EUROPEAN JOURNAL OF ONCOLOGY

GIORNALE EUROPEO DI ONCOLOGIA

Founded by / Fondato da

Leonardo Caldarola† Turin, Italy/*Torino, Italia*

Cesare Maltoni† Bologna, Italy/*Italia*

Ramazzini Institute
Istituto Ramazzini

Publisher
Mattioli 1885 - srl
Casa Editrice

Autorizzazione del Tribunale di Parma n. 14/97 del 11/6/1997 - ISSN 1128-6598
La testata fruisce dei Contributi Statali diretti di cui alla legge 7 agosto 1990, n. 250

The European Journal of Oncology is indexed by Excerpta Medica (EMBASE), the Elsevier BioBASE, Science Citation Index Expanded (SciSearch®), Journal Citation Report/Science Edition, ISI Web of Science
Il Giornale Europeo di Oncologia è recensito su Excerpta Medica (EMBASE), Elsevier BioBASE Science Citation Index Expanded (SciSearch®), Journal Citation Report/Science Edition, ISI Web of Science

European Journal of Oncology

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

Issue 1 **Bernardino Ramazzini - Biography and Bibliography** ESAURITO

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

Issue 3 **Ban on asbestos in Europe**
C. Bianchi, M. Soffritti, F. Minardi, J.V. Brazier (Editors) - 18 €

Issue 4 **Genetic testing at work - Ethical and legal implications**
K. Van Damme, M. Sorsa, M. Soffritti, F. Minardi, J.V. Brazier (Editors) - 18 €

Issue 5 **Non-thermal effects and mechanism of interaction between elecromagnetic fields and living matter**
L. Giuliani, M. Soffritti - (Editors) - 29 €

title (titolo)

European Journal of Oncology
Giornale Europeo di Oncologia

notes (note)

Issn 1128-6598

Issues/year 4 — *Periodicità* Trimestrale

Language Italian-English — *Lingua* Italiano-Inglese

Subscription rates (prezzi abbonamenti)

	Individual (Privati)			Institution (Istituzioni)	
	Italy	Europe	out of Europe Air Mail	Italy and Europe	out of Europe Air Mail
Annual subscription (Abb. annuo)	45,00 euro	50,00 euro	55,00 euro	55,00 euro	65,00 euro
Back Numbers (Arretrati)					
Single Issue (Numero singolo)	19,00 euro	24,00	28,00 euro	28,00 euro	32,00 euro
Complete Year (Annata compl.)	62,00 euro	78,00	91,00 euro	84,00 euro	106,00 euro

I would like to

☐ subscribe to the "**European Journal of Oncology**" year .....

☐ receive the following issues of "**Ramazzini Library**"
Issue: 2 ☐ 3 ☐ 4 ☐ 5 ☐

please send the issues to:

Mr/Dr/Prof. .....

.....

Address ..... N .....

Town .....

Postal Code ..... Country .....

e-mail .....

Phone/Fax .....

☐ (If italian) Codice Fiscale e Partita Iva (mandatory):

.....

Payment may be made:

☐ by cheque N° .....

☐ by Bank transfer made out to
Mattioli 1885 srl Strada di Lodesana 649/sx, Loc. Vaio,
43036 Fidenza (PR),
Bank: Cassa di Risparmio di Parma e Piacenza, Ag. 3 di Fidenza;
cod. IBAN: IT 39 S 06230 65732 000094186751
cod. BIC: CRPPIT2P487

☐ By credit card: ☐ Visa ☐ Mastercard

Card number ☐☐☐☐ ☐☐☐☐ ☐☐☐☐ ☐☐☐☐

Expiry Date ☐☐ ☐☐ ☐☐ CVV ☐☐☐

Cardholder's name (in capital letters): .....

Signature .....

Mattioli 1885 Casa Editrice
Strada di Lodesana 649/sx, Loc. Vaio
43036 Fidenza (Pr)

Mattioli 1885 Casa Editrice - srl - Strada di Lodesana 649/sx, Loc. Vaio - 43036 Fidenza (Pr) tel 0039/0524/530383 fax 0039/0524/82537 e-mail subscribe@mattioli1885.com
www.mattioli1885.com

EUROPEAN JOURNAL OF ONCOLOGY
GIORNALE EUROPEO DI ONCOLOGIA

CONTENTS/INDICE

VOLUME 16 - N. 3 - 2011

Eur. J. Oncol., vol. 16, n. 3, pp. 129-138, 2011

K-*ras* mutations in tumour specimens and peripheral blood samples of Hungarian patients with pancreatic adenocarcinoma

Mutazioni K-ras nei tessuti tumorali e nei campioni di sangue periferico in pazienti ungheresi affetti da adenocarcinoma pancreatico

Edit Nádasi* Tímea Varjas*, Ida Prantner*, Róbert Papp**, Ghodratollah Nowrasteh*, István Ember*, Dezső Kelemen**

* University of Pécs, Faculty of Medicine, Department of Public Health and Preventive Medicine, Pécs, Hungary
** University of Pécs, Faculty of Medicine, Department of Surgery, Pécs, Hungary

Summary

Aim: **Pancreatic cancer is a deadly disease, with a mortality rate approximately equal to the incidence rate. One prominent feature of the disease is the high frequency of K-*ras* mutations, associated with certain risk factors. Since patients are usually observed at an advanced stage, a less invasive screening test, allowing for early diagnosis, would be of great utility.** ***Patients and Methods:*** **Tumour and blood samples of 28 patients with histologically verified pancreatic adenocarcinoma were screened for K-*ras* mutations and K-*ras* gene expression. Known risk factors of pancreatic cancer were investigated by a questionnaire.** ***Results:*** **Significant relationships were found between polluted environment and K-*ras* gene mutations, alcohol consumption and codon 61 mutations and coffee consumption and codon 12 mutations. Of interest, meat consumption showed a protective effect against codon 61 mutations. Though K-*ras* status of the tumour and blood samples were the same in 71.4% of the**

Riassunto

Scopo: **Il tumore del pancreas è una malattia letale con un tasso di mortalità approssimativamente uguale al tasso di incidenza. Un tratto caratteristico della malattia è l'elevata frequenza delle mutazioni K-*ras* associate a fattori di rischio certi. Poiché di solito i pazienti si presentano ad uno stadio avanzato della malattia, un metodo analitico meno invasivo, che renda possibile una diagnosi precoce, sarebbe di grande utilità.** ***Pazienti e Metodi:*** **Sono stati esaminati campioni di tessuto tumorale e sangue di 28 pazienti con adenocarcinoma pancreatico confermato dagli esami istologici per le mutazioni e l'espressione del gene K-*ras*. I fattori di rischio noti del tumore pancreatico sono stati indagati con un questionario.** ***Risultati:*** **Sono state trovate correlazioni significative tra le mutazioni del gene K-*ras* e l'ambiente inquinato, mutazioni del codone 61 ed il consumo di alcool, mutazioni del codone 12 ed il consumo di caffè. Da notare che il consumo di carne è risultato un fattore protettivo contro le**

Received/Pervenuto 12.4.2011 - Accepted/Accettato 6.7.2011
Address/Indirizzo: Edit Nádasi MD, PhD, H-1225 Budapest, Batthyany u 54. 3/2, Hungary - Tel. +36/20/567-6073 - E-mail: edit.nadasi@gmail.com

cases, no significant relationship between the presence of the K-*ras* mutations and the gene expression were found either in the tumour or the blood samples. *Conclusions:* Our results indicate that K-*ras* mutations are often found in blood samples of patients with pancreatic adenocarcinoma. Due to the small number of patients of the present investigations, further studies are required to assess the relevance of our findings for the clinical practice. Eur. J. Oncol., 16 (3), 129-138, 2011

Key words: **pancreatic cancer, blood sample, K-*ras* mutation, gene expression**

mutazioni del codone 61. Nonostante il profilo delle mutazioni K-*ras* nei campioni di sangue e nei tessuti tumorali sia stato identico nel 71,4% dei casi, la presenza della mutazione e l'espressione del gene non hanno dimostrato una correlazione significativa né nei campioni di sangue, né in quelli tumorali. *Conclusioni:* I nostri risultati indicano che le mutazioni K-*ras* sono spesso presenti nei campioni di sangue di pazienti con adenocarcinoma pancreatico. A causa del basso numero di pazienti in questo studio sono necessarie ulteriori analisi per stabilire l'importanza delle nostre osservazioni nella pratica clinica. Eur. J. Oncol., 16 (3), 129-138, 2011

Parole chiave: **tumore del pancreas, campione di sangue, mutazione K-*ras*, espressione genica**

Introduction

Pancreatic cancer is a deadly disease, being among the ten most frequent malignancies worldwide (1-3). In spite of recent improvements in diagnosis and staging, with a mortality rate approximately equalling the incidence, it accounts for about 5% of all malignant tumours (1). Unfortunately, the symptoms are often vague, and the majority of patients present with advanced disease, which precludes curative therapy (4). Adjuvant therapy currently plays a limited rôle and is clearly ineffective in the majority of patients. Therefore, attention needs to be directed to earlier diagnosis and to the development of novel therapeutic approaches.

One prominent feature of the disease is the high frequency of K-*ras* mutations, associated with smoking, alcohol consumption, diet, and occupational exposure to certain chemicals (3, 5, 6). Since most pancreatic tumours are present at an advanced stage, a less invasive screening test, such as investigation of blood or stool samples, allowing for early diagnosis, would be of great utility. Several studies have already demonstrated that K-*ras* mutations can be found in pancreatic juice samples (7, 8) and plasma/sera of patients diagnosed with pancreatic cancer (9-11), though the origin of these mutations (cancer cells or naked DNA in circulation, general carcinogenic effect) is yet not clear. Therefore, in our study we have investigated 28 patients with histologically verified pancreatic adenocarcinoma for the presence of K-*ras* mutations in tumour tissue samples and peripheral blood samples. Since K-*ras* amplification was shown to negatively correlate to survival in pancreatic cancer patients (12), gene expression was also investigated in a subset of samples in order to determine the correlation of K-*ras* mutations and gene expression found in tumour tissue samples and blood samples of patients with pancreatic adenocarcinoma.

Patients and Methods

Twenty-eight patients (mean age=53.71 years, age range 37-76 years, 17 males and 11 females) with operable, histologically verified pancreatic adenocarcinoma treated between 2002 and 2005 at the Department of Surgery, University of Pécs were selected for the study. The study was approved by the University of Pécs Ethics Committee (registry number: 1601).

Epidemiology/Risk factors

After obtaining their informed consent, patients were asked to fill in a questionnaire regarding age, living circumstances, regular physical activity,

regular medical control, level of education, dietary and smoking habits. Regular alcohol consumption was defined as >30 g/day, regular coffee consumption was defined as ≥3 cups of coffee (>88 mg caffeine) a day, regular tea consumption was defined as ≥3 cups of tea a day, while regular consumption of hot/spicy food, fruits and vegetables, milk, meat and animal fat was defined as consumption of ≥1 serving a day.

Environmental pollution has also been assessed by the questionnaire (living in a big town versus a small city or village, vacationing place, using car/public transportation, feeling the environment polluted). No individual polluting factors have been assessed.

Since the role of chronic pancreatitis in the development of pancreatic cancer is not unambiguous (1), chronic pancreatitis has not been investigated among the risk factors.

DNA- and RNA-extraction

Ten ml of anti-coagulated peripheral blood taken right before the scheduled pancreatic surgery were used for DNA isolation according to Miller *et al* (13). During the surgery, a 5-gram tumour specimen was removed and used for DNA isolation as described elsewhere (14).

For real-time PCR analyses, total cellular RNA was isolated from 20 ml citrate-anti-coagulated peripheral blood taken prior to surgery and from the tumour removed during the operation by using TRIZOL Reagent (GIBCO, Grand Island, NY, USA) (15). RNA concentration and quality check was performed at 260/280 nm.

Mutant allele-specific PCR

Presence of K-*ras* mutations in codons 12, 13 and 61 of the K-*ras* gene were determined by mutant allele-specific PCR reactions using 7 different primer sets as described by Ichii *et al* (16). PCR products were visualized on 2% agarose gel containing 0.5 μg/ml ethidium-bromide. Normal DNA containing no K-*ras* mutations was used as negative control.

Quantitative real time-PCR

Gene expression of the codons 12, 13 and 61 were determined by real-time PCR (RT-PCR) on the 8 tumour samples and 8 corresponding blood samples available for analysis. The following primers were used for quantitative RT-PCR: forward primer 5'-ACT TGT GGT AGT TGG AGC-3' and reverse primer 5'-CTC ATG AAA ATG GTC AGA GAA ACC-3' for codons 12 and 13, and forward primer 5'-TAT TCT CGA CAC AGC Agg-3' and reverse primer 5'-ACT ATA ATT ACT CCT TAA TGT CAG C-3' for codon 61. Hypoxantin guanine phosphorobosil transferase (HGPT) gene expression was used as internal control (forward primer 5'-CAG GAC TGA AAG ACT TGC TC-3' and reverse primer 5'-TCA TAG GAA TGG ACC TAT CAC-3') (17).

Quantitative RT-PCR was performed using the Gene Amp® 5700 Sequence Detection System (Applied Biosystems, CA, USA) according to the manufacturer's instructions. All the primers were designed by Primer Express™ Software (Applied Biosystems, CA, USA) and synthesized by Integrated DNA Technologies. Relative gene expression values were calculated by the comparative threshold cycle (CT) method (MMCT method, Applied Biosystems, CA, USA). The comparative CT method gives the amount of target gene normalized to an endogenous reference gene and to a relative calibrator sample. Mean values and ranges were derived from quantitative results of mRNA gene expression measurement of three independent parallel experiments.

Statistical analysis

Relationships among the different risk factors (investigated by the questionnaire), the presence of K-*ras* mutations and the gene expressions in the tumour and blood samples were investigated through logistic regression.

Results

Epidemiology/Risk factors

Out of the 28 participating patients, 16 reported polluted environment. Thirteen patients lived in a big town so that their living environment was polluted, while the 8 patients living in a village reported no environmental pollution. Three patients living in town reported environmental pollution

while 4 patients being village residents reported no environmental pollution. Thirteen patients had higher education than primary school, another 17 reported regular medical control, while 13 reported regular physical activity. Twelve patients had been regular smokers for more than 5 years and 13 reported being a passive smoker for more than 5 years. Ten patients admitted regular alcohol consumption (>30 g/day), twelve drank more than 3 cups of coffee (>88 mg caffeine) a day, 4 regularly consumed hot-spicy food and 16 regularly used animal fat for cooking (Table 1). Out of the 14 risk factors investigated, the total number of risk factors present per patient varied between 3 and 9 (Table 1).

Table 1 - Risk factors in the 28 pancreatic cancer patients participating the study[a]

No	G	AGE	HB	PE	GP	SA	SP	ALC	COF	TEA	SPT	HSF	FV	MLK	MT	FAT	Total No of risk factors[b]
1	F	45	0	0	1	0	1	1	0	0	0	1	1	1	1	1	6
2	N	57	1	1	0	0	0	0	1	1	0	0	1	1	1	1	7
3	N	76	1	0	0	0	0	0	1	1	0	0	1	1	0	0	4
4	F	55	1	0	1	1	0	0	0	1	0	0	0	1	1	1	6
5	F	37	0	1	0	1	0	0	1	0	0	0	1	1	0	0	4
6	F	56	0	1	1	0	0	0	0	1	0	1	1	1	1	1	6
7	F	44	1	1	1	0	0	0	0	1	1	0	1	1	1	1	6
8	F	41	1	1	0	1	0	0	1	1	0	0	1	1	1	0	7
9	N	51	0	0	0	0	0	0	0	1	0	0	1	1	1	0	3
10	F	51	1	1	1	1	0	0	0	1	1	0	1	1	1	0	6
11	F	43	0	1	0	1	1	1	1	1	0	0	1	1	1	1	9
12	F	63	1	1	1	0	1	0	0	1	0	1	1	1	1	1	8
13	F	46	0	0	0	1	1	1	1	1	1	1	1	1	1	1	9
14	F	64	0	0	0	0	1	0	0	1	0	0	1	0	0	0	2
15	N	74	1	1	0	0	0	0	1	1	0	0	1	1	1	0	6
16	F	72	1	1	0	0	1	1	0	1	1	0	1	1	1	1	8
17	F	64	1	1	0	0	0	1	1	1	0	0	0	0	1	1	7
18	N	70	0	0	0	0	1	0	0	1	1	0	1	1	1	0	4
19	F	40	0	0	0	1	1	0	0	1	1	0	0	1	0	0	4
20	N	48	1	1	1	1	1	0	1	1	0	0	1	1	1	1	9
21	N	54	1	1	1	1	0	1	1	0	1	0	1	1	1	1	8
22	F	46	1	0	1	1	0	1	0	1	1	0	1	0	1	0	5
23	N	37	0	0	0	0	0	1	0	1	1	0	1	0	1	0	3
24	F	57	1	1	0	1	1	0	0	1	1	0	1	1	1	1	8
25	N	51	0	1	0	1	1	0	1	1	1	0	1	1	1	1	8
26	N	47	1	0	1	0	1	1	1	1	0	0	1	1	1	1	8
27	F	60	0	0	0	0	0	1	0	1	1	0	1	1	1	0	4
28	N	55	1	1	1	0	1	0	0	1	1	0	1	1	1	1	7

[a] 1: present, 0: absent, No: patient number, G: gender, AGE: age (yrs), HB: habitat (0: farm/village/small city, 1: town), PE: polluted environment (based on patient's subjective judgement), GP: regular (yearly) check-up by General Practitioner, SA: smoker – active, SP: smoker – passive, ALC: regular alcohol consumption (>30 g/day), COF: regular coffee consumption (>88 mg caffeine/day), TEA: regular tea consumption (≥3 cups of tea/day), SPT: regular (daily) sport/physical activity, HSF: regular (≥1 serving/day) consumption of hot/spicy food, FV: regular (≥1 serving/day) consumption of fruits and vegetables, MLK: regular (≥1 serving/day) milk consumption, MT: regular (≥1 serving/day) meat consumption, FAT: regular (≥1 serving/day) use/consumption of animal (pig) fat.

[b] Numbers are not the sum of numbers seen in the table, since presence of the protective factors (GPC, SPT and FV) have an inverse effect on the total sum.

Mutant allele-specific PCR

Of the 28 pancreatic carcinomas, 15 harbored codon 61 K-*ras* mutation and 9 exhibited codon 12/13 mutation, in 3 cases with simultaneous mutations in codons 61 and 12/13. Altogether, K-*ras* mutation in any of the investigated codons was found in 21 tumour tissue samples (75.0%). Fifteen out of the 28 blood samples contained a codon 61 K-*ras* mutation and 10 samples showed codon 12/13 mutation, with 5 cases exhibiting both codon 61 and codon 12/13 mutations. Altogether, K-*ras* mutation in any of the investigated codons was found in 20 blood samples (71.4%).

Among the 21 tumour samples exhibiting K-*ras* mutations, mutations in codon 61 were the most frequent (15 samples, 71.4% of cases with mutation), 11 harboring a CAA/CAT (Gln/His), and 4 having a CAA/CAC (Gln/His) mutation. Thirteen of the corresponding blood samples (86.7%) harbored the same K-*ras* mutations as the tumour samples, while no mutation could be demonstrated in 2 blood samples. Out of all the samples investigated, 2 cases presented codon 61 mutations in the blood sample with no corresponding mutation in the tumour sample. Nine tumour samples (42.8% of samples with mutation) showed mutation in codon 12, three harbored a GGT/CGT (Gly/Arg), three exhibited a GGT/TGT (Gly/Cys) and three contained a GGT/GAT (Gly/Asp) mutation. Seven of the corresponding blood samples (77.8%) exhibited the same K-*ras* mutation. Out of all the blood samples investigated, in 3 cases (10.7%) the mutation was present without a mutation in the tumour sample. No tumour sample had K-*ras* mutation in codon 13. Three of the cases showed K-*ras* mutation in both codons 12 and 61 in the tumour and blood samples as well (Table 2).

K-*ras* status of the tumour and blood samples was identical in 20 out of the 28 (71.4%) cases, meaning that the corresponding tumour and blood samples showed the same mutation status (present or absent) in all the investigated codons (Table 2). Namely, 4 sample sets exhibited mutations neither in the tumour, nor in the blood samples. Ten cases harbored codon 61 mutations both in the tumour and blood, with no mutations of codon 12/13 in the tumour or blood. Three sample sets contained codon 12/13 mutations in both the tumour and the blood with codon 61 mutation neither in the tumour nor in the blood. In 3 cases both codon 61 and codon 12/13 mutations were found in the tumour and the corresponding blood samples (Table 2).

Quantitative RT-PCR

Only in 8 cases tumour and blood samples available for analysis were matching. Out of the 32 samples, 10 harbored either codon 61 and/or codon 12/13 K-*ras* mutations. In 5 samples an elevated K-ras expression with regard to the control HGPT expression (111.20% - 404.18%) could be demonstrated, while among the 22 samples with no K-ras mutations only 1 sample showed elevated gene expression (425.75%). For 1 case no matching blood sample was available for the analysis of codon 12/13 mutation. No significant relationships between the presence of the K-*ras* mutations and the gene expression were found either in the tumour or the blood samples (p=0.69 and p=0.45 respectively).

Statistical analysis

Mutations in any of the investigated three codons were found in a significantly smaller number among patients who reported non-polluted environment (OR=0.15, CI: 0.03-0.77).

Higher than primary school education, regular medical check-ups and regular physical activity showed no correlation with the presence of any of the investigated K-*ras* mutations (OR=1.2, CI: 0.27-5.25; OR=0.34, CI: 0.07-1.65; and OR=2.33, CI: 0.52-10.48, respectively).

In our series of samples, neither active nor passive smoking were found to have a relationship with the presence of K-*ras* mutations (OR=0.76, CI: 0.16-3.42; and OR=2.4, CI: 0.54-10.69, respectively).

Alcohol consumption has significantly increased the presence of codon 61 mutation (OR=28.38, CI: 1.04-772.55).

Regular consumption of hot-spicy food and cooking with animal fat had no significant effect on the presence of the investigated K-*ras* mutations (OR=0.24, CI: 0.02-2.63 and OR=0.71, CI: 0.15-3.23, respectively).

Table 2 - K-ras mutations and gene expression in the 28 pancreatic cancer patients participating the study[a]

Patient number	Mutation Tumour codon 61	Mutation Blood codon 61	Mutation Tumour codon 12/13	Mutation Blood codon 12/13	RT-PCR Tumour GE codon 61	RT-PCR Blood GE codon 61	RT-PCR Tumour GE codon 12/13	RT-PCR Blood GE codon 12/13
1	0	0	1	0	39.23	425.75	404.18	91.38
2	1	0	0	0	111.212	20.03	0.84	0.85
3	0	1	0	1	0.01	194.53	0.21	317.11
4	0	0	0	1	1.15	2.7	55.48	77.38
5	1	1	0	0	N/A	N/A	N/A	N/A
6	0	0	1	0	N/A	N/A	N/A	N/A
7	0	0	0	0	N/A	N/A	N/A	N/A
8	0	0	0	0	N/A	N/A	N/A	N/A
9	0	0	0	0	0.62	N/A	1.45	N/A
10	1	1	0	0	N/A	N/A	N/A	N/A
11	0	0	0	1	N/A	N/A	N/A	N/A
12	1	1	0	0	N/A	N/A	N/A	N/A
13	0	0	0	0	0.28	0.02	0.21	10.77
14	1	1	0	0	N/A	N/A	N/A	N/A
15	1	1	0	0	N/A	N/A	N/A	N/A
16	1	1	1	1	48.47	40.33	0.18	338.7
17	1	1	0	0	N/A	N/A	N/A	N/A
18	1	1	1	1	N/A	N/A	N/A	N/A
19	0	0	1	1	N/A	8.84	N/A	15.12
20	1	0	0	0	5.42	6.72	1.17	6.45
21	1	1	0	0	N/A	N/A	N/A	N/A
22	1	1	0	0	N/A	N/A	N/A	N/A
23	1	1	1	1	N/A	N/A	N/A	N/A
24	0	0	1	1	N/A	N/A	N/A	N/A
25	1	1	0	0	N/A	N/A	N/A	N/A
26	0	1	1	1	N/A	N/A	N/A	N/A
27	0	0	1	1	N/A	N/A	N/A	N/A
28	1	1	0	0	N/A	N/A	N/A	N/A

[a] GE: relative gene expression as percentage of the expression of control gene (=100%), 1: present, 0: absent, N/A: no data (no sample available for analysis). Cases with identical tumour tissue and blood sample mutation status are marked with grey shading.

Of interest, meat consumption showed a protective effect against codon 61 mutations (OR=0.01, CI: 0.0002-0.9900).

Codon 12 mutations were significantly reduced by limited coffee consumption (OR=0.1, CI: 0.01-0.97).

Regular consumption of tea, vegetable/fruit and dairy products showed no correlation with the presence of the investigated K-*ras* mutations (OR=0.54, CI: 0.04-6.77; OR=0.22, CI: 0.02-2.81; and OR=2.35, CI: 0.23-24.05 respectively).

Discussion

Pancreatic cancer is a devastating disease with poor prognosis and short patient survival following diagnosis. One prominent feature of human pancreatic cancer is the high frequency of K-*ras* mutations at the early stage of the disease. The frequency of K-*ras* mutation has been related to smoking and alcohol consumption in patients with pancreatic cancer (3, 5, 6, 18). It is suspected that other environmental factors, such as diet and occupational

exposure to certain chemicals, are also involved in causing pancreatic cancer (1, 5, 6, 19). Generally, increased risk has been associated with animal protein and fat consumption (5, 20, 21), and decreased risk with intake of vegetables and fruits (3, 5). Since patients are usually observed at an advanced stage, a less invasive screening test, such as investigation of blood samples, allowing for early diagnosis, would be of great utility in selected population at higher risk.

Among our 28 pancreatic cancer tissue samples investigated, mutations of the codon 61 and/or codon 12 of the K-*ras* gene were present in 75.0%; this finding is in accordance with the literature, where the reported K-*ras* mutation rates are between 50% and 93% [50% (22), 56% (23), 70% (24), 79% (25), 90% (26), 93% (27)]. Original studies that examined the presence of K-*ras* mutations in the sera or plasma of patients with pancreatic cancer found that the frequency of K-*ras* mutations were 0% (24), 35% (27), 51% (28), 70% (29, 30), 70.9% (31), and 100% (32) which confirm our findings of 71.4%. Also in agreement with our results, a meta-analysis of 11 studies investigating K-*ras* mutations in colorectal and pancreatic cancer found that between 29% and 100% of patients with a K-*ras* mutation in the tumour presented the same mutation in the peripheral blood (33).

In our series of samples, a significant relationship was found between self-reported polluted environment and the presence of K-*ras* gene mutations in any investigated codon. Though no such statistical analysis has been carried out, self-reported polluted environment showed a correlation with self-reported place of living. Due to the self-reported nature of the data, over- or underestimation of the polluted environment cannot be excluded. According to the literature, environmental factors also play a rôle in causing pancreatic cancer (1, 5, 6, 19).

Alcohol consumption showed a significant correlation with codon 61 mutations while reduced or no coffee consumption had a significant relationship with the absence of codon 12 mutations. These findings are in accordance with the literature, where alcohol consumption was found to be a risk factor for codon 12 K-*ras* mutations (34), while coffee consumption in large quantities is a risk factor of pancreatic cancer (5).

Higher intake of meat and fat is also thought to be associated with an increased risk of pancreatic cancer (20, 21). Of interest, in our series of samples meat consumption showed a protective effect against codon 61 mutations. This unexpected finding may be explained by the fact that N-*nitroso* compounds (present in smoked, grilled or fried meat as well as in cigarette smoke) are prone to cause mutations and aberrant hypermethylation in codon 12 (34).

Most probably due to the low number of samples, no significant relationship between the presence of K-*ras* mutation in the pancreatic tumour tissue sample and the presence of K-*ras* mutation in the corresponding blood sample could be demonstrated in our investigations. Since to our knowledge no study has so far statistically analyzed the correlation between the presence of K-*ras* mutations in the tumour tissue and blood/plasma/serum samples of pancreatic cancer patients, it still remains to be clarified whether our result of no significance is in agreement with other groups' findings. However, the rate of blood samples harbouring the same mutation as the corresponding tissue samples in our investigations (71.4%) is within the range found by other studies [0% (24), 35% (27), 100% (32, 33)]. An original study with a similar sample number that have investigated the direct relationship between the presence of K-*ras* mutation in the pancreatic tissue and corresponding blood samples of patients with pancreas carcinoma found no K-*ras* mutations in the sera of 30 patients with pancreatic cancer (24). However, this study focused only on codon 12 mutations. In samples from 28 pancreatic cancer patients Uemura *et al* found the same K-*ras* mutations in the plasma DNA as in the pancreatic cancer in 9 out of the 26 (35%) K-*ras* mutated pancreatic cancers (27). A review by Sorenson *et al* (28) found that in 40 out of 79 patients (51%) with demonstrated mutated KRAS2 in their pancreatic carcinomas had positive plasma assays for the same mutation. A meta-analysis of 11 studies on colorectal and pancreatic cancer found that 29%-100% of patients with a K-*ras* mutation in the tumour presented the same mutation in the peripheral blood (33). Out of the 11 mentioned studies, only 3 included patients with pancreatic cancer. Mulcahy *et al* examined the plasma of 21 pancreatic cancer patients and found mutant K-*ras* in 17 samples (81%), all in codon 12. Biopsy tissues

were available for 10 patients and plasma and tumour DNA alterations corresponded in each case where K-*ras* mutation was present. The number of the mutant samples out of the 10 cases is not mentioned. No statistical analysis has been performed on the correlation of the tissue and plasma samples' mutation status (35). In a phase I clinical study (36) K-*ras* mutations were detected in 5 out of 11 (45%) pretreatment plasma samples of the enrolled patients and in the matched tumour tissues in 3 out of 4 (75%) patients. No statistical analysis has been performed on the correlation of the tissue and plasma samples' mutation status. Sorenson summarized the results of 144 pancreatic cancer patients, where 79 patients (54.9%) had K-*ras* mutation and of these, 51% had a positive serum/plasma assay for K-*ras* mutations. No statistical analysis has been performed on the correlation of the tissue and plasma samples' mutation status (37). Other studies investigating the K-*ras* mutations of plasma/sera of patients with pancreatic cancer did not control whether the K-*ras* mutations are also present in the pancreatic tumour tissues (29-31).

In our series of samples, no significant relationship between the presence of the K-*ras* mutations and the elevated gene expression were found either in the tumour or the blood samples. This result is not surprising when considering the low number of samples available for RT-PCR analysis. To our knowledge, no investigations have so far evaluated the possible relationships between the presence of K-*ras* mutation either in the pancreatic tumour tissue or in the corresponding blood sample and the K-*ras* gene overexpression. A study of 33 pancreatic cancer patients (12) analyzed the correlation between the K-*ras* gene copy number and survival. According to its results, K-*ras* was amplified in 18 patients (54.5%). However, the study did not investigate whether a K-*ras* mutation was present in these 18 cases, and they did not determine either whether gene amplification resulted in gene overexpression. Therefore, also considering the fact that our samples have not been investigated for the presence of gene amplification, our results on the relationship between the presence of K-*ras* mutations and the gene overexpression may currently not be confirmed or contradicted by any published data. Further investigations with a higher number of samples available for RT-PCR are needed to determine the possible correlation between the presence of K-*ras* mutation and altered gene expression.

Several studies have already been carried out to detect K-*ras* mutations in pancreatic cancer tissue and in peripheral blood samples, and to evaluate the value of K-*ras* mutation as serological marker of pancreatic cancer. Most of these studies have successfully detected K-*ras* mutations in the plasma or sera of pancreatic cancer patients (27-31, 34-36), though not all of them (24). In general, none of these studies proved that K-*ras* mutations of circulating DNA are an applicable stand-alone biologic marker of pancreatic cancer. However, each study was carried out only on a limited number of samples.

Conclusions

Our results indicate that K-*ras* mutation(s) harbored by the pancreatic carcinoma are very often present even in the blood of the patient(s) with pancreatic malignancy and that a less invasive, blood test-based assay may provide diagnostic information for clinicians in the future. Because of the small number of patients in the present study, further investigations on large cohorts are required to assess the exact relevance of our findings for the clinical practice. Consecutive analysis of the K-*ras* mutations origin found in blood samples with the application of an independent marker is also needed to determine whether the mutations are detected in the circulating cancer cells, naked DNA or leukocytes of the patients. The later would indicate a general carcinogenic effect in these patients.

Acknowledgements

The authors thank Zsuzsanna Brunnerné Bayer and Mónika Herczeg for their technical help. The present study was funded by an internal research grant from the University of Pécs (grant No: 114-1878/2000).

References

1. Zalatnai A. Pancreatic cancer a continuing challenge in oncology. Pathol Oncol Res 2003; 9 (4): 252-63.

2. Parker SL, Tong T, Bolden S, *et al*. Cancer statistics, 1996. CA Cancer Journal Clinic 1996; 46: 5-27.
3. van den Brandt PA, Goldboh RA. Nutrition in the prevention of gastrointestinal cancer. Best Pract Res Clin Gastroenterol 2006; 20 (3): 589-603.
4. Howe JR, Conlon KC. The molecular genetics of pancreatic cancer. Surg Oncol 1997; 6 (1): 1-18.
5. Li D, Firozi PF, Zhang W, *et al*. DNA adducts, genetic polymorphisms, and K-*ras* mutation in human pancreatic cancer. Mutat Res 2002; 513: 37-48.
6. Lowenfels AB, Maisonneuve P. Epidemiology and risk factors for pancreatic cancer. Best Pract Res Clin Gastroenterol 2006; 20 (2): 197-209.
7. Tada M, Omata M, Kawai S, *et al*. Detection of *ras* gene mutations in pancreatic juice and peripheral blood of patients with pancreatic adenocarcinoma. Cancer Res 1993; 53 (11): 2472-4.
8. Queneau PE, Adessi GL, Thibault P, *et al*. Early detection of pancreatic cancer in patients with chronic pancreatitis: diagnostic utility of K-*ras* point mutation in the pancreatic juice. Am J Gastroenterol 2001; 96 (3): 700-4.
9. Castells A, Puig P, Mora J, *et al*. K-*ras* mutations in DNA extracted from the plasma of patients with pancreatic carcinoma: diagnostic utility and prognostic significance. J Clin Oncol 1999; 17 (2): 578-84.
10. Theodor L, Melzer E, Sologov M, *et al*. Detection of pancreatic carcinoma – diagnostic value of K-*ras* mutations in circulating DNA from serum. Dig Dis Sci 1999; 44 (10): 2014-9.
11. Mulcahy H, Lyautey J, Lederrey C, *et al*. A prospective study of K-*ras* mutations in the plasma of pancreatic cancer patients. Clin Cancer Res 1998; 4: 271-5.
12. Nagy A, Kozma L, Kiss I, *et al*. Copy number of cancer genes predict tumor grade and survival of pancreatic cancer patients. Anticancer Res 2001; 21 (2B): 1321-5.
13. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleate cells. Nucleic Acid Res 1988; 16 (3): 1215.
14. Sato T, Tanigami A, Yamakawa K, *et al*. Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. Cancer Res 1990; 50: 7184-9.
15. Chomczynski P, Sacchi N. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162: 156-9.
16. Ichii S, Takeda S, Horii A, *et al*. Detailed analysis of genetic alterations in colorectal tumors from patients with and without familial adenomatous polyposis (FAP). Oncogene 1993; 8: 2399-405.
17. Varjas T, Nowrasteh G, Budán F, *et al*. Chemopreventive effect of *Panax ginseng*. Phytother Res 2009; 23 (10): 1399-403.
18. Fryzek JP, Garabrant DH, Schenk M, *et al*. The association between selected risk factors for pancreatic cancer and the expression of *p53* and K-*ras* codon 12 mutations. Int J Gastrointest Canc 2006; 37: 139-45.
19. Soliman AS, Lo AC, Banerjee M, *et al*. Differences in K-ras and p53 gene mutations among pancreatic adenocarcinomas associated with regional environmental pollution. Carcinogenesis 2007; 28 (8): 1794-9.
20. Thiebaut ACM, Jiao L, Silverman DT, *et al*. Dietary fatty acids and pancreatic cancer in the NIH-AARP diet and health study. J Natl Cancer Inst 2009; 101 (14): 1001-11.
21. Ghadirian P, Boyle P, Simard A, *et al*. Reported family agregation of pancreatic cancer within a population-based case-control study in the Francophone community in Montreal, Canada. Int J Pancreatol 1991; 10 (3-4): 183-96.
22. Pabst B, Arps S, Binmoeller K, *et al*. Analysis of K-ras mutations in pancreatic tissue after fine needle aspirates. Anticancer Res 1999; 19 (4A): 2481-3.
23. Roa JC, Anabalón L, Tapia O, *et al*. Frequency of K-ras mutation in biliary and pancreatic tumors. Rev Med Chil 2005; 133 (12): 1434-40.
24. Marchese R, Muleti A, Pasqualetti P, *et al*. Low correspondence between K-ras mutations in pancreatic cancer tissue and detection of K-ras mutations in circulating DNA. Pancreas 2006; 32 (2): 171-7.
25. Ren YX, Xu GM, Li ZS, *et al*. Detection of point mutation in K-ras oncogene at codon 12 in pancreatic diseases. World J Gastroenterol 2004; 10 (6): 881-4.
26. Bos JL. *Ras* oncogene in human cancer: a review. Cancer Res 1989; 49: 4682-9.
27. Uemura T, Hibi K, Kaneko T, *et al*. Detection of K-ras mutations in the plasma DNA of pancreatic cancer patients. J Gastroenterol 2004; 39: 56-60.
28. Sorenson GD. Detection of mutated KRAS2 sequences as tumor markers in plasma/serum of patients with gastrointestinal cancer. Clin Cancer Res 2000; 6: 2129-37.
29. Theodor L, Melzer E, Sologov M, *et al*. Detection of pancreatic carcinoma: diagnostic value of K-ras mutations in circulating DNA from serum. Dig Dis Sci 1999; 44 (10): 2014-9.
30. Theodor L, Melzer E, Sologov M, *et al*. Diagnostic value of K-*ras* mutations in serum of pancreatic cancer patients. Ann N Y Acad Sci 2000; 906: 19-24.
31. Dianxu F, Shengdao Z, Tianguan H, *et al*. A prospective study of detection of pancreatic carcinoma by combined plasma K-ras mutations and serum CA19-9 analysis. Pancreas 2002; 25 (4): 336-41.
32. Sorenson GD, Pribish DM, Valone FH, *et al*. Soluble normal and mutated DNA sequences from single-copy genes in human blood. Cancer Epidemiol Biomarkers Prev 1994; 3: 67-71.

33. Rogosnitzky M, Danks R. Validation of blood testing for K-ras mutations in colorectal and pancreatic cancer. Anticancer Res 2010; 30 (7): 2943-7.
34. Lin Y, Tamakoshi A, Kawamura T, *et al*. An epidemiological overview of environmental and genetic risk factors of pancreatic cancer. Asian Pac J Cancer Prev 2001; 2 (4): 271-80.
35. Mulcahy HE, Lyautey J, Lederrey C, *et al*. Plasma DNA K-*ras* mutations in patients with gastrointestinal malignancies. Ann N Y Acad Sci 2000; 906: 25-8.
36. Olsen CC, Schefter TE, Chen H, *et al*. Results of a phase I trial of 12 patients with locally advanced pancreatic carcinoma combining gefitinib, paclitaxel, and 3-dimensional conformal radiation. Am J Clin Oncol 2009; 32 (2): 115-21.
37. Sorenson GD. A review of studies on the detection of mutated KRAS2 sequences as tumor markers in plasma/serum of patients with gastrointestinal cancer. Ann N Y Acad Sci 2000; 906: 13-6.

Eur. J. Oncol., vol. 16, n. 3, pp. 139-148, 2011

Controlling pesticide poisoning at community level in Lake Eyasi Basin, Karatu District, Tanzania

Controllo dell'avvelenamento da pesticidi nella Comunità del Lago Eyasi Basin, Distretto di Karatu, Tanzania

Aiwerasia V.F. Ngowi, Innocent Semali

Muhimbili University of Health and Allied Sciences, Dar-es-Salaam, Tanzania

Summary

Efforts to increase food production and bring about economic empowerment should not adversely affect public health and the environment. Strategies geared towards increasing farmers' capacity to farm sustainably and productively can greatly enhance both public health and profits. In rural communities in Tanzania, poor pesticide management and hazardous practices are common due to a lack of capacity, economic empowerment, and untrained and uninformed farmers. To improve the health of the Lake Eyasi Basin community, we embarked on a project that aimed at empowering farmers to monitor pesticides health impacts on themselves and the community in general through a community-based surveillance and make decisions that will contribute to the sustainable reduction of the risks of pesticides. The outcome of a three months implementation (April – June 2010) of the project will be shared in this paper. Eur. J. Oncol., 16 (3), 139-148, 2011

Key words: **pesticide poisoning, community self-surveillance, pesticide regulation, obsolete stocks**

Riassunto

Gli sforzi per aumentare la produzione di cibo e per aumentare lo sviluppo economico, non devono avere ricadute negative per la sanità pubblica e l'ambiente. Strategie mirate ad aumentare la propensione degli agricoltori a coltivare in maniera sostenibile e produttiva possono ampiamente migliorare sia la salute pubblica che i profitti. Nelle comunità rurali della Tanzania, l'utilizzo improprio dei pesticidi e le pericolose applicazioni sono comunemente dovute ad una mancanza di capacità, di mano d'opera a buon mercato, e alla presenza di contadini inesperti e disinformati. Per migliorare la salute della Comunità del Lago Eyasi Basin, abbiamo intrapreso un progetto che intende responsabilizzare gli agricoltori nel monitoraggio dell'impatto che hanno i pesticidi non solo sulla loro salute ma anche sulla comunità in generale attraverso una sorveglianza comunitaria di prima istanza e prendendo decisioni volte a ridurre sostanzialmente i rischi dei pesticidi. I risultati di tre mesi di avvio (Aprile – Giugno 2010) del progetto verranno descritti in questo articolo. Eur. J. Oncol., 16 (3), 139-148, 2011

Parole chiave: **avvelenamento da pesticidi, autosorveglianza comunitaria, regolamentazione sui pesticidi, magazzini obsoleti**

Received/Pervenuto 2.5.2011 - Accepted/Accettato 12.7.2011
Address/Indirizzo: Aiwerasia VF Ngowi, PhD Muhimbili University of Health and Allied Sciences, P.O. Box 65015, Dar-es-Salaam, Tanzania

Introduction

Data on the incidence of pesticide poisoning in Tanzania are rare and unreliable (1). This occurs in spite of the increasing trends in import, distribution and application of pesticides in various settings in the country. In parallel, also dearth of information on the structures that could provide data on the quantities, safety, types and intensities of exposure to pesticides at all levels is also present. In poor countries, Tanzania included, an important source of exposures is the agricultural sector, which also employs about 90% of the population. Whereas it is important to initiate strategies to monitor health effects from pesticides exposures to facilitate mitigation of the negative impact of pesticide in poor countries like Tanzania, it is important to ensure the agricultural populations have a high level of awareness and skills on pesticides and its applications.

In addition to agriculture, accidental poisoning and employment in manufacturing and processing industries are also sources of exposures. However, owing to financial constraints and to competing research interests the monitoring of pesticides and their health impacts on farmers and the public in general, which is normally performed by qualified researchers and public health practitioners, is not neglected in many developing countries. The burden of pesticide related illness and injury is therefore difficult to determine since many cases of pesticide poisoning remain undiagnosed and/or unreported (1).

Pesticide poisoning

The World Health Organization (WHO) in the 1990s using hospital based data estimated that around 3 million people (two million suicides, one million accidental) are poisoned each year. However, it is recognized that only a small proportion of poisonings are reported to the health care system (usually severe cases of ingestion from suicide/homicide attempts) and that available figures represent only a small fraction of pesticide poisoning (2). Those few who present themselves for treatment also run the risk of being misdiagnosed because pesticide poisoning may mimic other health problems. On the other hand the mild to moderate pesticide poisoning is less likely to be reported to the health care system due to background ill health, costs, inaccessibility of health services and fear of losing employment in the case of employed workers (3). Therefore, lack of information flow structures leading to underestimated magnitude of pesticide poisoning may be the cause of ineffective pesticide policies (4).

Information flow structures are required at different levels including; national, intermediary and community as well as family which is also where exposures and subsequent impact mostly take place. There exist laws and regulations in respect to pesticide handling and management but enforcement of laws and regulations is in most cases inadequate, due to lack of capacity (human and equipment) and at times political will (1). At community level, participation of farmers is needed to provide adequate data on pesticide types, quantities, exposures, health and environmental impact, safety, and management. It therefore makes more sense to empower communities themselves to monitor the impact of pesticides and to take decisions that might reduce the risks to themselves and to their environment.

Accumulation of obsolete stocks

With about 1200 metric tons of obsolete pesticides stocks, scattered in over 350 stores countrywide, Tanzania is one of the seven countries, including Ethiopia, South Africa, Mali, Morocco, Tunisia and Nigeria, in Africa that are critically affected by accumulation of obsolete pesticides. The National Environment Management Council (NEMC) and collaborators in 1997/98 carried out a countrywide inventory to determine the extent of the problem and found that the obsolete stocks were posing a direct threat to human health and to the environment.

The Africa Stockpiles (ASP) Programme, funded by Global Environmental Facility (GEF) and other multilateral donors, was then initiated with the aims of eliminating publicly held obsolete pesticide stocks and associated waste, and implementing measures to reduce and prevent future accumulation and related risks. According to ASP Tanzania, small-scale farmers are at risk for accumulation small quantities of obsolete pesticides stocks

through agricultural inputs. These small quantities are normally poorly stored and handled and thus pose considerable threat to human health and the environment (1).

Pesticide usage in agriculture and public health in Tanzania has increased substantially over the years making pesticide poisoning a major public health concern (5). It is on record that "the launching of the economic recovery programmes and liberalization of trade in Tanzania resulted in three fold rise of amount of pesticides imported in Tanzania from 500 tons in year 2000 to 1,500 tons in year 2003" (6).

Pesticides suppliers are rewarded with increased earnings based on increased sales and would likely encourage higher pesticides use (7). Pesticide shortages and oversupply may lead to accumulation of stocks at family and community levels that end up becoming obsolete.

Community self-monitoring

In the absence of effective pesticide regulation and lack of infrastructure for controlling the availability and disposal of pesticides, users (farmers) rely on the pesticide suppliers for their choice of products as well as advice for safety in their use. As initial steps to address the problem in Tanzania scientists from the Southern Africa Development Community (SADC) and collaborators from Sweden received funding from Sida, Sweden to run the Work and Health in Southern Africa (WAHSA) programme (8, 9) and started the Action on Health Impact of Pesticides project. Through the pesticides project community capacity-building started in Ngarenanyuki, Tanzania reported elsewhere (10) and now Lake Eyasi Basin. The untimely end of WAHSA led to the start of TAPOHE a non-governmental organization (NGO) that now carries out the community based pesticide surveillance system in Arusha region. In order to improve performance in Lake Eyasi, an assessment was done to document awareness, practices, exposures and health effects among the farming communities. The information will facilitate understanding of the magnitude of exposures as well as strengthening the design and initiation of community-based pesticide surveillance in the area.

Materials and methods

Setting

This study was carried out in Arusha region which is one of the 27 regions of Tanzania with one and a half million inhabitants of whom 80 percent live in rural areas and depend on agricultural activities for their living. The population is located in five districts composed of 120 wards sub-divided into 360 villages. The main occupation of the people is agriculture, followed by animal husbandry and a few do business and mineral prospecting. Arusha region is among the regions with high pesticide use in both horticulture and other food crop farming and has a large number of pesticide retailers (about 111) (6).

Study area

Two Wards located at Lake Eyasi Basin in Karatu district (fig. 1) were selected for the study because extensive farming is performed in the areas, with intensive use of pesticides all year round. Crops grown and treated with pesticides are mostly onions, beans, vegetables (tomatoes, Chinese cabbage, and spinach). Maize and paddy/rice is also grown but not treated that much. Also, the Lake Eyasi community was among the invited stakeholders to a workshop organized through WAHSA project in Ngarenanyuki, Arumeru District, and Arusha Region, where they expressed the need to carry out a community monitoring in their area. The Lake Eyasi representatives at the workshop feared that their situation was worse than in Ngarenanyuki and requested assistance.

Four villages out of six, known to utilize pesticides intensively, were randomly selected from the two wards to participate in the study and these included: Quang'dend, Mbuga Nyekundu, Mang'ola Barazani and Maleckchand. TAPOHE was aiming to establish a community self-surveillance system in the Lake Basin so as to empower the community and help people to decide on safer and more reliable options for pest control.

1. Establishing surveillance system

Establishment of the surveillance system included preliminary discussions with the district authority,

Fig. 1. Map of studied area, Karatu District

ward and village leaders followed with selection of study population and follow up.

Establishment of the trial community based surveillance system included the following:

(a) Preparation of teaching materials

Materials were described in the Farmers Self surveillance system of pesticide poisoning manual by Helen Murphy of FAO in Asia (3). Flip charts and markers were used to draw body maps and write notes. Cut pieces of paper were used to note signs and symptoms, masking tapes for sticking papers on the body map and on the wall. Pesticide products for reading labels were borrowed from retail shops nearby. Forms for self reporting designed by Murphy (3) were translated into Kiswahili and used.

(b) Selection of surveillance population

With the help of village leaders, farmers known to use pesticides were approached and requested to participate in the study to establish the surveillance system. One hundred and sixty agreed to do self-reporting during the entire duration of study (4 months). In addition a management team composed of at least five community members and two school pupils was established in each of the study village. A community member qualified as a team member if she/he lived close to self-reporting farmers and was ready to volunteer.

(c) Training

Before the training, the majority of farmers were unaware of health and environmental impacts of the chemicals they use. They reported different poisoning symptoms that were not associated with pesticide exposure. Most of the farmers did not know the environmental impact of pesticides. The majority of the farmers could not understand what was written on the label. Some read the label to find out the trade name of the product and its expiry date only. After selection of the self-reporting farmers they were invited for a training meeting together with the management teams. The two-day training session was held in each village where the farmers and management teams were introduced to the relationship between health and environment, existing pesticide legislation, health hazards including possible signs, symptoms, direct and indirect costs of pesticides. They were then taken through exercises to explore signs and symptoms of pesticides poisoning using body maps and reading pesticide labels to identify trade and common names. Finally, they practiced how to fill in the data collection form (Form 1). The form asked for basic information about the farmer (name and contact of farmer, gender); spraying activities (spray session, crops sprayed, name of pesticide sprayed, quantity and spray duration) and then signs and symptoms of pesticide poisoning experienced within 24 hours of pesticide handling. The farmers were asked to circle the appropriate sign and symbol.

2. Operation of the pilot surveillance

At the end of the training, each farmer was given blank forms and asked to fill out a form after each time he or she sprays and hand it over to a respective Management team member or deposit it in the Village office. The information that was to be recorded included name; gender (if female specify if pregnant); address; date; spray event number (for the month); crops sprayed; list of pesticides used; number of tanks used, and hours sprayed. Any sign or symptom experienced during or up to 24 hours after spraying had to be circled on the body map that showed 31 potential signs and symptoms associated with pesticide poisoning (any other effects not on the body map could be written in). These signs and symptoms were classified by Murphy (c) as minor (a), moderate (b), or severe (c) as defined below:

(A) Minor: vague, ill defined, or results of the irritant effects of pesticides.

(B) Moderate: clearly defined potential neurological effect resulting from cholinesterase inhibition.

(C) Serious: serious neurological effects (loss of consciousness, seizure).

Reporting and data analysis

At the end of each week, the trained members of the community management teams used the forms collected from the households or village offices and checked the forms for completion, accuracy and consistency, helped the farmers to correct mistakes and summarized the results on weekly basis. Since then an educational pilot data analysis was kept simple, done manually by the management teams that included school pupils, aged 14-18 years. Once each month a community meeting was held with the self-reporting farmers to hear the results of their work. At this time farmers were asked about their experience with the reporting tool and were questioned about signs and symptoms to ensure they understood their meaning well. During the meetings the data were graphed to look for trends and associations. Here we present some of the results of the analysis of data generated during the four months of community self-reporting.

Results and discussion

The study trained 162 adults to use the surveillance tool whose age ranged from 20 to 60 years. Table 1 presents the distribution of the community trainees by village and gender. Number of trainees in the villages was almost equal ranging from 39 to 42.

At the baseline the majority (66.1%) of the 162 farmers who participated in the training could not read a pesticide label and neither could they complete in the self-reporting form. During the training sessions, farmers expressed keen interest in learning how to read the pesticide label and how to recognize the signs and symptoms of pesticides exposure. Farmers in Lake Eyasi Basin rely on pesticides dealers and peers to make decision on what type of pesticides to use, dosage and combination to apply to their crops. The agricultural extensionists were few, one per village with no transport to reach the farmers. The extensionists were also not sensitive to the impact of pesticides to health and the environment as they were basically trained to promote the use of pesticides to enhance agricultural production (11).

Table 1 - Trained Self-reporting farmers and Management team members, L. Eyasi, April 2010

SerNo	Village	Trained individuals*		Totals
		Male	Female	
1	Mbuga Nyekundu	32	8	40
2	Quang'nded	19	22	41
3	Maleckchand	28	14	42
4	Mang'ola Barazani	28	11	39
Total		107	55	162

*Self reporting farmers, community management teams

Pesticide labeling as a hazard communication methods is not effective because three out of four farmers, who are the end users in Lake Eyasi use the label to identify the products recommended to them and their expiry dates and are not familiar with hazard symbols or precautionary statements.

Farmers were shown how to read labels and asked to identify the common names/active ingredients on labels. The exercise was very difficult for most of them. About 55.6% farmers could not identify some common names of pesticides on the label (Table 2). Some came up with names such as "Active Ingre-

Table 2 - Farmers interpretation of trade and corresponding common names on pesticides labels

Trade name	Common name	Common names recorded by farmers
Dimethoate	Dimethoate	Organophosphorus 40% w/w
Dume	Dimethoate	2 PAM AU ATIOPINE
Helarat	Lamdacyahalothrin	Helm
Mukpar Dimethoate 40% EC	Dimethoate	- Organo Phosphorus Insecticide - Insecticide - Prosphiorous
Murphamine 72%	NOT ON 2007 REGISTERED	- Dichlorophenoxy - Amina elective weed - Selective weed killer
Ninja 5EC	Lamda cyahalothrin	- LAMDA - Contains 5%
Ogor	NOT ON 2007 REGISTERED	Emulsifiable concentrate
Profecron	Profenofos	Composition EC 720
Pyrinex 48 EC	Chloropyrifos	CLASS II GLARAJAIL
Rondopaz	Glyphosate	Herbicide - Kiuagugu
Roundup	Glyphosate	HE 150 GUARANTEE
Tafgor 40 EC	NOT ON 2007 REGISTER	Insecticide
Thionex 35 EC	Endosulfan	- Commercial and Agricultural P.D 5/09/E.D 05/11 - Symptomatically
Weedal 480SL	Glyphosate	Active Ingredients

dient, Composition EC 720, Emulsifiable Concentrate" suggesting a language problem. There was also a problem of the labeling format that was not standardized, making it difficult to train farmers on how to recognize the active ingredient or common names on the label. It was even more difficult for the trainers to locate the names that were either presented as Guarantee, Active Ingredient or Common name. When asked about the meaning of pictograms on labels and warning signs the participants (55.6%) also could not interpret them.

Application of pesticides

The types of pesticides used in Lake Eyasi have the potential to cause acute as well as chronic effects (Table 3). That notwithstanding the tools revealed that pesticides handling practices in Lake Eyasi was hazardous. The farmers were applying pesticides mixtures in combinations that were overdoses of insecticides with the potential for high exposures (Table 4). A common combination was Dursban + Selecron + Profectron + Fenom C that was actually chlorpyrifos + profenofos + profenofos + (profenofos + cypermethrin).

Exposure days

Based on the surveillance information, the farmers worked 52 risk days/year, since the majority (73%)

Table 3 - Pesticides reported in use, their WHO classification and health effects in Lake Eyasi Basin, April - July 2010

Trade name	Common name	Type	WHO class	Health effects*
Alpha	unknown	unknown	unknown	
Bayfolatan	unknown	fungicide		
Celcron	profenofos	exp	II	CI
Dip	chlorfenvinphos	acaricide		
Dithane M45	mancozeb	fungicide		SE, C
Dudual	cypermethrin+chlorpyrifos	Insecticide	II + II	SE, PC, CI
Dume	dimethoate	Insecticide		
Dursban	chlorpyrifos	Insecticide	II	CI
Endosulfan	endosulfan	Insecticide	II	SE
Fenom C	profenofos + cypermethrin	Insecticide	II + II	CI, SE, PC
Fenom Plus	profenofos + lamda cyhalothrin	Insecticide	II + II	CI, SE
Galgal	oxyfluorfen	herbicide		
Helarat	lamda cyhalothrin	Insecticide	II	SE
Karate	lamda cyhalothrin	Insecticide	II	SE
Mo-Selecron	profenofos	Insecticide	II	CI
Mukpar	unknown	unknown		
Polytrin	profenofos + cypermethrin	Insecticide	II	CI, SE
Profecron	profenofos	Insecticide	II	CI
Profit	profenofos	Insecticide	II	CI
Selecron	profenofos	Insecticide	II	CI
Suba	unknown	unknown		
Thionex	endosulfan	Insecticide	II	SE

*CI = Cholinesterase Inhibitor; C = Carcinogen; PC = Possible carcinogen; SE = Suspected endocrine disruptor (ILO, 2005)

Table 4 - Pesticide Mixtures commonly applied on onions, L. Eyasi, April – July 2010

Trade name	Common name	Type
Dursban + Selecron + Profectron + Fenom C	Chlorpyrifos + profenofos + profenofos + profenofos + cypermethrin	Insecticide
Thionex + Polytrin + Selecron	Endosulfan + profenofos + cypermethrin + profenofos	Insecticide
Fenom Plus + Profecron + Dursban	profenofos + lamda cyhalothrin + profenofos + chloropyrifos	Insecticide

Children working using sharp tools

Empty pesticides containers disposed in the farms

Containers dropped in the spray mixture

Sprayman fills his pump with container fished out of the spray mix with bare hands

Fig. 2. Hazardous practices in pesticide handling in Lake Eyasi Basin

of 162 farmers applied pesticides once a week and fewer (18%) applied the pesticides twice a week. Spraying at an average of 100 litres per hour for two to 10 hours in a day sprayers tend to risk high exposures. The long duration of spraying coupled with the weather conditions and the spraying frequencies created a high exposure potential. The farmers also showed hazardous practices (fig. 2) that could exacerbate the health and environmental problems

Signs and symptoms reported could be attributed to pesticides exposure (fig. 3). Exhaustion, dizziness, headache, itching were the most common throughout the study period and although they could be caused by other conditions such as malnutrition, malaria and other common ailments. Fig. 4 shows that the spraying and illness episodes follow the same pattern.

Conclusions

This six months study of community self-surveillance capacity showed that the capacity to identify pesticide hazards through community based surveil-

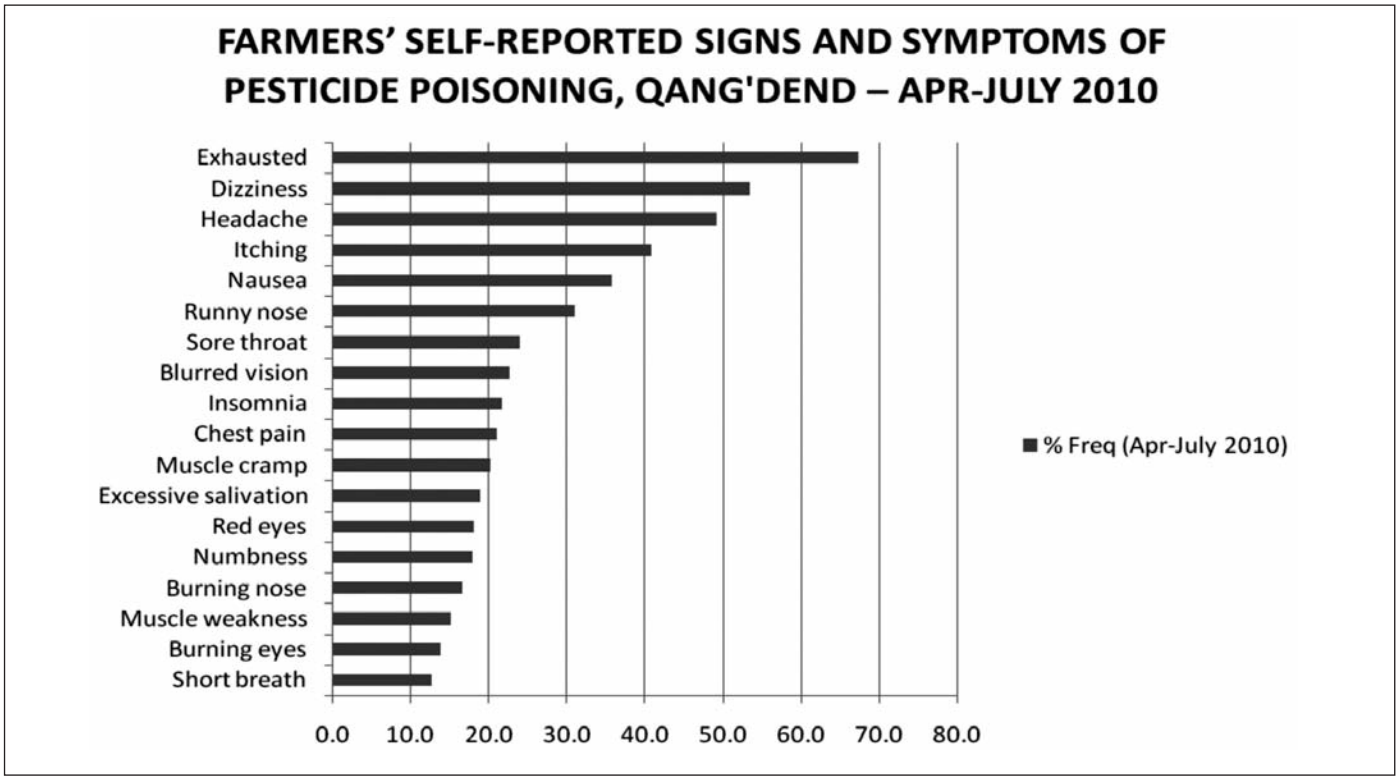

Fig. 3. Signs and symptoms of pesticide poisoning

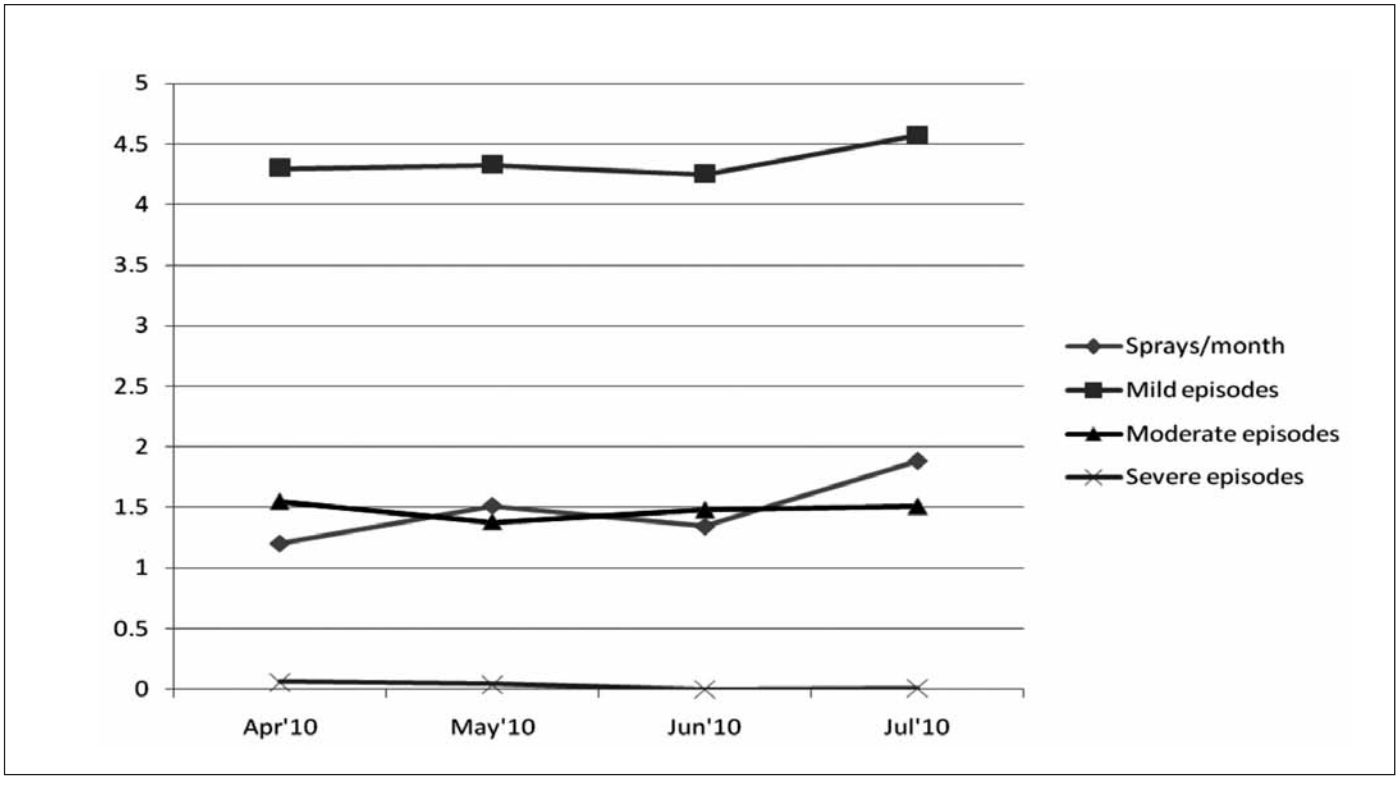

Fig. 4. Average monthly spray event and illness episodes per farmer

lance was low in the Lake Eyasi community and that there was an increased burden of illness due to indiscriminate pesticide use among farmers. Among the contributing factors, the labeling of the pesticide was inadequate: it creates communication hazards to the community since people could not decipher the label prints. On the other hand it revealed a high prevalence of mixing of same type of insecticide or different insecticides in one spray which is seriously hazardous and uneconomical.

A more firm action on pesticide hazards is required to compliment traditional approaches of pesticide legislation and safe use campaigns. The community self-surveillance approach was well received in the community and requires implementation for a longer time and on a wider scale so as to bring about change.

The frequency of self-reported pesticide poisoning signs and symptoms is high hence the need to draw up action plans in an effort to contribute to the reduction of pesticides use and improve awareness creation and campaigns for safe and sustainable pest control in Lake Eyasi Basin. The reporting of moderate and severe signs and symptoms is an indication of serious pesticide exposures.

Acknowledgements

This exercise received financial assistance from the UK Department For International Development (DFID) Civil Society Challenge Fund, 'Civil Society Preventing Obsolete Pesticides in Africa' through Pesticide Action Network (PAN UK). The training materials from the WAHSA and Africa Stockpile Programme (ASP) projects are acknowledged. We also extend our thanks to the District, wards and villages leaders for their assistance, as well as farmers and school pupils and their teachers who agreed to participate in the study. We are also grateful to the agricultural extension staff, Karatu and Lake Eyasi Basin for putting extra effort in organizing the farmers. The Collegium Ramazzini members at the Ramazzini Days in Carpi, October 2010 made the publication of this article possible through their interest when the paper was presented at the meeting.

References

1. Ngowi AVF. Health impact of exposure to pesticides in agriculture in Tanzania. PhD Thesis, Acta Universitatis Tamperensis 890, University of Tampere, 2002.
2. London L, Flisher AJ, Wesseling C, *et al*. Suicide and expoure to organophosphate insecticide: cause and effects. Am J Ind Med 2005; 47: 308-21.
3. Murphy HH, Hoan NP, Matteson P, *et al*. Farmers' self-surveillance of pesticide poisoning: a 12-month pilot in northern Vietnam. Int J Occup Environ Health 2002; 8 (3): 201-11.
4. London L, Bailie R. Challenges for improving surveillance for pesticide poisoning: policy implications for developing countries. Int J of Epidemiol 2001; 30: 564-70.
5. Ngowi AVF, Mbise TJ, Ijani ASM, *et al*. Smallholder vegetable farmers in Northern Tanzania: pesticides use practices, perceptions, cost and health effects. Crop Prot (2007), doi:10.1016/j.croprpro.2007.01.008.
6. AGENDA, Pesticides and Poverty: Case Study on trade and utilization of pesticides in Tanzania: implication to stockpile AGENDA Publication, 2006.
7. Konradsen F, Vander Hoek W, Cole DC, *et al*. Reducing acute poisoning in developing countries. Options for restricting the availability of pesticides. Toxicology 2003; 192: 249-61.
8. Hogstedt C, Elgstrand K, Ryan A, *et al*. The Work and Health in Southern Africa (WAHSA) Programme – overall experiences and the way forward, Occupational Health Southern Africa, Nov. 2009; Vol. 15 WAHSA Special Issue pg 2-6.
9. Rother H-A, Ngowi V, London L. WAHSA Action on Pesticides- building capacity to reduce hazardous pesticide exposures in the SADC. Occupational Health Southern Africa, Nov. 2009; Vol. 15 WAHSA Special Issue pg 36-45.
10. Ngowi AVF, Rongo LMB, Mbise TJ. Community-based monitoring of pesticide impact on Ngarenanyuki, Tanzania, Encyclopedia of Environmental Management. In Press
11. Ngowi AVF, Maeda DN, Partanen TJ. Knowledge, attitudes and practices among extensionists on prevention of health effects of pesticides in coffee and cotton growing areas in Tanzania. Med Lav 2002; 93 (4): 338-46.

Eur. J. Oncol., vol. 16, n. 3, pp. 149-161, 2011

Occupational cancer in the Czech Republic – a tip of the iceberg?

Cancro professionale nella Repubblica Ceca – la punta di un iceberg?

Daniela Pelclova, Zdenka Fenclova, Pavel Urban

Department of Occupational Medicine, 1st Medical Faculty, Charles University and General Teaching Hospital, Prague, Czech Republic

Summary

Aim: **The incidence of malignancies in the Czech Republic shows a long-term increasing trend. Therefore, occupational cancers and their proportion to the total numbers were studied.** ***Methods*****: The incidence and absolute counts of cancers from 1991 until 2008 were received from the National Cancer Registry of the Czech Republic. Statistics of mortality were provided by the Czech Statistical Office. Data concerning occupational cancer, acknowledged in the years 1991-2009 were gained from the database of the National Registry of Occupational Diseases.** ***Results:*** **In the years 1991-2009, a total of 1038 cases of occupational tumors (mean 58/year), were acknowledged in the Czech Republic. Among them, 94.7% involved men with a decreased trend. In the year 1991 out of a total of 47,313 tumors in the Czech Republic, 100 cases of cancer were notified as occupational (0.21%). Finally in 2008 from a total of 77,370 new tumors in the Czech Republic, only 24 new occupational tumors (0.03%) were observed. As a causing**

Riassunto

Finalità**. L'incidenza a lungo termine dei tumori maligni nella Repubblica Ceca mostra un andamento crescente. Pertanto, sono studiati i tumori professionali e la relativa proporzione rispetto al numero di tumori totali.** ***Metodi.*** **L'incidenza ed il numero assoluto dei tumori maligni dal 1991 al 2008 sono stati forniti dal Registro Tumori Nazionale della Repubblica Ceca. Le statistiche sulla mortalità sono state eseguite dall'Ufficio Statistico Ceco. I dati relativi ai tumori professionali relativi agli anni 1991-2009 sono stati acquisiti dal Registro Nazionale delle Malattie Professionali.** ***Risultati*****. Nel periodo 1991-2009 sono stati registrati 1038 casi di tumori professionali (media 58/anno). Tra questi il 94,7% ha coinvolto gli uomini con andamento decrescente. Nel 1991 sono stati registrati 100 casi di tumori professionali (0,21%) su un totale di 47313 tumori totali osservati nella Repubblica Ceca. Infine nel 2008 sono stati registrati solo 24 nuovi casi di tumori professionali (0,03%) su un totale di 77370 nuovi tumori osservati nella Repubblica Ceca. Tra gli**

Received/Pervenuto 4.5.2011 - Accepted/Accettato 8.7.2011
Address/Indirizzo: Prof. Daniela Pelclova, Department of Occupational Medicine, 1st Medical Faculty, Charles University and General Teaching Hospital, Na Bojisti 1, 120 00 Prague, Czech Republic - E-mail: daniela.pelclova@lf1.cuni.cz

agent, the ionizing radiation was the most commonly assigned factor (78.0%); asbestos (13.1%) and chemical agents (8.9%) were less frequent. *Conclusions:* The development in new occupational cancers acknowledged does not follow the trend in the cancer morbidity. Decrease may be caused by the elimination of almost all workplaces in uranium mines and asbestos manufacturing. However, the diagnosis of occupational cancer is not easy and an important factor seems the under estimation of the occupational cause of the tumor. More efficient education of physicians and workers is obviously needed. Eur. J. Oncol., 16 (3), 149-161, 2011

Key words: **occupational cancer, ionizing radiation, asbestos, chemicals, registry of occupational diseases**

agenti eziologici le radiazioni ionizzanti sono quelli osservati con maggiore frequenza (78,0%); l'asbesto (13,1%) e gli agenti chimici (8,9%) sono quelli meno frequenti. *Conclusioni.* L'andamento dei nuovi casi di tumori professionali osservati non segue quello del cancro. La diminuzione potrebbe essere dovuta alla eliminazione di quasi tutte le miniere di uranio e gli impianti di lavorazione dell'asbesto. Tuttavia le diagnosi di tumore professionale non sono semplici e un importante fattore è la tendenza a sottostimare la causa professionale del tumore. È necessaria una più efficiente formazione del personale sanitario e dei lavoratori. Eur. J. Oncol., 16 (3), 149-161, 2011

Parole chiave: **cancro professionale, radiazioni ionizzanti, asbesto, agenti chimici, registro delle malattie occupazionali**

Introduction

The contribution of occupational causes to cancer burden is estimated as about 2-10% (1). In France, cancer deaths were associated with occupation in 1.9-4% of men and 0.3-0.7% of women, depending on the association with their smoking status (mean 2.4% for both sexes) (2). Rushton et al. (3) estimate that occupation causes as much as 8.2% of cancer deaths in men and 2.3% in women with mean cancer incidence of 4.0% for carcinogens group 1 or 2A, according to the International Agency for Research on Cancer (4).

The incidence of malignancies in the Czech Republic shows a long-term increasing trend. In addition, the decrease in standardized mortality is relatively slow in comparison with France and U.S. data (2, 5). Malignant neoplasms represent the second most frequent cause of death, after cardiovascular disorders both in Czech men and women. Therefore, our aim was to compare the numbers and trends in occupational cancers.

The population of the Czech Republic totals about 10 million inhabitants, the working population about 5 million employees. Out of them, about 10% are working at high risk of various hazardous factors. The Registry of persons occupationally exposed to carcinogens (REGEX) contains data about 3,790 exposed persons and 5,253 entries, i.e. several workers are exposed to more than one carcinogen (6).

In the Czech Republic (and in the former Czechoslovakia), the first occupational diseases were collected in 1932, however until 1990, the statistical items were limited. In 1991, the National Registry of Occupational Diseases was founded (7). About twenty items are stored in the Registry on each case of occupational disease. Currently, the Registry contains data on more than 40 thousand cases of occupational diseases. In 2004, the Registry joined EUROSTAT/European Occupational Diseases Statistics (EODS).

In the Czech Republic, the recognition process of occupational diseases is carried out by occupational diseases specialists at the Departments for Occupational Diseases and of industrial hygienists of the Regional Public Health Authorities.

Any physician has to refer a patient whose disease might be related to his/her working activity, to a Department of Occupational Diseases for a qualified assessment whether or not the disease is an occupational one. The patient himself/herself may also contact the Department for consultation and exami-

nation. The patient is always informed where to appeal against the decision in case of the refusal of the claim for an occupational disease.

There may be several reasons for denying the claim to acknowledge the occupational disease. One reason is the absence of the agent on the List of Occupational Diseases (approximately 5% of refused cancers with suspected occupational origin until July 2011), and the second reason may be that the type of tumor and/or organ affected do not correspond to the IARC database (about 30% of refused cancers). Some patients do not even need to come to the Department for Occupational Diseases since the inquiry may be answered without the physical examination.

If the clinical diagnosis corresponds with the noxious agent (noxa), the rejection may result from the low exposure to the ionizing radiation, as proven by the State Institute or Nuclear Safety (50% of refused cancers). Finally, due to a long latency, there may be a difficulty to prove the contact of the patient with the carcinogen (10% of refused cancers). However, the most common reason is the ignorance of the treating physicians about the occupational history and the occupational hazard of their patient. Consequently, in the majority of cancers the occupational origin is never evaluated at the Departments of Occupational Diseases.

According to the Czech legislation, 18 specialized Centers of Occupational Diseases/Occupational Medicine are authorized by the Ministry of Health to acknowledge occupational diseases that are enumerated in the List of Occupational Diseases and meet the prescribed medical and exposure criteria.

For a disease to be recognized, two complementary processes have to occur. The first one deals with the patient's "clinical conditions" and the second one with his/her "hygienic conditions" at work.

As for the "clinical conditions" of the patient, only diseases caused by carcinogens group 1 according to the IARC, affecting organs, documented by the IARC might be acknowledged. As stated in the List of Occupational Diseases, lung cancer due to asbestos could be acknowledged only if associated with either pleural hyalinosis or asbestosis. A detailed study of the medical documentation of the patient is always performed.

In case the "clinical condition" is met, the industrial hygienists of the Regional Public Health Authorities evaluate the "hygienic condition", i.e. occupational exposure, in the relevant time interval prior to the appearance of the disease. For malignancies due to the ionizing radiation, a calculation of the prevailing probability of the causal relationship with the exposure is performed by the State Institute on Nuclear Safety, based on the previous exposure measurements, and data from historical cohorts (8). The worker's inhalational exposure to radon daughters is expressed based on the Working Level units (WL). One WL is defined as any combination of short-lived radon decay products in one liter of air that will result in the ultimate emission of 1.3×10^5 MeV of potential alpha energy. One Working Level Month (WLM) corresponds to 1 WL exposure for 170 hours. Recent pooled analysis of the French and Czech miner cohorts demonstrated statistically significant associations between cumulative radon exposure and lung cancer mortality at levels of exposure as low as 50 WLM (9).

In Czech uranium miners with lung cancers who reached the cumulative exposure of 100 WLM (and were employed until 1970), the condition of sufficient exposure is always fulfilled and the probability of causality is considerably higher than 50%, i.e. sufficient to be acknowledged. In all other miners with a lower and later exposure, the probability must be exactly calculated. The cumulative WLM is multiplied by a factor that considers both the age of the miner, when he entered the mine and the latency period until the diagnosis (9). The probability of causality must reach 50% or more percent. In case, a borderline probability (40-50%) is found, the smoking status of the patient is considered. When the probability is lower than 40%, occupational disease is not acknowledged.

In general, the claim for recognition of an occupational disease is either accepted when both clinical and hygienic results are positive, or refused when any one of the requirements is not fulfilled.

When all the criteria are met, the occupational disease is recognized and compensation granted. Final decisions always fall within the competence of the Department for Occupational Diseases.

The company where the victim last worked under the hazardous working conditions before the disease appeared is responsible for the compensation.

Anyway, all employers have to be insured for the

case of occupational injuries and diseases, and the insurance companies cover almost all expenses for pain and suffering and impaired life capacity of their employees with occupational diseases. The insurance companies do not usually disclaim the acknowledgement of the Departments of Occupational Disease (they sometimes withhold the level of compensation). However, in case the employers do not agree with the decision concerning the occupational disease, similarly as the patients, they both may appeal to the director of the Hospital and to the Committee of Appeal of the Ministry of Health or Regional Health Department.

In total, more than 95% of all occupational diseases acknowledged by the Departments of Occupational diseases are finally also compensated. The Committees of Appeal re-evaluate less than 10% decisions of Departments of Occupational Diseases per year and most of their verdicts confirm the primary decision. A low percentage of cases (around 1-5%) may finally be decided by the court. The whole system is well established, the criteria are well settled, and one advantage is the independence from the side of the employer, employee and insurance companies.

Recognized occupational diseases data are compiled and analyzed by the National Institute of Public Health.

List of Occupational Diseases, governmental regulation no. 290/1995 valid until June 30, 2011 did not allow acknowledging all diseases due to chemical agents, such as silica, cytostatics and their mixtures, similarly as cancer of the larynx due to asbestos dust. On the other hand, any disease due to ionizing and electromagnetic radiation, and biological agents could be compensated, supposing a sufficient proof of the causality provided. New List, governmental regulation no 114/2011, valid from July 1, 2011, extends the possibilities, as shown in Table 1.

Methods

Information on the absolute counts and the incidence of oncological diseases in the Czech Republic was received from the National Cancer Registry of the Czech Republic. Statistics of mortality was provided by the Czech Statistical Office. Last available data was obtained in the year 2008.

Data concerning cancer, acknowledged as an occupational disease in the Czech Republic in the years 1991-2009 were gained from the database of the National Registry of Occupational Diseases at the Center of Occupational Health (www.szu.cz/chpnp/index.php) of the National Institute of Public Health in Prague. The following parameters were analyzed: appointed noxious agent, diagnosis, sex, age, occupation, economy sector and duration of exposure. Since 2004, the latency period was also registered, considering as latency from the first contact with noxious agent to the disease manifestation.

Results

The incidence of malignant neoplasms in men and women in the Czech Republic according to the data from the National Cancer Registry of the Czech Republic in the long term trend has increased, as can be obseved in fig. 1.

At the beginning of the studied period, in the year 1991, from a total of 47,313 tumors in the Czech Republic, 100 cases of cancer were notified as occupational (0.21%). Finally in 2008 from a total of 77,370 new tumors in the Czech Republic, only 24 new occupational tumors were diagnosed (0.03%).

Mild decrease of the standardized mortality can be observed from the second half of the 90ies, especially in men, as shown in fig. 2. However, the absolute mortality did not decrease so rapidly and in 2008, an elevation, related also to the ageing Czech population, was noted. From a total of 27,359 in 2007, total cancer deaths increased to 27,571 subjects in 2008 (1% in men and 0.5% in women).

In the years 1991-2009 a total of 1038 occupational tumors were acknowledged in the Czech Republic (58 cases annually on average). Among them, 986 (94.7%) involved men and 52 (5.3%) women. The numbers in every years gradually decreased, as can be seen observed in fig. 3.

As a causing agent, most commonly assigned factor was ionizing radiation (78.0%), less frequently asbestos (13.1%) and chemical agents (8.9%). No case of cancer, attributable to a biological agent (hepatitis B and C virus) was acknowledged in that time interval. The numbers and years of the notification are shown in Table 2.

Table 1 - Czech List of Occupational Diseases 2011, Governmental regulation n. 114/2011

Exposure criteria are a necessary condition and must be proven for every patient. Basic criteria are also given in the list

Chapter I Conditions caused by chemical agents

1 Conditions caused by lead or its compounds
2 Conditions caused by mercury or its compounds
3 Conditions caused by arsenic or its compounds
4 Conditions caused by antimony or its compounds
5 Conditions caused by berrylium or its compounds
6 Conditions caused by cadmium or its compounds
7 Conditions caused by chromium or its compounds
8 Conditions caused by manganese or its compounds
9 Conditions caused by nickel or its compounds
10 Conditions caused by phosphorus or its inorganic compounds
11 Conditions caused by vanadium or its compounds
12 Conditions caused by fluorine or its compounds
13 Conditions caused by chlorine or its compounds
14 Conditions caused by other halogenated hydrocarbons and their compounds
15 Conditions caused by zinc or its compounds
16 Conditions caused by copper or its compounds
17 Conditions caused by carbon monoxide
18 Conditions caused by nitrogen oxides
19 Conditions caused by sulphur oxides
20 Conditions caused by hydrogen cyanide of cyanides
21 Conditions caused by isocyanates
22 Conditions caused by phosgene
23 Conditions caused by boranes
24 Conditions caused by carbon disulfide
25 Conditions caused by hydrogen sulfide and sulfides
26 Condition caused by amoniak
27 Conditions caused by halogenated hydrocarbons
28 Conditions caused by alifatic and alicyclic hydrocarbons
29 Conditions caused by alcohols
30 Conditions caused by glycols
31 Conditions caused by ethers and ketones
32 Conditions caused by formaldehyde and other alifatic aldehydes
33 Conditions caused by acrylonitrile and other nitriles
34 Conditions caused by alifatic nitroderivatives
35 Conditions caused by benzene
36 Conditions caused by homologues of benzene
37 Conditions caused by naphthalene
38 Conditions caused by vinylbenzene or divinylbenzene
39 Conditions caused by phenols, their homologues or halogenated derivatives
40 Conditions caused by aromatic nitro- or amino compounds
41 Conditions caused by polychlorinated bifenyles, dibenzodioxines and dibenzofuranes
42 Conditions caused by polycyclic aromatic hydrocarbons
43 Conditions caused by synthetic pyrethrines
44 Conditions caused by dipyridiles
45 Conditions caused by carbamates
46 Conditions caused by platinum compounds
47 Conditions caused by thallium or its compounds
48 Conditions caused by baryum or its compounds
49 Conditions caused by tin compounds

(continued)

Table 1 ***(continued)*** **-** Czech List of Occupational Diseases 2011, Governmental regulation n. 114/2011

Exposure criteria are a necessary condition and must be proven for every patient. Basic criteria are also given in the list

50 Conditions caused by selenium and telurium
51 Conditions caused by uranium and it´s compounds
52 Conditions caused by nitric acid esters
53 Conditions caused by inorganic acids
54 Conditions caused by ethylene oxides and other oxiranes
55 Conditions caused by halogenated alkyl ethers or arylethers (bischloromethylether)
56** **Conditions caused by organic acids
57** **Conditions caused by alkalis
58** **Conditions caused by other substances and the mixtures of substances

Chapter II Conditions caused by physical agents

1 Conditions caused by ionizing radiation
2 Conditions caused by electromagnetic radiation
3 Cataract caused by thermal radiation
4 Noise-induced perceptive hearing loss 40% according to Fowler up to 30 years of age, increasing by 1% by 2 years of age, i.e. 50% after 50 years of age
5 Conditions caused by working in hyperbaric or hypobaric conditions
6 Secondary Raynaud's fenomenon (of at least 4 phalanges during cold-provocation test) caused by working with vibrating tools
7 Ischemic and pressure-induced nerve damage due to exposure to vibrations
8 Bones and joints damage due to exposure to vibrations
9 Tendons, tendovaginal, muscles or joints conditions due to physical overload
10 Pressure-induced nerve damage due to physical overload
11 Chronic conditions of the mucous bursae caused by constant pressure
12 Meniscus lesions due to work in the kneeling or crouching position

Chapter III Conditions of the respiratory tract, lungs, pleura and peritoneum

1 Silica-containing dust induced lung diseases (silicosis, coal worker's pneumoconiosis, pneumoconioses with tbc)
1.a with typical radiographic signs starting from p3, q2, r2 (ILO classification)
1.b with active tuberculosis starting from p1, q1, r1
1.c considering the dynamics of the development starting from p2, q1, r1
2 Asbestos dust-related diseases
2.a Asbestosis with radiographic signs starting from s2, t2, u2 (ILO classification)
2.b Pleural hyalinosis with restrictive lung functions impairment
2.c Mesothelioma of the pleura, or of the peritoneum
2.d. Lung cancer or* larynx cancer combined with asbestosis s1, t1, u1 (ILO classification) or with pleural hyalinosis
3 Pneumoconiosis due to the dust in the production or processing of hard metals
4 Welder' s pneumoconiosis with radiographic signs starting from p3, q2, r2 (ILO classification)
5 Conditions due to the inhalation of Co, Sn, Ba, graphite, Al gama oxide, Be, St or TiO2 containing dust
6 Lung cancer due to radioactive substances
7 Malignant neoplasms of the respiratory tract and the lungs caused by crude coke oven gas
8 Cancer of the nasal cavities and sinuses caused by wood dust*
9 Extrinsic allergic alveolitis
10 Bronchial asthma and allergic conditions of the upper airways
11 Conditions of the lower respiratory tract and the lungs caused by raw cotton, tow, hemp or sugarcane dust (byssinosis)
12** **Lung cancer in subjects with silicosis with radiographic signs starting from p3, q2, r2 (ILO classification)
13** **COPD (stage III) in underground black coal miners exposed to 90% a maximum allowed cumulative dose of coal dust, until 2 years after the end of exposure

(continued)

Table 1 ***(continued)*** **-** Czech List of Occupational Diseases 2011, Governmental regulation n. 114/2011

Exposure criteria are a necessary condition and must be proven for every patient. Basic criteria are also given in the list

Chapter IV Skin conditions

1. Skin conditions due to physical, chemical or biological agents

Chapter V Conditions caused by infective agents or parasites

1. Infectious and parasitic diseases
2. Diseases transmitted to human beings from animals
3. Tropical infectious and parasitic diseases

Chapter VI Conditions due to other causes

1. Severe hyperkinetic dysphonia, vocal cords nodes and severe insufficiency of the vocal cords

* New disorders comparing the List of Occupational Diseases n° 290/1995

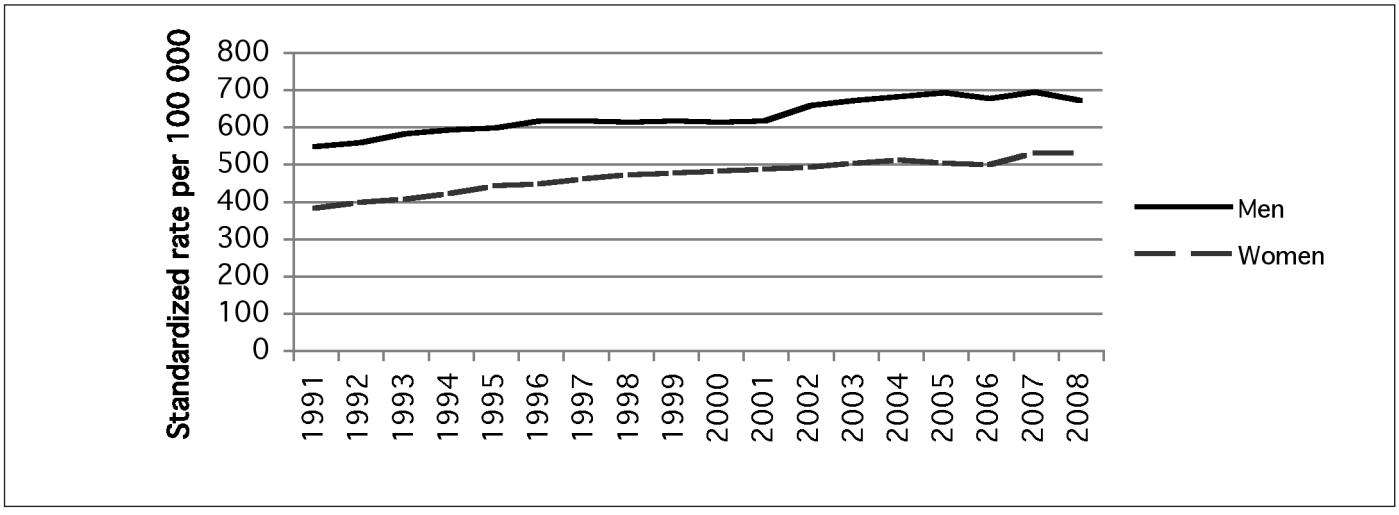

Fig. 1. Incidence of cancer in the Czech Republic in the years 1991-2008

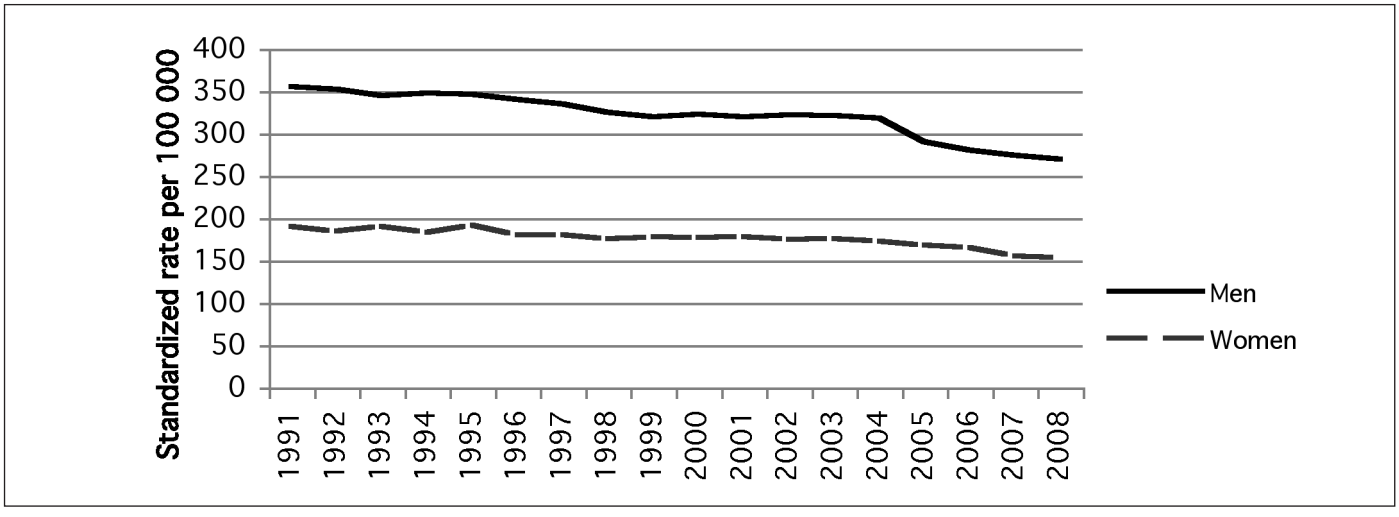

Fig. 2. Mortality due to cancer in the Czech Republic in the years 1991-2008

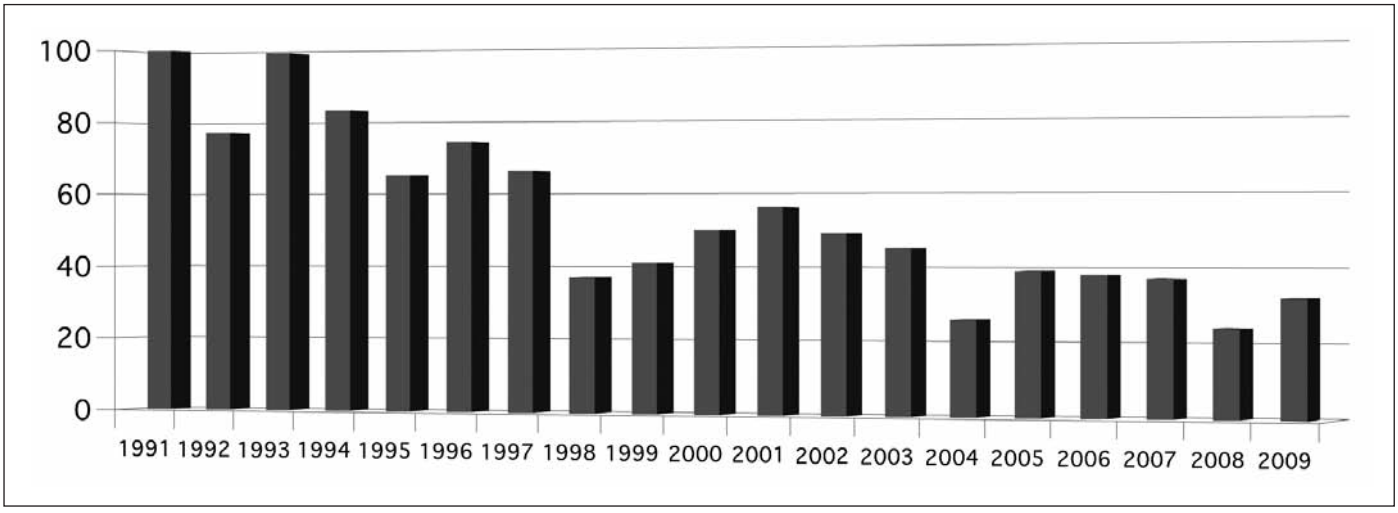

Fig. 3. Occupational cancer in the Czech Republic in the years 1991-2009

Table 3 presents occupational tumors acknowledged in the Czech Republic in the years 1991-2009, according to the organs affected. As can be observed, lung cancer was the prevailing occupational malignancy with 854 cases (82.3%).

Ionizing radiation

The ionizing radiation was the most frequent cause of lung cancer with 764 (89.5%), almost exclusively in men (761 men and 3 women). The highest number, 92 cases, was observed in the year 1993.

Geographical site of the domicile of the patients with occupational lung cancer corresponded to the proximity of uranium mines: 71.9% in Central Bohemia, 15.6% in Western Bohemia and 12.5% in Southern Moravia. Most frequent occupations were in the underground of uranium mines: miner (681x), locksmith (12x), worker (17x), supervisor (10x), electrician (8x), locomotive transport worker (8x), technician (4x), blaster (3x); other jobs were less frequent.

Table 4 presents the mean age of the subjects diagnosed with most frequent occupational cancer due to noxious agents, the mean duration of exposure, and

Table 2 - Noxae attributed to occupational cancer in the Czech Republic in the years 1991-2009

Noxa	1991	1992	1993	1994	1995	1996	1997
Chemicals + wood	9	1	3	3	4	3	11
Ionizing radiation	86	72	92	77	55	67	45
Asbestos	5	4	4	3	6	4	10
Total	100	77	99	83	65	74	66

Noxa	1998	1999	2000	2001	2002	2003	2004
Chemicals + wood	3	3	6	9	6	3	1
Ionizing radiation	27	30	36	33	35	36	18
Asbestos	7	8	8	14	8	6	7
Total	37	41	50	56	49	45	26

Noxa	2005	2006	2007	2008	2009	Total	%
Chemicals + wood	4	7	6	8	2	92	8,9
Ionizing radiation	25	21	22	11	22	810	78,0
Asbestos	10	10	9	5	8	136	13,1
Total	39	38	37	24	32	1038	100,0

Table 3 - Organs affected by occupational cancer in the Czech Republic in the years 1991-2009

ICD	Organ	Noxa	Total
C01	Tongue	PAH	1
C09	Tonsils	PAH 2x, ION	3
C30	Nasal cavity	Oak wood	1
C32	Larynx	ION, 3 chromium, H_2SO_4	
C34	Lungs	ION 764x Asbestos 55x Coke oven gas 18x PAH 11x Chromium 2x Bis(chloromethyl) ether 2x Nickel Cobalt Total	854
C44	Skin	ION 37x, 40 PAH 3x	40
C45	Mesothelioma of pleura 80x, of peritoneum 1x	Asbestos	81
C64	Kidney	PAH 2x, TCDD	3
C67	Bladder	Aromatic amines 33x, PAH 8x	41
C71	Brain	ION	1
C73	Thyroid	TCDD	1
C21	Anorectum	TCDD	1
C65	Kidney pelvis	PAH	1
C92	Leukemia	ION 6x, benzene	7
C	Total		1038

PAH: polycyclic aromatic hydrocarbons, ION: ionizing radiation, TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin

Table 4 - Age, length of exposure and latency in occupational cancer in the Czech Republic in the years 1991-2009

	N	Age (years)		Available data	Exposure (years)		Available data	Latency (years)		Available data
Noxae		median	range	%	median	range	%	median	range	%
Ionizing radiation										
Lung cancer	764	68	36-87	100	9	0.75-38	87.8	52	34-63	14.8
Skin cancer	37	76	45-83	100	13.5	1.6-53.0	100	52	10-58	86.5
Leukemia	6	66.5	52-77	100	11	2-17.3	100	50	47-53	50.0
Asbestos										
Mesothelioma	81	62	33-87	100	18	0.4-43	97.5	39	27-58	39.5
Lung cancer	55	64	47-79	100	19	2.5-49.0	94.5	40.5	18-52	32.7
Chemicals										
All agents	92	59	38-81	100	20	0-08-44.0	98.9	35	10-55	31.5

the latency time since the first exposure (if available).

Skin cancer due to ionizing radiation was diagnosed in 37 subjects, all men. All but one were employees of the uranium mines (19x miner, 9x underground workers, 2x rescue personnel, etc.). Only one person was a radiologist. The body localization of the skin cancer was 17x the head, 12x the thorax, 1x lower extremity, 7x the skin area was not described. Histologically, 30x basalioma, 4x spinalioma were obsered; 3x type was not specified.

Occupational leukemia caused by ionizing radiation was acknowledged in 6 men, 4x acute and 2x chronic myeloid leukemia. Their occupation was 5x miner and 1x electrician in uranium mines.

Asbestos

Mesothelioma has been diagnosed in 81 subjects (50 men and 31 women). Eighty mesotheliomas originated from the pleura, only one from the peritoneum. The most frequent occupation of the victims was a worker in asbestos-manufacturing plant (41x), locksmith (12x), maintenance man (5x), turner of asbestos-cement pipes (4x), electrician (2x), and stove fitter (2x). Other occupations were observed only once.

Distribution of mesotheliomas and lung cancers due to asbestos in the years of diagnosis is shown in fig. 4.

Lung cancer caused by asbestos, acknowledged in 55 subjects, has also been more frequent in men (43x) than in women (12x). More common was the association with asbestosis (45x), than with pleural hyalinosis (10x). Again, the most common occupation was a worker in asbestos-manufacturing plant (25x), locksmith (6x), maintenance man (3x), but also asbestos spinner (3x).

Chemicals

Among 92 subjects, diagnosed with occupational cancer due to chemicals (including wooden dust), only 7 subjects (7.6%) were women. The spectrum and yearly distribution of agents is shown in Table 5. Among aromatic amino compounds, 2-naphthylamine (12x) and benzidine (12x) were more frequently involved.

As regards occupations, in addition to worker (23x) and locksmith (11x), the most frequent jobs were coke worker (11x), chemist (6x), machinist (5x), craftsman (5x), maintenance man (3x), and asphalt worker (2x).

Discussion

The diagnostics of occupational cancer is a very difficult one. In general, in more than half of the cancers, the etiology is unknown (2). Even the epidemiology brings some inconsistent and

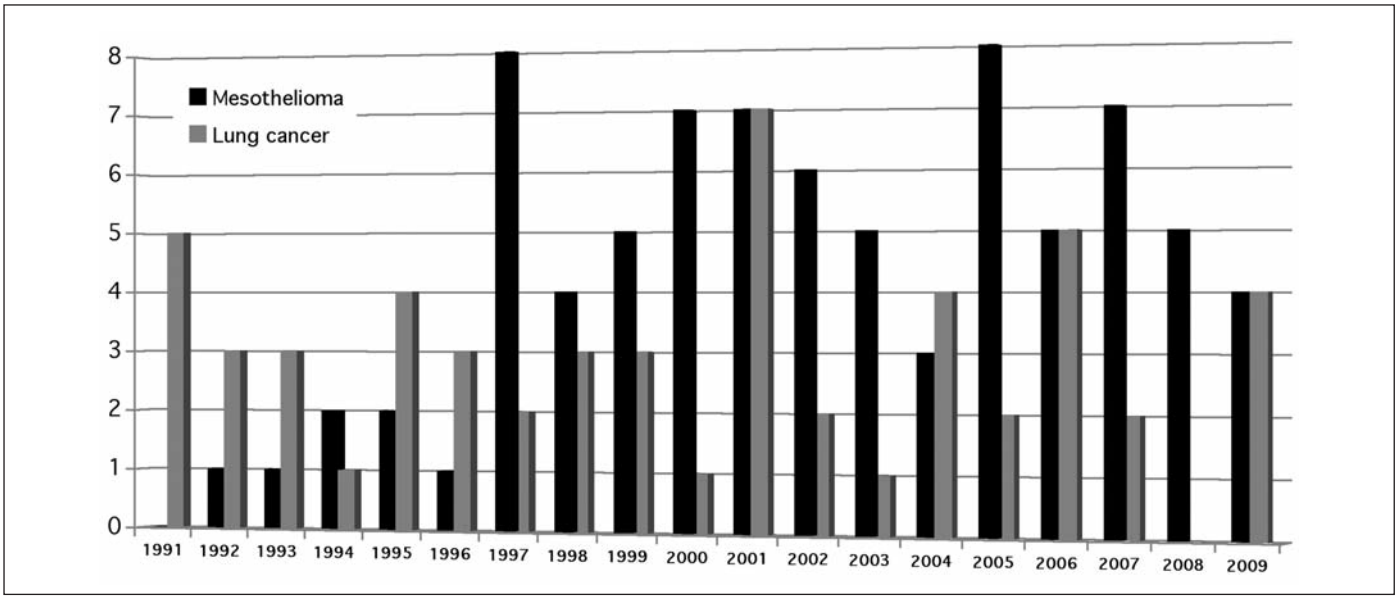

Fig. 4. Occupational tumors due to asbestos in the Czech Republic in the years 1991-2009

Table 5 - Chemical agents and wood dust attributed to occupational cancer in Czech Republic in the years 1991-2009

Agent/Year	Total
Aromatic Amines	33
Polycyclic Aromatic Hydrocarbons	28
Coke Oven Gas	18
2,3,7,8-TDDD*	3
Chromium	3
Bis(chloromethyl) ether	2
H_2SO_4	1
Cobalt	1
Nickel	1
Benzene	1
Wood (oak)	1
Total	92

* 2,3,7,8-tetrachlordibenzo-p-dioxin

misleading evidence that may have impact on approaches to diagnose diseases (10).

The decrease in occupational cancer in the Czech Republic during the past decades may on one side positively reflect the closing of hazardous workplaces several years ago.

Uranium production in the Czech Republic amounted to about 110 thousand tons which placed the country in 2004 to the 7th place in the world (11). The largest employment in the uranium mining was in the 1955, with about 47 thousand employees, including political prisoners of the communist regime, working and living in extremely hard conditions. Six out of seven uranium mines have been closed in the years 1962-1996 in the Czech Republic (11) and only one mine in Southern Moravia is still working with about 200 at risk workers (6).

Asbestos manufacture in the Czech Republic has been substantially depressed in the year 1999 and completely banned in 2007. Anyway, demolition and removal of asbestos will continue on the long term basis to be the source of occupational asbestos exposure (12), and REGEX still registers about 60 workers. The proportion of Czech women with occupational mesothelioma was very high, especially when comparing with the incidence in the Czech population, which is slightly higher in men than in women (0.4/100,000 and 0.3/100,000 in the year 2008, respectively). The male to female ratio of mesothelioma incidence in the US population was about 5:1 in men and women (13), and 2.6:1 in Japan (14). The reason for it is unknown, and may be related to the type of occupation with a high proportion of women, such as asbestos textile manufacture and asbestos-cement tiles production, and no asbestos mining in the Czech Republic.

However, the proportion of occupational cancer in Czech Republic seems considerably underreported. If the attributable fraction of 2.4% (2) would be applied to the Czech Republic, a total of 1861 occupational cancers in 2008 would be expected, instead of only 24 cases registered.

Obviously, several other factors may be responsible for this discrepancy.

In the Czech Republic, only established carcinogens of the IARC group 1 are attributable to occupational cancer. Additionally, the proof of adequate exposure must be given for every patient, which is not always feasible. Due to the long latency between exposure and appearance of the malignancies, including mesothelioma, a difficulty appears in order to prove exposure by the Public Health Authorities for occupations, where contact with asbestos is not a requisite part of the work, such as locksmith, maintenance man, etc., especially in case the employer no longer exists.

This discrepancy is the most obvious in mesothelioma, where the cause is almost explicit. The incidence of mesothelioma in the Czech population is about 0.5/100.000 inhabitants; however, less than 10% of these malignancies have been compensated as an occupational disease (15).

Interestingly, the proportion of lung cancer to mesothelioma two-thirds to one, as brought by Darnton (16), is in good agreement with the Czech data of compensated subjects. More commonly associated lung cancer with asbestosis than with hyalinosis may relate to a higher exposure of subjects with asbestosis. Another explanation may be the neglecting of the importance of smaller asbestos-induced hyalinosis by the radiologists for the cause finding and compensation.

However, the main reason for the low numbers of occupational cancer seems to be the low awareness of both general practitioners and specialists, not taking a detailed occupational history of their patients, especially pensioners. Similarly, the subjects themselves have not been clearly informed about possible symptoms and health consequences

of their occupations.

Additionally, the cause may be administrative, since the Czech List of Occupational Diseases (1995) did not enable to acknowledge some malignances. It has recently been updated by a new Governmental regulation no. 114/2011, valid starting July 1, 2011 (Table 1). As concerns occupational cancer, several extensions of the list have been added, the main are the following ones: 1) any disease due to chemical agents and their mixtures, 2) lung cancer in the patients with silicosis (17), and 3) cancer of the larynx following the inhalation of asbestos dust. These changes might lead to a small increase of the numbers in the future.

Three malignant tumors (kidney, thyroid, and anorectum) were attributed to 2,3,7,8-tetrachlordibenzo-*p*-dioxin (TCDD) related to exposure in the years 1965-1968 as a consequence of the production of the butylester of 2,4,5-trichlorophenoxyacetic acid, where TCDD originated as an intermediate product (18). Recent findings support the classification of IARC from 1997 as established carcinogen group 1, with increased probability of any cancer type (19).

Vinyl chloride monomer exposure started in the Czech Republic in 1975; currently, about 70 workers are registered in REGEX. No angiosarcoma of the liver has yet been diagnosed in the workers as the air concentrations have been maintained at a low level.

Hard wood exposure was registered in 130 workers, however only one case of an occupational cancer was acknowledged in this traditional exposure. The low number points to the probable underestimation and low awareness of the risk. Similarly, no case of liver cancer due to hepatitis virus B or C was acknowledged, although the list enabled this.

Rather surprising is the long latency period of about 50 years for tumors attributed to ionizing radiation, including 6 leukemias. The low number of leukemias might be the explanation. On the other hand, the median latency for asbestos-induced diseases was shorter, around 40 years. Obviously, an increase in those tumors due to their long latencies can be expected in the next decades.

What trends in occupational cancer can we expect in the future? Data from REGEX show that at present, the most frequent exposure to carcinogens is in the healthcare, where about 1650 nurses and other healthcare workers are exposed to cytostatics. Until 2011, cancer due to cytostatics could not be compensated as it was not included in the listed 55 groups of chemical agents. Nowadays, occupational cancer due to any pharmaceuticals could be acknowledged, however much uncertainty exists concerning the type of cancer and exposure, especially due to the changing spectrum of cytostatics used.

Benzene exposure is the second most common chemical carcinogen with about 800 registered workers, work in steel foundries with more than 530, coke production with about 300, and gas production with about 220 employees at risk. Exposure to benzo(a)pyrene was registered in about 130 workers and cadmium exposure in about 190 workers.

As to other chemicals, the majority of subjects is exposed to one carcinogen (3,854 subjects): exposure to 2 agents was registered in 498 subjects (mostly cytostatics + ionizing radiation), in 102 subjects to 3 agents, in 76 subjects to 4 agents (6).

Of course, new sources of exposure may come into question, as, for example, little is known about the effects of nanoparticles in humans (20, 21). Therefore, tracing new occupational disorders and presentation of suspected associations appears extremely important (22).

Conclusion

Statistical data show that the incidence of malignancies in the Czech general population is increasing, while the incidence of the registered cases of occupational cancer in on decrease. This discrepancy has multiple causes. On the one hand, the decrease in the proportion of occupational malignancies to the general oncological morbidity is relative, connected with advances made in the diagnostics, among others with the use of screening methods capable of detecting incipient, clinically silent cases, which inflates the total cancer morbidity. On the other hand, the numbers of the registered occupational malignancies are definitely underestimated.

The diagnosis of an occupational cancer is extremely difficult. Our knowledge of causal associations between exposure to potential carcinogens and subsequent malignancies is imperfect. Nowadays compensated cases apparently represent only a

tip of the iceberg. Although new evidence is continuously introduced; we will probably never know the full range of agents that can cause cancer.

However, even if a particular causal relation has been proved, the awareness of such a possibility is low, not only in workers but also in physicians providing occupational health services. Moreover, in the Czech Republic, an occupational cancer can be acknowledged only if the causal agent has been included in the List of occupational diseases.

Occupational cancer belongs to at least partially preventable diseases. Therefore, the primary prevention of occupational cancer occupies a priority position in the health care programs of industrially developed countries. Doll and Peto estimated that in the Great Britain the occupational etiological fraction represents about 2% (with a range of 1-5%) of all malignancies and they suggested that almost 1% is avoidable by practicable ways (23). The occupational cancer burden appears sufficiently high to justify all efforts to prevent this type of exposure.

Acknowledgements

Supported by the grant MSM0021620807.

References

1. Rugo HS, Fischman ML. Occupational cancer. In LaDou J. Occupational and Environmental medicine. III Ed, New York: McGraw-Hill Comp, Inc, 2004, 229-68.
2. Boffetta P, Tubiana M, Hill C, *et al.* The causes of cancer in France. Ann Oncol 2009; 20: 550-5.
3. Rushton L, Bagga S, Bevan R, *et al.* Occupation and cancer in Britain. Br J Cancer 2010; 102: 1428-37.
4. International Agency for Research on Cancer. IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans. Vol. 1-91, Lyon, France: IARC 1971-2007, http://monographs.iarc.fr (31 July 2011 last assessed)
5. Clapp RW, Jacobs MM, Loechler EL. Environmental and occupational causes of cancer: new evidence 2005-2007. Rev Environ Health 2008; 23: 1-37.
6. REGEX Registr profesionálních expozic karcinogenům http://www.szu.cz/publikace/registr-profesionalnich-expozic-karcinogenum (1 August 2011 last assessed)
7. Urban P, Cikrt M, Hejlek A, *et al.* The Czech National Registry of Occupational Diseases. Ten years of existence. Cent Eur J Public Health 2000; 8: 210-2.
8. Tomášek L, Swerdlow AJ, Darby SC, *et al*. Mortality in uranium miners in west Bohemia: a long-term cohort study. Occup Environ Med 1994; 51: 308-15.
9. Tomášek L, Rogel A, Tirmarche M, *et al*. Lung cancer in French and Czech 1246 uranium miners – risk at low exposure rates and modifying effects of time since exposure and age at exposure. Radiat Res 2008; 169: 125-37.
10. Boffetta P, McLaughlin JK, La Vecchia C, *et al.* False-positive results in cancer epidemiology: a plea for epistemological modesty. J Natl Cancer Inst 2008; 100: 988-95.
11. Proatom http://proatom.luksoft.cz (31 July 2011 last assessed)
12. Collegium Ramazzini. Asbestos is still with us: repeat call for a universal ban. Eur J Oncol 2010; 15: 69-75.
13. Weill H, Hughes JM, Churg AM. Changing trends in US mesothelioma incidence. Occup Environ Med 2004; 61: 438-41.
14. Kanazawa N, Ioka A, Tsukuma H, *et al.* Incidence and survival of mesothelioma in Osaka, Japan. Jpn J Clin Oncol 2006; 36: 254-7.
15. Pelclová D, Fenclová Z, Urban P. Asbestos exposure, legislation and diseases in the Czech Republic. Cent Eur J Public Health 2007; 15: 99-102.
16. Darnton AJ, McElvenny DM, Hodgson JT. Estimating the number of asbestos-related lung cancer deaths in Great Britain. Ann Occup Hyg 2006; 50: 29-38.
17. Pelucchi C, Pira E, Piolatto G, *et al.* Occupational silica exposure and lung cancer risk: a review of epidemiological studies 1996-2005. Ann Oncol 2006; 17: 1039-50.
18. Pelclová D, Urban P, Preiss J, *et al.* Adverse health effects in humans exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Rev Environ Health 2006; 21: 119-38.
19. Steenland K, Bertazzi P, Baccarelli A, *et al.* Dioxin revisited: developments since the 1997 IARC classification of dioxin as a human carcinogen. Environ Health Perspect 2004; 112: 1265-8.
20. Castranova V. Overview of current toxicological knowledge of engineered nanoparticles. J Occup Environ Med 2011; 53: S14-7.
21. Van der Laan G. Tracing new occupational diseases in nano-workers. J Biomed Nanotechnol 2011; 7: 18.
22. Bonneterre V, Faisandier L, Bicout D, *et al.* Programmed health surveillance and detection of emerging diseases in occupational health: contribution of the French national occupational disease surveillance and prevention network (RNV3P). Occup Environ Med 2010; 67: 178-86
23. Doll R, Peto R. Epidemiology of cancer. In Warren DA, Cox TM, Firth JD (Eds), Oxford Textbook of Medicine, I Ed, Oxford: Oxford University Press, 2005:193-218.

Eur. J. Oncol., vol. 16, n. 3, pp. 163-169, 2011

Struma ovarii. Case report and revision of the literature

Struma ovarii. Caso clinico e revisione della letteratura

Attilio Ignazio Lo Monte*, Giuseppe Damiano*, Carmelo Zumbino*, Vincenzo Davide Palumbo*, Maurizio Bellavia*, Gabriele Spinelli*, Maria Concetta Gioviale*, Gaspare Cucinella**, Renato Venezia**, Antonino Perino**, Giuseppe Buscemi*

* Dipartimento di Discipline Chirurgiche ed Oncologiche (DICHIRON), Università degli Studi di Palermo, Palermo, Italy
** Dipartimento Materno Infantile Facoltà di Medicina e Chirurgia, Università degli Studi di Palermo, Palermo, Italy

Summary

Struma ovarii is a rare form of ovarian neoplasm, entirely or predominantly composed of thyroid tissue. This tumour generally has a benign biological behavior, although some cases of malignant transformation have been reported (5-10%). The tumour usually presents as an asymptomatic mature mass that may measure as much as 10 cm in diameter. The presence of ascites is possible (15-20%), and in a few cases the association of ascites and hydrothorax has been recorded (Pseudo-Meigs Syndrome); a close relationship between ascites and high levels of Ca-125, that may wrongly induce to a diagnosis of ovarian carcinoma is present. Other possible clinical consequences are compressive symptoms on adjacent structures and symptoms of estrogenic-progestinal hyperproduction such as menometrorrhagia, dysmenorrhoea, infertility, and skin rash.

Riassunto

Lo struma ovarii è una rara forma di neoplasia ovarica, composta interamente o prevalentemente da tessuto tiroideo. Questo tumore presenta, generalmente, un comportamento biologico benigno, nonostante siano stati riportati alcuni casi di trasformazione maligna (5-10%). Il tumore di solito si presenta con una massa matura e asintomatica che può raggiungere il diametro di 10 cm. È possibile la presenza di ascite (15-20%), e in qualche caso è stata riportata l'associazione di ascite e idrotorace (Sindrome Pseudo-Meigs). Vi è una stretta correlazione fra ascite e alti livelli di Ca-125, che possono erroneamente indurre alla diagnosi di carcinoma ovarico. Altre possibili conseguenze cliniche sono i sintomi compressivi sulle strutture adiacenti e i sintomi di iperproduzione estro-progestinica, come menometrorragia, dismenorrea, infertilità e rash cutaneo. Riportia-

Received/Pervenuto 10.6.2011 - Accepted/Accettato 23.8.2011
Address/Indirizzo: Attilio Ignazio Lo Monte, Associate Professor of General Surgery, Dipartimento di Discipline Chirurgiche ed Oncologiche (DICHIRON), Università degli Studi di Palermo - Facoltà di Medicina e Chirurgia, Via del Vespro 129 - 90127 Palermo (Italy)
Tel. +39 091 655 26 66 - Fax +39 091 655 28 36 - E mail: ailomonte@unipa.it

Authors contributions: AILM, MCG and CZ contributed to the conception of the work. Data were collected by GC, RV, MB, GS and AP. CM contributed in the critical revision of the intellectual contents. GD and VDP were involved in drafting the manuscript and revising the literature. GB and AILM gave the final approval of the version to be published. All authors commented on the manuscript, data and conclusions before submission.

Here we report the case of a young woman presenting, at her admission, a voluminous abdomino-pelvic mass (max. diam. 30 cm), histologically represented by a mature teratoma prevalently composed of thyroid tissue. Eur. J. Oncol., 16 (3), 163-169, 2011

Key words: **struma ovarii, ovarian germinal tumours, hyperthyroidism, mature cystic teratoma**

mo il caso di una giovane donna che presentava, al momento del ricovero, una voluminosa massa addomino-pelvica (diam. max. 30 cm) istologicamente rappresentata da un teratoma maturo con predominanza, nel suo contesto, di tessuto tiroideo. Eur. J. Oncol., 16 (2), 163-169, 2011

Parole chiave: **struma ovarii, tumori germinali dell'ovaio, ipertiroidismo, teratoma cistico maturo**

Introduction

The struma ovarii is a rare form of ovarian neoplasm, representing less than 3% of all other forms of teratoma, entirely or predominantly composed of thyroid tissue (1). Described for the first time by Von Kalden in 1895 (2-3), the struma ovarii is histologically classified among mature teratomas, and thus among tumours that arise from ovarian germ cells (Table 1) (4). This tumour has usually, a benign biological behavior and, despite its possible carcinomatous transformation, reported in 5-10% of cases, the risk of metastatic diffusion is rather low (5). Most patients undergo surgery after the detection of a pelvic neoplasm, often incidentally diagnosed or as a consequence of compressive symptoms on the adjacent structures (6). In fact, these tumours are usually clinically silent, until they reach or exceed 10 cm in maximum diameter. Possible symptoms may be usually related to ascites (15-20%), whose formation is probably linked to three different mechanisms: compression exerted by the mass on abdomino-pelvic lymphatic vessels; partial torsion of the cystic peduncle around its axis; peritoneal reaction to the progressive development of the neoplasia (7). The presence of ascites may be associated with hydrothorax, making out the so-called Pseudo-Meigs Syndrome. In this case, the pleural effusion can be explained by the passage of ascitic liquid from the abdominal cavity to the pleural space through the trans-diaphragmatic abdomino-thoracic connections, or through the draining action exerted by the abdomino-thoracic lymphatic vessels. Furthermore, an ovarian cancer might be often wrongly suspected because of the presence of elevated serum levels of Ca-125, whose values are related to the presence of ascites and are rapidly halved after the removal of the lesion (8). Moreover, it is also possible to record a symptom complex characterized by menometrorrhagia, dysmenorrhoea, infertility, and skin rash, associated with hormonal estro-progestinic hyperproduction. Despite the presence of thyroid tissue, symptoms and signs of hyperthyroidism are present only in 5% of cases (7-9).

Table 1 - Ovarian germinal tumours

- Dysgerminoma;
- Endodermal sinus tumour;
- Embryonal carcinoma;
- Polyembrioma;
- Choriocarcinoma;
- Gonadoblastoma;
- Mixed forms;
- Teratoma:
  1. Immature;
  2. Mature:
     - Solid
     - Cystic
       a) Dermoid cyst
       b) Dermoid cyst with malignant transformation
  3. Monodermal:
     - Struma Ovarii
     - Carcinoid
     - Struma Ovarii and Carcinoid

Case report

We report the case of a 33-year-old woman who referred, at her admission, a progressive abdominal

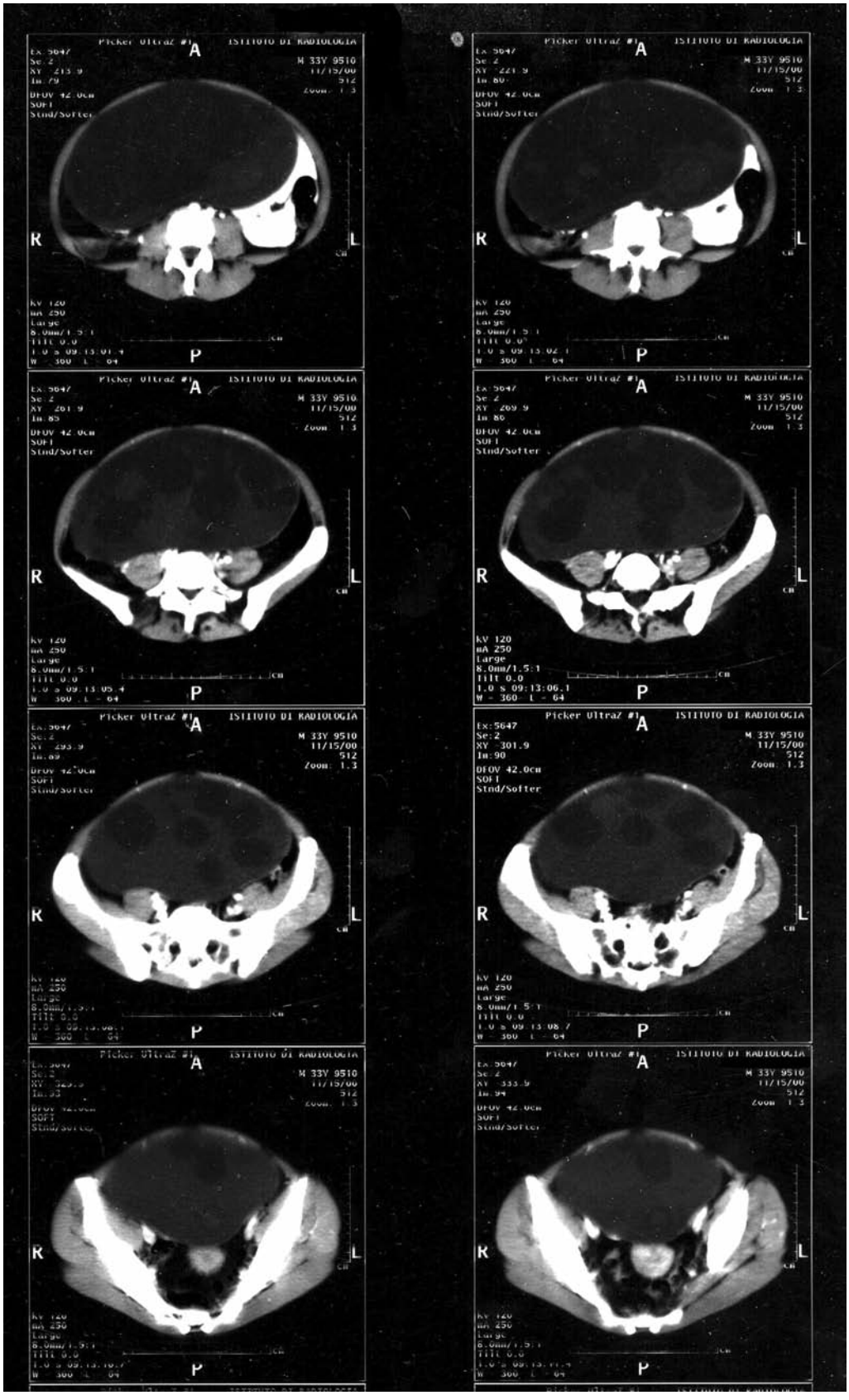

Fig. 1. Contrast enhanced Computerized Tomography of the lower abdomen. Images show a plurichambered and well encapsulated oval neoformation (transverse diameter 23 cm, cephalo-caudal diameter 29 cm), in close relations of contiguity with jejunum-ileo-colic ansae, uterus and bladder, the presence of free effusion in the abdominal cavity, and the absence of volumetric increase in the abdominal-pelvic lymph node stations

volumetric increase dating back to the previous three months. The patient suffered from constipation and occasional abdominal colic pain. Only ten days before admission, the patient reported ingravescent abdominal pain and dysuria. Local objective examination clearly revealed the presence of a voluminous neoplasm with abdomino-pelvic extension, parenchymatous consistency, and undefined margins. The mass was neither aching nor tender, it was mobile with respect to planes above, and it took up all abdominal quadrants. Routine preoperative laboratory tests showed values within normal limits, whereas the oncologic markers pointed out the presence of high levels of Ca-125 (1,570 U/mL vs 0-35 U/ml normal value). Imaging (abdomen and pelvis ultrasonography, abdominal computerized tomography) showed a plurichambered and well encapsulated oval neoplasm (transverse diameter 23 cm, cephalo-caudal diameter 29 cm), in close relation of contiguity with jejunum-ileo-colic ansae, uterus and bladder, and the presence of free effusion in the abdominal cavity; abdomino-pelvic lymph node stations resulted exempt from the illness (fig. 1). The patient underwent the surgical intervention: the median xifo-umbilical-pubic laparotomy was followed by the simple mobilization of the voluminous neoformation from the abdominal cavity, thanks to the presence of a clear cleavage plane between the mass and the adjacent structures (fig. 2). The right adnexal origin of the neoformation was evident; so a right salpingo-oophorectomy was carried out, allowing the complete removal of the neoplasm from the abdomen (fig. 3). It had a cystic appearance, with plentiful yellow-brown dense sebaceous material, mixed with hairs (fig. 4).

Histological samples showed a mature tridermal teratoma, presenting tissues derived from all three embryonal layers, mixed in a chaotic and bizarre way (*salad of tissues*), with predominant expressions of dermoid cyst and "struma ovarii" (with follicular, trabecular and oxyphilic areas), and with the association of small cartilage areas (Figs. 5-8). The patient was discharged four days after surgery, after Ca-125 blood levels showed a return to normal values.

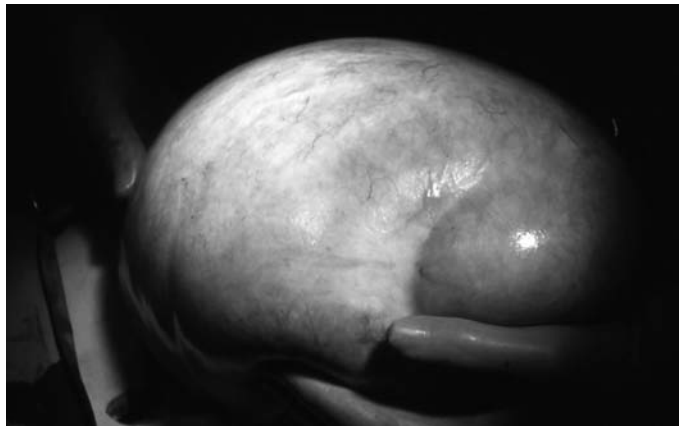

Fig. 3. Struma ovarii. Macroscopic external aspect

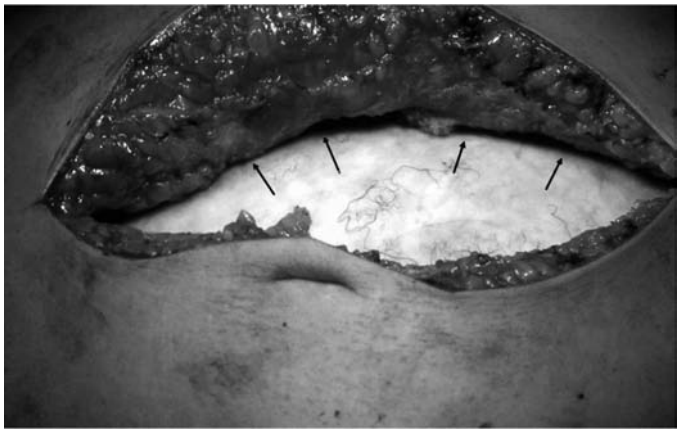

Fig. 2. Intraoprative image of the big ovarian neoformation raising from the median xifo-umbilical-pubic laparotomy. Note the clear cleavage plane between the mass and the adjacent structures (arrows)

Fig. 4. Struma ovarii, macroscopic examination. The mass presents a cystic appearance, with plentiful yellow-brown dense sebaceous material, mixed with hairs

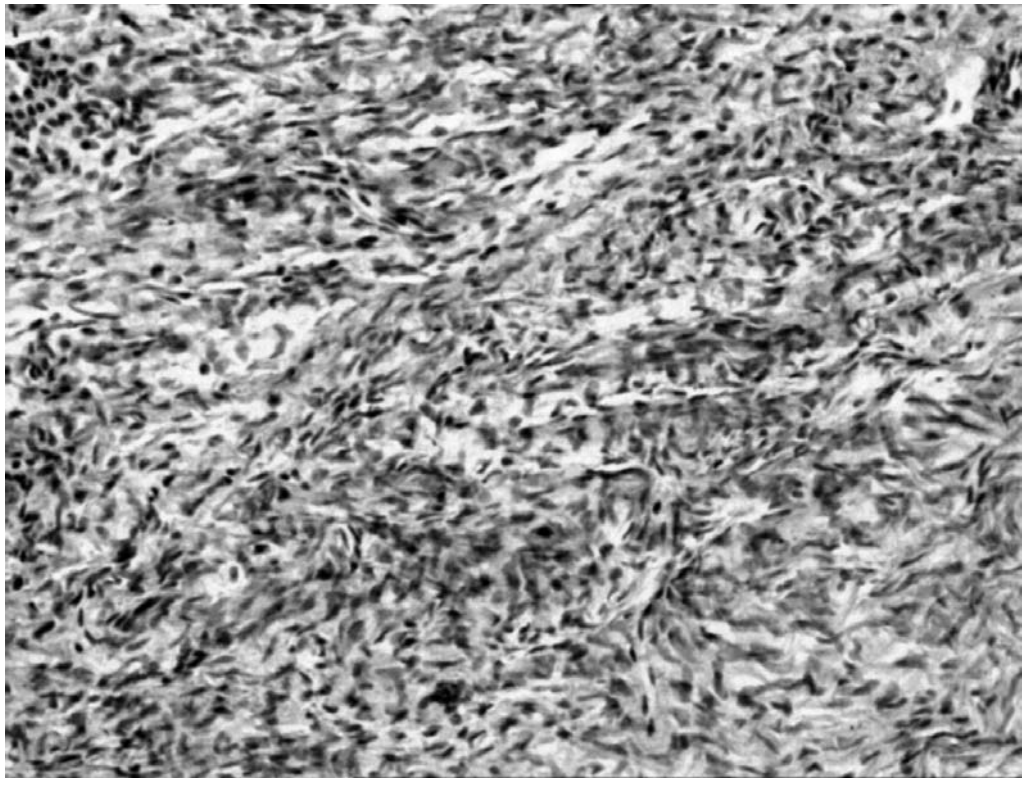

Fig. 5. Struma ovarii, microscopic examination.
In this image a cluster of indifferentiated connective tissue may be recognized (H&E staining) (40x)

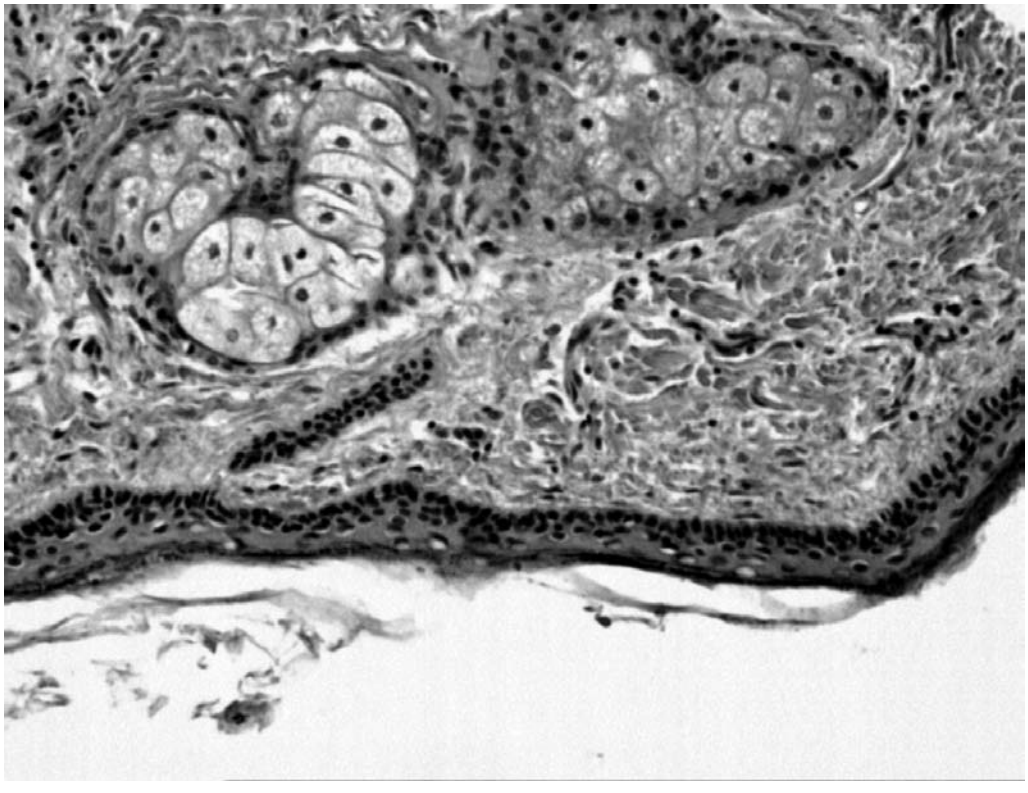

Fig. 6. Struma ovarii, microscopic examination. The image shows sebaceous cysts in the context of the dermoid cyst (H&E staining) (25x)

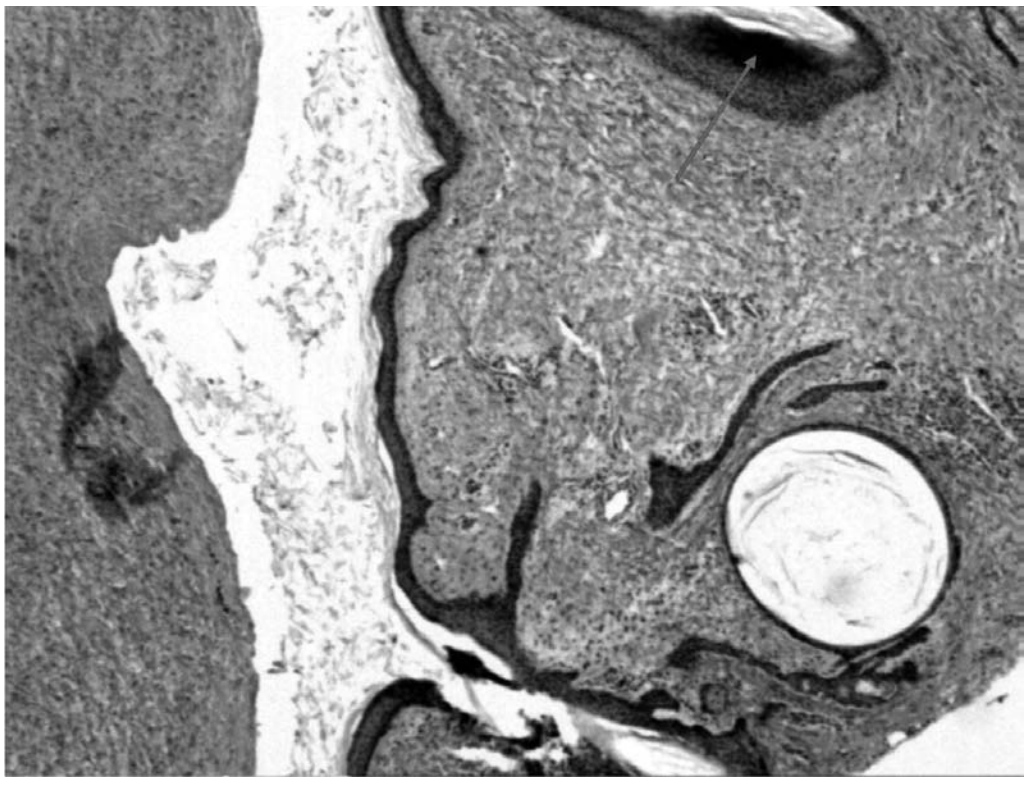

Fig. 7. Struma ovarii, microscopic examination. This section shows an epitelial squamous tissue with different pattern of organization, from pilifer bulb (see arrow) to that cyst in the bottom of the picture (H&E staining) (10x)

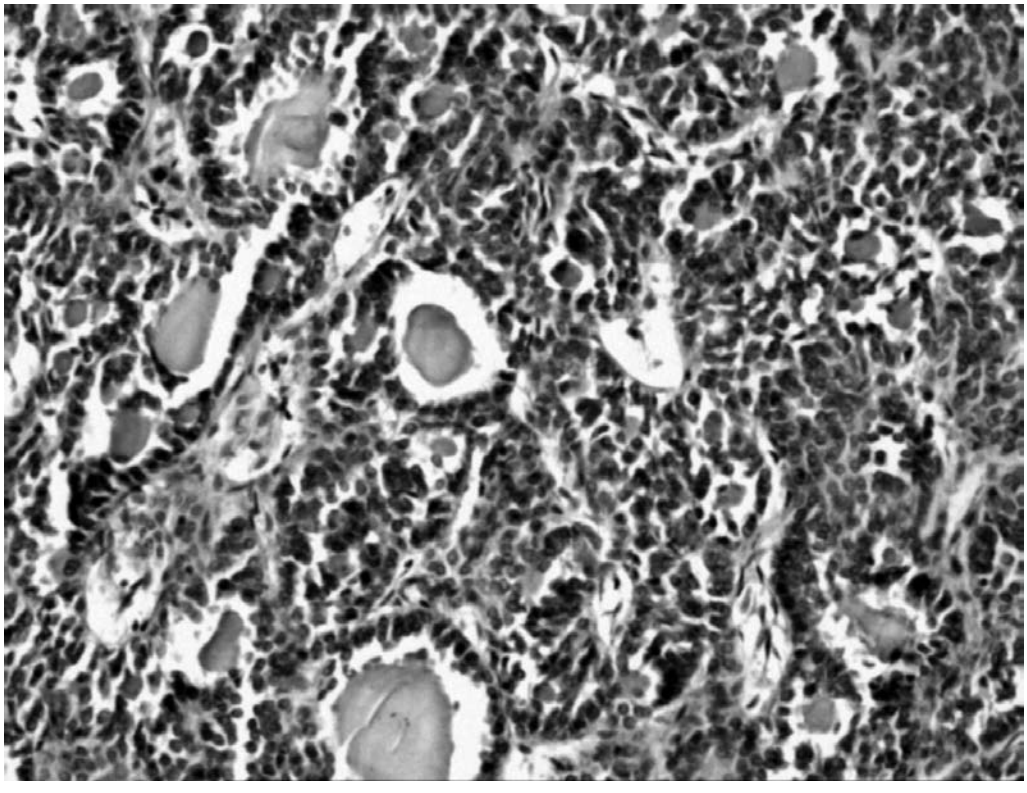

Fig. 8. Struma ovarii, microscopic examination. A typical thyroid tissue with follicles filled of colloid is evident in this picture (H&E staining) (25x)

Discussion

Patients with a discharge diagnosis of "struma ovarii" need to undergo a standardised post-surgery follow-up, which includes the execution of consecutive serial pelvic echotomographies. For these patients the onset of hyperthyroidism is also possible, and it is probably due to the release of autoantibodies against thyroid, following the surgical manipulation of the lesion (10). Surgical manipulation may also be the cause of the release of high levels of thyroid hormones (*thyroid storm*). Any carcinomatous transformation requires the execution of scintigraphy with ^{131}I in order to detect the possible presence of metastases (11). Their presence needs a radio-metabolic treatment, rarely associated with excisional surgery. However, both ensure a high recovery rate (12-13).

Conclusions

As previously mentioned, ovarian teratomas are classified in mature, immature and monodermal. Monodermal teratoma is composed of highly specialized tissues also including struma ovarii. This type of neoplasm is uncommon, representing only 0.3% of all ovarian tumours, and it develops, in 85% of cases, in women in premenopausal stage.

Clinically asymptomatic, in most cases it might cause an undefined clinical picture, usually referable to symptoms of compression on the adjacent structures. Symptoms and signs of overproduction of thyroid hormones are rare.

On the basis of what is above reported, in 80% of cases the diagnosis of struma ovarii is only histological. The possible presence of carcinomatous foci does not compromise the prognosis, because the surgical treatment alone, or associated with metabolic radiotherapy with ^{131}I, is able to ensure the complete healing in the majority of cases.

References

1. Kunstmann L, Fénichel P. Struma ovarii, a rare form of ovarian tumor. Gynecol Obstet Fertil 2007; 35: 49-54.
2. Volpi E, Ferrero A, Nasi PG, *et al*. Malignant struma ovarii: a case report of laparoscopic management. Gynecol Oncol 2003; 90: 191-4.
3. Yannopoulos D, Yannopulos K, Ossowoski R. Malignant struma ovarii. Pathol Annu 1999; 11: 410-3.
4. Seroy SF, Scully RE, Robin IH. Hystological typing of ovarian tumors: international histological classification of tumors. World Health Organization, Geneve, 1973.
5. Gamoudi A, Farhat K, Hechiche M, *et al*. Struma ovarii. Three malignant cases and one benign case. Rev Fr Gynecol Obstet 1995; 90: 542-7.
6. Seung-Chul Y, Ki-Hong C, Mi-Ok L, *et al*. Clinical characteristics of struma ovarii. J Gynecol Oncol 2008; 19: 135-8.
7. Zhang X, Axiotis C. Thyroid-type carcinoma of struma ovarii. Arch Pathol Lab Med 2010; 134: 786-91.
8. Jiang W, Lu X, Zhu ZL, *et al*. Struma ovarii associated with pseudo-Meigs' syndrome and elevated serum CA 125: a case report and review of the literature. J Ovarian Res 2010; 29: 18.
9. March DE, Desai AG, Park CH, *et al*. Struma ovarii: hyperthyroidism in a postmenopausal woman. J Nucl Med 1988; 29: 263-5.
10. Morrissey K, Winkel C, Hild S, *et al*. Struma ovarii coincident with Hashimoto's thyroiditis: an unusual cause of hyperthyroidism. Fertil Steril 2007; 88: 497. e15-7.
11. Willemse PH, Oosterhuis JW, Aalders JG, *et al*. Malignant struma ovarii treated by ovariectomy, thyroidectomy, and ^{131}I administration. Cancer 1987; 60: 178-82.
12. Brenner W, Bohuslavizki KH, Wolf H, *et al*. Radiotherapy with iodine-131 in recurrent malignant struma ovarii. Eur J Nucl Med 1996; 23: 91-4.
13. Dardik RB, Dardik M, Westra W, *et al*. Malignant struma ovarii: two case reports and a review of the literature. Gynecol Oncol 1999; 73: 447-51.

Eur. J. Oncol., vol. 16, n. 3, pp. 171-172, 2011

Short report of the International Conference on "Emerging Trends in Preventing Occupational Respiratory Diseases and Cancer in the Workforce" held in New Delhi, March 22-24, 2011

Breve resoconto della Conferenza Internazionale su "Strategie Emergenti nella Prevenzione delle Malattie Respiratorie Occupazionali e del Cancro nei Lavoratori" tenutasi a Nuova Delhi, 22-24 marzo 2011

Arthur Frank*, Yael Stein**, Tushar Kant Joshi***

* Drexel University School of Public Health, Philadelphia, USA
** Unit of Occupational and Environmental Medicine, Hebrew University, Hadassal School of Medicine, Jerusalem, Israel
*** Occupational and Environmental Medicine Programme, Centre for Occupational and Environmental Health (COEH), New Delhi, India

The Maulana Azad Medical College, and its Centre for Occupational and Environmental Health, in conjunction with various Indian government agencies as well as the Collegium Ramazzini, the Drexel University School of Public Health, and the Heart of England NHS Foundation Trust organized and presented an International Conference entitled: "Emerging Trends in Preventing Occupational Respiratory Diseases and Cancer in the Workforce" on March 22-24, 2011. There was also a special one day symposium on Safety and Health in Building and Construction held during the meeting.

The Collegium Ramazzini was well represented at this International Conference. Led by Dr. T.K. Joshi, a fellow who heads the Centre for Occupational and Environmental Health, there were many other Collegium fellows present. Dr. Arthur Frank was the representative of the Drexel University School of Public Health, and Drs. Knut Ringen and Anders Englund were the leaders of the Safety and Health Symposium on Building and Construction. Also in attendance were Drs. Elihu Richter, Carl Cranor, James Melius and Kurt Straif. Other fellows had participated in the scientific committee organizing the meeting including Drs. McDiarmid, Rice, Brautbar, Landrigan, Ruchirawat, Soffritti, Zhao and Songnian.

The Dean of Maulana Azad, Dr. A.K Agarwal was also instrumental in assisting with the meeting.

The inaugural session included greetings from the Health and Family Welfare Department as delivered by the secretary Mr. Rajendra Kumar and an address by Dr. R.K. Srivastava, the Director General for Health Services of the Government of India. Also giving greeting were Dean Agarwal, Dr. Joshi, Dr. Frank, who spoke on behalf of Drexel and sent greetings from the Collegium, and other dignitaries.

The first day of the meeting included four concurrent workshops relating to occupational lung disease including the clinical evaluation of occupational

Received/Pervenuto 17.5.2011 - Accepted/Accettato 9.8.2011
Address/Indirizzo: Dr. Arthur L. Frank, Drexel University of Public Health, 1505 Race Street, 13th floor MS 1034, PA19102, Philadelphia, USA
Tel. 001/2157623930 - Fax 001/2157628846 - E-mail: alf13@drexel.edu

respiratory diseases, pulmonary function testing, the use of the ILO classification of X-rays, and industrial hygiene assessment. These sessions were limited but were all very well attended. The clinical workshop addressed the clinical skills needed by physicians to identify occupational lung diseases, and how to link disease with exposure. This was conducted by Drs. Frank and Joshi.

Dr. R. Mukerjee, a consultant at the Birmingham Heartland Hospital, another sponsor, together with Dr. P. Jugnandan of Canada and Dr. S.S. Waghe of Mumbai, conducted the session on pulmonary function testing and interpretation of the results.

Dr. P.K. Sishodiya, the director of the Indian National Institute of Miner's Health and Dr. A.D. Vellore of the UK reviewed the ILO classification and the use of CT scans in occupational respiratory disease.

Dr. H. Perez of Drexel University and Mr. Pal of Mumbai did a workshop on industrial hygiene issues. They reviewed such topics as dust, noise, heat, radiation and ergonomic stress.

The general session included reviews of such relevant topics as exposures to coal, silica, and asbestos, as well as discussion of occupational asthma, hypersensitivity pneumonitis, and an excellent presentation on nanotechnology from a colleague of Dr. Richter's from Israel. The latter part of the meeting had to do with occupational cancers including a detailed overview of occupational carcinogens given by Dr. Straif, and sessions on hematopoietic cancers and benzene, occupational lung cancers, head and neck cancers, and a discussion of policy, ethical and moral issues as well as issues of prevention and control as well as regulation.

A major point made from these sessions was that occupational asthma was likely to become more prevalent in India as new processes were undertaken. Many cases were thought to be preventable if suitable precautions were taken.

The subject of nanomaterials was address by Dr. Y. Stein of Israel. A colleague of Dr. Richter, she covered this topic in a forward looking manner.

The symposium on "Safety and Health in Building and Construction" included sessions on implementation of construction safety and health in India, the potential for an updated statutory framework, and the science of construction safety and health.

The meeting was well attended by some 200 individuals drawn not only from the international arena but many from India as well as individuals from a number of other countries supported to attend from Bhutan, Indonesia, and Burma, giving a Southeast Asian perspective to the meeting. Coming as a follow-up to the December 2009 international meeting previously sponsored at Maulana Azad with the assistance of the Collegium and Drexel University, there was universal agreement that this meeting was as strong, if not stronger, than the prior meeting which had gone extremely well.

Outcomes for India included recommendations for (1) creating a unit of occupational and environmental health, (2) addressing gaps in reporting, (3) improving the education of physicians in occupational and environmental safety and health, (4) strengthening and creating laboratories for industrial hygiene, and (5) implementing awareness campaigns and ethical norms. There was also the suggestion of promoting international collaboration.

The staff of the Centre for Occupational and Environmental Health should be commended for once again working so diligently to put in place this significant meeting which was of great service to many in South Asia. It was also notable that many government officials from various parts of the Indian government were also in attendance and participated in the meeting, giving hope to long-term benefit for those in attendance and those that they care for.

As usual, Dr. Joshi arranged for excellent social events and for the international visitors who wished, important sightseeing activities to compliment this important scientific program.

Eur. J. Oncol., vol. 16, n. 3, pp. 173-174, 2011

Louis S. Beliczky
1925 - 2011

Louis S. Beliczky passed away January 5, 2011, at the Eliza Jennings Home, Cleveland Ohio.

Born Aug. 23, 1929, in Cleveland, Ohio, he had been a Wickliffe, Ohio resident for 51 years.

Louis ("Lou") Beliczky grew up on the West Side of Cleveland and graduated in 1943 from St. Ignatius High School. Immediately upon graduation he enlisted in the US Marines, completed basic training at Parris Island, received advanced training at Bucknell University and became a demolition instructor. He was then selected to be the Marines' Hollywood film representative, but chose to serve with the 1st Marines. He fought as a combat Marine, Headquarters Company, in numerous battles including the battle of Kenushi Ridge at Okinawa. After the victory at Okinawa, he then continued serve with the 1st Marines in China repatriating Japanese soldiers and was honorably discharged in April, 1946. Under the GI Bill he attended and graduated with a BS from John Carroll University in 1949. He went on to to attend graduate school at Kent State University and at the University of Pittsburgh receiving a MS and a Master's of Public Health, respectively.

He began his professional career as an industrial hygiene consultant and also worked with The Cleveland Clinic and Case Western Reserve University. In 1971 he was hired by the United Rubber Workers ("URW") as the first, full-time industrial hygienist employed by an industrial union in the US. Mr. Beliczky continued with the URW as their Director of Industrial Hygiene until his retirement in 1993. He was widely recognized as a pioneer in his field. He was the author of numerous workplace safety and health standards, was a consultant to and advised the US DOL, OSHA, CDC, NIOSH, NIEHS, and the World Health Organization and traveled extensively throughout the US, Europe and Asia. He was appointed Emeritus Fellow of the Collegium Ramazzini, an international academy of noted worldwide experts in the field of occupational and environmental health. He was an honorary member of the American Industrial Hygiene Association, recipient of the J. William Lloyd Award for outstanding

service in the cause of worker safety and health and was also named an Honorary Kentucky Colonel.

When not traveling the globe or promoting the cause of worker safety and health, he spent time with his family, played the violin, squash and tennis, sang with the Cleveland Singers' Association, was an avid gardener and woodworker, and enjoyed hunting and fishing. He was also very active with the Marine Corps League at the local and state levels and was very proud of is Hungarian heritage, also being fluent in Hungarian and German.

He is survived by his children, Stephanie (Donald) Szabo of Mentor, L. Stephen (Paula) Beliczky of Richmond, Va., Mark (Cindy) Beliczky of Glenwood, Md., Claudia (James Connolly) Beliczky of Andover, Ann (Raymond) Petrick of Philadelphia, Roberta (Dennis) Thomas of Ponte Vedra, Fla. and Monica (Tom) Irwin of Lakewood; grandchildren, Allison, Braeden, Emily, Erin, Ian, John, Kyle, Laine, Leslie, Mairin, Meagan, Melissa, Nicholas, Nicole, Stephen and Will; one great-grandchild, Cadence. He was preceded in death by his wife of 50 years Bertie Lou (Griffith) Beliczky.

Mark W. Beliczky

SOCIETÀ ITALIANA TUMORI *prevenzione diagnosi e terapia*

XXXVII Congresso Nazionale SIT-Società Italiana Tumori

CESARE MALTONI: IL RICORDO DELLA SIT A 10 ANNI DALLA SCOMPARSA

"Attualità in Oncologia: dal laboratorio alla cura del paziente"

24-26 Novembre 2011

Hotel Savoia Regency - Bologna

in collaborazione con Istituto Ramazzini

Istituto "B. Ramazzini" Coop. Soc. ONLUS

Con il patrocinio di: Ministero della Salute, Inca-Cgil Nazionale, INAIL, Regione Emilia-Romagna, ARPA Emilia-Romagna, Comune di Bologna, Comune di Bentivoglio, Comune di Carpi, Comune di Faenza, Università di Bologna, AUSL di Bologna.

Hanno aderito e parteciperanno al Congresso SIT 2011 molti tra i maggiori rappresentanti dell'oncologia italiana e di altre discipline interessate al problema dei tumori e delle malattie ambientali.

L'inaugurazione del Congresso e la Cerimonia commemorativa dedicata a Cesare Maltoni si terranno il 24 Novembre al pomeriggio nel Municipio di Bologna, Palazzo d'Accursio.

Il Congresso si aprirà giovedì 24 Novembre con una sessione dedicata alla Prevenzione primaria e secondaria.

Nella giornata di venerdì 25 Novembre si svolgeranno sessioni parallele dedicate rispettivamente ai rischi cancerogeni ambientali, ai tumori professionali, alla biologia molecolare, e ai progressi terapeutici per i tumori del colon retto e del polmone.

Sabato 26 il congresso tratterrà di Medicina Palliativa, con particolare riguardo agli Hospice, e dei progressi terapeutici per i tumori della mammella.

Attraverso la partecipazione al Congresso è possibile acquisire crediti ECM; è aperta una sessione "Comunicazioni" a cui è possibile partecipare, previo invio di un abstract sull'argomento trattato. Tutte le istruzioni sono indicate nel programma del Congresso, che è disponibile su:

www.societaitalianatumori.it o www.ramazzini.it

EUROPEAN JOURNAL OF ONCOLOGY
GIORNALE EUROPEO DI ONCOLOGIA

The European Journal of Oncology (Eur. J. Oncol.), Official Organ of the Italian Society of Tumours (SIT)/Prevention, Diagnosis and Therapy, is promoted by the
Ramazzini Institute
via Libia, 13/a - 40138 Bologna, Italy
telephone: for Italy 051/302252; for abroad 0039/051/302252
fax: for Italy 051/302245; for abroad 0039/051/302245
and published by:
Mattioli 1885 - Casa Editrice
Strada di Lodesana 649/sx, Loc. Vaio - 43036 Fidenza (PR) - Italy
telephone: for Italy 0524/530383; for abroad 0039/0524/530383
fax: for Italy 0524/82537; for abroad 0039/0524/82537
All editorial mail must be addressed to the:
Editorial Office of the European Journal of Oncology
Cancer Research Centre,
Castle of Bentivoglio, Via Saliceto 3, 40010 Bentivoglio (Bologna), Italy
telephone: for Italy 051/6640460; for abroad 0039/051/6640460
fax: for Italy 051/6640223; for abroad 0039/051/6640223

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

The journal is a quarterly publication

INFORMATION AND INSTRUCTIONS TO AUTHORS

INFORMATION

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

Contributions may be in the form of:
- editorials;
- general reviews;
- original studies and research;
- clinical case reports;
- brief communications;
- letters to the Editors.

The official languages of the journal are Italian and English.

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

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

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

INSTRUCTION FOR MANUSCRIPTS

Mailing

Manuscripts must be sent to the Editorial Office (at the Cancer Research Centre, Bentivoglio) via e-mail to events@ramazzini.it. Texts must be written in Word 6 (or earlier versions) for Windows or Mac.

All relations that might raise a conflict of interests for the Authors with reference to the topic of the report must be clearly stated.

Texts

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

Pages must be numbered sequentially.

EDITORIALS, GENERAL REVIEWS AND ORIGINAL STUDIES AND RESEARCH

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

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

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

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

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

Bibliography style

Journal report, up to 3 Authors:

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

Journal report, more than 3 Authors:

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

Complete book:

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

Chapter of book:

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

Chapter of book that forms the proceedings of a meeting:

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

Abstract:

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

Supplement:

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

Editorial:

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

Letter to the Editor:

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

Scientific or technical report:

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

Newspaper article:

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

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

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

CASE REPORTS

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

BRIEF COMMUNICATIONS

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

LETTERS TO THE EDITOR

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

PROOF READING

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

REPRINTS

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

ANNOUNCEMENTS

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

PUBLICITY

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

TRANSFER OF AUTHOR COPYRIGHT

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

EUROPEAN JOURNAL OF ONCOLOGY
GIORNALE EUROPEO DI ONCOLOGIA

Il Giornale Europeo di Oncologia, Organo Ufficiale della
Società Italiana Tumori (SIT)/Prevenzione, Diagnosi e Terapia, è promosso dalla:
Istituto Ramazzini
via Libia, 13/a - 40138 Bologna, Italia
telefono: per l'Italia 051/302252; per l'estero 0039/051/302252
fax: per l'Italia 051/302245; per l'estero 0039/051/302245
e pubblicato presso la:
Mattioli 1885 - Casa Editrice
Strada di Lodesana 649/sx, Loc. Vaio - 43036 Fidenza (PR) - Italia
telefono: per l'Italia 0524/530383; per l'estero 0039/0524/530383
fax: per l'Italia 0524/82537; per l'estero 0039/0524/82537
Tutta la corrispondenza editoriale deve essere indirizzata a:
Redazione Giornale Europeo di Oncologia
Centro di Ricerca sul Cancro,
Castello di Bentivoglio, Via Saliceto 3, 40010 Bentivoglio (Bologna), Italia
telefono: per l'Italia 051/6640460; per l'estero 0039/051/6640460
fax: per l'Italia 051/6640223; per l'estero 0039/051/6640223

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

La rivista esce con periodicità trimestrale

INFORMAZIONI E ISTRUZIONI PER GLI AUTORI

INFORMAZIONI

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

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

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

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

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

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

ISTRUZIONI PER I MANOSCRITTI

Invio

I testi completi devono essere inviati alla Redazione (presso il Centro di Ricerca sul Cancro, Bentivoglio), via e-mail a events@ramazzini.it. I testi devono essere in Word 6 (o versione inferiore) per Windows o Mac.

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

Stesura

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

Le pagine devono essere numerate sequenzialmente.

EDITORIALI, RIVISTE GENERALI E STUDI E RICERCHE ORIGINALI

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

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

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

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

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

Stile della bibliografia

Articolo in rivista, fino a 3 autori:

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

Articolo in rivista, più di 3 Autori:

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

Libro completo:

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

Capitolo di libro:

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

Capitolo di libro che costituisce gli atti di un convegno:

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

Abstract:

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

Supplemento:

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

Editoriale:

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

Lettera all'Editore:

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

Resoconto scientifico o tecnico:

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

Articolo di giornale:

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

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

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

RESOCONTI SU CASI CLINICI

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

COMUNICAZIONI BREVI

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

LETTERE ALL'EDITORE

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

CORREZIONE DELLE BOZZE

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

ESTRATTI

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

ANNUNCI

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

PUBBLICITÀ

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

TRASFERIMENTO DEL COPYRIGHT

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