Significance of serum mesothelin in an asbestos-exposed population in the Czech Republic

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Aims. Pleural mesothelioma is a highly aggressive and difficult-to-treat form of cancer induced by asbestos in 80-90% of cases. The population group most at risk of the condition are asbestos-exposed workers. Mesothelin or soluble mesothelin-related protein (SMRP) is studied as a potential marker of mesothelioma in the at-risk population.
Methods. The study comprised 239 subjects with a mean duration of occupational exposure to asbestos of 19.9 years. In all of them, a complete medical history was taken, focused on exposure duration and a physical examination, a chest X-ray or other imaging investigations and a lung function test were performed. Their serum SMRP levels were measured and biopsy samples were taken to diagnose pleural disease. Based on the above examinations, the subjects were classified into subgroups and serum SMRP concentrations were statistically analyzed with respect to individual parameters.
Results. In asbestos-exposed individuals, mesothelin levels were significantly higher in those with pathological X-ray findings than in those with normal X-ray results (0.78 ± 0.63 vs. 0.50 ± 0.35, $P<0.0001$). The group of patients with benign disease had statistically significantly higher mesothelin levels than those with normal X-ray findings (0.755 ± 0.543 vs. 0.50 ± 0.35, $P<0.001$). In the group with present malignant processes, mesothelin levels were higher than in individuals with benign disease (1.19 ± 0.89 vs. 0.76 ± 0.54, $P=0.015$). Only a weak correlation was found between mesothelin levels and asbestos exposure duration. There were relatively high sensitivity and high specificity (75% and 90.6%, respectively) of serum mesothelin for pleural mesothelioma. However, given the small number of mesothelioma cases in the group, the results cannot be considered as statistically significant.
Conclusions. In persons followed up for asbestos exposure, increased mesothelin levels signalize pathological processes in the chest and correlate with severity of the disease. The study suggests that mesothelin cannot be considered a reliable marker for the early stage of malignant degeneration of pleural disease but only an additional criterion for examination of the followed-up individuals.

Key words: mesothelin, asbestos-exposed population, malignant pleural mesothelioma

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INTRODUCTION

Mesothelioma is a highly aggressive form of cancer developing from mesothelial cells of serous membranes. It affects the pleura, peritoneum, pericardium, tunica vaginalis testis and ovarian surface epithelium. Histologically, it is classified into three types: epithelioid, sarcomatoid and mixed. Approximately 75-80% of mesothelioma cases develop in the pleural cavity, making it the most common primary pleural tumor. There are marked geographical variations in the incidence of mesothelioma[1]. The rates range from 7 cases per 1,000,000 population in Japan to 40 cases per 1,000,000 in Australia, with approximately 20 cases per 1,000,000 being reported in Europe[2,3]. There are also differences in incidence between males and females, with 10-66 cases and 1-2.5 cases per 1,000,000, respectively[4]. In the Czech Republic, the incidence of mesothelioma in 2005 was 8 cases per 1,000,000 males and 3 cases per 1,000,000 females[5]. Worldwide, it is estimated to cause 15,000-20,000 deaths each year[6]. The incidence has been increasing globally and the rates are expected to soar in the next 10-20 years[7-10].

Exposure to asbestos, the main causative factor for pleural mesothelioma, is reported in 80-90% of patients and its negative impact has been known for over 50 years[11]. Asbestos is a fibrous type of metamorphosed silicate minerals, naturally occurring in two basic forms, serpentines and amphiboles, with higher carcinogenic potential. Exposure to asbestos is mainly occupational, with asbestos workers having a risk of developing the disease of 5-10% (ref.[7]). Para-occupational exposure has been noted in family members sharing households with these workers[3]. The third group at risk is people living close to asbestos mines and asbestos manufacturing plants[12,13]. Other risk factors are simian virus 40, asbestos-like materials (erionite), deposition of alpha particles (ionizing radiation, Thorotrast) and as yet unexplained genetic abnormalities, as suggested by the existence of high-risk families in Turkey, Canada and the USA. Typically, there is a median delay between asbestos exposure and develop-

ment of the disease of more than 40 years, ranging from less than 10 years to over 80 years[3,14-17].

Mesothelioma is very difficult to treat and its prognosis is rather poor; the survival is approximately 12 months from diagnosis[9,18-21]. The advanced stages of the disease and sarcomatoid type of mesothelioma have worse prognosis[20,22-24]. Recent data indicate median survival according to TNM classification for 21 months stage I, stage II 19 months, 16 months stage III and stage IV 12 months. According to histological type, then the median survival for the type epithelioid 19 months, biphasic 13 months, and sarcomatoid 8 months[25]. In the Czech Republic there are data available in patients treated with standard chemotherapy cisplatin + pemetrexed. Median survival for epithelioid type is 29.4 months, for sarcomatoid + biphasic type is 14.9 months. Stage I hasn't achieved median survival, 1-year survival rate is 93.3%, stage II-III have a median survival 29.4 months and 1-year survival rate 70.2% (ref.[26,27]). The median survival rates are 5-9 months in palliative care, 10-16 months in chemotherapy and 20-24 months in small groups of patients undergoing radical surgery[2,9,28,29]. This is due to late diagnosis of advanced mesothelioma and relatively high resistance to radiotherapy and chemotherapy[30]. Early diagnosis of mesothelioma is thought to lead to prolonged survival and better prognosis of the patients. Currently, there is no mesothelioma marker routinely used in clinical practice[31]. In recent years, however, new biomarkers have been discovered, with mesothelin being the most promising one.

Mesothelin is a glycosylphosphatidylinositol-anchored glycoprotein expressed on mesothelial cells[32]. It results from a precursor mesothelin protein cleaved by furin-like protease and is also referred to as C-ERC/mesothelin (ref.[33]). It performs numerous physiological functions in the organism[34]. It is overexpressed in many cancers; most frequently in mesothelioma, less frequently in ovarian, pancreatic and lung cancers (especially adenocarcinoma) and rarely in breast, colon, endometrial and kidney cancers and sarcoma[35-40]. In pleural biopsies, the immunohistochemical positivity of mesothelin has a sensitivity of 75-100% and specificity of 90% for pleural mesothelioma[38,41].

In certain situations, mesothelin or its fragments are released into body fluids where it is detected as the so-called soluble mesothelin-related peptides (SMRP) (ref.[42,43]). There are three variants of these proteins, with variant 1 being most common[44-46]. These result from proteolytic cleavage of a part of a mesothelin molecule, probably by the action of a phospholipase or protease or by gene mutation[47,48]. Increased SMRP levels are common in mesothelioma, especially epithelioid, less frequent in pancreatic, ovarian and lung cancers, and rare in other malignancies. SMRP may be mildly to moderately increased in benign cases such as those with asbestos exposure, arterial hypertension, congestive heart failure or renal insufficiency[36]. Serum SMRP levels may be affected by age, weight (body mass index < 25), high glucose levels, smoking and a history of malignancy or asbestos-related pleural involvement[49-52].

Given the fact that asbestos is the main causative factor for mesothelioma and asbestos workers are the most threatened population group with a significantly increased risk for development of the disease, they are suitable for monitoring and examinations, including assessment of the clinical significance of mesothelin, in particular with respect to early detection of pleural mesothelioma.

MATERIALS AND METHODS

The group comprised individuals with occupational exposure to asbestos followed up at the Department of Occupational Medicine of the General University Hospital in Prague and the Department of Occupational Medicine of the University Hospital Olomouc. At least once a year, they underwent clinical examination, a chest X-ray or CT scan and a lung function test. In all participants, a complete medical history was taken and a physical examination, a chest X-ray and a lung function test were performed. Their age, asbestos exposure duration, history and findings of asbestos-related pleural and/or lung disease or malignancy were noted. Venous blood samples were collected to assess the level of serum mesothelin. The MESOMARK kit (Fujirebio Diagnostics, Inc.), a two-stage enzyme-linked immunosorbent assay (ELISA), was used. It utilizes two monoclonal antibodies, with 4H3 binding soluble mesothelin variants 1, 2 and 3 and OV569 detecting soluble mesothelin variants 1 and 3. Color changes are caused by the reagent substrate tetramethylbenzidine. When catalyzed with peroxidase, it produces a blue color; addition of "stop" solution (1% hydrochloric acid) changes the color from blue to yellow to be read at a wavelength of 450 nm. This is compared with a 6-point calibration curve (range, 0-32 nmol/L) to determine SMRP values in the tested samples. The cut off was set at 1.5 nmol/L. Mesothelin levels > 1.5 nmol/L were classified as increased and positive. Those ≤ 1.5 nmol/L were referred to as normal and negative.

Individuals with new or aggravated clinical symptoms (dyspnea, cough, chest pain) or abnormal chest X-ray findings underwent further investigations – a CT scan of the lungs or an ^{18}F-fluorodeoxyglucose PET/CT scan. In those with confirmed pathological findings, a morphologic diagnosis was developed to make a definite diagnosis. More detailed investigations were also carried out in persons with increased mesothelin levels.

Subsequently, based on the findings, the subjects were classified into a group of healthy persons, i.e. those with no radiological abnormalities, and individuals with abnormal radiological findings. The latter were subdivided into groups with hyalinosis, asbestosis, mesothelioma, other malignancies or combinations of these conditions. The relationship between serum mesothelin levels and exposure duration and individual groups of findings was assessed. Sensitivity and specificity of high serum mesothelin levels were calculated for the mesothelin, other malignancies and all malignancies groups.

Statistical analysis was carried out using the SPSS, version 15 software. All tests were performed at a sig-

nificance level of 0.05. Descriptive statistics comprised the arithmetic mean and standard deviation (SD). The Spearman's rank correlation coefficient, Mann-Whitney U test, Kruskal-Wallis test and Fisher's exact test were also used.

RESULTS

The group comprised 239 persons with work-related exposure to asbestos. Their mean age was 63.84 years (± 9.920 years). The mean asbestos exposure was 19.91 years (± 10.736). Of those, 90 (37.7%) were healthy, i.e. with no pathological findings, and 149 had one or more pathological findings (Table 1).

Statistically, there was a weak positive correlation between mesothelin levels and exposure duration (r = 0.230, *P*=0.0003) (Fig. 1), but no statistically significant difference in exposure duration between patients with normal and those with high mesothelin levels (*P*=0.185).

Mesothelin levels for individual groups are shown in Table 2. Persons with abnormal X-ray findings had statistically significantly higher mesothelin levels than healthy subjects (0.78 ± 0.63 vs. 0.50 ± 0.35, *P*<0.0001) (Fig. 2). In those with abnormal X-ray findings, there was a statistically significantly higher proportion of individuals with positive mesothelin, as compared with the group with no findings (14.8% vs. 2.2%, *P*=0.001). The group with benign findings only had statistically significantly higher mesothelin levels than the one with no abnormal X-ray scans (0.755 ± 0.543 vs. 0.50 ± 0.35, *P*<0.001). In the group with just benign findings (pleural hyalinosis, asbestosis), significantly higher mesothelin levels were found in asbestosis patients (*P*=0.027) (Fig. 3). Comparing the group with just benign findings and the group with malignant (as well as benign) findings, significantly higher mesothelin levels were found in the malignancy group (1.19 ± 0.89 vs. 0.76 ± 0.54, *P*=0.015) (Fig. 4). Persons with malignancies had statistically significantly higher presence of positive mesothelin as compared with the group with just benign findings (42.9% vs. 10.2%, *P*=0.001). In the malignancy group, there was no statistically significant difference in mesothelin levels between individuals with mesothelioma and those with other malignancies (*P*=0.107; mesothelioma found in 4 subjects only).

Increased mesothelin levels of > 1.5 nmol/L were detected in 24 individuals (10.0%), of whom 3 were diagnosed with mesothelioma, 6 with other malignancies and 15 with benign processes. The sensitivity and specificity of serum mesothelin for mesothelioma are shown in Table 3.

Table 1. Distribution patients according to pathologic finding.

Finding	Number	%
Healthy, without finding	90	37.7
Asbestosis and pleural hyalinosis	80	33.5
Pleural hyalinosis	31	13.0
Asbestosis	17	7.1
Asbestosis, pleural hyalinosis, other malignancy	9	3.8
Other malignancy	4	1.7
Asbestosis, other malignancy	3	1.3
Asbestosis, pleural hyalinosis, pleural mesothelioma	2	0.8
Asbestosis, pleural mesothelioma	1	0.4
Pleural hyalinosis, pleural mesothelioma	1	0.4
Pleural hyalinosis, other malignancy	1	0.4
Total	239	100.0

DISCUSSION

There are numerous studies suggesting mesothelin as a possible marker of asbestos exposure, with significantly higher SMRP levels being seen in asbestos-exposed persons as compared with those without the exposure. Already in their first study of mesothelin, Robinson et al. found higher levels in individuals exposed to asbestos than in healthy subjects[53]. Pass et al. reported significantly higher SMRP levels in asbestos-exposed persons than in controls (0.99 ± 0.09 vs. 0.39 ± 0.02, *P*<0.001) (ref.[6]). Similar results were published in a 2011 Italian study showing significantly higher SMRP levels in asbestos-exposed individuals compared with controls (0.93 ± 0.49 vs. 0.54 ± 0.28) (ref.[54]). Also Portal et al. reported similar results for mesothelin in asbestos-related and control subjects (0.41 ± 0.15 vs. 0.23 ± 0.07, *P*<0.001 (ref.[55]). The only exceptions are studies by Creaney et al. and Hollevoet et al. showing similar SMRP levels in both asbestos-exposed individuals (median levels of 0.638 and 0.820, respectively) and controls (median levels of 0.701 and 0.950, respectively) (ref.[56,57]).

The correlation between mesothelin levels and asbestos exposure duration was assessed but not proved by a 2009 Spanish study (r = 0.194, *P*=0.50) (ref.[55]). Similarly, other authors found no correlations between SMRP levels

Table 2. Value of SMRP in the groups of patients.

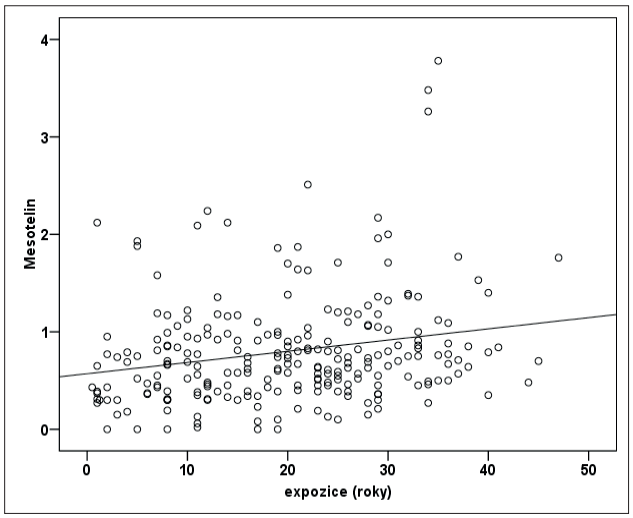
Group	Number	Median	Mean	SD
All	239	0.6900	0.7991	0.56523
Without finding	90	0.5000	0.5709	0.34767
With finding	149	0.7800	0.9370	0.62471
With benign finding	128	0.7550	0.8685	0.54307
With malignant finding	21	1.1900	1.3543	0.89574

Table 3. Sensitivity a specificity of SMRP for malignant diseases.

	Mesothelioma	Other malignancies	All malignancies
Sensitivity	75.0%	35.3%	42.9%
Specificity	90.6%	91.9%	93.1%

and asbestos exposure duration or numbers of asbestos fibers present in the lungs[6,49,56,58-60]. This study found only a weak correlation between mesothelin levels and exposure duration (r=0.230, *P*=0.0003) but no differences in exposure duration between persons with normal and increased mesothelin levels. Naturally, asbestos exposure duration alone is not indicative of the severity of exposure. More significant is dustiness expressed as airborne fiber concentration. This, however, was not regularly measured at workplaces in the mid-20th century. Increased SMRP levels may be contributed to by other pathological processes as well, including specific processes in tumor cells[58].

Most studies reported significantly higher mesothelin levels in asbestos-exposed individuals with benign pleural and pulmonary involvement than in healthy persons exposed to asbestos. Park et al. found a mean SMRP level of 0.79 ± 0.45 in healthy subjects exposed to asbestos as compared with 1.06 ± 0.92 in those with benign disease (*P*<0.01) (ref.[61]). The same results were obtained in a 2012 Italian study, with mean SMRP levels of 0.51 nmol/L in benign disease and 0.43 nmol/L in healthy exposed population[62]. Similar findings were documented in numerous other studies[6,52,57,63-66]. The presented study also showed statistically significantly higher mesothelin levels in a group of persons with just benign findings than in a group with no abnormalities detected by X-ray (0.755 ± 0.543 vs. 0.50 ± 0.35, *P*<0.001). A recent Australian study even showed an association between SMRP levels and the severity of



exposure (years)

Fig. 1. Correlation between value of mesothelin and exposure length mesothelin (nmol/L).

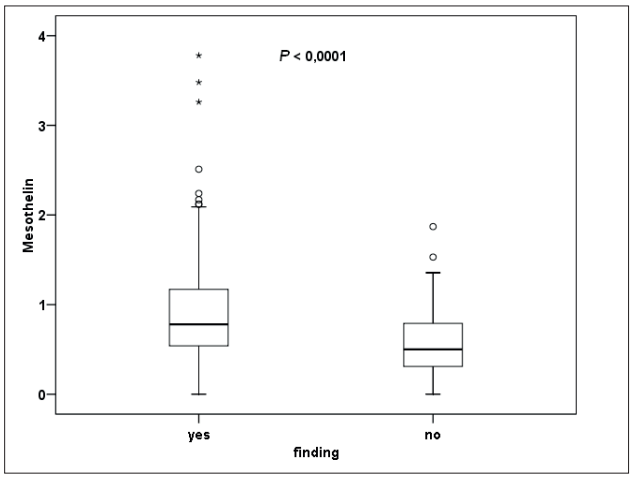


Fig. 2. Comparison of value of mesothelin in patients with and without pathologic finding

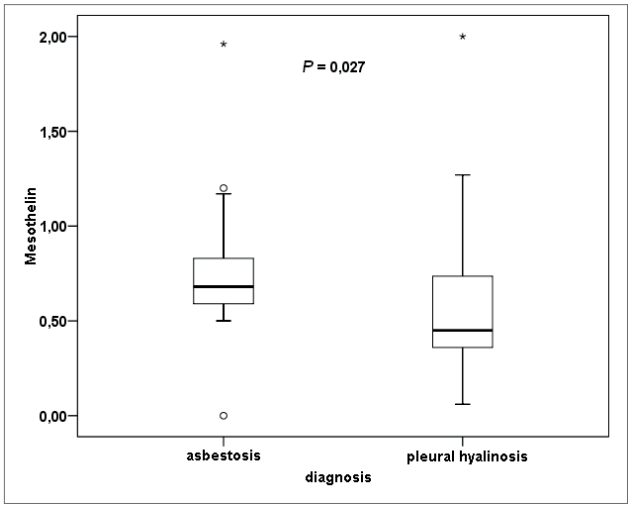


Fig. 3. Comparison of value of mesothelin in patients with benign pathologic findings (asbestosis vs. pleural hyalinosis).

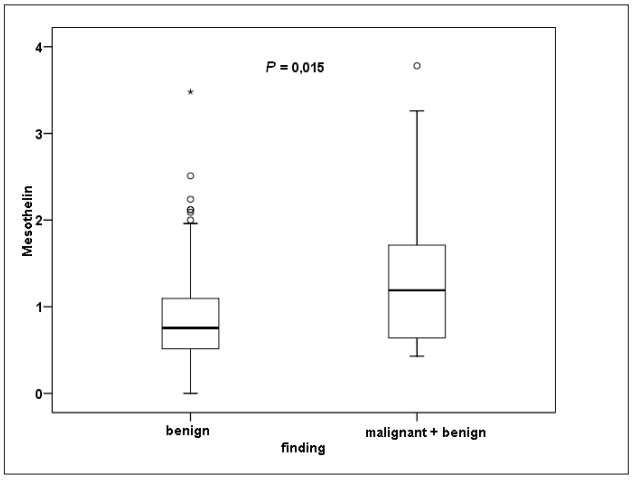


Fig. 4. Comparison of value of mesothelin in patients with benign and malignant pathologic findings.

asbestos-induced respiratory disease[67]. Exceptional are studies by Portal et al. and Felten et al. carried out in asbestos-exposed subjects. They showed no differences between individuals with benign pleural involvement and a group of healthy subjects (0.40 ± 0.14 and 0.706, respectively vs. 0.41 ± 0.15 and 0.796, respectively; *P*=0.038) (ref.[49,55]). Surprisingly, the presented study comparing serum mesothelin levels in benign asbestos-induced respiratory disease showed significantly higher mesothelin levels in asbestosis than in pleural hyalinosis. This finding may be related to higher cumulative doses of asbestos fibers (fiber-years) assumed to be involved in the development of asbestosis. A recent study, however, reported no increase in serum SMRP in asbestosis[61].

The first impulse for investigating SMRP as a potential predictive factor for mesothelioma was a 2003 study by Robinson et al. providing a primary assessment of the utilization of mesothelin in the diagnosis of pleural mesothelioma as compared with healthy controls exposed to asbestos and patients with other inflammatory or malignant lung and pleural diseases. Over a five-year follow-up, however, three out of seven asbestos-exposed healthy individuals with increased concentrations of soluble mesothelin were diagnosed with pleural mesothelioma; another person developed lung cancer[53].

Other studies produced less optimistic results. An Australian study assessed 538 asbestos-exposed subjects comprising a heterogeneous group of individuals with or without radiologically confirmed asbestos-induced pleural or lung disease. The cut-off was set at 2.5 nmol/L. Increased concentrations were found in 15 persons who underwent further examinations including PET/CT scans. One of them was diagnosed with lung carcinoma but none of them had pleural mesothelioma[61].

Roe et al. investigated SMRP in serum samples collected 1-30 years prior to the diagnosis of pleural mesothelioma from 47 persons with the disease and analyzed serum samples in 141 controls. The SMRP cut-off was 2.3 nmol/L. The authors observed neither elevated serum SMRP levels prior to the diagnosis of pleural mesothelioma nor differences in SMRP concentration between the mesothelioma group and controls[36].

Conversely, this study showed significantly higher mesothelin levels in persons with pathological findings as compared with healthy individuals (0.78 ± 0.63 vs. 0.50 ± 0.35, *P*<0.0001). Among those with pathological X-ray findings, persons with malignancies had significantly higher mesothelin levels than the group with just benign findings (1.19 ± 0.89 vs. 0.76 ± 0.54, =0.015).

In a 2010 Australian study, SMRP was repeatedly assessed in archived serum samples obtained from 106 persons with pleural mesothelioma, 99 asbestos-exposed individuals and 109 healthy controls. The median SMRP concentrations were 1.556 nmol/L, 0.638 nmol/L and 0.701 nmol/L, respectively. With a cut-off of 2.5 mmol/L, an absolute increase in SMRP was observed in 15% of mesothelioma patients with a median of 8 months prior to the diagnosis. During longitudinal follow-up, SMRP was relatively increased in 40% of pleural mesothelioma patients[56].

A recent German study of serum SMRP with a cut-off of 1.5 nmol/L found that in asbestos-exposed subjects, the specificity and sensitivity for mesothelioma were 91.8% and only 10.0%, respectively[8]. Unlike other recent studies on pleural mesothelioma, the presented study found relatively high sensitivity and high specificity of serum mesothelin (75.0% and 90.6%, respectively). However, the result is most likely to be caused by a small, statistically insignificant number of patients with mesothelioma of the pleura (n=4). After all malignancies were included in the analysis, the results corresponded with recent data, showing high specificity but markedly lower sensitivity. Given the small number of pleural mesothelioma cases, mesothelin levels could not be compared between pleural mesothelioma and other malignancies. Some studies claim that there is no increase in SMRP at 12 months or more before the diagnosis of mesothelioma[8,36]. On the other hand, it is suggested that at least six months prior to the development of clinical manifestations of mesothelioma, SMRP levels are significantly increased[49]. It seems, however, that rather than using absolute SMRP concentrations, assessing SMRP change dynamics (marker velocity) or time to doubling of SMRP levels (doubling time) could be more useful[49]. But recent studies recommend that SMRP should not be used in the screening for mesothelioma in asbestos-exposed persons[3,61].

Numerous studies have been performed to assess the utilization of mesothelin in the diagnosis of mesothelioma, reporting a sensitivity of 50-84% and specificity of 72-95% and statistically significantly higher SMRP levels in mesothelioma as compared with healthy exposed individuals or those with benign asbestos-induced conditions[30,31,35,55,68-72]. Recent meta-analysis reported a sensitivity and specificity of SMRP for mesothelioma of 32-64% and 88-100%, respectively[73,74].

Another finding in many studies was statistically significantly higher mesothelin levels in mesothelioma than in metastatic involvement of the pleura but also statistically significantly higher mesothelin concentrations in metastatic pleural disease compared with asbestos-exposed individuals (healthy and/or with benign disease) (ref.[37,63,65,70]).

Large differences in results between individual studies are probably due to different cut-off values used. Higher cut-offs mostly lead to higher sensitivity and lower specificity. There have been studies measuring pleural SMRP, the absolute values of which tend to be higher than those of serum SMRP, showing similar sensitivity and specificity[6,37,58,75].

Most studies have shown higher SMRP levels in epithelioid mesothelioma than in other mesothelioma types, with an SMRP sensitivity of only 10% in sarcomatoid mesothelioma and 40% in the mixed type[30,36,37,53,69-71,76,77]. Also in the presented group of subjects examined for pleural effusions of unknown etiology, the sensitivity, specificity, negative predictive value, accuracy and median of serum mesothelin were higher in epithelioid mesothelioma than in the group of all mesothelioma types[78]. Serum SMRP levels are assumed to be directly correlated with the proportion of the epithelial component in the tumor[37]. Higher

clinical stages of mesothelioma (stages II and III-IV) are associated with higher SMRP concentrations[6,58,69,71]. Other data suggest that serum mesothelin levels increase as the tumor grows[53,70,71]. On the other hand, surgical tumor reduction is followed by a decrease in SMRP levels[6,35,68].

Numerous studies have reported increased SMRP to be an independent prognostic factors for mesothelioma, with mesothelin levels negatively correlating with survival[31,36,37,63,71,74]. Another area of application of serum mesothelin measurement is monitoring of mesothelioma progression and its response to therapy, with SMRP being increased in disease progression and stable or decreasing SMRP levels being a sign of positive response to therapy[30,53,63,64,70,71,79].

CONCLUSIONS

Finding a sensitive marker for the early diagnosis of mesothelioma is a challenge. It is expected that the number of cases of the disease will continue to rise due to a widespread use of asbestos in the last century. Moreover, the association with occupational exposure to asbestos is not considered in many mesothelioma patients. As a result, approximately 90% of these patients are not compensated for having an occupational disease[80]. The presented study found significantly higher mesothelin levels in individuals with pathological chest X-ray findings, significantly more frequently in those with malignant disease. Only a weak correlation between mesothelin levels and asbestos exposure duration was found. There was a relatively high sensitivity of serum mesothelin for pleural mesothelioma. However, the finding cannot be considered as statistically significant due to a small number of mesothelioma patients in the study. Thus, more large studies are needed to confirm the significance of mesothelin for asbestos-exposed persons or asbestos-induced diseases.

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Author contributions: PJ, DP, VK, MK: literature search; PJ, DP, VK: study design; PJ: manuscript writing; all authors: data collection and data interpretation; PJ: data analysis; statistical analysis, figures.

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REFERENCES

1. Lin RT, Takahashi K, Karjalainen A, Hoshuyama T, Wilson D, Kameda T, Chan CC, Wen CP, Furuya S, Higashi T, Chien LC, Ohtaki M. Ecological association between asbestos-related diseases and historici asbestos consumption: an international analysis. Lancet 2007;369:844-9.
2. Robinson BW, Lake RA. Advances in malignant mesothelioma. N Engl J Med 2005;353:1591-603.
3. Scherpereel A, Astoul P, Baas P, Berghmans T, Clayson H, de Vuyst P, Dienemann H, Galateau-Salle F, Hennequin C, Hillerdal G, Le Péchoux C, Mutti L, Pairon JC, Stahel R, van Houtte P, van Meerbeeck J, Waller D, Weder W, European Respiratory Society/European Society of Thoracic Surgeons Task Force. Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleural mesothelioma. Eur Respir J 2010;35:479-95.
4. Palatka K, Kolek V, Tichý T, Musilová K. Zkušenosti s diagnostikou a léčbou maligního mezoteliomu, srovnání období 1990-1999 a 2000-2004. Stud Pneumol Phthiseol 2007;67:188-93.
5. Pelclová D, Fenclová Z, Urban P. Asbestos exposure, legislation and diseases in the Czech Republic. Cent Eur J Public Health 2007;15(3):99-102.
6. Pass HI, Wali A, Tang N, Ivanova A, Ivanov S, Harbut M, Carbone M, Allard J. Soluble Mesothelin-Related Peptide Level Elevation in Mesothelioma Serum and Pleural Effusions. Ann Thorac Surg 2008;85:265-72.
7. Pass HI, Carbone M. Current Status of Screening for Malignant Pleural Mesothelioma. Semin Thorac Cardiovasc Surg 2009;21:97-104.
8. Gube M, Taeger D, Weber DG, Pesch B, Brand P, Johnen G, Müller-Lux A, Gross IM, Wiethege T, Weber A, Raithel HJ, Kraus T, Brüning T. Performance of biomarkers SMRP, CA125, and CYFRA 21-1 as potential tumor markers for malignant mesothelioma and lung cancer in a kohort of workers formerly exposed to asbestos. Arch Toxicol 2011;85:185-92.
9. Robinson BW, Musk AW, Lake RA. Malignant mesothelioma. Lancet 2005;366:397-408.
10. Hodgson JT, McElvenny DM, Darnton AJ, Price MJ, Peto J. The expected burden of mesothelioma mortality in Great Britain from 2002 to 2050. Br J Cancer 2005;92:587-93.
11. Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. Br J Ind Med 1960;17:260-71.
12. Bourdes V, Boffetta P, Pisani P. Environmental exposure to asbestos and risk of pleural mesothelioma: review and meta-analysis. Eur J Epidemiol 2000;16:411-7.
13. Maule MM, Magnani C, Dalmasso P, Mirabelli D, Merletti F, Biggeri A. Modeling mesothelioma risk associated with environmental asbestos exposure. Environ Health Perspect 2007;115:1066-71.
14. Stahel RA. Malignant pleural mesothelioma: A new standard of care. Lung Cancer 2006;54(S2):S9-S14.
15. Lanphear BP, Buncher CR. Latent period for malignant mesothelioma of occupational origin. J Occup Med 1992;34:718-21.
16. Ohar J, Sterling DA, Bleecker E, Donohue J. Changing patterns in asbestos-induced lung dinase. Chest 2004;125(2):744-53.
17. Marinaccio A, Binazzi A, Cauzillo G, Cavone D, Zotti RD, Ferrante P, Gennaro V, Gorini G, Menegozzo M, Mensi C, Merler E, Mirabelli D, Montanaro F, Musti M, Pannelli F, Romanelli A, Scarselli A, Tumino R. Italian Mesothelioma Register (ReNaM) Working Group. Analysis of latency time and its determinants in asbestos related malignant mesothelioma cases of the Italian register. Eur J Cancer 2007;43(18):2722-8.
18. Roberts HC, Patsios DA, Paul NS, DePerrot M, Teel W, Bayanati H, Shepherd F, Johnston MR. Screening for malignant pleural mesothelioma and lung cancer in individuals with a history of asbestos exposure. J Thorac Oncol 2009;4:620-8.
19. West SD, Lee YC. Management of malignant pleural mesothelioma. Clin Chest Med 2006;27:335-54.
20. Flores RM, Pass HI, Seshan VE, Dycoco J, Zakowski M, Carbone M, Bains MS, Rusch VW. Extrapleural pneumonectomy versus pleurectomy/decortication in the surgical management of malignant pleural mesothelioma: results in 663 patients. J Thorac Cardiovasc Surg 2008;135:620-6.
21. Hyland RA, Ware S, Johnson AR, Yates DH. Incidence trends and gender differences in malignant mesothelioma in New South Wales, Australia. Scand J Work Environ Health 2007;33:286-92.
22. O'Byrne KJ, Edwards JG, Waller DA. Clinico-pathological and biological prognostic factors in pleural malignant mesothelioma. Lung Cancer 2004;45(S1):S45-S48.
23. Neumann V, Löseke S, Nowak D, Herth FJF, Tannapfel A. Malignant Pleural Mesothelioma**.** Incidence, Etiology, Diagnosis, Treatment, and Occupational Health. Dtsch Arztebl Int 2013;110(18):319-26.
24. Kao SC, Vardy J, Chatfield M, Corte P, Pavlakis N, Clarke C, van Zandwijk N, Clarke S. Validation of prognostic factors in malignant pleural mesothelioma: a retrospective analysis of data from patients seeking compensation from the New South Wales Dust

Diseases Board. Clin Lung Cancer 2013;14(1):70-7. doi: 10.1016/j.cllc.2012.03.011
25. Rusch VW, Giroux D, Kennedy C, Ruffini E, Cangir AK, Rice D, Pass HI, Asamura H, Waller D, Edwards J, Weder W, Hoffmann H, van Meerbeeck JP. IASLC Staging Committee. Initial analysis of the international association for the study of lung cancer mesothelioma database. J Thorac Oncol 2012;7(11):631-9.
26. Kolek V, Zatloukal P, Pešek M, Salajka F, Marel M, Roubec J, Skřičková J, Sixtová D, Petruželka L, Grygárková I, Koubková L, Štícha M. Pemetrexed in the first line chemotherapy of malignant pleural mesothelioma. A multicentre prospective study. 14th World Conference on Lung Cancer. Journal of Thoracic Oncology 2011;6(6):1360-1.
27. Kolek V, Pešek M, Zatloukal P, Salajka F. Pemetrexed in the first line chemotherapy of malignant pleural mesothelioma. the Czech prospective study. 24th International congress on anti-cancer treatment. A World leading educational congress. Abstract book, Paris, France 5-7 February 2013.(IC/AB967),p.314-5.
28. Antman K, Pass H, Sciff P. Benign and malignant mesothelioma. In: deVita, VT, Jr., Hellman, S, Rosenberg, S, editors. Cancer: principles and practice of oncology. 6th ed. Philadelphia: Lippincott-Raven, 2001, p.1943-70.
29. Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, Gatzemeier U, Boyer M, Emri S, Manegold C, Niyikiza C, Paoletti P. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003;21:2636-44.
30. Schneider J, Hoffmann H, Dienemann H, Herth FJ, Meister M, Muley T. Diagnostic and Prognostic Value of Soluble Mesothelin-Related Proteins in Patients with Malignant Pleural Mesothelioma in Comparison with Benign Asbestosis and Lung Cancer. J Thorac Oncol 2008;3:1317-24.
31. Creaney J, Christansen H, Lake R, Musk AB, de Klerk N, Robinson BW. Soluble Mesothelin Related Protein in Mesothelioma. J Thorac Oncol 2006;1:172-4
32. Chang K, Pai LH, Batra JK, Pastan I, Willingham MC. Characterization of the Antigen (CAK1) Recognized by Monoclonal Antibody K1 Present on Ovarian Cancers and Normal Mesothelium. Cancer Res 1992;52:181-6.
33. Maeda M, Hino O. Molecular tumor markers for asbestos-related mesothelioma: Serum diagnostic markers. Pathology International 2006;56(11):649-54.
34. Rump A, Morikawa I, Tanaka M, Minami S, Umesaki N, Takeuchi M, Miyajima A. Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. J Biol Chem 2004;279:9190-8.
35. Hassan R, Remaley AT, Samson ML, Zhang J, Cox DD, Pingpank J, Alexander R, Willingham M, Pastan I, Onda M. Detection and Quantitation of Serum Mesothelin, a Tumor Marker for Patients with Mesothelioma and Ovarian Cancer. Clin Cancer Research 2006;12:447-53.
36. Roe OD, Creaney J, Lundgren S, Larsson E, Sandeck H, Boffetta P, Nilsen TI, Robinson B, Kjaerheim K. Mesothelin-related predictive and prognostic factors in malignant mesothelioma: A nested case-control study. Lung Cancer 2008;61:235-43.
37. Scherpereel A, Grigoriu B, Conti M, Gey T, Grégoire M, Copin MC, Devos P, Chahine B, Porte H, Lassalle P. Soluble Mesothelin-related Peptides in the Diagnosis of Malignant Pleural Mesothelioma. Am J Respir Crit Care Med 2006;173:1155-60.
38. Ordonez,NG. Immunohistochemical diagnosis of epithelioid mesothelioma. An update. Arch Pathol Lab Med 2005;129:1407-14.
39. Watanabe H, Okada G, Ohtsubo K, Yamaguchi Y, Mouri H, Motoo Y, Wakabayashi T, Sawabu N. Expression of mesothelin mRNA in pure pancreatic juice from patiens with pancreatic carcinoma, intraductal papillary mucinous neoplasm of the pankreas, and chronic pankreatitis. Pancreas 2005;30(4):349-54.
40. Ho M, Bera TK, Willingham MC, Onda M, Hassan R, FitzGerald D, Pastan I. Mesothelin expression in human lung cancer. Clin Cancer Rev 2007;13(5):1571-5.
41. Yaziji H, Battifora H, Barry TS, Hwang HC, Bacchi CE, McIntosh MW, Kussick SJ, Gown AM. Evaluation of 12 antibodies for distinguishing between epithelioid mesothelioma from adenocarcinoma: Identification of a free-antibody immunohistochemical panel with maximal sensitivity and specificity. Mod Pathol 2006;19:514-23.
42. Shiomi K, Miyamoto H, Segawa T, Hagiwara Y, Ota A, Maeda M, Takahashi K, Masuda K, Sakao Y, Hino O. Novel ELISA systém for detection of N-ERC/mesothelin in the sera of mesothelioma patients. Cancer Sci 2006;97:928-32.
43. Scholler N, Fu N, Yang Y, Ye Z, Goodman GE, Hellström KE, Hellström I. Soluble member(s) of the mesothelin/megakaryocyte potentiating factor family are detectable in sera from patients with ovarian carcinoma. Proc Natl Acad Sci USA 1999;96:11531-6.
44. Muminova ZE, Strong TV, Shaw DR. Characterization of human mesothelin transcripts in ovaria nand pancreatic cancer. BMC Cancer 2004;4:19-29.
45. Hassan R, Bera TK, Pastan I. Mesothelin: a new target for immunotherapy. Clin Cancer Rev 2004;10:8751-3.
46. Hellstrom I, Raycraft J, Kanan S, Sardesai NY, Verch T, Yang Y, Hellstrom KE. Mesothelin Variant 1 Is Released from Tumor Cells as a Diagnostic Marker. Cancer Epidemiology, Biomarkers Prev 2006;15:1014-20.
47. Ho M, Onda M, Wang Q, Hassan R, Pastan I, Lively MO. Mesothelin Is Shed from Tumor Cells. Cancer Epidemiology, Biomarkers Prev 2006;15:1751.
48. Sapede C, Gauvrit A, Barbieux I, Padieu M, Cellerin L, Sagan C, Scherpereel A, Dabouis G, Grégoire M. Aberrant splicing and protease involvement in mesothelin release from epithelioid mesothelioma cells. Cancer Science 2008;99:590-4.
49. Felten MK, Khatab K, Knoll L, Schettgen T, Müller-Berndorff H, Kraus T. Changes of mesothelin and osteopontin levels over time in formerly asbestos-exposed power industry workers. Int Arch Occup Environ Health 2014;87(2): 195-204. doi 10.1007/s00420-013-0853-1
50. Park EK, Thomas PS, Creaney J, Johnson AR, Robinson BW, Yates DH. Factors affecting soluble mesothelin related protein levels in an asbestos-exposed population. Clin Chem Lab Med 2010;48:869-74.
51. Hollevoet K, Nackaerts K, Thas O, Thimpont J, Germonpré P, De Vuyst P, Bosquée L, Legrand C, Kellen E, Kishi Y, Delanghe JR, van Meerbeeck JP. The effect of clinical covariates on the diagnostic and prognostic value of soluble mesothelin and megakaryocyte potentiating factor. Chest 2012;141:477-84.
52. Filiberti R, Marroni P, Mencoboni M, Mortara V, Caruso P, Cioè A, Michelazzi L, Merlo DF, Bruzzone A, Bobbio B, Del Corso L, Galli R, Taveggia P, Dini G, Spigno F. Individual predictors of increased serum mesothelin in asbestos-exposed workers. Med Oncol 2013;30:422. doi 10.1007/s12032-012-0422-6
53. Robinson BW, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, Winzell P, Hellstrom KE, Hellstrom I. Mesothelin-family proteins and diagnosis of mesothelioma. Lancet 2003;362:1612-6.
54. Marini V, Michelazzi L, Cioé A, Fucile C, Spigno F, Robbiano L. Exposure to asbestos: Correlation between blood levels of mesothelin and frequency of micronuclei in peripheral blood lymphocytes. Mutat Res 2011;721(1):114-7.
55. Portal JAR, Becerra ER, Rodríguez D, Michavila IA, Martínez AQ, Roza CD, Jiménez AL, Montes II, Rivas PC. Serum levels of soluble mesothelin-related peptides in malignant and nonmalignant asbestos-related pleural disease: relation with past asbestos exposure. Cancer Epidemiol Biomarkers Prev 2009;18:646-50.
56. Creaney J, Olsen NJ, Brims F, Dick IM, Musk AW, de Klerk NH, Skates SJ, Robinson BW. Serum Mesothelin for Early Detection of Asbestos-Induced Cancer Malignant Mesothelioma. Cancer Epidemiol Biomarkers Prev 2010;19(9):2238-46.
57. Hollevoet K, Nackaerts K, Thimpont J, Germonpré P, Bosquée L, De Vuyst P, Legrand C, Kellen E, Kishi Y, Delanghe JR, van Meerbeeck JP. Diagnostic Performance of Soluble Mesothelin and Megakaryocyte Potentiating Factor in Mesothelioma. Am J Respir Crit Care Med 2010;181:620-5.
58. Fujimoto N, Gemba K, Asano M, Wada S, Ono K, Ozaki S, Kishimoto T. Soluble mesothelin-related protein in pleural effusion from patients with malignant pleural mesothelioma. Experimental Therapeutic Med 2010;1:313-7.
59. Amati M, Tomasetti M, Mariotti L, Tarquini LM, Valentino M, Santarell, L. Assessment of biomarkers in asbestos-exposed workers as indicators of cancer risk. Mutat Res 2008;655:52-8.
60. Foddis R, Vivaldi A, Filiberti R, Puntoni R, Mutti L, Ambrosino N, Chella A, Guglielmi G, Gattini V, Buselli R, Perretta S, Cristaudo A. Serum mesothelin dosages in follow-up of previously exposed workers. Ital Med Lav Ergon 2007;29(3):342-5.
61. Park EK, Sandrini A., Yates DH, Creaney J, Robinson BW, Thomas PS, Johnson AR. Soluble Mesothelin-Related Protein in an Asbestos-Exposed Population. The Dust Diseases Board Cohort Study. Am J Respir Crit Care Med 2008;78:832-7.

62. Mencoboni M, Michelazzi LA, Cioé A, Bruzzone A, Delcorso L, Mortara V, Marroni P, Dini G, Marcenaro S, Spigno F. Mesothelin and individual characteristics in a cohort of asbestos exposed workers. Abstract no.2412, ESMO 2012
63. Grigoriu B, Scherpereel A, Devos P, Chahine B, Letourneux M, Lebailly P, Grégoire M, Porte H, Copin MC, Lassalle P. Utility of Osteopontin and Serum Mesothelin in Malignant Pleural Mesothelioma Diagnosis and Prognosis Assessment. Clin Cancer Res 2007;13(10):2928-35.
64. Grigoriu BD, Chahine B, Vachani A, Gey T, Conti M, Sterman DH, Marchandise G, Porte H, Albelda SM, Scherpereel A. Kinetics of Soluble Mesothelin in Patients with Malignant Pleural Mesothelioma during Treatment. Am J Respir Crit Care Med 2009;179:950-4.
65. Creaney J, Yeoman D, Demelker Y, Segal A, Musk AW, Skates SJ, Robinson BW. Comparison of Osteopontin, Megakaryocyte Potentiating Factor, and Mesothelin Proteins as Markers in the Serum of Patients with Malignant Mesothelioma. J Thorac Oncol 2008;3(8):851-7.
66. Creaney J, Bruggen I, Hof M, Segal A, Musk AW, de Klerk N, Horick N, Skates SJ, Robinson BW. Combined CA125 and Mesothelin Levels for the Diagnosis of Malignant Mesothelioma. Chest 2007;132:1239-46.
67. Park EK, Yates DH, Creaney J, Thomas PS, Robinson BW, Johnson AR, Association of Biomarker Levels with Severity of Asbestos-Related Diseases. Safety and Health at Work 2012;3(1):17-21. doi: 10.5491/SHAW.2012.3.1.17
68. Robinson BW, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, Winzell P, Hellstrom KE, Hellstrom I. Soluble mesothelin-related protein – A blood test for mesothelioma. Lung Cancer 2005;49(Suppl 1):109-11.
69. Creaney J, Robinson BW. Serum and pleural fluid biomarkers for mesothelioma. Curr Opin Pulm Med 2009;15(4):366-70.
70. Beyer HL, Geschwindt RD, Glover CL, Tran L, Hellstrom I, Hellstrom KE, Miller MC, Verch T, Allard WJ, Pass HI, Sardesai NY. MESOMARK™: A Potential Test for Malignant Pleural Mesothelioma. Clinical Chemistry 2007;53:666-72.
71. Cristaudo A, Foddis R, Vivaldi A, Guglielmi G, Dipalma N, Filiberti R, Neri M, Ceppi M, Paganuzzi M, Ivaldi GP, Mencoboni M, Canessa PA, Ambrosino N, Chella A, Mutti L, Puntoni R. Clinical Significance of Serum Mesothelin in Patients with Mesothelioma and Lung Cancer. Clin Cancer Res 2007;13:5076-81.
72. Di Serio F, Fontana A, Loizzi M, Capotorto G, Maggiolini P, Mera E, Bisceglia L, Molinini R. Mesothelin family proteins and diagnosis of mesothelioma: analytical evaluation of an automated immunoassay and preliminary clinical results. Clin Chem Lab Med 2007;45:634-8.
73. Luo L, Shi HZ, Liang QL, Jiang J, Qin SM, Deng JM. Diagnostic value of soluble mesothelin-related peptides for malignant mesothelioma: A meta-analysis. Respir Med 2010;104(1):149-56.
74. Hollevoet K, Reitsma JB, Creaney J, Grigoriu BD, Robinson BW, Scherpereel A, Cristaudo A, Pass HI, Nackaerts K, Rodríguez Portal JA, Schneider J, Muley T, Di Serio F, Baas P, Tomasetti M, Rai AJ, van Meerbeeck JP. Serum mesothelin for diagnosing malignant pleural mesothelioma: an individual patient data meta-analysis. J Clin Oncol 2012;30:1541-9.
75. Davies HE, Sadler RS, Bielsa S, Maskell NA, Rahman NM, Davies RJ, Ferry BL, Lee YC. Clinical Impact and Reliability of Pleural Fluid Mesothelin in Undiagnosed Pleural Effusions. Am J Respir Crit Care Med 2009;180:437-44.
76. Grigoriu BD, Grigoriu C, Chahine B,Gey T, Scherpereel A. Clinical utility of diagnostic markers for malignant pleural mesothelioma. Monaldi Arch Chest Dis 2009;71:31-8.
77. Zervos MD, Bizekis C, Pass HI. Malignant mesothelioma 2008. Curr Opin Pulm Med 2008;14:303-9.
78. Jakubec P, Grygárková I, Kolek V, Cwiertka K, Kapustová M. Mezotelin v detekci mezoteliomu pleury v diagnostice pohrudničního výpotku. Studia pneumol phthiseol 2010;70(6):243-8.
79. Wheatley-Price P, Yang B, Patsios D, Patel D, Ma C, Xu W, Leighl N, Feld R, Cho BC, O'Sullivan B, Roberts H, Tsao MS, Tammemagi M, Anraku M, Chen Z, de Perrot M, Liu G. Soluble mesothelin-related Peptide and osteopontin as markers of response in malignant mesothelioma. J Clin Oncol 2010;28(20):3316-22.
80. Pelclova D, Fenclova Z, Urban P. Occupational cancer in the Czech Republic – a tip of the iceberg? Eur J Oncol 2011;16:149-61.