to having only one high-risk allele.

Table 1 - Distribution of p53 codon 72 genotypes among colorectal cancer patients and controls

	Colorectal cancer patients	Controls
Arg/Arg	259 (51.8%)	349 (69.8%)
Arg/Pro	188 (37.6%)	123 (24.6%)
Pro/Pro	53 (10.6%)	28 (5.6%)

Table 2 - Distribution of XRCC1 Arg399Gln genotypes among colorectal cancer patients and controls

	Colorectal cancer patients	Controls
Arg/Arg	186 (37.2%)	219 (43.8%)
Arg/Gln	236 (47.2%)	216 (43.2%)
Gln/Gln	78 (15.6%)	65 (13.0%)

Since the analysis of allelic combinations suggested a possible interaction between the studied polymorphisms, we extended this type of investigation to further allelic polymorphisms. In an earlier study we tested the effect of certain metabolizing enzymes (GSTM1, GSTT1, GSTP1, NAT1, NAT2) on the risk of colorectal cancer, using the same group of patients and controls as in the present study. By combining the genotype data from the present study (p53 and XRCC1) with our previous results (GSTM1, GSTT1, GSTP1, NAT1, NAT2), two tables were constructed with the number of putative high-risk alleles per person among cases and controls (Tables 3 and 4). The tables illustrate that simultaneous presence of several high-risk alleles in a person is a more frequent state of affairs among colorectal cancer patients than in the control group (OR: 6.39, 95% CI: 2.73-14.92, for carrying at least 6 high risk alleles). The distribution, as illustrated by fig. 1, is shifted to the right for the colorectal cancer patients, compared to the control group ($p<0.001$, chi-square goodness of fit).

Table 3 - Distribution of GSTM1, GSTT1, GSTP1, NAT1 and NAT2 genotypes among healthy controls

	+	0	Ile/Ile	Ile/Val	Val/Val	Slow	Rapid
GSTM1	258	242	-	-	-	-	-
GSTT1	392	108	-	-	-	-	-
GSTP1	-	-	214	212	74	-	-
NAT1	-	-	-	-	-	305	195
NAT2	-	-	-	-	-	318	182

Table 4 - Distribution of GSTM1, GSTT1, GSTP1, NAT1 and NAT2 genotypes among colorectal cancer patients

	+	0	Ile/Ile	Ile/Val	Val/Val	Slow	Rapid
GSTM1	209	291	-	-	-	-	-
GSTT1	369	131	-	-	-	-	-
GSTP1	-	-	200	212	88	-	-
NAT1	-	-	-	-	-	289	211
NAT2	-	-	-	-	-	267	233

Discussion

Human carcinogenesis is a complex, multistep process involving several environmental and genetic factors. In spite of the several mechanisms (including genetic and epigenetic components) taking part in the process of malignant transformation, the DNA repair has been considered to be one of the most important anticancer defense mechanisms by protecting the integrity of the cellular genetic material. Well-working DNA repair mechanisms keep the risk of unrepaired DNA damage low, thus preventing the malignant transformation of the cells. Efficacy of DNA repair pathways shows an interindividual variation, measured by different phenotyping methods (16). Recently, genetic factors have been demonstrated to play an important role in the variability mentioned. SNPs have been found in almost all of the repair-related genes, causing alterations in the amino acid sequence of the encoded proteins (17, 18).

Our study focused on polymorphisms in two genes, XRCC1 and p53. XRCC1 is a DNA repair gene, taking part in the base excision repair. The p53 tumour suppressor gene, often called as "the guardian of the genome", possesses several functions including cell cycle control, apoptosis induction, and it also has a very strong connection to DNA repair (19). Previous studies on the XRCC1 Arg399Gln polymorphism and the risk of cancer in humans failed to determine the effect of this polymorphism (20-24). One possible cause of the controversial results was that the populations studied were not always comparable; they had different genetic background and different environmental or occupational exposures. The phenotype-genotype relation-

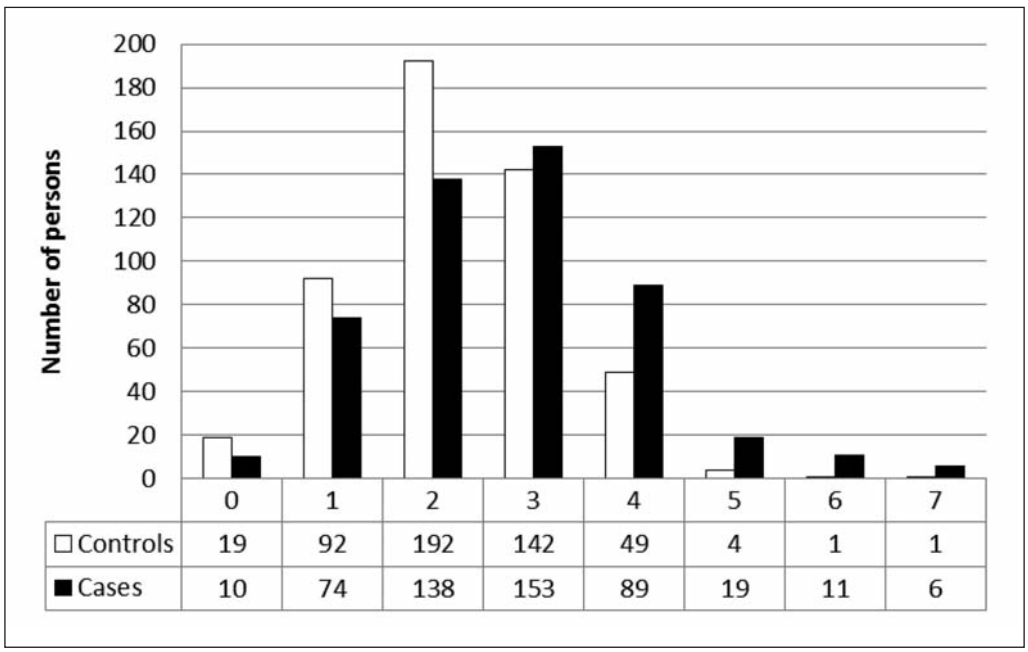

Fig. 1. Number of high-risk alleles per person among colorectal cancer patients and controls

ship in this allelic polymorphism is not completely understood, however, most of the studies suggest a possibly less effective repair function of the protein encoded by the Gln allele (25, 26). Our results are in accordance with these data, confirming the association between elevated cancer risk and occurrence of the Gln allele. The results provide a mechanistic link between the slower DNA repair in individuals carrying the Gln allele, and their elevated colorectal cancer risk.

Several studies have investigated the biological properties of the p53 alleles. Differences were observed in the electrophoretic mobility (27), apoptotic potential (28, 29) according to genotypes. These data are in agreement with the results of molecular epidemiological studies on the effect of p53 Arg/Pro polymorphism on lung cancer (30-32). In the case of other tumours, however, the relationship between the allelic polymorphism and risk of cancer is not fully established (33-36). The present study found quite a strong connection between the Pro allele and an elevated risk of colorectal tumours (OR: 2.15). Our results underline the importance of DNA repair functions and cell cycle control in human carcinogenesis. These findings are in accordance with the results of similar studies: Ivkovic *et al.* found that an allelic polymorphism in the H-ras oncogene significantly affected the risk of colorectal cancer (37). These studies also confirm the risk-modifying rôle of low-penetrance genetic factors like allelic polymorphisms of oncogenes and tumour suppressor genes.

The so-called low-penetrance genetic factors typically cause a risk increase of 1.2-2. These allelic polymorphisms cannot be directly used for individual risk assessment, since their effect on the absolute risk of a particular cancer is relatively low. However, they have a much stronger impact at a population level, because of their high population attributable risk (PAR), due to their frequent occurrence, in contrast to the hereditary tumours. A possible approach to "bringing down" low penetrance cancer susceptibility genes from the population level to the individual risk assessment is to study them in combination with each other. While the strength of the effect of XRCC1 and p53 genes alone was moderate (OR: 1.32 and OR: 2.00, respectively), their combined analysis resulted in a stronger effect (OR: 3.04). Combining our present results with those of our previous studies demonstrated the possibilities of further improving this approach.

In an earlier study we examined the effect of glutathione-S-transferase and N-acetyltransferase enzymes on the risk of colorectal cancer. GST enzymes belong to the so-called phase II metabolizing enzymes, they take part in the detoxification of carcinogenic molecules by conjugating their substrates (e.g. polycyclic aromatic hydrocarbons, epoxides, halomethanes) with glutathione. Similarly, the N-acetyltransferase enzymes also metabolize known carcinogenic molecules (e.g. aromatic and heterocyclic amines) thus influencing the amount of active carcinogenic molecules in our body. Since these polymorphisms may also have an influence on the risk of colorectal cancer, we combined these earlier results with our present findings, because studying several allelic polymorphisms might improve the accuracy of the risk assessment, and, hopefully already in the near future, can even lead to individual risk characterization. Our combined analysis contained 7 allelic polymorphisms, 4 of them (XRCC1, p53, GSTM1, NAT2) were found to be statistically and significantly associated with an increased colorectal cancer risk when analyzed separately. Our results suggest (OR: 6.39, 95% CI: 2.73-14.92 for at least 6 high-risk alleles) that this approach is a promising possibility to identify high-risk individuals.

The main limitation of our study is the involvement of only 7 allelic polymorphisms in the combined analysis, while there are possibly hundreds or thousands of similar factors which have an influence on cancer susceptibility. All the polymorphic genes directly or indirectly involved in the malignant transformation might contribute to the actual risk status. At present, however, no direct individual risk estimation can be given, and the practical implementation of the research results requires more time and work in this field. However, with the development of our knowledge concerning the effect of low-penetrance genetic factors, after cost-benefit analyses screening or pre-screening programs will be worked out, and hopefully the allelic status will more directly contribute to cancer risk assessment.

References

1. Jemal A, Siegel R, Ward E, *et al*. Cancer statistics, 2008. CA Cancer J Clin 2008; 58(2): 71-96.
2. Hall JM, Lee MK, Newman B, *et al*. Linkage of early-onset familial breast cancer to chromosome 17q21. Science 1990; 250: 1684-9.
3. Wooster R, Neuhausen SL, Mangion J, *et al*. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. Science 1994; 265: 2088-90.
4. Bodmer WF, Bailey CJ, Bodmer J, *et al*. Localization of the gene for familial adenomatous polyposis on chromosome 5. Nature 1987; 328: 614-6.
5. Cannon-Albright LA, Goldgar DE, Meyer LJ, *et al*. Assignment of a locus for familial melanoma, MLM, to chromosome 9p13-p22. Science 1992; 258: 1148-52.
6. Barbujani G, Colonna V. Human genome diversity: frequently asked questions. Trends Genet 2010; 26(7): 285-95.
7. Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997; 88: 323-31.
8. Fan R, Wu MT, Miller D, *et al*. The p53 codon 72 polymorphism and lung cancer risk. Cancer Epidemiol Biomarkers Prev 2000; 9: 1037-42.
9. Papadakis EN, Dokianakis DN, Spandidos DA. p53 codon 72 polymorphism as a risk factor in the development of breast cancer. Mol Cell Biol Res Commun 2000; 3: 389-92.
10. Perez-Perez GI, Bosques-Padilla FJ, Crosatti ML, *et al*. Role of p53 codon 72 polymorphism in the risk of development of distal gastric cancer. Scand J Gastroenterol 2005; 40: 56-60.
11. Zdzienicka MZ. Mammalian mutants defective in the response to ionizing radiation-induced DNA damage. Mutat Res 1995; 336: 203-13.
12. Shen MR, Jones IM, Mohrenweiser H. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res 1998; 58: 604-8.
13. Kiss I, Németh Á, Bogner B, *et al*. Polymorphisms of glutatione-S-transferase and arylamine N-acetyltransferase enzymes: susceptibility to colorectal cancer. Anticancer Res 2004; 24: 3965-70.
14. Storey A, Thomas M, Kalita A, *et al*. Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. Nature 1998; 393: 229-34.
15. Ito H, Matsuo K, Hamajima N, *et al*. Gene-environment interactions between the smoking habit and polymorphisms in the DNA repair genes. Carcinogenesis 2004; 25: 1395-401.
16. Norbury CJ, Hickson ID. Cellular responses to DNA damage. Ann Rev Pharmacol & Toxicol 2001; 41: 367-401.
17. de Boer JG. Polymorphisms in DNA repair and environmental interactions. Mutat Res 2002; 509: 201-10.
18. Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 2002; 11: 1513-30.
19. Smith ML, Seo YR. p53 regulation of DNA excision repair pathways. Mutagenesis 2002; 17: 149-56.
20. Wang J, Zhao Y, Jiang J, *et al*. Polymorphisms in DNA repair genes XRCC1, XRCC3 and XPD, and colorectal cancer risk: a case-control study in an Indian population. J Cancer Res Clin Oncol 2010; 136(10): 1517-25.
21. Jelonek K, Gdowicz-Klosok A, Pietrowska M, *et al.* Association between single-nucleotide polymorphisms of selected genes involved in the response to DNA damage and risk of colon, head and neck, and breast cancers in a Polish population. J Appl Genet 2010; 51(3): 343-52.
22. Wang B, Wang D, Huang G, *et al*. XRCC1 polymorphisms and risk of colorectal cancer: a meta-analysis. Int J Colorectal Dis 2009; 25: 313-21.
23. Improta G, Sgambato A, Bianchino G, *et al*. Polymorphisms of the DNA repair genes XRCC1 and XRCC3 and risk of lung and colorectal cancer: a case-control study in a Southern Italian population. Anticancer Res 2008; 28(5B): 2941-6.
24. Sliwinski T, Krupa R, Wisniewska-Jarosinska M, *et al*. No association between the Arg194Trp and Arg399Gln polymorphisms of the XRCC1 gene and colorectal cancer risk and progression in a Polish population. Exp Oncol 2008; 30: 253-4.
25. Hu JJ, Smith TR, Miller MS, *et al*. Amino acid substitution variants of APE1 and XRCC1 genes associated with ionizing radiation sensitivity. Carcinogenesis 2001; 22: 917-22.
26. Wang Y, Spitz MR, Zhu Y, *et al*. From genotype to phenotype: correlating XRCC1 polymorphisms with mutagen sensitivity. DNA Repair 2003; 2: 901-8.
27. Thomas M, Kalita A, Labrecque S, *et al*. Two polymorphic variants of wild-type p53 differ biochemically and biologically. Mol Cell Biol 1999; 19: 1092-100.
28. Dumont P, Leu JI, Della Pietra AC, *et al*. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet 2003; 33: 357-65.
29. Pim D, Banks L. p53 polymorphic variants at codon 72 exert different effects on cell cycle progression. Intl J Cancer 2004; 108: 196-9.
30. Liu G, Miller DP, Zhou W, *et al*. Differential association of the codon 72 p53 and GSTM1 polymorphisms on histological subtype of non-small cell lung carcinoma. Cancer Res 2001; 61: 8718-22.
31. Miller DP, Liu G, De Vivo I, *et al*. Combinations of the variant genotypes of GSTP1, GSTM1, and p53 are associated with an increased lung cancer risk. Cancer Res 2002; 62: 2819-23.

32. Murata M, Tagawa M, Kimura M, *et al*. Analysis of a germ line polymorphism of the p53 gene in lung cancer patients; discrete results with smoking history. Carcinogenesis 1996; 17: 261-4.
33. Katiyar S, Thelma BK, Murthy NS, *et al*. Polymorphism of the p53 codon 72 arg/pro and the risk of HPV type 16/18-associated cervical and oral cancer in India. Mol Cell Biochem 2003; 252: 117-24.
34. Chen X, Liu F, Li B, *et al*. p53 codon 72 polymorphism and liver cancer susceptibility: a meta-analysis of epidemiologic studies. World J Gastroenterol 2011; 17(9): 1211-8.
35. Hsieh YY, Lin CS. P53 codon 11, 72, and 248 gene polymorphisms in endometriosis. Int J Biol Sci 2006; 2: 188-93.
36. Tang NP, Wu YM, Wang B, *et al*. Systematic review and meta-analysis of the association between P53 codon 72 polymorphism and colorectal cancer. Eur J Surg Oncol 2010; 36(5): 431-8.
37. Ivkovic CT, Loncar B, Spaventi R, *et al*. Association of H-ras polymorphisms and susceptibility to sporadic colon cancer. Int J Oncol 2009; 35: 1169-73.

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Mortality associated with liver diseases in Egypt (1986-2005)

La mortalità associata alle malattie epatiche in Egitto (1986-2005)

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Summary

Background: **Liver diseases including hepatitis C are an important health problem in Egypt. There are few available studies about their burden and mortality rates.** ***Objectives:*** **We carried out this study to address the magnitude of liver diseases in Egypt by measuring their mortality rates and number of years of life lost (YLL) over the period 1986-2005.** ***Methods:*** **The data were obtained from mortality records of both the Central Agency of Public Mobilization and Statistics (for years 1986-1999) and the National Information Center for Health and Population of Ministry of Health and Population (for years 2000-2005). The age adjusted death rates for four major liver diseases were measured (hepatitis B and C virus infection, liver cirrhosis and liver cancer). The YLL due to these diseases were calculated.** ***Results:*** **The study showed little variations in the total mortality rates for the total liver diseases and mortality rates due to hepatitis B alone and their number of YLL during the period 1986-1999 then started to increase from year 2000 up to year 2005. No deaths due to hepatitis C infec-**

Riassunto

Contesto: **Le malattie epatiche compresa l'epatite C sono un importante problema sanitario in Egitto. In questo contesto e per quanto riguarda gli indici di mortalità, esistono pochi studi disponibili.** ***Obiettivi:*** **Lo studio è stato condotto per affrontare la vastità delle malattie epatiche in Egitto attraverso la misurazione degli indici di mortalità e del valore AVP (anni di vita persi) nel periodo esaminato 1986-2005)** ***Metodi:*** **I dati sono tratti dai registri di mortalità dell'Agenzia Centrale per la Mobilitazione Pubblica e la Statistica (per gli anni 1986-1999) e dal Centro Nazionale di Informazione sulla Salute e la Popolazione del Ministero della Salute e della Popolazione (per gli anni 2000-2005). Sono stati misurati i tassi di mortalità aggiustati per età per quattro principali malattie epatiche (infezione da virus di epatite B e C, cirrosi epatica e cancro del fegato). È stato calcolato il numero di anni di vita persi (AVP) a causa di queste malattie.** ***Risultati:*** **Lo studio ha mostrato variazioni minime negli indici di mortalità totale per tutte le malattie epatiche, negli indici di mortalità dovuti alla sola epatite B e nel lo-**

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tion during the period 1986-1999 and also in year 2003 were recorded. On the other hand from year 2000, an upward trend for mortality rates and number of YLL due to hepatitis C infection was observed. Mortality rates and number of YLL due to liver cancer and liver cirrhosis showed a gradual increase from year 1986 up to year 2005. Males showed higher mortality from liver diseases than females. Also the rates were higher in old age groups than in the young ones. ***Conclusions:*** **The study showed that the total mortality rates and YLL for liver diseases are steadily increasing with time with higher mortality from liver diseases among males than females all through the study period. Eur. J. Oncol., 16 (4), 211-220, 2011**

Key words: **mortality, liver diseases, Egypt**

ro numero di AVP durante il periodo 1986-1999, dopo il quale si è registrato un incremento a partire dal 2000 fino al 2005. Non si sono registrate morti per l'infezione da epatite C durante il periodo 1986-1999 ed anche nell'anno 2003. D'altra parte, dal 2000, c'è stata una tendenza al rialzo degli indici di mortalità e del numero di anni di vita persi dovuti all'infezione da epatite C. Gli indici di mortalità e il numero di AVP dovuti al cancro del fegato e alla cirrosi epatica hanno mostrato un graduale aumento dal 1986 fino al 2005. Per le malattie epatiche la mortalità maschile si è dimostrata maggiore rispetto a quella femminile. Inoltre gli indici erano più alti in gruppi di persone anziane rispetto a quelle giovani. ***Conclusioni:*** **Lo studio ha dimostrato che gli indici di mortalità totale e gli anni di vita persi per malattie epatiche stanno progressivamente aumentando col passare del tempo, con una più alta mortalità tra i maschi rispetto alle femmine durante tutto il periodo di studio. Eur. J. Oncol., 16 (4), 211-220, 2011**

Parole chiave: **mortalità, malattie epatiche, Egitto**

Introduction

Very little scientifically based information is available on the causes of specific mortality rates for many developing countries. What information does exist is often out of date, applicable only to major urban areas, and not sufficiently disaggregated to differentiate between important population sub-groups (1). Although liver diseases are important health problem in Egypt, few studies about their burden and mortality rates are available. In Egypt, it has been estimated that liver diseases could contribute to more than 10% of over-all mortality and is the second most common cause of death after heart diseases in Egypt (2). It has been estimated that 7% of all Egyptian died annually were suffering from either liver cirrhosis or hepatocellular carcinoma (HCC); 75-85% of persons with these conditions present either chronic hepatitis B virus infection or hepatitis C virus infection as a contributing cause (3). Exposure to hepatitis viruses is extensive among the rural population in Egypt. Antibodies against hepatitis A virus (HAV) are detected in nearly 100% of both children and adults. Antibodies to hepatitis B virus (HBV) are present in 40-65% in most community based studies (4). Hepatitis C virus (HCV) related mortality (due to hepatocellular carcinoma and liver cirrhosis) is expected at least to double in the next 20 years (5). HCC was reported to account for about 4.7% of chronic liver disease patients (6). More than 80% of the risk of HCC is attributable to chronic infections with hepatitis B and C viruses worldwide (7). We carried out this study to address the magnitude of liver diseases in Egypt by measuring their mortality rates and the years of life lost (YLL) over the period 1986-2005. These informations are necessary to evaluate the impact of health policies regarding liver diseases and to justify the adoption of new ones.

Aim of work

The aim of the study was to calculate mortality rates of some conditions related to liver disease in

Egypt and their trend over the period 1986-2005 and to calculate YLL due to some liver diseases in Egypt.

Subjects and methods

Mortality study was performed using mortality records of Egypt during the period 1986-2005 from two main sources; the first source was mortality records of the Central Agency of Public Mobilization and Statistics (CAPMAS) for years 1986-1999. In CAPMAS, coding of death follows ICD-9. The second source was mortality records of the National Information Center for Health and Population of Ministry of Health and Population (NICHP-MOHP) for years 2000-2005 in which coding of death follows ICD-10.

These death records include different items (e.g. sex of dead person, job of dead person, date of occurrence of the event, etc). Census data obtained from CAPMAS are as follows: 1986 and 1996 censuses, annual estimates of population age-sex composition for the years in-between census years and the projected estimates from year 1997 up to year 2005. In estimation of cause specific death rate according to age and sex, burden of disease list for causes of death of WHO was used (replacing ICD-9 and ICD-10 codes by codes of burden of disease). Cause specific death rates for the causes of death related to liver diseases (hepatitis B, hepatitis C, liver cancer and liver cirrhosis), direct standardization of cause specific death rates was done using the population of year 2005 (the most recent year) as the standard population, moreover the trend of mortality due to liver diseases over this period was observed. This method was performed for each year from 1986 up to 2005.

YLL are the mortality component of Disability Adjusted Life Years (DALY). They determined by the average life expectancy at age of death while discounting future years by three per cent. The mean life expectancy for each age category and sex was estimated from the observed mean age at death in the age interval and the life expectancy figures at the exact ages defining the age interval. The mean life expectancy in each age interval was then discounted at three per cent using the formula: YLL=(1-exp (-0.03L)/ 0.03); where L is the life expectancy. Thus, YLL time discounted were calculated for each age group and sex then was multiplied by the observed deaths to derive the YLL by cause, age and sex (8).

Ethical considerations

The study was carried out after the consent of both Ministry of Health and Population and Central Agency for Public Mobilization and Statistics.

Results

As shown in fig. 1, age adjusted death rates of liver diseases in Egypt during the period 1986-1999 were fluctuating between 5.76/100,000 and

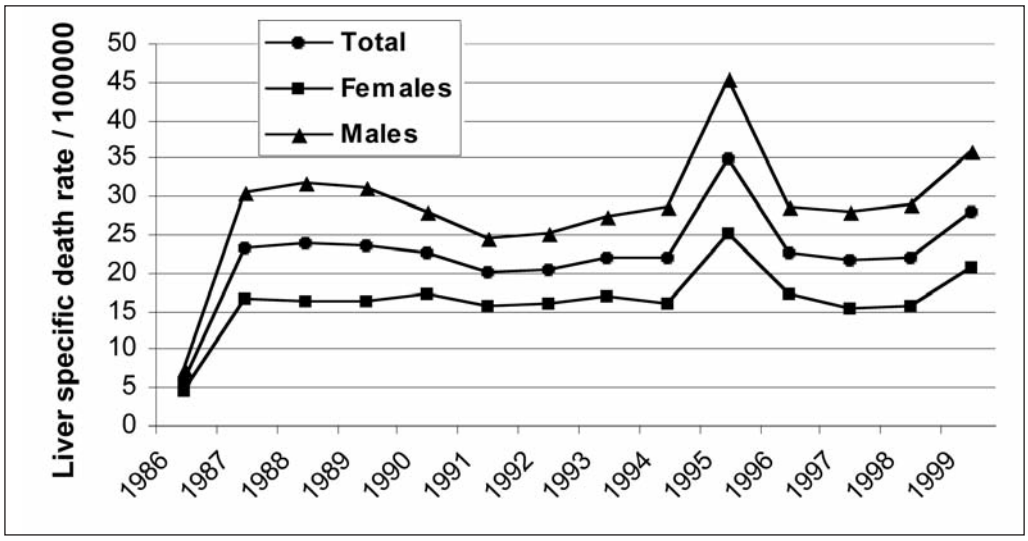

Fig. 1. Mortality trend of liver diseases, Egypt 1986-1999

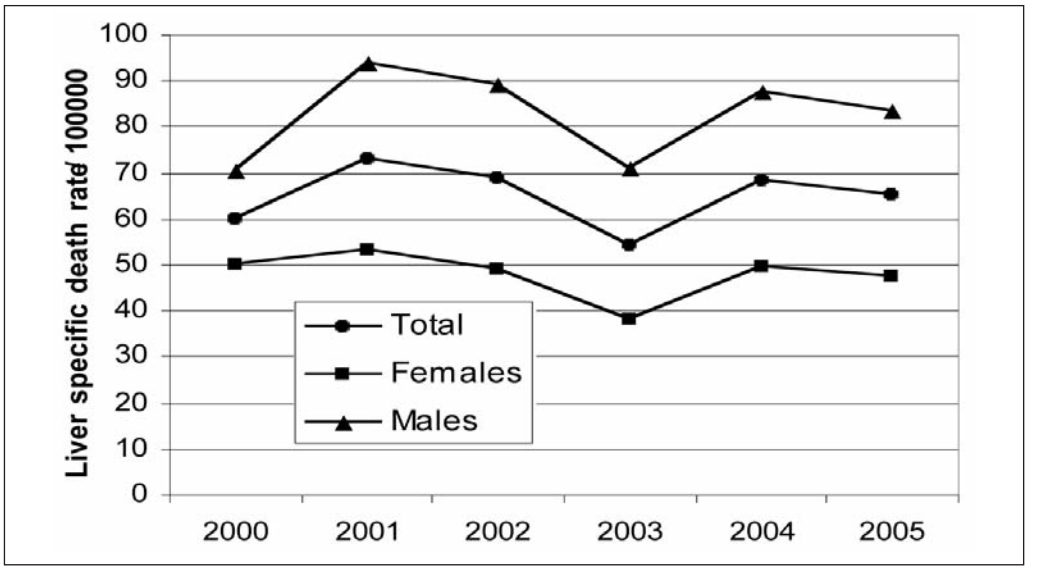

Fig. 2. Mortality trend of liver diseases, Egypt 2000-2005

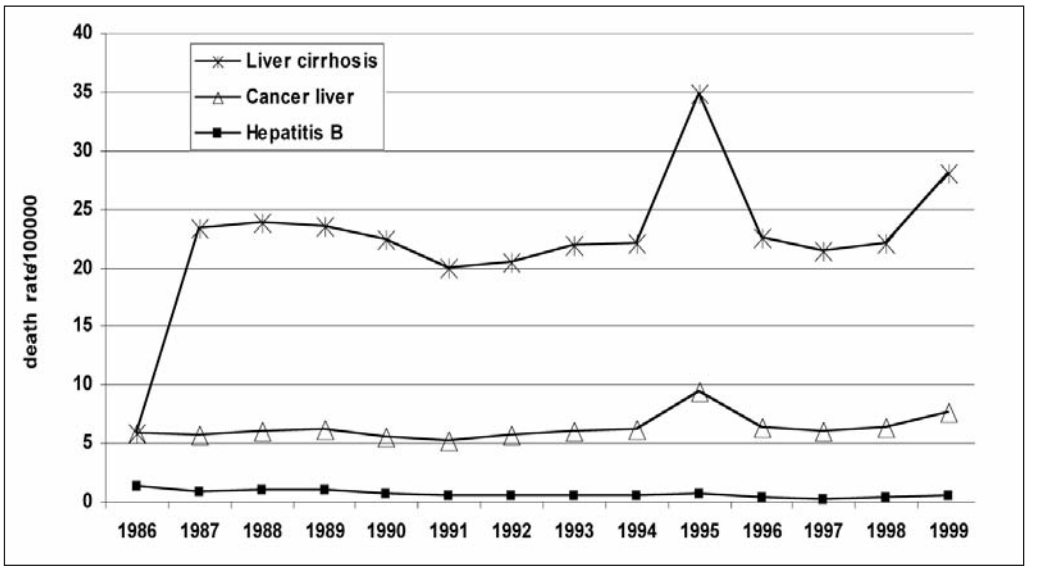

Fig. 3. Comparative pattern for mortality trend of different liver diseases, Egypt 1986-1999

34.85/100,000. But from year 2000 up to year 2005, the mortality rates markedly increased and fluctuating to reach highest level 73.09/100,000 in year 2001 (fig. 2). The liver specific death rates were higher in males than females.

Figs. 3 and 4 compare the mortality trends of the four liver diseases (hepatitis B, hepatitis C, liver cancer and liver cirrhosis) in Egypt during the period 1986-2005. It shows that liver cirrhosis had the highest mortality rates among liver diseases in Egypt during this period, followed by liver cancer, hepatitis B infection and hepatitis C infection.

Figs. 5, 6, and 7 describe liver diseases specific death rates for different age groups in both males and females for years 1986, 1996 and 2005. It is obvious that old age groups showed higher mortality from liver diseases than young ones during the three years and the highest rate was at age group above 75 years in both males and females.

Fig. 8 represents YLL due to all liver diseases for different age groups for both males and females in year 2005. The pattern is the same for all studied liver diseases and for males and females with a maximum of YLL at age group 50-54 in males and 55-59 in females.

Table 1 represents YLL due to liver diseases (hepatitis B, hepatitis C, liver cancer and liver cirrhosis) for different age groups for both males and females in year 2005. The pattern was the same in males and females with a maximum of YLL at age group 50-54 in males and 55-59 in females in all liver diseases.

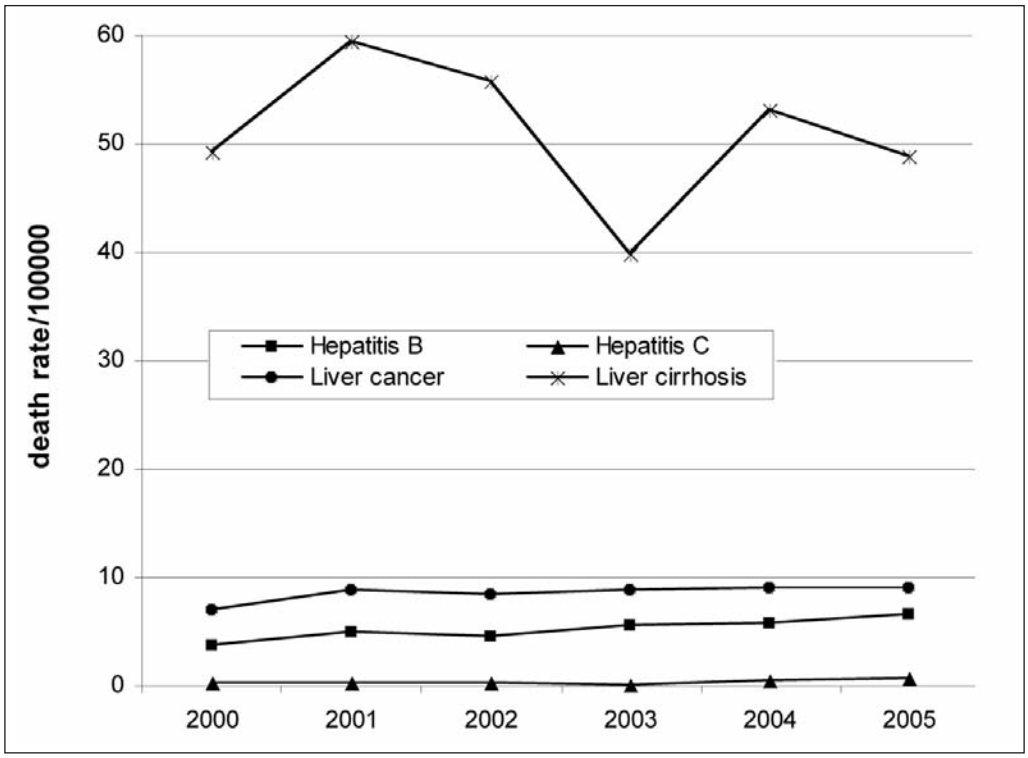

Fig. 4. Comparative pattern for mortality trend of different liver diseases, Egypt 2000-2005

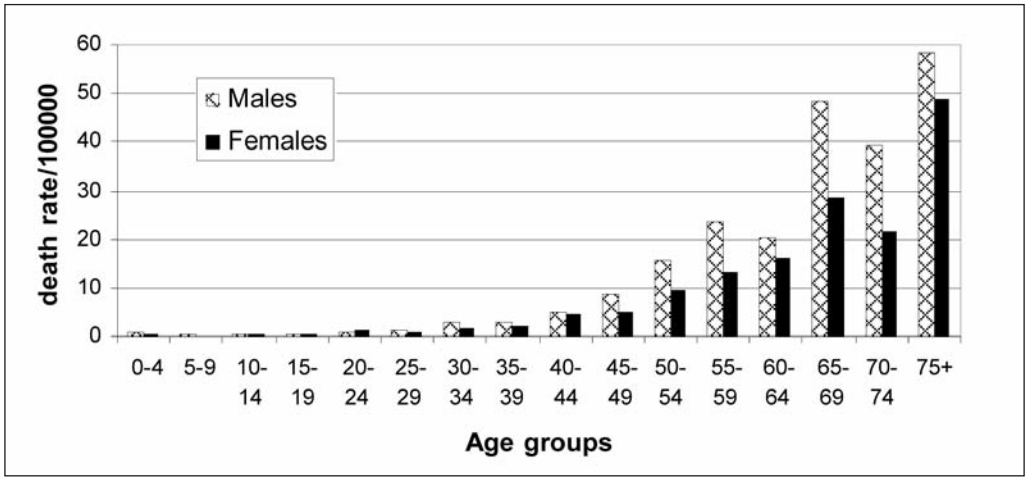

Fig. 5. Liver diseases specific death rates for different age groups (per 100,000), Egypt 1986

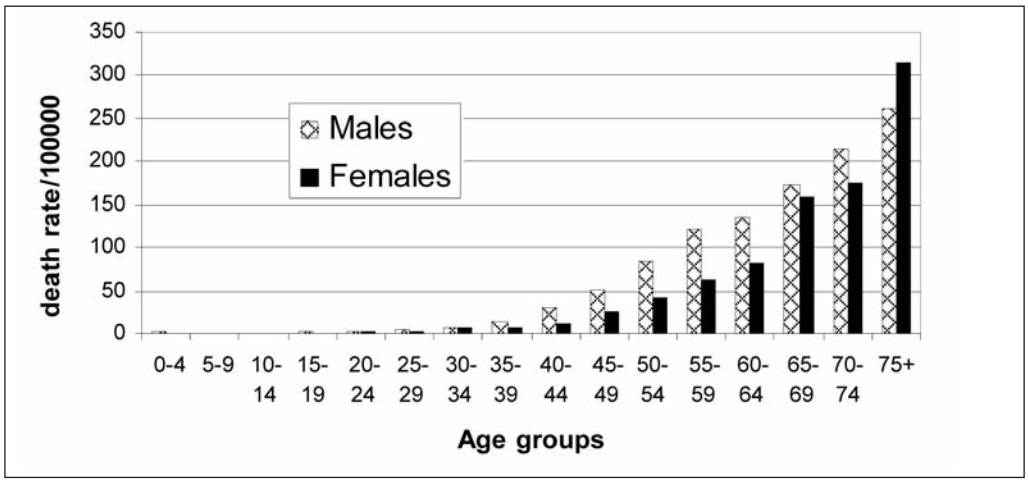

Fig. 6. Liver diseases specific death rates for different age groups (per 100,000), Egypt 1996

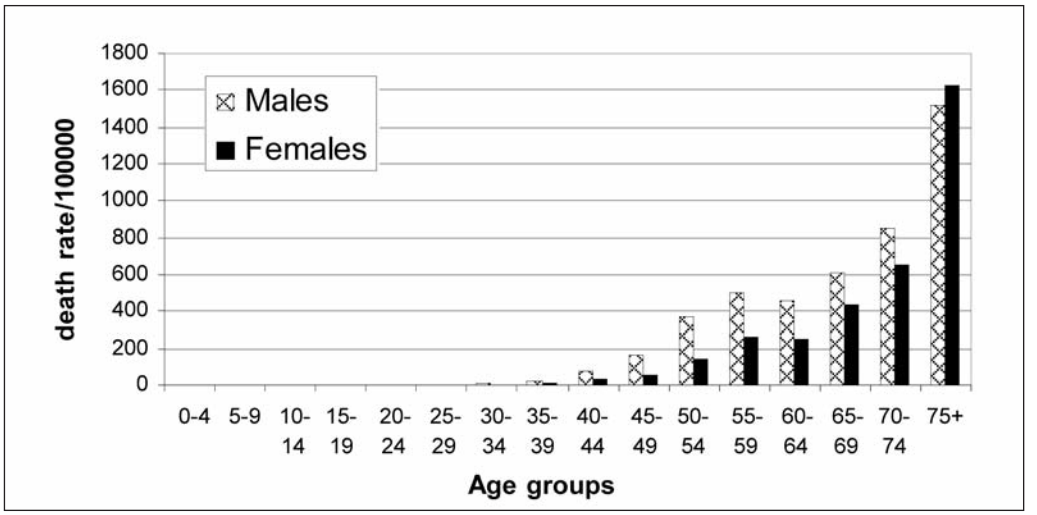

Fig. 7. Liver diseases specific death rates for different age groups (per 100,000), Egypt 2005

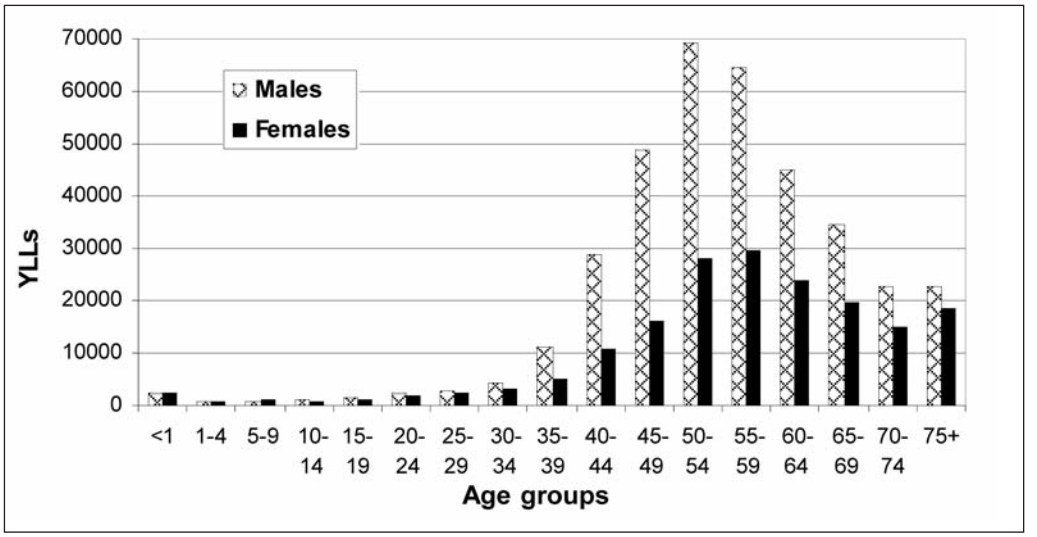

Fig. 8. Years of life lost due to liver diseases for different age groups, Egypt 2005

Discussion

Marked increase in mortality rates started from year 2000 may be due to increased number of deaths from liver cirrhosis and liver cancer due to accumulation of elder people who had old hepatitis infection. This indicates poor management and treatment of these diseases in Egypt. Also, better methods for diagnosis introduced may share in the spurious increase of the number of deaths due to liver diseases in this period.

It could be also explained by the change in coding system of deaths from ICD-9 to ICD-10 which started in Egypt in year 2000 with more details about the actual cause of death. Change of the source of the analyzed data from CAPMAS (1986-1999) to NICHP of MHOP (2000-2005) mortality records may also explain the upward trend of mortality rates related to liver diseases from year 2000 up to year 2005.

Although bilharziasis is considered as one of the most endemic liver diseases in Egypt for a long time, deaths due to bilharziasis couldn't be identified. This is explained by the fact that bilhariziasis is a very common disease especially in rural Egypt, in case of death due to bilharziasis, the cause of death is not registered as bilharizsiasis in death records, but instead the complications of bilharziasis like bleeding oseophagial varices, liver cell failure, liver cell carcinoma or bladder cancer are recorded in death certificates as the cause of death. So mortality rates due to bilharziasis alone couldn't be measured and included in this study.

Males showed higher mortality from liver diseases than females all over the period 1986-2005. This

Table 1 - Years of life lost due to liver diseases for different age groups, Egypt 2005

	Hepatitis B		Hepatitis C		Liver cancer		Liver cirrhosis	
Age group	Males	Females	Males	Females	Males	Females	Males	Females
<1	440	692	0	0	0	0	2041	1462
1-4	316	192	39	0	0	0	592	538
5-9	69	69	0	0	113	147	733	805
10-14	136	71	0	0	182	151	750	532
15-19	168	234	33	0	328	183	1007	906
20-24	387	398	32	0	548	214	1321	1427
25-29	253	191	31.62	0	527	310	1898	1816
30-34	332	482	60	30	769	587	3104	1930
35-39	1276	701	311	112	1368	1107	8187	3200
40-44	2999	879	286	51	3926	1954	21621	7966
45-49	5090	1480	564	211	7064	3044	35967	11331
50-54	7126	2922	585	317	10361	4297	51205	20549
55-59	6562	2976	742	280	9650	4166	47608	22375
60-64	4533	2377	354	258	7084	2760	32853	18590
65-69	3270	1881	310	155	5905	2473	24943	15079
70-74	2012	1553	188	145	3428	1856	17101	11589
75+	2288	1863	141	51	2828	1579	17379	14988

means that the exposure to different liver diseases is more common among males than females (as bilharziasis, viral hepatitis and subsequent liver cirrhosis and liver cancer). This may be due to increased high risk behaviors among males (as drug abuse and contaminated sharps like shaving blades).

Similar results were noticed in England, where mortality rates for chronic liver diseases more than doubled during the period 1979-2005 and this increase in mortality rates may be due to increased amount of alcohol-induced liver diseases in England (9).

However, in the United States, mortality due to chronic liver diseases during the period 1979-1989 declined by about 22% for both sexes (10), and during the period 1990-1994 declined by about 5% (12.1 to 11.6/100,000). But during the period 1995-1998, the rates remained unchanged and were higher among males (47.6/100,000) than females (32.2/100,000) (11).

In the current study, it is obvious that old age groups showed higher mortality from liver diseases than young ones and the highest rate was at age group above 75 years in both males and females.

This age distribution of liver diseases mortality could be attributed to the fact that mortality in general is higher among old age groups than young ones and not specific only for liver diseases. Also, deaths due to hepatitis B and C occur as a result of its long term complications which usually occur at old ages.

It is obvious that liver cirrhosis had the highest mortality rates among liver diseases in Egypt during this period, followed by liver cancer, hepatitis B infection and then hepatitis C infection.

This could be attributed to the fact that liver cirrhosis is the most common complication following hepatitis B and C infection in Egypt.

Similar results between the periods 1987-1991 and 1997-2001, were observed in Scotland where cirrhosis mortality in men more than doubled (104% increase) and in England and Wales rose by over two-thirds (69%). Mortality in women increased by almost half (46% in Scotland and 44% in England and Wales) (12). This increase in rates of mortality due to liver cirrhosis in these countries was mainly due to increase in alcohol consumption and the subsequent alcoholic cirrhosis.

In contrast to these results, liver cirrhosis mortality in United States declined during the period 1970-2002 by 46.1 percent (from 17.8 to 9.6/100,000) and the mortality was higher in males than females during this period (13). This decrease in liver cirrhosis mortality rates in United States may

be due to decrease in its risk factors, improvement in the diagnostic tools and treatment options of viral infections than in developing countries and successful management of cirrhotic patients.

As regards mortality due to hepatitis C virus infection, there were no deaths recorded due to hepatitis C infection during the period 1986-1999 and also in year 2003. This does not mean that there were no deaths due to hepatitis C during this period but this may be due to the fact that death due to hepatitis C infection occurs mainly as a result of its long term complications (as liver cirrhosis and liver cancer) and rapid fulminant liver failure associated with HCV infection is a rare event (14). Also the absence of deaths due to hepatitis C in year 2003 may be due to false data entry in death records in this year.

From year 2000 up to year 2005 (except for year 2003), the age adjusted death rates of hepatitis C gradually increased. The start of recording hepatitis C as a cause of death from year 2000 may be due to more awareness about hepatitis C virus and its complications. This increase in hepatitis B and C mortality from year 2000 up to year 2005 may be due to increased exposure to the virus (as transfusion of infected blood, contaminated sharps, drug abuse using contaminated syringe, perinatal transmission, tattooing, hospitalization, surgical operations, suturing for cut wounds and dental care). Also, past history of injection treatment for schistosomiasis as a potential risk factor for previous episode of hepatitis C infection (15).

The compulsory vaccination for hepatitis B was introduced in Egypt in 1996 (16). The age of vaccinated cohorts now is about 13 years, so the impact of these efforts on YLL and mortality rates related to hepatitis B will be delayed for more years till the vaccinated children become adults.

In Canada, hepatitis B mortality rates increased from 0.03/100,000 in 1979 to 0.26/100,000 in 1997 and from 0.12/100,000 to 0.41/100,000 for hepatitis C. Males also show higher mortality than females (17). These rates are to some extent similar to the rates in Egypt during this period 1979-1997 which indicates that the exposure rate to the virus was nearly the same in both countries. During this period no deaths due to hepatitis C in Egypt were recorded, this may be explained by the little awareness and lack of diagnostic tools during this period.

In the United States, hepatitis C virus mortality rates increased 220% from 1993 to 1998 (0.57 to 1.67/100,000) (11), and during the period 1995-2004 increased from 1.09/100,000 to 2.44/100,000 (18).

Although hepatitis C is more prevalent in Egypt than in the United States, the mortality rates are higher in the United States. This is mainly due to improved awareness of and testing for HCV infection among chronic liver disease patients in the United States (11), and due to under estimation of hepatitis C mortality in Egypt as mentioned before.

As regard mortality due to liver cancer in Egypt, the age adjusted death rates for liver cancer nearly doubled from year 1986 up to year 2005. This may be attributed to increased risk factors for liver cancer in Egypt which include several biological (e.g. hepatitis B and C virus infection) and environmental factors (e.g. aflatoxin). Other factors such as cigarette smoking, occupational exposure to chemicals such as pesticides, and endemic infections in the community, such as schistosomiasis, may have additional roles in the etiology or progression of the disease (19). This increase in liver cancer death rates may be also due to false recording of deaths with secondaries in liver as deaths due to liver cancer. On the other hand, these increased rates of hepatocellular carcinoma may be related to better detection due to technologically improved diagnostic testing, such an effect would be expected to plateau at some point in the future (20).

A study carried out in Egypt in year 2004 by NICHP found that, during the period 1987-2000 liver cancer death rates showed a significant increase from 2.4/100,000 up to 5.2/100,000 and the rates were higher in males than in females (8). These findings are consistent with the findings of the current study.

Similar results were recorded in the United States, Maryland (21) and in Australia (22) where mortality from hepatocellular carcinoma increased, and also it was higher in males than in females.

While simple death rates represent an important measure of public health, deaths at younger ages may be considered of greater public health concern than deaths at older ages. YLL, the mortality component of the DALY, give greater value to deaths of young people, thus emphasizing the concept of premature death (23). For this reason

YLL was used in the current study as a sensitive measure of the magnitude and importance of liver diseases in Egypt.

The number of YLL due to liver diseases was fluctuating during the period 1986-1999 except for year 1995 where the number of YLL markedly increased. But from year 2000 up to year 2005, it markedly increased to reach 543,867 in year 2005. The number of YLL was higher in males than in females all over the period 1986-2005. YLL are gradually increasing with the peak at age group 50-54 in males and 55-59 in females then gradually declined; the pattern was the same in males and females.

This upward trend of the number of YLL due to liver diseases reflects the increasing magnitude of liver diseases in Egypt over this period and indicates priorities for interventions against life threatening liver diseases through the implementation of universal precautions and safe injection practices, screening of blood donation and blood products and modification of certain personal risky behaviors. Also, prevention and intervention approaches directed to risk factors of liver cancer as viral hepatitis infection and prevention of exposure to aflatoxins may be achieved either at community (via good agriculture practices) or individual levels (treatment or dietary interventions) (19).

References

1. Workshop on Evidence for Health Policy. Burden of diseases, cost effectiveness, health system performance, and poverty and health. Module 1, Workshop 2, Burden of disease Unit 2001, Harvard Center for Population and Development Studies, World Health Organization, Geneva.
2. Mohamed MK. Epidemiology of HCV in Egypt. The Afro-Arab Liver Journal 2004; 3 (2): 41-52.
3. Ministry of Health and Population (MOHP). National guide lines for communicable diseases surveillance. Ministry of Health and Population, Preventive Sector, Epidemiology and Surveillance Unit. Cairo 2001.
4. Darwish MA, Faris R, Clemens ID, *et al.* High seroprevalence of hepatitis A,B,C and E viruses in residents in Egyptian village in the Nile Delta: a pilot study. Am J Trop Med Hyg 1996; 54: 554-8.
5. Burban SD, Mohamed MK, Larouze B, *et al*. Expected increase in hepatitis C-related mortality in Egypt due to pre-2000 infections. J Hepatol 2006; 44: 455-61.
6. El-Zayadi A, Abaza H, Shawky S, *et al*. Prevalence and epidemiological features of hepatocellular carcinoma in Egypt - a single center experience. Hepatol Res 2001; 19: 170-9.
7. Chen CJ, Yu MW, Liaw YF. Epidemiological characteristics and risk factors of hepatocellular carcinoma. J Gastroenterol Hepatol 1997; 12 (9-10): S294-S308.
8. The Burden of Disease and Injury in Egypt. National Information Center for Health and Population (NICHP), MOHP, Cairo, August, 2004.
9. Thomson SJ, Westlake S, Rahman TM, *et al.* Chronic liver disease an increasing problem: a study of hospital admission and mortality rates in England, 1979-2005, with particular reference to alcoholic liver disease. Alcohol Alchol 2008; 43 (4): 416-22.
10. Hurwitz ES, Holman RC, Strine TW, *et al*. Chronic liver disease mortality in the United States, 1979 through 1989. Am J Pub Health 1995; 85: 1256-60.
11. Vong S, Bell BP. Chronic liver disease mortality in the United States, 1990-1998. Hepatology 2004, 39 (2): 476-83.
12. Leon D, McCambridge J. Liver cirrhosis mortality rates in Britain from 1950 to 2002: an analysis of routine data. Lancet 2006; 367 (9504): 52-6.
13. National Institute on Alcohol Abuse and Alcoholism. Liver cirrhosis mortality in the United States 1970-2002. US Department of Health and Human Services, Public Health Service, National Institute of Health, National Institute on Alcohol Abuse and Alcoholism, August 2005.
14. Houghton M. Hepatitis C viruses. In: Fields BN, Knipe DM, Howley PM, eds. Fields Virology, 3rd ed. Philadelphia, Lippincott – Raven, 1996, 1035-58.
15. Mohamed MK, Massoud AA, Aoun S, *et al.* "Seminar" Hepatitis C in Egypt: Epidemiological profile and magnitude of the problem. Luxor, Egypt 1999.
16. Kane M. Global Program on Immunization, World Health Organization, 1999.
17. Pohani G, Zou S, Tepper M. Trends of hepatitis B and hepatitis C mortality in Canada, 1979-1997. Can J Public Health 2001; 92 (4): 250-4.
18. Wise M, Bialek S, Finelli L, *et al.* Changing trends in hepatitis C-related mortality in the United States, 1995-2004. Hepatology 2008; 47 (4): 1128-35.
19. Anwar WA, Khaled HM, Amra HA, *et al.* Changing pattern of hepatocellular carcinoma (HCC) and its risk factors in Egypt: possibilities of prevention. Mutat Res 2008; 659 (1-2): 176-84.
20. Hashem B, El-Serag HB, Jessica A, *et al.* The continuing increase in the incidence of hepatocellular carcinoma in the United States: an Update. Ann Int Med 2003; 139 (10): 817-23.
21. Nair S, Shiv Kumar K, Thuluvath PJ. Mortality from hepatocellular carcinoma and biliary cancers: changing

epidemiological trends. Am J Gastroenterol 2002; 97 (1): 167-71.
22. Law MG, Roberts SK, Dore GJ, *et al.* Primary hepatocellular carcinoma in Australia, 1978-1997: increasing incidence and mortality. Med J Aust 2000; 173 (8): 403-5.
23. Murray CJ, Lopez AD. A comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020 (The Global Burden of Disease and Injury). Harvard School of Public Health, 1996.

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Acute hyperleukocytosis: a medical emergency in pediatric oncology

Iperleucocitosi: un'emergenza medica in oncologia pediatrica

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Summary

Hyperleukocytosis is a leukemia-related condition characterized by an extremely high white blood cell count. Symptomatic leukostasis represents a medical emergency which needs to be promptly recognized and adequately managed in order to prevent early mortality and severe morbidity. Any organ may be affected, but intracranial hemorrhage and respiratory failure are the most frequent life-threatening complications of leukostasis. Since there are not clear guidelines on hyperleukocytosis treatment, this condition remains a challenge for physicians. Our review focuses on clinical manifestations of hyperleukocytosis and possible therapeutic approaches in order to provide a practical guide for early diagnosis and proper management of hyperleukocitic leukemia. Eur. J. Oncol., 16 (4), 221-231, 2011

Key words: **hyperleukocytosis, leukostasis, leukemia, children**

Riassunto

L'iperleucocitosi è una possibile complicanza della leucemia e può rappresentare la sintomatologia d'esordio. La leucostasi, quadro clinico derivante dalla iperleucocitosi, è da considerarsi un'emergenza medica e necessita, pertanto, di un precoce riconoscimento e di un adeguato e pronto intervento. Ogni organo o apparato può essere colpito ma, più frequentemente, le manifestazioni cliniche sono a carico dell'apparato respiratorio e del sistema nervoso centrale. Nonostante la gravità clinica di questa condizione, non sono, ad oggi, disponibili linee guida condivise riguardo la gestione di questi pazienti. La nostra review analizza gli strumenti essenziali per un pronto riconoscimento e le possibilità terapeutiche per un adeguato trattamento della sindrome da leucostasi così da poter prevenire sia la mortalità che la severa morbidità imputabili a questa condizione. Eur. J. Oncol., 16 (4), 221-231, 2011

Parole chiave: **iperleucocitosi, leucostasi, leucemia, bambini**

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Introduction

Hyperleukocytosis is a leukemia related condition defined as circulating blast count >100,000 blasts/mm^3. It is a negative prognostic indicator in children with acute leukemia. Several authors have found a significant association between hyperleukocytosis and age <1 year, T cell immunophenotype, cell ploidy ≤50 chromosomes, central nervous system (CNS) leukemia at diagnosis and some clinical features like hepatosplenomegaly, high serum levels of lactic dehydrogenase and presence of a mediastinal mass (1, 2). Moreover, some cytogenetic abnormalities are often found in patients with hyperleukocytic acute lymphoblastic leukemia (ALL), like 11q23 rearrangements and t(9;22, Philadelphia chromosome) (1, 3).

Even if a significant relationship between white blood cell (WBC) count and clinical manifestations is recognised, the number of blasts is neither the only, nor the most important element that enter in the determination of leukostasis risk. Clinical manifestations of acute hyperleukocitic leukemia depend, first of all, on the cell lineage of the leukemic blasts. Acute myeloid leukemia (AML) has a higher incidence of symptomatic hyperleukocitosis if compared to ALL, despite a higher incidence and degree of hyperleukocytosis in ALL *vs* AML (from 10% to 30% and from 5% to 13%, respectively).

Inaba *et al.* described how more than 50% of patients with AML and hyperleukocytosis were FAB M4 and M5 where these two FAB subtypes proved to be at higher risk of early complications, such as early death, respiratory, renal or bleeding events and coagulopathy (4).

Acute hyperleukocytosis and leukostasis should be considered a medical emergency and their clinical implications are extremely relevant for patient's prognosis, both in terms of morbidity and mortality. Leukostasis – which is not only caused by hyperleukocitosis but also by adhesive interactions between leukemic blasts and endothelial cells, as well as by the expression of adhesion molecules on lymphoblast cell surface – may lead to vascular occlusion, perivascular leukemic cell infiltration, vascular damage and reduction of blood flow which, in turn, can result in multi-organ failure (pulmonary failure, intra-cranial bleeding, renal failure), severe coagulopathy, metabolic abnormalities and tumor lysis syndrome. All these conditions often occur in hyperleukocitic leukemia determining the high early mortality and morbidity rate (20-40%) observed in these patients (1, 5-8). However, the best management of patients with acute leukemia and hyperleukocytosis is still unclear and, for this reason, represents a challenge for physicians.

Pathogenetic mechanism of leukostasis

Litchmann *et al.* studied leukostasis mechanism in depth with a view to explaining this difference between AML and ALL (9). They suggest that – in addition to cell number – the mean corpuscular volume (MCV) is crucial for determining a critical "leukocrit" (fractional leukocytes volume) that would result in an increased blood viscosity. Myeloblasts have larger volume than lymphoblasts and this leads to a different incidence of leukostasis in AML and ALL for the same degree of leucocytosis.

Blood viscosity is not the only element determining leukostasis. As described by Steinberg *et al.* (10) in hyperleukocitic leukemia WBC number usually does not reach such high levels to determine a sensible increase in the whole blood viscosity and lead to leukostasis. Moreover, in leukemias, as leukocytes rise, erythrocytes usually decrease and protect from hyperviscosity. In any case, leukocytes are less deformable than red blood cells (RBC), thus leukocrit has a higher impact on blood viscosity than haematocrit.

It is important to point out that in these patients RBC transfusion should not be performed with haemoglobin values higher than 7-8 g/dl, if not symptomatic. A transfusion, in fact, may increase the risk of leukostasis.

What is interesting to notice is that blast aggregates and blast thrombi are not equally distributed throughout the circulation. They are more frequent in brain, lungs and heart, which suggests some organ specificity. It is not yet completely clear what is the mechanism regulating blasts adhesion to microvascular endothelium and the formation of microthrombi but, what is known, is that several elements cooperate together. Local released chemoattractant

factors seem to play a role in determining distribution and severity of the damage (11-12). On the other hand, several adhesion molecules on leukemic cells, like ICAM-1 and selectins, have been investigated and seem to be part of the mechanism leading to leukostasis. In a recent study Stucki *et al.* (13) show how myeloblasts, through the secretion of tumor necrosis factor-α and interleukin-1β, are able to induce the expression of several adhesion molecules, such as intracellular adhesion molecule-1, vascular cell adhesion molecule-1, CD54, CD62E, CD62P, CD106, E-selectin, in endothelial cells and that this up-regulation is associated with an increased number of blasts attached to the endothelium. According to this theory, in a short time, a self-perpetuating loop among cells could be activated and it is clear why a prompt and rapid cytoreduction should be performed to reduce the risk of early morbidity and mortality.

Even if the molecular mechanism that regulate cell adhesion and leukostasis is not completely understood, it seems clear that there are some differences among different subsets of leukemia. A correlation between CD56/NCAM expression and development of leukostasis has been described in myelomonocitic subtypes of AML. Novotny *et al.* suggest that detection of CD56 at baseline may help to identify patients at higher risk of developing fatal leukostasis in AML (FAB M4 and M5) (14). Not only CD56 expression seems to be a good predictor of leukostasis but also a predictor of bad response to leukoapheresis. All these molecular data could have important clinical and therapeutic implications: the management of blocking antibodies against TNF-α, anti-IL-1β, and against CD54, CD106, CD62E may lead to a block of this time-dependent accumulation of myeloblasts on the endothelium and could be a valid weapon against leukostasis.

Clinical manifestations of hyperleukocytosis

Leukostasis is the pathological mechanism underlying the development of clinical manifestations in hyperleukocytic leukemia. However, there are not clinical criteria to define this syndrome, thus it is often empirically diagnosed when patients present with acute leukemia, hyperleukocytosis, and respiratory or neurological symptoms (1,7). Acute hyperleukocytosis and leukostasis should be considered as a medical emergency since mortality rate may be up to 40% if it is not recognized and quickly treated (15).

Despite a higher incidence and degree of hyperleukocytosis in ALL *vs* AML (from 10% to 30% and from 5% to 13%, respectively), symptomatic hyperleukocytosis is not commonly observed in ALL. The clinical picture at presentation includes metabolic abnormalities, coagulopathy and multiple organ failure, and it often mimics infectious and hemorrhagic complications of acute leukemia (16). Although leukostasis may affect any organ, the most dramatic and frequent symptoms arise from the involvement of respiratory and central nervous systems, and the earliest deaths are due to respiratory failure and intra-cranial hemorrhage (17) (Table 1).

Respiratory complications are largely described in clinical studies (18, 19), and the risk of pulmonary involvement increases with leukocyte count, thus suggesting that together with infection, edema and hemorrhage, leukostasis is also responsible for the respiratory symptoms experienced by patients.

The sludging of leukemic blasts into the pulmonary microcirculation determines pulmonary leukostasis syndrome, which may include respiratory distress, hypoxemia, as well as diffuse interstitial or alveolar infiltrates on chest radiographs (20). However, due to a very rapid leukocytic oxygen consumption, extreme leukocytosis might be associated with fictitious hypoxemia. Therefore, refrigeration on ice of blood samples should be promptly performed to reduce the risk of pseudohypoxemia (21, 22). Diagnosis of hypoxemia in patients without obvious respiratory symptoms and pulmonary pathology needs caution and careful evaluation, with pulse oximetry able to asses oxygenation status more accurately in this setting (23). Tachypnea and exertional dyspnea are not specific symptoms of pulmonary leukostasis and clinical presentation can range from mild to severe respiratory impairment which require, in extreme cases, mechanical ventilation.

Cerebral microvasculature is also prone to leukostasis, and hemorrhage represents one of the most important causes of early deaths in acute leukemia. Since it was first described more than 40 years ago, CNS hemorrhage is the most threatening

Table 1 - Clinical manifestations of leukostasis

Site	Signs and symptoms
Respiratory system	Tachypnea Exertional dyspnea Respiratory distress Hypoxemia Diffuse interstitial or alveolar infiltrates on chest X rays
Central nervous system	CNS hemorrhage Confusion, somnolence, stupor, delirium and coma Headache, dizziness, tinnitus, gait instability Blurred vision and visual field defects Cranial nerve defects and neck stiffness
Cardiovascular system	ECG signs of right ventricular overload Neck vein distention Gallop rhythm Myocardial ischemia Priapism Acute limb ischemia Bowel infarctions Renal vein thrombosis
Eyes	Papilledema Retinal vein distention and retinal hemorrhages
Blood	Coagulopathy Thrombocytopenia CID
Systemic and metabolic alterations	Hyperkalemia Hyperphosphatemia Hypocalcemia High fever (>39°C)

and feared complication in the management of children with hyperleukocytosis (24, 25). Even though intra-cerebral hemorrhage may occur in either ALL or AML, the incidence of this condition is much higher in patients with AML, especially with leukocyte count ≥300,000/mm^3, and its frequency is estimated to be 5-33% in AML with hyperleukocytosis (8). Extremely high white blood counts, increased blood viscosity and decreased blast deformation lead to leukocyte thrombosis followed by hemorrhage. Postmortem examinations in patients with hyperleukocytosis who died in the acute phase of their diseases showed hemorrhagic lesions limited to the white matter of the brain, centered at intravascular microscopic leukemic nodules. Studies demonstrated that leukemic cells accumulate in the capillaries of the white matter leading to leukostasis, leukemic nodule formation, capillary wall disruption, and ultimately petechial hemorrhage. Coalescence of such microhemorrhages results in the larger hemorrhages observed and in the massive and refractory cerebral swelling. Brain magnetic resonance imaging (MRI) may be useful to identify progressive white matter pathology. Serial imaging demonstrates features of hemorrhagic lesions and edema followed by rapidly progressive cerebral atrophy and ventricular enlargement. Differential diagnoses include cytotoxic edema secondary to

ischemia, vasogenic edema secondary to leukemic infiltration, leukoencephalopathy secondary to chemotherapy, or diffuse effects of central nervous system infections (26).

Clinicians should know that higher blood viscosity is directly related to an increased risk of adverse events, such as CNS hemorrhage (27). This consideration presents important clinical implications because red cell transfusions and high hemoglobin concentrations have been associated with increased morbidity and mortality, which should be avoided (28).

Confusion, somnolence, stupor, delirium and coma are all conditions related to important CNS involvement. Nevertheless, patients may present with more subtle neurological symptoms, such as headache, dizziness, tinnitus, gait instability, blurred vision or visual field defects. Cranial nerve defects and neck stiffness may also be found at the physical examination. Continuous monitoring of neurological status of the patient is essential to recognize subtle and precocious signs of ongoing leukostasis and clinicians should always perform careful clinical history and physical examination (29). Neurological signs and symptoms of hyperleukocytosis in children are not largely described, and most of reports come from adult cases. However it has been demonstrated that incidence of CNS complications is 3.6% in children with initial WBC <400,000/mm^3 and 17.9% for children with WBC counts >400,000/mm^3 (19).

Vascular and rheological alterations due to hyperleukocytosis create a prothrombotic state which occasionally may lead to neck vein distention, gallop rhythm and ECG signs of right ventricular overload, myocardial ischemia, priapism, acute limb ischemia, bowel infarctions, and renal vein thrombosis (30-32). In addition, the correlation has been studied between hematological and ocular findings, such as papilledema, retinal vein distention and retinal hemorrhages, thus highlighting the importance of opthalmological evaluation of patients with acute leukemia (33).

Another common problem is coagulopathy induced by procoagulant substances in the cytoplasm of leukemic blast cells. This leads to thrombin activation and complicates thrombocytopenia with acute hemorrhage or thrombosis. Disseminated intravascular coagulation occurs in 30% to 40% of patients with AML and in 15% to 25% of patients with ALL. Though more common in promyelocytic leukemia, disseminated intravascular coagulation (DIC) may occur in all subtypes of acute leukemia. Careful assessment for thrombocytopenia and coagulopathy must be included in laboratory evaluation; platelets should be manually counted, since a spurious elevation of the automated platelet count may occur due to the presence of fragments of white and red blood cells, underestimating thrombocytopenia (34).

Other observed complications that may be related to hyperleukocytosis are metabolic abnormalities such as hyperkalemia, hyperphosphatemia and hypocalcemia with tetany (19).

High fever (>39°C) of not infectious origin is very common, showing how leukostasis syndrome represents a generalized systemic condition of great impact on the organism.

Patients with high WBC are also especially prone to tumor lysis syndrome (TLS) as chemotherapy is started. TLS is caused by an excessive release of purine metabolites with consequent hyperuricemia, hyperkalemia, hyperphosphatemia and uremia. This is life-threatening and may lead to renal failure in 10% to 15% of AML whereas in ALL only rarely.

Management of acute hyperleukocytosis

Correlation between the degree of leukocytosis and long-term survival is very strong in pediatric ALL. High WBC counts (>50,000/mm^3) are associated with poor prognosis, shorter duration of remission and overall survival (2, 35, 36). On the other hand, prognostic value of hyperleukocytosis in AML has not been entirely defined, even though studies report lower complete remission rate, and shorter disease-free and overall survival in patients with WBC higher than 100,000/mm^3 (37).

Prompt leukocitoreduction is mandatory to prevent leukostasis in patients with acute hyperleukocytosis and to treat patients who have already experienced symptoms of leukostasis, even if all the complications, including death, may occur despite significant reduction in leukemic blast cell count.

However, the optimal management of children with leukemia and hyperleukocytosis is not

completely clear. Though a prompt initiation of chemotherapy remains a mainstay of treatment of acute hyperleukocytic leukemia, several physicians manage urgent leukapheresis to induce a rapid cytoreduction while planning for the administration of chemotherapy.

Leukapheresis, consisting in the removal of circulating WBC with reinfusion of leukocytes-poor plasma, leads to a rapid blast reduction. Nevertheless, there are not evidence-based guidelines for its use and there is only a paucity of data demonstrating the real efficacy of a prompt cytoreduction in children with acute leukemia (1, 17, 19, 29). This lack of data on leukapheresis usefulness is probably due to the fact that it is usually started after clinical presentations of leukostasis and, for this reason, it does not prevent hyperleukocytosis complications. In addition, it is not clear if it may actually reverse the clinical consequences of leukostasis, once established, or if it may prevent early deaths. Eguiguren *et al.* (2) reported that the difference in the occurrence or severity of complications between patients treated with leukapheresis and those who did not receive it is not significant. On the other hand, Maurer et al. (17) observed a significant lower incidence of metabolic abnormalities in patients who were treated with leukapheresis.

Hematological disorder, such as coagulation abnormalities, are less frequent in patients who received leukapheresis because it also allows the infusion of blood products (eg platelets or coagulation factors) in order to correct these alterations. As Koenig *et al.* (26) reported, an early leukapheresis, in association with other interventions such as cranial radiotherapy, could be useful to prevent intracranial hemorrhage (ICH) in patients with already known signs of leukostasis. However, the debate on this point is still open: a retrospective study on 75 adult patients with hyperleucocytic AML treated with leukapheresis or prophylactic cranial irradiation did not show any benefit in reducing the incidence of ICH or early death (38). All the authors agree in suggesting a different management of patients with ALL or AML according to the fact that the incidence of leukostasis is higher in patients with myeloid leukemia. In AML leukapheresis is usually started with WBC count more than 100,000/mm^3 or in presence of symptoms of leukostasis irrespective of the blasts count (39, 40). In ALL leukapheresis should be, instead, reserved for symptomatic patients or for those with blast count exceeding 300,000/mm^3 (19, 29, 39, 41). A single session of this procedure may reduce the WBC count by 20-50%; however, by removing circulating blasts, it could recruit marginated blasts into the intravascular space, determining a sort of rebound effect (1). Therapeutic effects of leukapheresis are only temporary and a daily repetition of the procedure is often needed until manifestations of hyperviscosity resolve or the initial WBC count is substantially reduced (1, 42). Moreover, the placement and maintenance of a central venous catheter to perform the procedure may cause complications and sometimes is an insurmountable obstacle to the execution of leukapheresis. In patients with excessive weight loss (more than 10 Kg) or in patients with an inadequate venous access exchange transfusion could be preferred to leukapheresis (2) but, as reported by Eguiguren *et al.* (2), with less brilliant results (62% of WBC reduction with leukapheresis *vs* 52% with exchange transfusion). In any case, the administration of exchange transfusion has been recently reduced because of the risk of contamination or transfusion related complications for patients who receive several transfusion cycles (2, 43).

Cytoreduction through leukapheresis is a good option in case of hyperleukocitosis because it allows a prompt reduction of blasts count (20-50% of WBC count) and a correction of hematological disorder. However, it is an invasive procedure, time consuming, expansive, with considerable risks since it could cause a delay in chemotherapy initiation. For these reasons, leukapheresis should be reserved for patients with extremely high WBC count or for those with clinical manifestations of leukostasis at presentation (19).

Besides leukapheresis, cytoreduction may be achieved by induction chemotherapy and hydroxyurea.

Induction chemotherapy represents the most important step in the management of hyperleukocytosis and it should be started as soon as possible. Leukapheresis should never interfere with or delay the initiation of therapy, because while a few hours may not be clinically significant for some patients, they could be critical for others, especially for those

with a mediastinal mass. Reduction of the number of circulating leukemic blast cells must be accompanied by supportive measures in order to prevent tumor lysis syndrome and renal complications. Vigorous hydration with intravenous fluids must be included, though with a careful monitoring of the fluid balance, especially in patients with coexisting cardiopulmonary comorbidities. Hydration should also guarantee alkalinization of the urine through sodium bicarbonate. Allopurinol orally or intravenously administered, with a dosage of 10-20 mg/Kg body weight/day up to a maximum of 400 mg/day (44), should be given to prevent hyperuricemia. The alternative drug for patients who cannot tolerate allopurinol is recombinant urate oxidate (rasburicase), which converts uric acid to allantoin, which is 5 to 10 times more soluble than uric acid and therefore is rapidly excreted by the kidneys. The European Medicines Agency recommends that rasburicase be administered once a day in a 30-minute intravenous (IV) infusion at 0.2 mg/kg/day for both adults and children (44). Conservative management based on supportive measures has been suggested as an alternative to cytoreduction in patients with no very high WBC count (mostly >200,000/mm^3). However, the relatively small number of patients treated only with conservative approach does not allow any definitive conclusion.

Some authors proposed treatment with 24-hour continuous infusion of low-dose prednisone. This treatment is both a cytoreductive and an induction chemotherapy. It is cheap, simple and noninvasive and requires only infusion and close monitoring. Twenty four hours continuous infusion allows the body's metabolism to have sufficient time for the clearance of metabolic products and catabolism, thus avoiding risk of tumor lysis syndrome. Low-dose prednisone continuous infusion may be chosen instead of more invasive cytoreductive procedures in ALL patients presenting with WBC counts between 100,000 and 400,000/mm^3 (5).

Efficacy of hydroxyurea has also been described in the management of hyperleukocytosis. Hydroxyurea 50-100 mg/kg/day, orally given in 3-4 divided doses, reduces the WBC count by 50% to 80% without causing tumor lysis pneumopathy or worsening the DIC (45, 46). However, nowadays hydroxyurea is not routinely administered, mostly because leukapheresis is generally preferred for its presumed advantages.

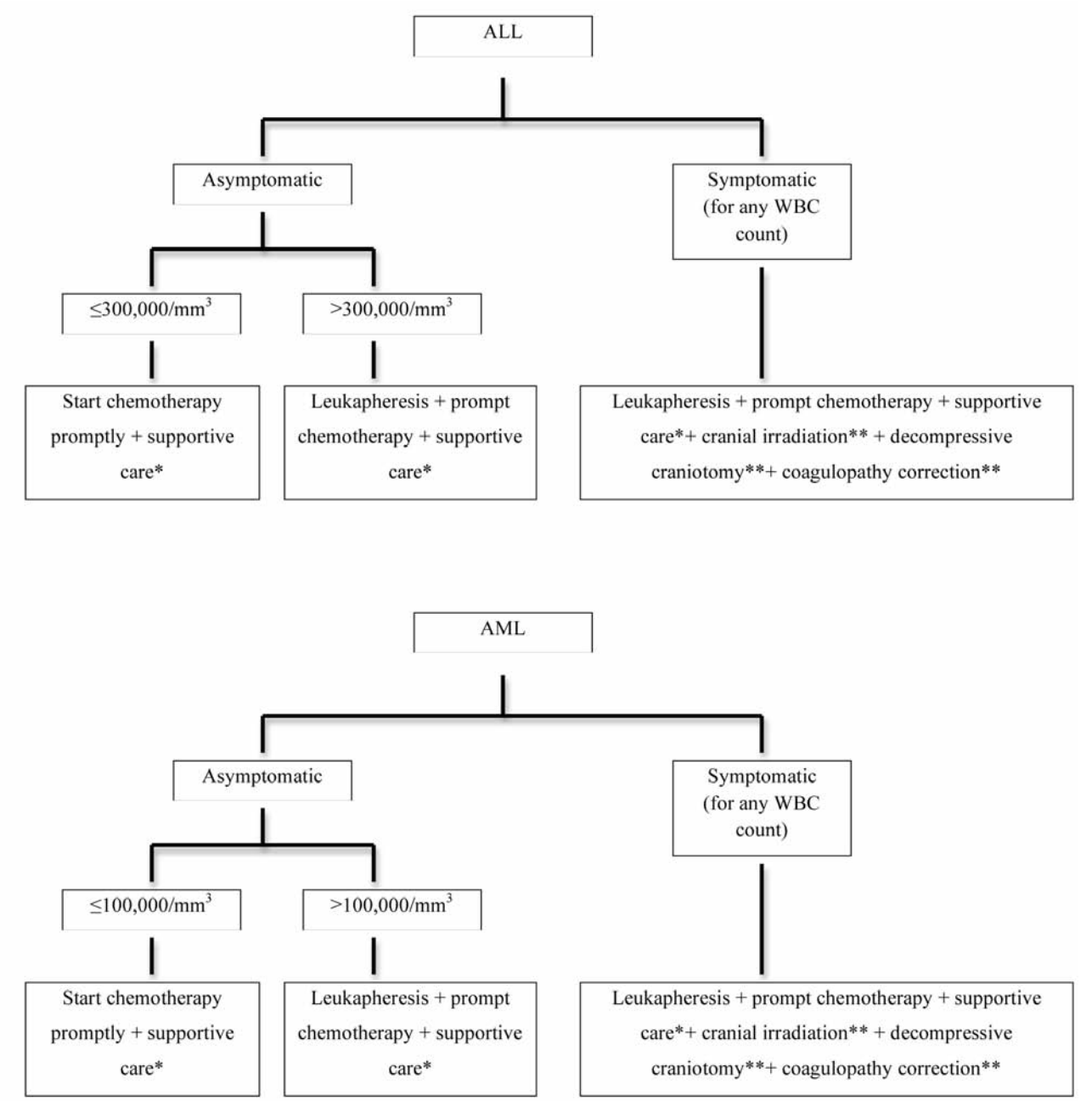
The association of blastic crisis, hyperleukocytosis and intra-cranial hemorrhage is a rare complication in children with acute leukemia, but due to its high probability of mortality it requires an adequate and sometimes aggressive clinical intervention. First of all, diagnosis of brain leukostasis should be promptly performed as soon as neurological involvement is suspected. Although brain MRI features of blastic hyperleukocytosis have not been described, retrospective studies have shown that sequential images correlate with the progression of blastic hyperleukocytosis. Initially imaging demonstrates multifocal, nonhemorrhagic areas of FLAIR T2 intensity without contrast enhancement, afterwards progression with new gaudolinium enhancement of these lesions and development of diffusion restriction in adjacent tissues and finally multiple hemorrhagic lesions and edema followed by progressive cerebral atrophy and ventricular enlargement. Because of the early abnormalities detectable with MRI, it is argued whether MRI of the brain should be part of the initial staging for ALL patients presenting with blastic hyperleukocytosis. Brain MRI defines focal pathology for prognostic and management decisions in blastic hyperleukocytosis. It allows clinicians to observe the presence of leukostasis prior to administration of chemotherapy as well as to early identify patients at risk of intracranial hemorrhage (26).

Cranial irradiation with 400-600 rads has been suggested on an emergency basis to control neurological complications due to leukostasis (47, 48). However, lack of controlled trials demonstrating the real benefit and the significant toxicity related to whole-brain irradiation precludes its routine use in children with hyperleukocytosis (49).

Moreover, it has been established that, since CNS complications arise from involvement of microvasculature without leukemic cells transgressing the blood-brain barrier, intrathecal chemotherapy is not necessary for the management of this condition, and that systemic chemotherapy appears to be the treatment of choice (26).

Aggressive measures, such as decompressive craniectomy or ventriculoperitoneal shunt placement, should be performed to control the massive

Table 2 - Therapeutic approaches suggested based on different hyperleukocytosis presentations



WBC: white blood cell; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia;
*Supportive care: hyperhydration, alkalination, allopurinol or rasburicase administration; **Only selected cases.

and refractory cerebral swelling due to multiple intraparenchymal hemorrhages, and may be life-saving, thus increasing the chances of cure and survival of these children (29).

DIC or thrombocytopenia, if present, should be corrected and platelet transfusions should be reserved when platelet count falls under $50x10^9$/l (27).

Transfusions should be avoided unless the patient presents symptoms of anemia and the hemoglobin is less than 7 to 8 g/dl. Increasing the erythrocyte (fractional erythrocyte volume) and, consequently, the

whole blood viscosity may lead to the development and worsening of leukostasis if the leukocrit is already high in patients with acute hyperleukocytosis.

Future therapies for leukostasis will specifically target cytokines and cell membrane adhesion molecules that mediate bast-blast and blast-endothelium interactions. It has been demonstrated how anti-TNF-α and anti-IL-1β antibodies can inhibit upregulation of several adhesion molecules (CD54, CD62E, CD62P, CD106) in endothelial cells. Furthermore, exposure to blocking antibodies to CD54, CD106, and CD62E may block time-dependent accumulation of myeloblasts on the endothelium. Therefore, future therapies may improve the outcome in patients with acute hyperleukocytosis. They will also allow experimental validation to the clinical principle that leukocytoreduction must be very rapidly obtained because the longer leukemic blasts interact with the endothelium, the stickier they become (1).

Conclusions

Hyperleukocytosis secondary to acute leukemia is a medical emergency that requires prompt clinical intervention. Although different therapeutic approaches are available, evidence-based guidelines for the management of hyperleukocytosis have not been defined. Moreover, there has been very little improvement in the treatment of this syndrome in the last 20-30 years, both in terms of morbidity and mortality. The involvement of respiratory and CNS is responsible for the majority of clinical manifestations of leukostasis and early deaths are mainly due to intra-cranial hemorrhages. Standard care for acute hyperleukocytosis should aim to cytoreduction and prevention of tumor lysis syndrome. In most ALL patients cytoreduction can be achieved through quick initiation of induction chemotherapy and supportive care as intravenous fluids, urine alkalinisation and allopurinol or rasburicase. If patients are symptomatic or when WBC >300,000/mm^3 in ALL and >100,000/mm^3 in AML, leukapheresis, if available, should be performed, but without delaying chemotherapy and the other treatment measures (Table 2). Early diagnosis of brain hemorrhage and edema through serial imaging is important to enable a prompt intervention. The management of cranial irradiation or of aggressive measures, such as decompressive craniectomy or ventriculoperitoneal shunt placement, could be necessary in case of severe and refractory cerebral swelling due to multiple intraparenchymal hemorrhages. Haematological abnormalities, if present, should be promptly corrected, too.

Clinicians should be aware of the fact that adequate management and, when needed, aggressive therapies are essential in the cure of these critically ill patients in order to prevent late-morbidity and early death as a result of acute hyperleukocytosis.

References

1. Porcu P, Farag S, Marcucci G, *et al*. Leukocytoreduction for acute leukemia. Ther Apher 2002; 6: 15-23.
2. Eguiguren JM, Schell MJ, Crist WM, *et al.* Complications and outcome in childhood acute lymphoblastic leukemia with hyperleukocytosis. Blood 1992; 79: 871-5.
3. Pui CH, Frankel LS, Carroll AJ, *et al.* Clinical characteristics and treatment outcome of childhood acute lymphoblastic leukemia with the t(4;11)(q21;q23): a collaborative study of 40 cases. Blood 1991; 77: 440-7.
4. Inaba H, Fan Y, Pounds S, *et al.* Clinical and biologic features and treatment outcome of children with newly diagnosed acute myeloid leukemia and hyperleukocytosis. Cancer 2008; 113 (3): 522-9.
5. Ozdemir MA, Karakukcu M, Patiroglu T, *et al.* Management of hyperleukocytosis and prevention of tumor lysis syndrome with low-dose prednisone continuous infusion in children with acute lymphoblastic leukemia. Acta Haematol 2009; 121: 56-62.
6. Haase R, Merkel N, Diwan O, *et al.* Leukapheresis and exchange transfusion in children with acute leukemia and hyperleukocytosis. A single center experience. Klin Padiatr 2009; 221: 374-8.
7. Porcu P, Cripe LD, Ng EW, *et al.* Hyperleukocytic leukemias and leukostasis: a review of pathophysiology, clinical presentation and management. Leuk Lymphoma 2000; 39: 1-18.
8. Bunin NJ, Pui CH. Differing complications of hyperleukocytosis in children with acute lymphoblastic or acute nonlymphoblastic leukemia. J Clin Oncol 1985; 3: 1590-5.
9. Litchmann MA. Rheology of leukocytes, leukocytes suspensions and blood in leukemia. J Clin Invest 1973; 52: 350-8.

10. Steinberg MH, Charm SE. Effect of high concentrations of leukocytes on whole blood viscosity. Blood 1971; 38: 299-301.
11. Van Buchem MA, Hogendoorn PC, Levelt CN, *et al.* Development of pulmonary leukostasis in experimental myelocytic leukemia in the Brown-Norway rat. Leukemia 1992; 6: 142-9.
12. Van Buchem MA, Hogendoorn PC, Bruijn JA, *et al.* Endothelial activation antigens in pulmonary leukostasis in leukemia. Acta Haematol 1993; 90: 29-33.
13. Stucki A, Rivier AS, Gikic M, *et al.* Endothelial cell activation by myeloblasts: molecular mechanisms of leukostasis and leukemic cell dissemination. Blood 2001; 97: 2121-9.
14. Novotny JR, Nückel H, Dührsen U. Correlation between expression of CD56/NCAM and severe leukostasis in hyperleukocytic acute myelomonocytic leukaemia. Eur J Haematol 2006; 76: 299-308.
15. Van Buchem MA, te Vlde J, Willemze R, *et al.* Leucostasis, an underestimated cause of death in leukemia. BLUT 1988; 56: 39-44.
16. Majhail NS, Lichtin AE. Acute leukemia with a very high leukocyte count: confronting a medical emergency. Cleve Clin J Med 2004; 71: 633-7.
17. Maurer HS, Steinherz PG, Gaynon PS, *et al.* The effect of initial management of hyperleukocytosis on early complications and outcome of children with acute lymphoblastic leukemia. J Clin Oncol 1988; 6: 1425-32.
18. Inaba H, Fan Y, *et al.* Clinical and biologic features and treatment of outcome of children with newly diagnosed acute myeloid leukemia and hyperleukocytosis. Cancer 2008; 113: 522-9.
19. Lowe EJ, Pui CH, Hancock ML, *et al.* Early complications in children with acute lymphoblastic leukemia presenting with hyperleukocytosis. Pediatr Blood Cancer 2005; 44: 1-6.
20. Van Buchem MA, Wonderdergem JA, Kool LJ, *et al.* Pulmonary leukostasis: radiologic pathologic study. Radiology 1987; 165: 739-41.
21. Chillar RK, Belman MJ, Farbstein M. Explanation for the apparent hypoxemia associated with extreme leukocytosis: leukocytic oxygen consumption. Blood 1980; 55: 922-4.
22. Gartrell K, Rosenstrauch W. Hypoxemia in patients with hyperleukocytosis: true, spurious, and clinical implications. Leukemia Res 1993; 17: 915-6.
23. Loke J, Duffy TP. Normal arterial oxygen saturation with the ear oximeter in patients with leukemia and leukocytosis. Cancer 1984; 53: 1767-9.
24. Freireich EJ, Thomas LB, Frei E III, *et al.* A distinctive type of intracerebral hemorrhage associated with ‘‘blastic crisis’’ in patients with leukemia. Cancer 1960; 13: 146-54.
25. Fritz RD, Forkner CE, Jr., Freireich EJ, *et al.* The association of fatal intracranial hemorrhage and blastic crisis in patients with acute leukemia. N Engl J Med 1959; 261: 59-64.
26. Koenig M, Sitton C, Wang M, *et al.* Central nervous system complications of blastic hyperleukocytosis in childhood acute lymphoblastic leukemia: diagnostic and prognostic implications. J Child Neurol 2008; 23: 1347-52.
27. Lichtman MA, Rowe JM. Hyperleukocytic leukemias: rheological, clinical, and therapeutic considerations. Blood 1982; 60: 279-83.
28. Harris AL. Leukostasis associated with blood transfusion in acute myeloid leukaemia. Br Med J 1978; 1: 1169-71.
29. Ruggiero A, Attinà G, Piastra M, *et al.* Severe hyperleukocytosis and multifocal intracranial haemorrhage: not always a fatal outcome. Int J Hematol 2009; 90: 87-90.
30. Murray JC, Dorfman SR, Brandt ML, *et al.* Renal vein thrombosis complicating acute myeloid leukemia with hyperleukocytosis. J Pediatr Hematol Oncol 1989; 18: 327-30.
31. Mataix R, Gomez-Casares MT, Campo C, *et al.* Acute leg ischemia as a presentation of hyperleukocytosis syndrome in acute myeloid leukemia. Am J Hematol 1994; 46: 250-1.
32. Campbell J, Mitchell CA. Acute leg ischemia as a manifestation of the hyperleukocytosis syndrome in acute myeloid leukemia. Am J Hematol 1994; 46: 167.
33. Karesh JW, Goldman EJ, Reck K, *et al.* A prospective ophthalmologic evaluation of patients with acute myeloid leukemia: correlation of ocular and hematological findings. J Clin Oncol 1989; 10: 1528-32.
34. Armitage JO, Goeken JA, Feagler JR. Spurious elevation of the platelet count in acute leukemia. JAMA 1978; 239: 433-4.
35. Reaman GH, Sposto R, Sensel M, *et al.* Treatment outcome and prognostic factors for infants with acute lymphoblastic leukemia treated on two consecutive trials of the Children’s Cancer Group. J Clin Oncol 1999; 17: 445-55.
36. Smith M, Arthur D, Camitta B, *et al.* Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. J Clin Oncol 1996; 14: 18-24.
37. Dutcher JP, Schiffer CA, Wiernik PH. Hyperleukocytosis in adult acute non-lymphocytic leukemia: impact on remission rate and duration and survival. J Clin Oncol 1987; 9: 1364-72.
38. Chang MC, Chen TY, Tang JL, *et al.* Leukapheresis and cranial irradiation in patients with hyperleukocytic acute myeloid leukemia: no impact on early mortality and intracranial hemorrhage. Am J Hematol 2007; 82 (11): 976-80.

39. Porcu P, Danielson CF, Orazi A, *et al.* Therapeutic leukapheresis in hyperleucocytic leukaemias: lack of correlation between degree of cytoreduction and early mortality rate. Br J Haematol 1997; 98: 433-6.
40. Giles FJ, Shen Y, Kantarjian HM, *et al.*. Leukapheresis reduces early mortality in patients with acute myeloid leukemia with high white cell counts but does not improve long- term survival. Leuk Lymphoma 2001; 42: 67-73.
41. Lane TA. Continuous-flow leukapheresis for rapid cytoreduction in leukemia. Transfusion 1980; 20: 455-7.
42. Grima KM. Therapeutic apheresis in hematological and oncological diseases. J Clin Apher 2000; 15: 28-52.
43. Basade M, Dhar AK, Kulkarni SS, *et al.* Rapid cytoreduction in childhood leukemic hyperleukocytosis by conservative therapy. Med Pediatr Oncol 1995; 25: 204-7.
44. Pession A, Masetti R, Gaidano G, *et al.* Risk evaluation, prophylaxis, and treatment of tumor lysis syndrome: consensus of an Italian expert panel. Adv Ther 2011; 28 (8): 684-97.
45. Schwartz JH, Canellos GP. Hydroxyurea in the management of the hematologic complications of chronic granulocytic leukemia. Blood 1975; 46: 11-6.
46. Grund FM, Armitage JO, Burns CP. Hydroxyurea in the prevention of the effects of leukostasis in acute leukemia. Arch Int Med 1977; 137: 1246-7.
47. Gilchrist GS, Fountain KS, Dearth JC, *et al.* Cranial irradiation in the management of extreme leukemic leukocytosis complicating childhood acute lymphocytic leukaemia. J Pediatr 1981; 98: 257-9.
48. Flasshove M, Schuette J, Sauervein W, *et al.* Pulmonary and cerebral irradiation for hyperleukocytosis in acute myelomonocytic leukemia. Leukemia 1994; 8:1792.
49. Butler RW, Hill JM, Steinherz PG *et al.* Neuropsychologic effects of cranial irradiation, intrathecal methotrexate, and systemic methotrexate in childhood cancer. J Clin Oncol 1994; 12: 2621-9.

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Metastasi polmonari: update di trattamento con Nd:YAG laser 1318 nm

Lung metastases: update of treatment with Nd:YAG laser 1318 nm

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Riassunto

Finalità: **Nei pazienti affetti da metastasi polmonari, al fine di rendere possibili resezioni polmonari con il massimo risparmio parenchimale, sono stati sviluppati laser che permettono di effettuare multiple resezioni anche a livello polmonare profondo, di ottenere un risparmio parenchimale e la riduzione degli interventi maggiori.** ***Materiali e metodi:*** **Lo studio ha valutato i risultati a lungo termine del trattamento chirurgico resettivo mediante Nd:YAG laser 1318 nm in 77 pazienti affetti da metastasi polmonari sottoposti a trattamento chirurgico dal 2005 al 2010. Il tumore primitivo era una neoplasia intestinale in 49 casi, un tumore del rene in 18, un sarcoma extratoracico in 5, una neoplasia mammaria in 3, un tumore maligno della lingua ed una neoplasia del laringe nei restanti due casi. 16 pazienti presentavano alla TAC torace una singola neoformazione, 40 2-3 noduli polmonari e 21 erano affetti da 3 o più noduli (*range* 3-15), di diametro medio 2,1 cm (*range* 0,7-5,5 cm). La resezione è stata effettuata in 73 casi (94,8%) mediante l'utilizzo del laser Nd:YAG 1318 nm con potenza 40W, in 4 (5,2%) casi è stato necessario eseguire una resezione maggiore.** ***Ri-***

Summary

Aim: **In order to permit multiple resections and to improve lung-sparing in people affected by lung metastases, different wavelength surgical lasers have been developed. Some of them achieved multiple radical lung resections and parenchymal-sparing efficacy, reducing major lung resections.** ***Material and methods:*** **This study evaluated long term results of surgical resection in 77 patients affected by lung metastases, treated from 2005 to 2010. The primary tumour was intestinal in 49 of them, kidney in 18, extrathoracic sarcoma in 5, breast in 3, tongue and larynx in the last two. At CT scan 16 patients had only 1 lung nodule, 40 had 2-3 lung neoplasms and 21 had 3 or more nodules (range 3-15) with an average diameter of 2.1 cm (range 0.7-5.5 cm). Resection was performed by means of Nd:YAG laser 1318 nm with a power of 40 W in 73 (94.8%) patients, with a major resection in 4 (5.2%) of them.** ***Results:*** **168 lung nodules have been removed, 127 of them were lung metastases, 41 were benign nodules. We had no mortality. Major complications were a pleural empyema treated surgically; minor complications were**

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sultati: **Sono stati asportati completamente 168 noduli polmonari, 127 dei quali sono risultati istologicamente delle metastasi polmonari, 41 delle lesioni benigne. Non abbiamo osservato mortalità. Tra le complicanze maggiori abbiamo osservato un caso di empiema trattato con *toilette* chirurgica. Complicanze minori sono state perdite aeree persistenti in 15 pazienti; un solo caso ha necessitato di revisione chirurgica per via videotoracoscopica. La degenza media è stata di 5,5 giorni (*range* 4-11). Tutti i pazienti sono inseriti in un programma di *follow-up* trimestrale mediante controllo clinico e radiologico. La sopravvivenza attuariale globale dei pazienti inseriti nello studio è stata del 82%, 67% e 32% a 1, 3 e 5 anni rispettivamente. *Conclusioni:* Il nostro studio dimostra che la metastasectomia polmonare con LASER è un trattamento sicuro ed efficace poiché permette di eseguire resezioni polmonari multiple, anche in caso di noduli profondi con indubbio risparmio parenchimale. Tale tecnica per gli indubbi vantaggi esposti sembra quindi destinata a divenire un trattamento specifico e standardizzato nei pazienti affetti da metastasi polmonari. Eur. J. Oncol., 16 (4), 233-240, 2011**

Parole chiave: **metastasectomia, resezione, laser Nd:YAG, sopravvivenza**

prolonged air leak in 15 patients; one patient required videothoracoscopic surgical correction of the air leakage. Mean hospital stay was 5.5 days (range 4-11). All patients are part of a program of three-monthly follow-up under a clinical and radiological control. Patients treated have reached 82%, 67% and 32% overall survival after 1, 3 and 5 years respectively. *Conclusions:* Our study has demonstrated that LASER-assisted lung metastasectomy is safe and effective in terms of lung and parenchyma-sparing resections, reducing major lung resections. This technique could become a specific and standardized treatment for patients affected by lung metastases. Eur. J. Oncol., 16 (4), 233-240, 2011

Key words: **metastasectomy, lobe sparing resection, laser Nd:YAG, survival**

Introduzione

L'efficacia terapeutica della metastasectomia polmonare si è gradualmente affermata in questi ultimi 20 anni ed attualmente rappresenta una procedura chirurgica universalmente accettata ed eseguita in numerosi centri con indicazioni ben precise (1). Fin dagli albori della chirurgia toracica, circa 100 anni fa, si è sempre ricercata la migliore procedura resettiva per il parenchima polmonare, utilizzando varie metodiche, il più delle volte efficaci nella resezione ma deficitarie per ciò che riguarda l'areostasi e l'emostasi (2, 3). L'avvento delle suturatrici meccaniche ha sopperito in gran parte a tali carenze. Tuttavia il loro utilizzo nel trattamento delle metastasi polmonari multiple o centroparenchimali richiede un importante sacrificio di tessuto sano (4) e non ha modificato il ricorso alle resezioni maggiori che nelle varie casistiche risultano necessarie nel 20-30% dei casi (1, 4). A tale limitazione hanno cercato risposta gli studi condotti da Rolle *et al.* (4, 5) alla fine degli anni '90 sull'utilizzo del Nd:YAG laser 1318 nm nelle resezioni polmonari per lesioni metastatiche. Questi studi hanno dimostrato che è possibile asportare con la tecnica laser metastasi multiple e/o profonde con importante risparmio parenchimale ed identica radicalità oncologica. Da ciò deriva anche la possibilità di trattare pazienti non eleggibili per la chirurgia maggiore e quella di eseguire interventi reiterati in caso di recidiva di malattia (4-6).

Scopo di questa pubblicazione è quello di presentare la nostra esperienza nell'utilizzo della metastasectomia polmonare laser valutando i risultati a lungo termine di tale trattamento.

Materiali e metodi

Nel quinquennio 2005-2010 presso il nostro Istituto sono stati selezionati, per essere sottoposti a trattamento chirurgico resettivo, 77 pazienti affetti da metastasi polmonari, 46 maschi e 31 femmine di età media 60,5 anni (*range* 37-78). Tutti i pazienti soddisfacevano ai criteri di eligibilità per la chirurgia delle metastasi evidenziati dall'*International Registry of Lung Metastases* del 1996 (assenza del tumore primitivo e di metastasi extratoraciche, assenza di N2 bilaterale) (1, 4). In 33 pazienti le metastasi erano bilaterali, 44 presentavano interessamento monolaterale. In 49 di essi il tumore primitivo era una neoplasia intestinale, in 18 una neoplasia renale, in 5 un sarcoma, in 3 casi una neoplasia mammaria; una neoplasia della lingua ed una del laringe erano presenti nell'anamnesi dei 2 rimanenti casi. La mediana dell'intervallo libero da malattia era di 21 mesi (*range* 10-35). Tutti i pazienti sono stati sottoposti a studio funzionale respiratorio e cardio-vascolare per valutarne l'operabilità e le riserve funzionali e ad uno specifico *staging* della malattia di base comprendente una TAC *total body*, una PET in 50 casi ed una colonscopia di controllo nei pazienti precedentemente affetti da neoplasia intestinale.

16 pazienti presentavano alla TAC del torace una metastasi singola, 40 da 2 a 3 noduli polmonari, mentre i restanti 21 pazienti erano affetti da più di 3 metastasi (*range* 3-15) (fig. 1). Il diametro medio dei noduli polmonari era di 2,1 cm (*range* 0,7-5,5 cm).

In 60 pazienti una diagnosi pre-operatoria di neoplasia metastatica è stata ottenuta mediante agobiopsia TC guidata od agobiopsia trans-bronchiale. 17 pazienti sono andati all'intervento senza una diagnosi di natura delle neoformazioni, che erano risultate PET positive in 10 casi. Il riscontro PET ha evidenziato una neoformazione polmonare captante in 40 pazienti ed una captazione linfonodale N1 in 10 casi. Non erano evidenti captazioni linfonodali delle stazioni N2. Tutti i pazienti sono stati sottoposti ad anestesia generale con intubazione selettiva a doppio lume. L'accesso utilizzato è stato sempre quello toracotomico laterale con incisione ascellare, bilaterale e sequenziale in 31 casi, con intervallo tra la prima e la seconda procedura di circa 30-40 giorni. Attraverso tale accesso si è provveduto all'accurata palpazione bimanuale del parenchima polmonare. In 73 casi (94,8%), mediante Nd:YAG laser di lunghezza d'onda 1.318 nm, abbiamo asportato tutti i noduli polmonari rinvenuti, utilizzando una potenza laser di 40 W e mantenendo un margine libero dalla neoplasia superiore ai 10 mm (fig. 2).

In 4 di essi ci siamo serviti di un ausilio, un repere metallico in 2 casi, un filo di seta negli altri 2, per esteriorizzare noduli particolarmente profondi. Nello

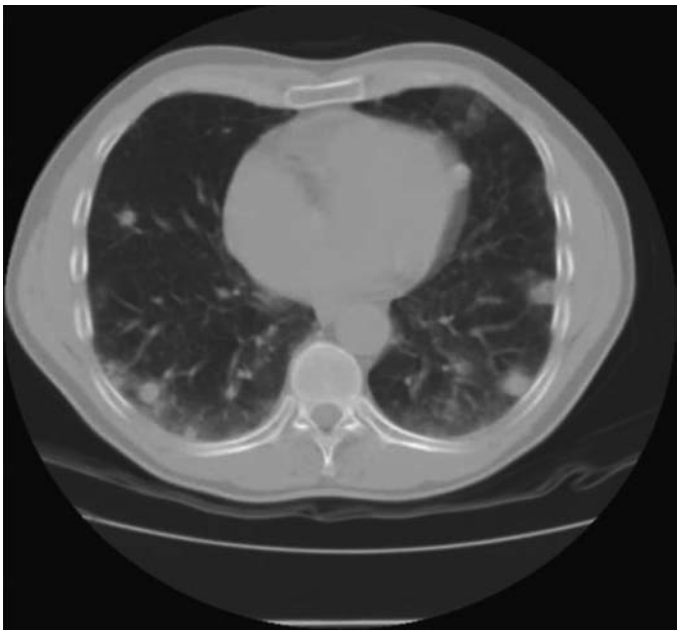

Fig. 1. Scansione TC torace che evidenzia metastasi multiple da neoplasia del colon

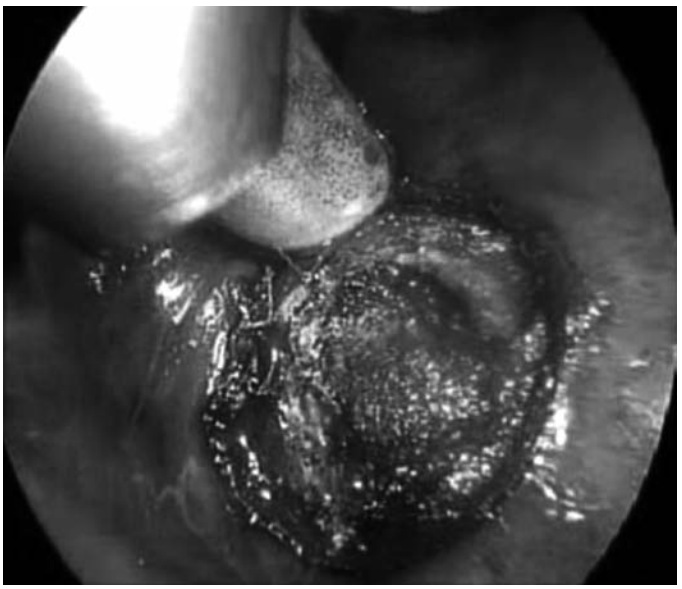

Fig. 2. Resezione con 1.318 nm Nd:YAG laser di una neoformazione metastatica

specifico abbiamo resecato 168 noduli polmonari, di cui 97 a carico del polmone destro, 71 a carico di quello sinistro. 91 lesioni erano periferiche, 77 centro-parenchimali. Una volta eseguita l'asportazione delle metastasi abbiamo sempre provveduto a completare l'emostasi e l'areostasi mediante sutura manuale della breccia parenchimale con pleurizzazione della superficie polmonare e, in presenza di perdite aeree persistenti, abbiamo associato l'utilizzo di sigillanti biologici o sintetici (fig. 3).

In 4 casi (5,2%) è stato necessario eseguire una lobectomia per la presenza di un interessamento neoplastico peribronchiale prossimale (2 casi di lobectomia inferiore) o per le dimensioni importanti della neoplasia (5 e 4,5 cm) che interessava il lobo medio (2 casi).

Alla fase resettiva è sempre seguito un *sampling* linfonodale mediastinico con un numero medio di stazioni linfonodali asportate di 3 (*range* 1-5). Il tempo operatorio medio per la procedura laser è stato di 152 minuti (*range* 100-248) ed è stato determinato in gran parte dal numero delle metastasi piuttosto che dall'esecuzione della procedura; quello dei casi sottoposti a resezione maggiore è stato di 210 minuti (*range* 170-260).

Abbiamo anche voluto valutare, prima di cominciare la nostra esperienza, il costo reale di questa nuova metodica e l'eventuale risparmio ottenibile, utilizzando una simulazione che prendeva in considerazione un *pool* virtuale di 100 pazienti trattati con tecnica laser vs *stapler* nell'arco di un quadriennio. Dall'analisi dei dati di tale simulazione abbiamo riscontrato un risparmio utilizzando la tecnologia laser di circa il 95% rispetto all'esecuzione di resezioni meccaniche con stapler, con ammortizzamento del costo di acquisto dell'apparecchio laser (150.000 €) nell'arco di un triennio (Tabella 1).

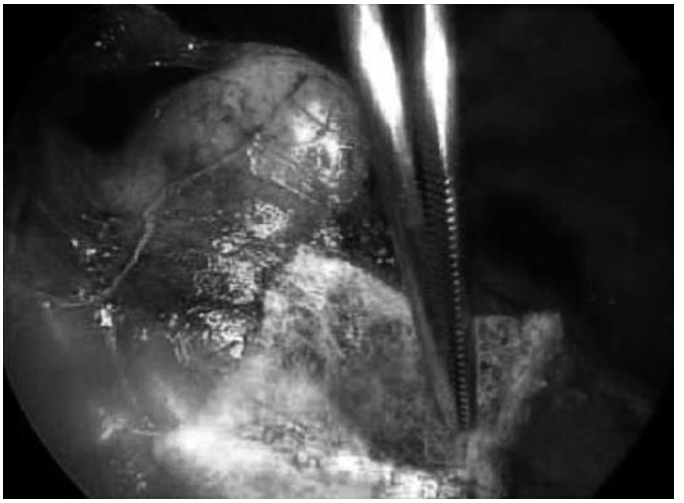

Fig. 3. Dopo la resezione laser si completa l'emostasi e l'areostasi con biosigillante

Risultati

In tutti i pazienti l'intervento è stato macroscopicamente radicale. I margini di resezione chirurgica non sono stati sempre facilmente valutabili dal punto di vista istologico per la presenza di lesioni coagulative determinate dall'azione del laser. Delle 168 lesioni asportate, 127 sono risultate essere metastasi polmonari attive, mentre 41 noduli sono risultati lesioni non neoplastiche (28 linfonodi antracotici intraparenchimali, 7 amartomi condroidi, 6 noduli flogistici), 28 delle quali erano associate ad almeno una lesione neoplastica. 70 noduli presentavano un margine libero da neoplasia di 11 mm, 23 di 9 mm, 20 7-8 mm e nelle restanti 14 neoformazioni 5-6 mm. L'esame istologico dei linfonodi ha evidenziato che

Tabella 1 - Simulazione virtuale su 100 pazienti trattati in un quadriennio con resezione laser a diodi *vs stapler* (i valori sono in euro)

Periodo	N. pazienti	Costo fibre ottiche	Fibre/anno (5 interventi/ fibra)	Costo totale/anno/ fibra/laser	N. medio cariche x procedura	Costo ricarica	Costo pistola	Costo totale *stapler*	Costo totale/anno *stapler*
1° anno	25	120	5	600	3	100	350	650	16250
2° anno	25	120	5	600	3	100	350	650	16250
3° anno	25	120	5	600	3	100	350	650	16250
4° anno	25	120	5	600	3	100	350	650	16250
Totale	**100**	**480**	**20**	**2400**				**2600**	**65000**

58 pazienti sono risultati N0, 14 N1 e 5 N2. Non abbiamo riscontrato mortalità intra e post-operatoria a 30 giorni. La durata media dei drenaggi toracici è stata di 4,5 giorni (*range* 2-10) sia per le resezioni laser che per quelle maggiori, così come la degenza media si è attestata sui 5,5 giorni (*range* 4-11) per entrambi i gruppi. Le complicanze minori sono state le perdite aeree prolungate osservate in 15 casi, soprattutto all'inizio della nostra esperienza in pazienti sottoposti a resezione di numerose metastasi; un solo caso ha richiesto la revisione chirurgica in videotoracoscopia. Fra quelle maggiori annoveriamo un caso di empiema pleurico post-operatorio risolto con *toilette* videotoracoscopica. Tutti i pazienti sono stati inseriti in un programma di *follow-up* mediante controllo clinico trimestrale e strumentale-radiologico con TC torace ogni 6 mesi. Abbiamo osservato un'incidenza di ripresa di malattia nel 35% dei pazienti in assenza di recidiva locale. La sopravvivenza attuariale globale della nostra casistica è del 82%, 67% e 32% rispettivamente a 1, 3 e 5 anni.

Discussione

Dopo un lungo periodo di scetticismo, a partire dal report dell'*International Registry of Lung Metastases* (1), la metastasectomia polmonare si è affermata universalmente come il trattamento che permette un significativo miglioramento della sopravvivenza a distanza soprattutto in gruppi selezionati di pazienti (1, 3, 4).

Argomento di discussione rimane invece la migliore tecnica di resezione delle metastasi polmonari, nel rispetto della radicalità oncologica e del risparmio parenchimale per la possibilità di recidive locali e quindi di interventi reiterati (1, 4, 6). La rimozione chirurgica delle metastasi polmonari mediante *stapler* viene considerata la procedura standard normalmente utilizzata, indicata soprattutto nei pazienti con lesioni periferiche (circa 1/3 dei casi), ma condizionata dalla necessità della presenza di un numero limitato di metastasi e dalla difficoltà nel resecare i noduli intraparenchimali profondi (7). In tali casi, ascrivibili circa al 20% dei pazienti (1, 4), in era pre-laser era necessario sacrificare l'intero lobo polmonare o l'intero polmone. Nel tentativo di riuscire a perfezionare una tecnica resettiva efficace e che comportasse un risparmio parenchimale, negli anni '80 ed inizio di quelli '90 numerosi Autori proposero l'utilizzo del laser Nd:YAG con una lunghezza d'onda di 1.064 nm (fig. 4, 5). Tale strumento era in grado di rimuo-

Fig. 4. Laser Nd:YAG 1.064 nm (Elettronica Valseriana)

Fig. 5. Monitor e fibra ottica della strumentazione laser Nd:YAG 1.064 nm (Elettronica Valseriana)

vere, con moderata istolesività, le metastasi, pur non soddisfacendo in termini di emostasi ed areostasi post-resezione (2, 3, 6, 9). Gli Autori riportavano però che l'utilizzo del laser Nd:YAG non modificava la sopravvivenza se paragonato ai trattamenti convenzionali e confermavano che in termini di risparmio tissutale il laser produceva eccellenti risultati (3, 6, 9).

Gli studi di A. Rolle *et al.* (4, 5) sull'utilizzo di un laser Nd:YAG a lunghezza d'onda di 1.318 nm hanno determinato una svolta decisiva nel progresso della chirurgia laser del parenchima polmonare (fig. 6, 7). Questa lunghezza d'onda si è dimostrata particolarmente efficace nell'ottenere una buona aerostasi ed emostasi, mantenendo l'efficacia resettiva e la radicalità oncologica (10). Rolle ha presentato una serie di 328 pazienti affetti da metastasi polmonari ed operati dal Gennaio 1996 al Dicembre 2003, descrivendo la rimozione di 3267 noduli (una media di 10 noduli/paziente); 2546 di essi risultarono essere delle metastasi all'esame istologico definitivo (8/paziente). Nel 15% dei pazienti la resezione è risultata incompleta, non per un difetto di tecnica chirurgica quanto per la presenza inaspettata di metastasi a sviluppo miliariforme o di carcinosi pleurica. Nonostante il 40% dei noduli fosse localizzato a livello centrale ed avessero dimensioni comprese tra i 3 e gli 80 mm, è stato possibile eseguire una resezione efficace nel 93% dei casi, riducendo la necessità di una lobectomia a solo il 7% dei pazienti (5).

Anche nella nostra esperienza seppur limitata come numero di pazienti abbiamo trattato con resezione laser il 94,8% dei pazienti, dovendo ricorrere alla resezione maggiore nel 5,2% dei casi. Non abbiamo osservato casi di resezione incompleta, probabilmente per l'esiguità della casistica.

L'utilizzo del Nd:YAG laser a lunghezza d'onda di 1318 nm dunque permette di eseguire una resezione di precisione difficilmente ottenibile mediante sutura con *stapler*, che richiede il sacrificio di gran parte del parenchima contiguo compreso nella resezione meccanica ed ancor più difficile risulta con tale metodica aggredire lesioni centro-parenchimali che quindi devono essere trattate con una resezione maggiore (4, 5, 8, 10). Altri vantaggi della tecnica laser a 1318 nm sono la capacità emostatica che giunge fino a 4 mm dal margine di resezione ed il potere aero-sigillante nei bronchioli <3 mm di diametro, determinato dal collasso dei setti alveolari e dalla creazione di una sottile membrana multistrato a tenuta aerea. Nei bronchi di maggiori dimensioni rimane invece indicato eseguire una sutura tradizionale di rinforzo (10).

Fig. 6. Laser Nd:YAG 1318 nm

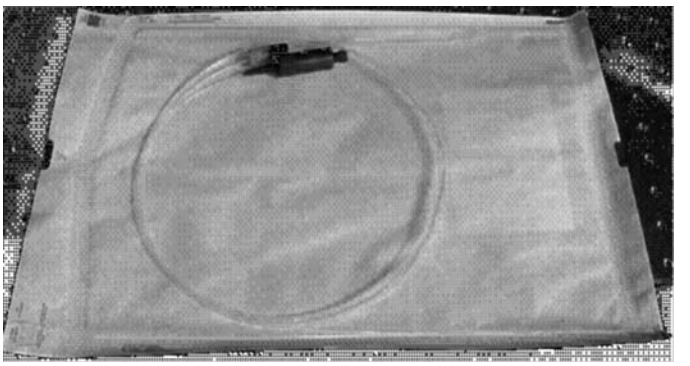

Fig. 7. Fibre ottiche della strumentazione laser

Il vantaggio principale della resezione laser è dato dal risparmio parenchimale, fondamentale soprattutto nei pazienti non eleggibili a chirurgia maggiore per le scadute condizioni respiratorie o portatori di multifocalità della patologia, a cui resterebbe solo l'opzione terapeutica palliativa (3-5). Rolle e collaboratori hanno dimostrato nella loro estesa serie come con tale tecnica si possa eseguire una resezione completa della neoplasia e ridurre l'incidenza delle lobectomie per malattia polmonare metastatica, al solo 7% contro il 22% delle precedenti casistiche (4, 5).

Proprio la completezza della resezione viene descritta in alcune serie come il fattore prognostico fondamentale. Rolle evidenzia una differenza di sopravvivenza statisticamente significativa nei pazienti sottoposti a resezione completa rispetto a quelli trattati con resezione incompleta (41% vs 7% a 5 anni) (5).

L'*International Registry of Lung Metastases* nel 1996 evidenziava altri fattori prognostici: l'intervallo libero da malattia, il numero delle metastasi, il tipo istologico, la bilateralità della patologia, l'impegno linfonodale (1).

Gli studi più recenti evidenziano come vi sia stata una riduzione d'importanza di tali fattori, fatta eccezione per l'intervallo libero da malattia, se considerati nell'ottica di una resezione completa. Rolle come altri Autori dimostra come la sopravvivenza di pazienti con 4 metastasi completamente resecate sia sovrapponibile a quella di pazienti affetti da 20 o più metastasi (36 vs 32%) (5, 11).

La resezione laser rende inoltre possibile l'esecuzione di interventi reiterati in caso di recidiva metastatica. Tale evento ha un'incidenza che oscilla fra il 34 ed il 60% nelle varie casistiche (4, 5, 7, 10). Il trattamento chirurgico delle recidive in alcuni tipi istologici determina una sopravvivenza compresa tra il 20 ed il 59 % a 3 anni che ne giustifica l'utilizzo (3-5, 8).

In generale quindi la sopravvivenza dopo resezione completa di metastasi polmonari è da considerarsi buona, variando nelle maggiori serie dal 70 all'85% ad un anno fino al 20-45% a 5 anni (4, 5, 11) e raggiungendo il 67% in casi selezionati (11). Nella nostra esperienza di 73 casi trattati con tecnica laser abbiamo ottenuto un valore di sopravvivenza a 5 anni del 32% in linea con le maggiori casistiche internazionali.

Sono di recente stati proposti un laser a diodi a lunghezza d'onda sovrapponibile a quella del Nd:YAG laser ed uno al Thulium di lunghezza d'onda superiore (2010 mm) che sembrano, dalle prime osservazioni, possedere i medesimi requisiti d'efficacia terapeutica del precedente modello, ma una maggiore maneggevolezza, rapidità e potenza, potendo giungere fino a 120 Watt contro i 40 Watt dello Nd:YAG. Saranno comunque necessari ulteriori studi anche su base multicentrica per valutarne le effettive potenzialità ed vantaggi rispetto al Nd:YAG laser.

Conclusioni

In conclusione, il laser Nd:YAG a 1.318 nm ha rappresentato una importante innovazione tecnologica e permette attualmente di eseguire qualunque tipo di resezione parenchimale polmonare. Facilita la resezione completa anche di metastasi multiple, centro-parenchimali e recidive con importante risparmio parenchimale. La metastasectomia laser o laser assistita rappresenta un trattamento efficace, diffuso ed ormai standardizzato che arricchisce l'arsenale terapeutico del chirurgo toracico ad un costo di gestione del tutto vantaggioso.

Bibliografia

1. Pastorino U, Buyse M, Friedel G, *et al.* The International Registry of Lung metastases: Long-term results of lung metastasectomy: prognostic analyses based on 5206 cases. J Thorac Cardiovasc Surg 1997; 113: 37-49.
2. Minton JP, Andrews NC, Jesseph JE. Pulsed laser energy in the management of multiple metastases. J Thorac Cardiovasc Surg 1967; 54: 707-13.
3. Branscheid D, Krysa S, Wollkopf G, *et al.* Does Nd:YAG laser extend the indictions for resection of pulmonary metastases? Eur J Cardiothorac Surg 1992; 6: 590-7.
4. Rolle A, Koch R, Alpard SK, *et al.* Lobe-sparing resection of multiple pulmonary metastases with a new 1318-nm Nd:YAG laser--first 100 patients. Ann Thorac Surg 2002; 74 (3): 865.
5. Rolle A, Pereszlenyi A, Koch R, *et al.* Is surgery for multiple lung metastases reasonable? A total of 328 consecutive patients with multiple-laser metasatasectomies with a new 1318-nm Nd:YAG laser. J Thorac Cardiovasc Surg 2006; 131: 1236-42.

6. Kodama K, Doi O, Higashiyama M, *et al.* Surgical management of lung metastases. J Thorac Cardiovasc Surg 1991; 101: 901-8.
7. Venuta F, Rolle A, Marco A, Martucci N, Bis B, Rocco G. Techniques used in lung metastasectomy. J Thorac Oncol 2010; 5 (Suppl 2): S145-50.
8. Borasio P, Gisabella M, Billé A, *et al.* Role of surgical resection in colorectal lung metastases: analysis of 137 patients. Int J Colorect Dis 2011; 26 (2): 183-90.
9. Mineo TC, Ambrogi V, Pompeo E, *et al.* The value of the Nd:YAG laser for the surgery of lung metastases in a randomized trial. Chest 1998; 113: 1402-7.
10. Rolle A, Pereszlenyi A, Koch R, *et al.* Laser Resection Technique and results of Multiple Lung Metastasectomies Using a New 1,318 nm Nd:YAG Laser System. Lasers in Surgery and Medicine 2006; 38: 26-32.
11. Rosenberg C, Puls R, Hegenscheid K, *et al.* Laser ablation of metastatic lesions of the lung: long-term outcome. AJR 2009; 192: 785-92.

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Mesotelioma peritoneale da amianto in lavoratore di uno stabilimento petrolchimico

Peritoneal mesothelioma in petrochemical plant worker exposed to asbestos

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Riassunto

Viene descritto un caso di mesotelioma in un lavoratore esposto ad amianto, addetto ai computer di processo in uno stabilimento petrolchimico. Il tempo di latenza, periodo intercorso fra l'inizio dell'esposizione e la comparsa dei primi sintomi e segni della neoplasia, è stato di 36 anni. Questo è il secondo caso di mesotelioma, di una serie di sette, pervenuto alla nostra attenzione su segnalazione dell'Unità Operativa di Chirurgia dell'Ospedale di Bentivoglio, trattato con chirurgia citoriduttiva e chemioipertermia intraoperatoria (HIPEC). Il paziente, a tre anni circa dall'intervento, gode di buona salute. L'iniziale diagnosi clinico-strumentale di carcinosi peritoneale è stata perfezionata in mesotelioma peritoneale in seguito ad esame istologico e caratterizzazione immunoistochimica. Eur. J. Oncol., 16 (4), 241-246, 2011

Parole chiave: **mesotelioma, amianto, industria petrolchimica, chirurgia citoriduttiva con chemioipertermia**

Summary

This work reports a case of mesothelioma in a worker exposed to asbestos, attending the computer process in a petrochemical facility. The latency time, i.e. the period elapsing between the start of exposure and the onset of early symptoms and signs of the neoplasia, was 36 years. This is the second case of seven which was brough to our attention by a report by the Operational Unit of Surgery of the Hospital of Bentivoglio, treated with cytoreductive surgery and with hyperthermic intraperitoneal chemotherapy (HIPEC). After two years from the surgical treatment, the patient is still in good health. Following histology and immunohistochemical characterization, the initial clinical diagnosis of peritoneal carcinosis was optimized in peritoneal mesothelioma. Eur. J. Oncol., 16 (4), 241-246, 2011

Key words: **mesothelioma, asbestos, petrochemical plant, cytoreductive surgery with chemo-hyperthermia**

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Introduzione

Il mesotelioma è un tumore che origina dal mesotelio, lo strato di cellule che costituisce le membrane sierose: pleura, peritoneo, pericardio, tunica vaginale del testicolo. La neoplasia origina quasi esclusivamente in seguito ad esposizione a fibre di amianto, con una latenza temporale (cioè il periodo che intercorre tra l'inizio della esposizione e la diagnosi clinica) particolarmente elevata, che va dai 15 ai 50 anni e più. La prognosi è infausta; caratterizzata da aggressività e resistenza alle comuni terapie, questa neoplasia comporta una sopravvivenza media di 11 mesi (range da 4 a 18) dal momento della diagnosi (1). In uno studio di Peto *et al.* (1999) si stima che circa 250.000 nuovi casi di mesotelioma correlati all'esposizione ad amianto insorgeranno nell'Unione Europea entro il 2035 (2). La quasi totalità dei casi rilevati è costituita da mesoteliomi della pleura. In Italia, dal 1993 al 2004 sono stati registrati 9.166 casi di mesotelioma di cui 8.485 della pleura, 614 del peritoneo, 36 del pericardio e 31 della tunica vaginale del testicolo (3). I casi di mesotelioma della pleura con diagnosi certa registrati sono aumentati dal 1993 al 2001, passando da 52 a 176 casi l'anno nelle donne e da 115 a 507 casi l'anno negli uomini (4). In un lavoro di Marinaccio *et al.* del 2005 è stato previsto, sulla base del consumo di amianto pro-capite dal 1970 al 1999 in Italia, un incremento di mesoteliomi negli uomini con un picco di circa 800 casi l'anno fra il 2012 e il 2024 (5). Per quanto riguarda il mesotelioma peritoneale dal 1993 al 2001, nelle donne i casi registrati sono passati da 9 a 16 e negli uomini da 8 a 41 (4). Il mesotelioma peritoneale è più raro di quello pleurico ed il rapporto tra il numero di casi osservati di queste due forme di neoplasia sembra essere in relazione al grado di esposizione. Dodson *et al*. hanno riportato che il 10-15% dei mesoteliomi origina nel cavo peritoneale perchè fibre di amianto possono migrare in questo distretto, così come confermato da casi clinici di pazienti affetti da mesotelioma peritoneale nei quali il tessuto tumorale contiene fibre di amianto. Ciò si verifica soprattutto nelle situazioni in cui l'esposizione ad amianto è molto elevata (6).

Data la rarità di questa neoplasia e le incertezze correlate alla reale efficacia dei trattamenti chirurgici e chemioterapici, appare di grande interesse il caso qui descritto che riguarda un paziente vivo a più di tre anni dall'intervento ed in buona salute; si tratta del secondo dei 7 casi di mesotelioma peritoneale pervenuti alla nostra osservazione attraverso l'Unità Operativa di Chirurgia dell'Ospedale di Bentivoglio. In questo presidio infatti, fin dal 2001, si è creata un'equipe dedicata alla cura delle carcinosi peritoneali di varia origine. In particolare in questa Unità Operativa sono pervenuti 338 pazienti affetti da carcinosi, di cui 223 sono stati trattati con chirurgia citoriduttiva e chemioipertermia intraoperatoria (HIPEC), una moderna metodica codificata da Sugarbacker (7) che prevede, oltre all'asportazione chirurgica, anche un ciclo intraoperatorio di chemioterapia associato al calore. Questa combinazione agisce in maniera sinergica per eliminare il residuo microscopico, non visibile dal chirurgo, causa di recidiva di malattia. I rimanenti 115 pazienti sono stati trattati esclusivamente con chirurgia citoriduttiva, senza HIPEC, per l'impossibilità di raggiungere una citoriduzione ottimale. La casistica di mesoteliomi peritoneali del centro chirurgico di Bentivoglio, uno dei principali centri di riferimento d'Italia per questo tipo di patologia, include 7 casi, provenienti sia dall'Emilia Romagna che da altre regioni del sud e del nord Italia, tutti trattati con chirurgia citoriduttiva associata ad HIPEC. L'esperienza e il razionale dell'U.O. di Chirurgia sono state riportate da Virzì *et al.* nel 2009 (8).

Resoconto del caso

R.E., nato nel 1948 in provincia di Foggia dove risiede.

Ex fumatore. Ha fumato circa 10-15 sigarette al giorno dall'età di 14 anni all'età di 57 anni.

Beve vino ai pasti.

Anamnesi familiare

- Padre, coltivatore diretto, deceduto a 77 anni per leucemia acuta.
- Madre, casalinga, deceduta a 80 anni per causa sconosciuta.
- 2 sorelle e 2 fratelli viventi ed in buona salute.
- Coniugato, con 2 figli in buona salute.

Anamnesi lavorativa

- Ha lavorato per 8 mesi, fra il 1970 e il 1971, presso l'azienda GBF Costruzioni Meccaniche di Bresso (MI). Riferisce che effettuava impianti elettrici su presse idrauliche, e che l'ambiente di lavoro era pulito e privo di polveri, gas o vapori e che non erano presenti strutture o coibentazioni contenenti amianto.
- Dal 1972 al 1998, per ben 26 anni, ha lavorato presso l'azienda ANIC S.p.A. IV Centro Petrolchimico Puglia, zona industriale Monte S. Angelo (FG), come addetto ai computer di processo. Da queste sale computer venivano monitorate le varie fasi di produzione di composti destinati al settore agricolo, al settore fibre artificiali e tecnopolimeri ed al settore degli intermedi aromatici. Ogni impianto di produzione aveva una sala di controllo dove erano collocati i computer. Gli impianti, dove il sig. R.E. prestava servizio, erano massivamente coibentati con amianto, i tetti erano costituiti da coperture in eternit, gli ascensori avevano pareti e tetto coibentati con amianto.
- Dal 1998 in mobilità protetta fino al 01/04/05.

Storia clinica

- In data 16/06/08, in seguito a comparsa di gonfiore addominale senza ulteriore sintomatologia, il paziente esegue ecografia addominale presso l'Ospedale di Manfredonia che evidenzia: "abbondante liquido ascitico diffuso in tutto l'addome. Microcalcoli della colecisti".
- In data 18/06/08 EGDS presso Ospedale di Manfredonia: "Gastrite acuta erosiva antrale"; pancolonscopia: polipo sessile del discendente (esame istologico: adenoma tubulare con displasia di grado moderato).
- In data 19/06/08 presso Ospedale di Manfredonia esegue TC addome da cui risulta: "Massiva ascite diffusa. In sede peritoneale si apprezza la presenza di formazioni pseudo nodulari a maggiore localizzazione mesenteriale che orientano per carcinosi peritoneale. Ispessimento della parete della colecisti e piccola lacuna (subcentimetrica) persistentemente ipodensa in sede epatica a livello del V-VI segmento. Piccole aree lacunari ipodense in sede renale bilaterale, alcune francamente liquide".
- In data 27/06/2008 ricovero presso Ospedale San Grado di Monza; esame citologico di liquido peritoneale: "presenza di aggregati di elementi epiteliali con atipie. Quadro suggestivo per adenocarcinoma".
- In data 26/07/08 si ricovera presso l'ospedale di Bentivoglio con diagnosi di carcinosi peritoneale di n.d.d., in data 28/07/08 viene sottoposto a laparoscopia diagnostica: " Si aspirano circa 4000 cc di liquido ascitico e si ispeziona il cavo addominale rilevando una carcinosi diffusa a tutti i settori con interessamento anche del meso e della matassa ileale. Si effettua prelievo di omento interessato dalla malattia che viene inviato per esame istologico e di chemiosensibilità".
- In data 01/08/08 referto esame istologico della biopsia omentale: "tessuto adiposo omentale con diffusa infiltrazione di adenocarcinoma scarsamente differenziato con aspetti solidi e cistico-papillari"; referto aggiuntivo del 19/08/08: "L'indagine immunoistochimica ha evidenziato positività intensa e diffusa degli elementi proliferanti per calretinina, CK 5/6 e CK 7 e negatività per CK 20. Il reperto morfologico ed immunoistochimico è compatibile con mesotelioma maligno".
- Test di chemio sensibilità eseguito presso l'IRST di Meldola: tessuto neoplastico resistente a 5-fluorouracile, irinotecan, mitomicina c, taxotere, oxaliplatino, alimta, cisplatino.
- 16/09/08 intervento chirurgico presso l'ospedale di Bentivoglio: "Laparotomia mediana xifo-pubica. Aperto il peritoneo riscontro di carcinosi peritoneale diffusa micronodulare che interessa anche il colon *in toto* ed il peritoneo del meso ileale. Isolamento degli ureteri bilateralmente, resezione anteriore del retto con Cointour. Resezione del colon traverso con GIA 80, asportazione in blocco di milza, omento, colon sinistro, peritoneo pelvico, parietale e diaframmatico sinistro. Emicolectomia destra con asportazione del peritoneo parietale destro. Completamento della citoriduzione con: asportazione del piccolo omento e del legamento rotondo epatico, colecistectomia, linfoadenectomia iliaco-otturatoria bilaterale. … HIPEC con 140 mg di cisplatino, 80 di Adriblastina per 90 min a 42,5°C. Relaparotomia. Confezionamento di ileostomia di protezione dell'anastomosi" (fig. 1).

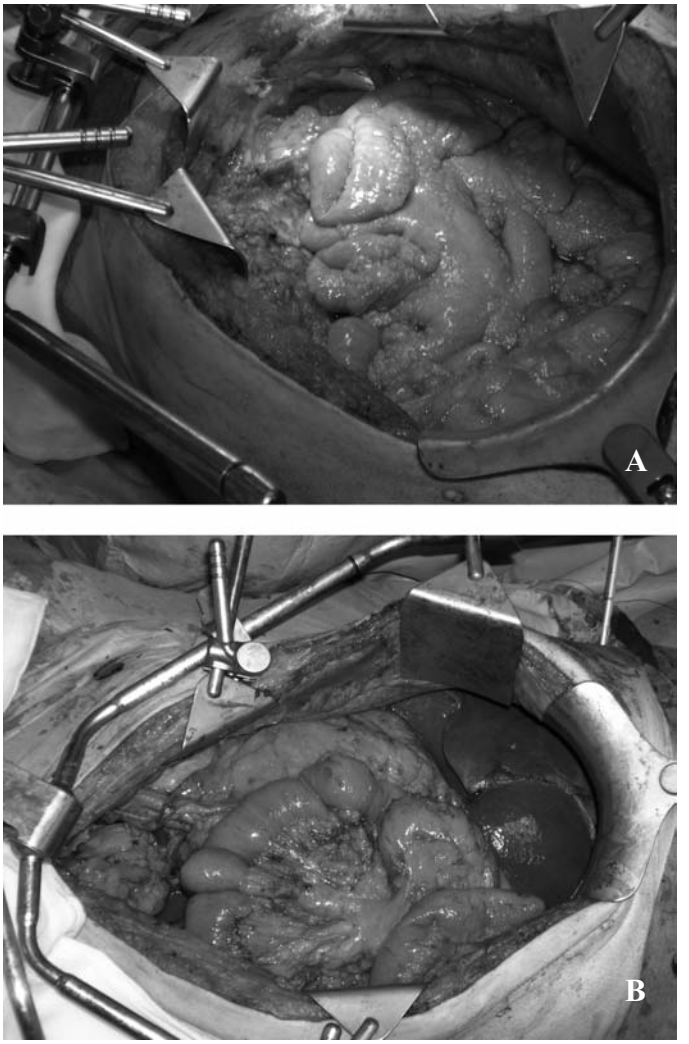

Fig. 1. Mesotelioma peritoneale, prima (A) e dopo (B) citoriduzione

- 16/09/08 esame istologico c/o U.O. di Anatomia e Istologia Patologica Policlinico S. Orsola-Malpighi, Bologna: mesotelioma maligno di tipo epiteliale diffuso con pattern tubulo-papillare, moderatamente differenziato (fig. 2). La lesione è in forma di noduli multipli, confluenti e diffusi a livello della sierosa peritoneale, della sierosa intestinale, dell'appendice vermiforme, della capsula splenica, del meso ileale e del ligamento rotondo del fegato; la neoplasia infiltra diffusamente il tessuto fibro-adiposo omentale e peripancreatico. Liberi da neoplasia il parenchima splenico, la mucosa intestinale e la sua tonaca muscolare. L'indagine immunoistochimica ha evidenziato positività degli elementi proliferanti per citocheratina (CK5/6), calretinina (fig. 3) ed EMA di membrana e negatività per CEA. Linfonodi reattivi, colecistite cronica litiasica con focolai di

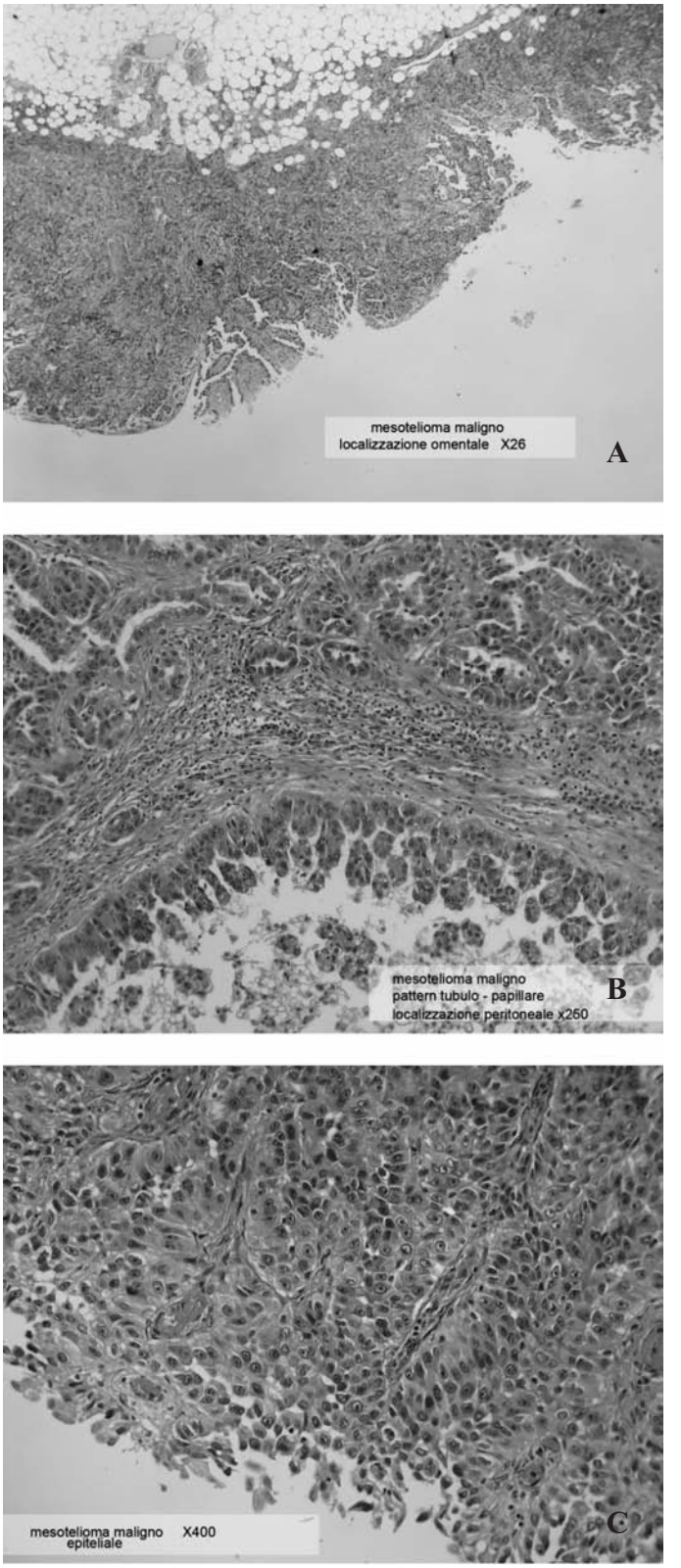

Fig. 2. Immagini istopatologiche del mesotelioma peritoneale con caratteristiche tubulo-papillari, a diversi ingrandimenti (A, B, C)

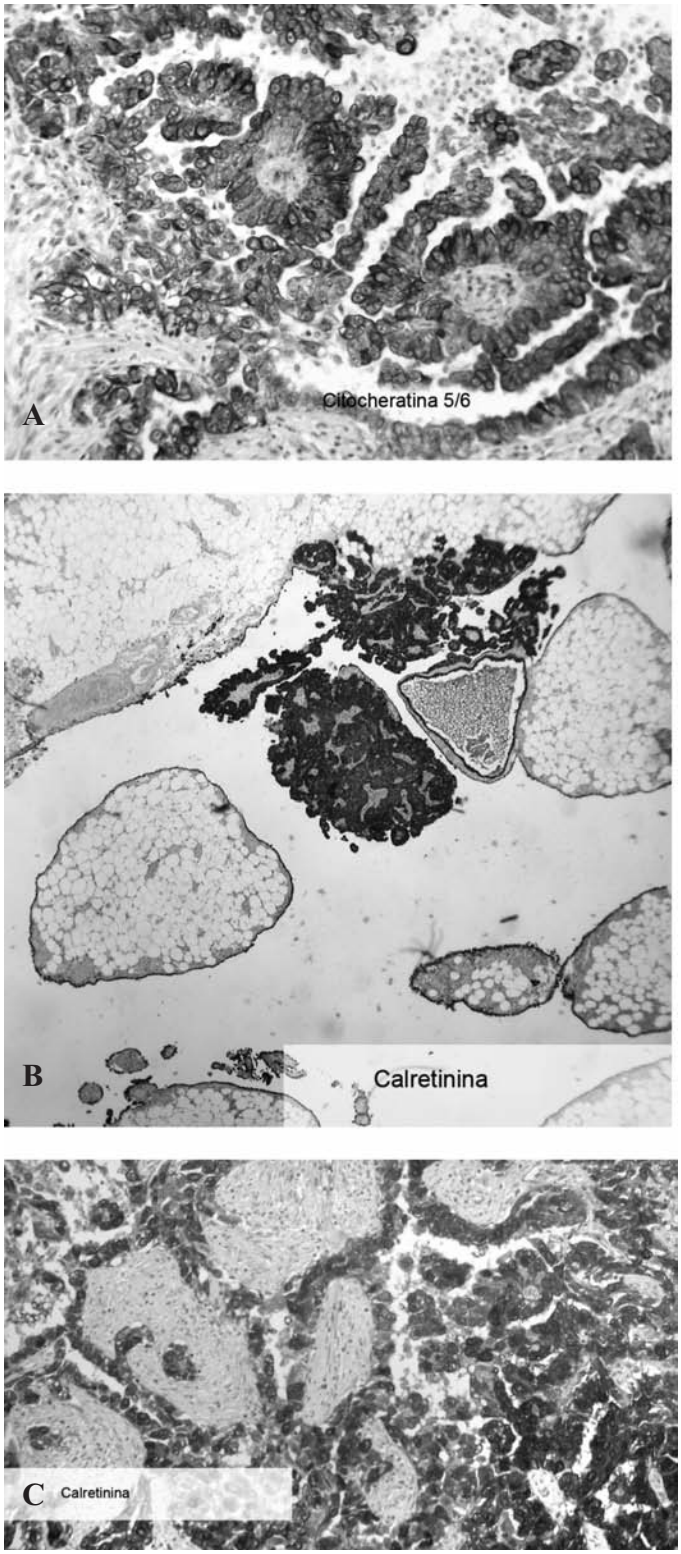

Fig. 3. Immagini immunoistochimiche che evidenziano positività degli elementi proliferanti per citocheratina 5/6 (A) e calretinina (B, C), diagnostici per mesotelioma peritoneale

adenomiosi e noduli neoplastici a livello della sierosa; due linfonodi del colletto reattivi.
- Immediato decorso post operatorio regolare.
- Decorso post operatorio tardivo complicato da ascesso endoaddominale, trattato con drenaggio chirurgico presso la chirurgia d'urgenza degli Ospedali Riuniti di Foggia.
- Controlli di follow up del 16 settembre 2009 e 30 novembre 2009: PET negativa.
- Controllo follow up gennaio 2011 incremento di accumulo del radiofarmaco nel contesto del muscolo obliquo interno e traverso di destra.
- Controllo ecografico della zona ipercaptante segnalata alla PET nel gennaio 2011: due aree ipoecogene di 1 cm e di 0,5 cm di diametro massimo nel contesto del muscolo.
- Intervento chirurgico di rimozione delle lesioni nel maggio 2011 (esame istologico: mesotelioma maligno epiteliale).
- Giugno 2011 inizia terapia sistemica adiuvante.

Discussione e conclusioni

Un esame superficiale del mesotelioma peritoneale descritto, insorto in un lavoratore addetto ai computer di processo in uno stabilimento petrolchimico, avrebbe potuto ignorare la correlazione con l'esposizione ad amianto. Una precisa ed attenta anamnesi lavorativa ha invece messo in evidenza una esposizione consistente e prolungata nel tempo. Tutti gli impianti, dove il sig. R.E. prestava servizio, erano massivamente coibentati con amianto, i tetti delle strutture erano costituiti da coperture in eternit, gli ascensori avevano pareti e tetto coibentati con amianto; le bocche di aspirazione dell'aria condizionata si trovavano sopra o erano attigue agli impianti di produzione. Va considerato che le sale di controllo non erano separate dalle sale di lavorazione; inoltre erano luogo di passaggio obbligato per il personale addetto agli impianti come i manutentori (meccanici, elettricisti, edili, strumentisti, ecc.) che portavano le fibre di amianto all'interno dei locali con gli attrezzi o gli indumenti di lavoro contaminati. Possiamo quindi ritenere che vi sia stata una forte esposizione ad asbesto per l'elevata contaminazione ambientale dovuta all'utilizzo massivo di amianto nelle varie strutture degli impianti.

Il tempo di latenza, cioè il tempo intercorso fra l'inizio dell'esposizione e l'osservazione dei primi segni e sintomi, è stato di 36 anni. La latenza osservata nel caso qui descritto è coerente con quanto riportato in letteratura (2, 9-11). L'intervento chirurgico secondo il protocollo adottato dalla Unità Operativa di Chirurgia dell'Ospedale di Bentivoglio ha avuto un esito favorevole, essendo il paziente in vita ed in buona salute a circa tre anni dall'intervento. I controlli effettuati negli ultimi mesi hanno messo in evidenza una ripresa di malattia, con due piccoli noduli localizzati esclusivamente a livello muscolare della parete addominale, che è stata prontamente trattata con un intervento di minima. Ricordiamo a tale proposito che la sopravvivenza media per il mesotelioma peritoneale è di 7-17 mesi (12). Date le buone condizioni cliniche del paziente, gli oncologi hanno ritenuto opportuno iniziare una chemioterapia a scopo "prudenziale" dopo l'escissione dei piccoli noduli risultati positivi per mesotelioma. Il comportamento biologico abbastanza lento e poco aggressivo permette inoltre di ipotizzare un *second look* chirurgico nel caso la malattia si ripresenti, nonostante la chemioterapia adiuvante. È inoltre importante ricordare come la caratterizzazione immunoistochimica sia stata utile per ottimizzare la diagnosi differenziale fra carcinosi peritoneale e mesotelioma epiteliomorfo, favorendo così la correlazione della neoplasia peritoneale con l'esposizione ad amianto.

Bibliografia

1. American Cancer Society. Survival Rates for Mesothelioma 2009. Available at: http://www.cancer.org/docroot/CRI/content/CRI_2_2_3X_Survival_rates_for_meosothelioma_29.asp?sitearea
2. Peto J, Decarli A, La Vecchia C, *et al*. The European mesothelioma epidemic. Br J Cancer 1999; 79 (3/4): 666-72.
3. ISPESL Istituto Superiore per la Prevenzione e la Sicurezza del Lavoro. Dipartimento di Medicina del Lavoro: III Rapporto Re.Na.M. 2010. Available at: http://www.ispesl.it/renam/Report.asp
4. ISPESL Istituto Superiore per la Prevenzione e la Sicurezza del Lavoro. Dipartimento di Medicina del Lavoro: II Rapporto Re.Na.M. 2006. Available at: http://www.ispesl.it/renam/Report.asp
5. Marinaccio A, Montanaro F, Mastrantonio M, *et al*. Predictions of mortality from pleural mesothelioma in Italy: a model based on asbestos consumption figures supports results from age-period-cohort models. Int J Cancer 2005; 115: 142-7.
6. Dodson RF, O'Sullivan MF, Brooks DR, *et al*. Asbestos content of omentum and mesentery in nonoccupationally exposed individuals. Toxicol Ind Health 2001; 17: 138-43.
7. Sugarbaker PH. Comprehensive management of peritoneal surface malignancy using cytoreductive surgery and perioperative intraperitoneal chemotherapy: the Washington Cancer Institute approach. Expert Opin Pharmacother 2009; 10(12):1965-77.
8. Virzì S, Iusco D, Bonomi S, *et al*. Chirurgia citoridutiva e chemioipertermia: un valido trattamento per la carcinosi peritoneale. Eur J Oncol , 2010; 15(1): 25-32. [Article in Italian]
9. Albin M, Magnani C, Krstev S, *et al*. Asbestos and cancer: An overview of current trends in Europe. Environ Health Perspect 1999;107(Suppl 2): 289-98.
10. Gruppo di lavoro per la sorveglianza del mesotelioma in Romagna. Analysis of occupational exposure to asbestos in cases of mesothelioma registered in Romagna (1986-1998). Med Lav 2000; 91(6): 575-86. [Article in Italian]
11. Haber SE, Haber JM. Malignant Mesothelioma: A Clinical Study of 238 Cases. Ind Health 2011; 49: 166-72.
12. Mussa B, Goss M, Moscato R, *et al*. Mesotelioma peritoneale in operaia addetta alla tessitura di manufatti a base di asbesto. Eur J Oncol, 1999; 4(1): 57-64. [Article in Italian]

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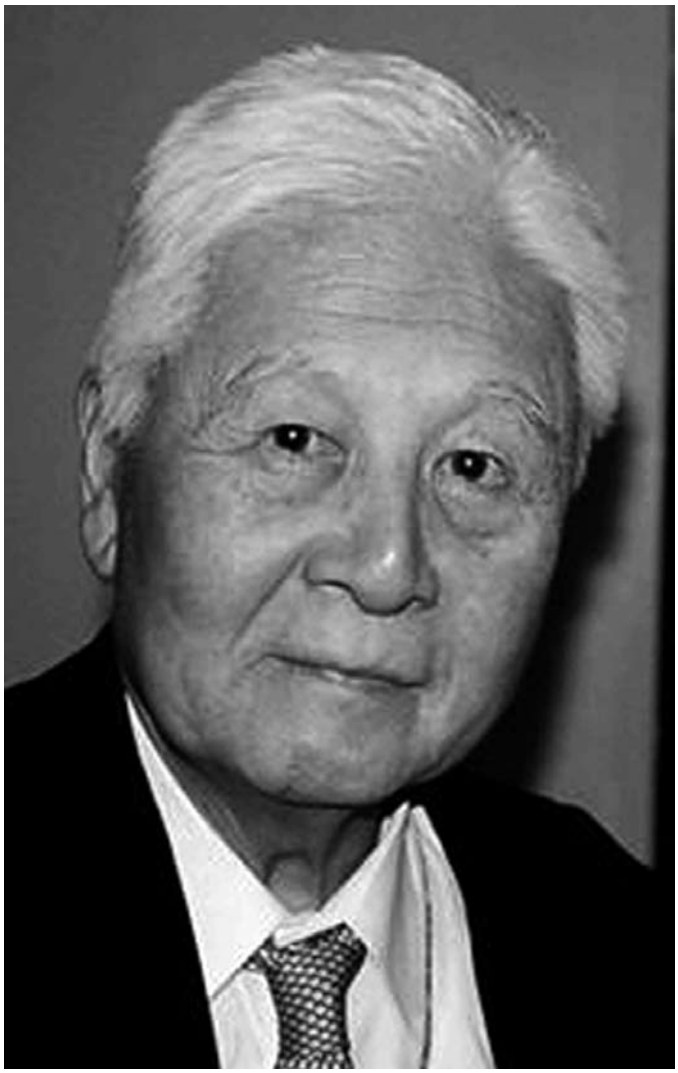
Dr. Yasunosuke Suzuki
1929 - 2011



Dr. Yasunosuke Suzuki, a world wide leader in the research of asbestos and environmental carcinogenesis and a friend of workers, died on August 9, 2011 at the age of 82. Dr. Suzuki received his medical degree from Keio University School of Medicine in Tokyo in 1953 and completed a one year internship in Tokyo at the Setagaya National Hospital. He was licensed in 1954 by the Japanese Government. In 1954 he was appointed Assistant of Pathology in the Keio University School of Medicine. He was awarded a Doctorate of Medical Sciences in Pathology in 1959 and received a post doctoral research fellowship from the National Institutes of Health (NIH) for training at the New York University School of Medicine under Professor Johannes Rhodin in 1960.

After completing his NIH fellowship, he joined the Renal Pathology Division under Professor Churg at the Mount Sinai Hospital School of Medicine where he studied pathology of renal disease in 1961. The following year, he returned to Keio University as a faculty member. He returned to Mount Sinai School of Medicine as a Research Associate working with Dr. Churg in renal pathology and with Dr. Irving Selikoff on asbestos-caused diseases.

In 1973, Dr. Suzuki returned to Japan as Chairman and Professor of Anatomy at Fujita-Gakuen University School of Medicine. He returned to Mount Sinai School of Medicine as a Research Professor of Community Medicine and Research Associate Professor of Pathology. From 1975 to 2006 when he retired, he devoted his time primarily to the investigation of the pathology of asbestos related diseases. He worked with Irving Selikoff and examined slides from pathological autopsy and biopsy material taken from about 5,000 cases of insulation workers and confirmed the diagnosis of asbestos-caused diseases. He was promoted to Professor of Pathology in 1989, and in 1991 to Professor of Community and Preventive Medicine.

He was elected as a Fellow of the Collegium Ramazzini in 1983 and, in 1993 he received the coveted Ramazzini Award for his significant contributions to the scientific knowledge of the pathology of mesotheliomas among asbestos-exposed workers. In 2006, the Collegium Ramazzini again honored Dr. Suzuki with the Irving J. Selikoff Award for his many years of work on diseases caused by asbestos. This award was presented by Professor Philip Landrigan, present Chair of the Department of Community and Preventive Medicine of the Mount Sinai School of Medicine.

During his career, Dr. Suzuki published 171 scientific papers and examined over 538,000 individual slides of tissue from workers who were exposed to asbestos.

The research findings of Dr. Suzuki place him in a select group of scientists dedicated to health in the workplace. This group includes Irving J. Selikoff, Cesare Maltoni, and Bernardino Ramazzini. Dr. Suzuki will long be remembered.

Myron A. Mehlman
Philip J. Landrigan

EUROPEAN JOURNAL OF ONCOLOGY
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