THE CONCENTRATION OF SELECTED CANCER MARKERS (TPA, TPS, CYFRA 21-1, CEA) IN WORKERS OCCUPATIONALLY EXPOSED TO ARSENIC (As) AND SOME HEAVY METALS (Pb, Cd) DURING A TWO-YEAR OBSERVATION STUDY

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Abstract

Objective: Molecular epidemiology studies have lately been focused on occupational cancer associated with exposure to chemical carcinogens in work environment. Measuring serum levels of tumour markers i.e. the substances produced in transformed cells, is a modern method used in the prevention or early detection of cancer. A two-year observation study was constructed to investigate the relationship between arsenic, lead, and cadmium concentrations and the levels of cancer markers: TPA (tissue polipeptide antigen), TPS (tissue polipeptide specific antigen), and CYFRA 21-1 in 69 male workers occupationally exposed to As and Pb, and environmentally exposed to Cd via tobacco smoking. Results: Significant correlations were found between CEA and blood Cd concentrations or between CEA and period of work under exposure. Multiple regression tests revealed also relationships between some cancer markers and the work period, and thereby the lifetime exposure to heavy metals. Duration of work under exposure significantly influenced TPA and TPS concentrations in these models. All the metals examined were found to have influence on the concentration of cancer markers, except for CYFRA 21-1, but the direction of this influence varied. Lead (especially FEP level) and cadmium were also among the metals affecting TPA concentration profile, although the multiple regression ratio for Cd-TPA correlation was negative. Conclusions: The strong positive correlation between blood concentrations of Cd and CEA, the marker of abnormal cellular differentiation, may reflect neoplastic transformation of normal cells stimulated by some carcinogens (e.g. cadmium). All the examined carcinogenic, or potentially carcinogenic metals (As, Cd, Pb) belong to the group of factors having impact on serum TPS and/or TPA concentrations in exposed workers. No correlation was found between CYFRA 21-1 and any metal studied but it is plausible that changes in the concentration level of this marker might be revealed after a longer observation period.

Key words: Cancer markers, Heavy metals, Arsenic, Occupational exposure

INTRODUCTION

In arsenic exposure, the potential mutagenic activity and proven carcinogenicity due to clastogenic properties (inducing chromosomal aberrations in peripheral blood lymphocytes) and enhancement of sister chromatid exchange seem to be the most essential problems. Arsenic carcinogenicity refers mainly to inhalation exposure and brings about the development mostly of lung and skin cancers (squamous cell cancer and basalioma). However, cancers of the bladder, liver and kidney were also reported [1–4].

The results of experimental trials indicate that arsenic might interfere with enzymes and the so-called transfer molecules, modifying the expression of genes that determine proper cell maturation and proliferation. For instance, sodium arsenite activates transcription of AP-1 factor (activating protein 1) and increases activity of its mitogenic components (c-fos and c-jun). These processes
are accompanied by kinase activation, which plays an important role in phosphorylation of the transcription factors enhancing the expression of some genes [5,6].

On the other hand, arsenic blocks transduction of inflammatory proteins, e.g. the transcription factor NFκB which participates in the pathogenesis of hematologic neoplasm, mainly multiple myeloma, by regulating expression of IL-6 and adhesive particles [5–7].

Moreover, arsenic induces cellular apoptosis (or programmed cell death), a basic mechanism for maintaining the tissue balance. Disturbed balance between cellular death and proliferation may lead to neoplastic processes. Kaspases, cysteine proteases that account for condensation and fragmentation of cellular nuclei, participate in apoptosis. Arsenic activation of kaspases was observed both in vivo and in vitro, in the studies using cancer cells of multiple myeloma, T-cell leukemia or neuroblastoma [5,6,8].

In arsenic exposure, an indirect mechanism leading to cell apoptosis consists in decreased telomerase activity which results in genetic instability of the cell and consequently in chromosomal aberrations [4,5].

A disorder of the normal redox status, changing the gene expression of AP-1, NFκB, p53, or p21<sup>ras</sup>, may also lead to cellular apoptosis [9]. Therefore, arsenic may either help prevent or promote cancer. After arsenic exposure, the production of cellular nitric oxide rapidly increases, and is immediately followed by NADH<sup>+</sup> oxygenation and ADP ribosilation, which results in DNA single strand breaks, micronuclei formation and cell proliferation. Activation of flavoprotein-dependent enzymes and accumulation of intracellular hydrogen peroxide is the result of exposure to arsenic and subsequent generation of free radicals in the cell [8,10]. These processes affect the membrane potential of the mitochondria as well as their aggregation and, finally, apoptosis.

Glutathione peroxidases and catalase are vital enzymes to maintain the normal redox status in mammals. The activity of these enzymes is inhibited by arsenic and its methyl derivatives (potentially non-toxic). Therefore, the antiproliferative and proapoptotic potential of arsenic compounds increases. This property has been made use of in the treatment of some neoplasms [5–7,10].

In cellular response to arsenic, a major part is played by the heat shock proteins, e.g. Hsp90 or HO-1, that are actively involved in the regulation of the redox status and apoptosis. Interactions between cytochrome c and Hsp90 are inhibited by elevated intracellular concentration of arsenic which is at the same time a strong inductor of cytoprotective protein HO-1 [5,6].

Finally, apoptosis might be induced by arsenic activating some oncogenes, e.g. ras, or suppressor gene mutation, e.g. p53. The cytoskeleton, and particularly its main part, tubuline, rich in sulphhydryl groups, seems to be another potential target of arsenic activity.

Apoptosis of normal and neoplastic cells may be initiated by the degradation of microtubular structure, inhibition of GTP-dependent polymerisation of tubuline monomers and formation of the mitotic spindle. Apoptosis can also result from inhibition of tissue angiogenesis by arsenic, a property that is used in cancer therapy [6].

The co-mutagenic effect plays an important role in the pathogenesis of arsenic. When in combination with other metals, alkylating compounds or UV radiation, all of which are mutagens, arsenic can initiate tumour development at lower concentrations than are normally necessary to trigger the neoplastic process [1,2].

The epidemiological data on the potential carcinogenicity of lead compounds are relatively novel, contrary to the well established hematological, neurological and nephrological effects of long-term exposure to lead. Comprehensive population studies conducted in the US, Scandinavian countries and the UK have provided evidence for arsenic as a potential carcinogen. A large two-stage study on 7,000 workers occupationally exposed to lead revealed a significant relationship between exposure and the lung, stomach, liver and kidney cancers.

The kinetic properties of lead, especially its prompt elimination in the lung (within 24 hours) following inhalation exposure, prevent lead accumulation in the lung; the exception being lead sulphate. The erythrocytes, liver and kidney (the so-called exchange compartment) are the tissues of the highest index of early lead accumulation.
 Redistribution concerns also the musculo-skeletal system. The period of lead elimination from this system may be even as long as 20 years. The studies conducted thus far provide inadequate evidence to consider lead a human carcinogen, as classified by IARC [2].

Although some of cadmium may be absorbed via the gastrointestinal system (1–7%), inhalation of aerosol particles remains the most prevalent route of cadmium exposure. Taking into account that cadmium is the predominant component of tobacco smoke (approximately 12%), and its retention in the lung reaches 50–80%, one may easily conclude that occupational exposure is not the leading source of cadmium intoxication in the general population.

The search for the relationship between the irritant activity of cadmium and its carcinogenicity has yielded a hypothesis that cadmium carcinogenicity is a derivate of its toxicity, and for the development of cancer, a long-term contact of the tissue with metal (even at non-toxic doses) is more important than a single intoxication at the maximal dose [11].

In 1993, IARC announced cadmium as a carcinogenic substance in view of the epidemiologically proven risk of increased prevalence of lung cancer in occupationally exposed populations [2].

Cadmium can bind both to the base and the phosphate groups of DNA, and tends to destabilize the DNA helix. Cadmium-metallothionein complex is able to induce DNA strand breaks. There is also evidence that the genotoxic effects of Cd may be mediated by oxidative DNA damage. Cadmium reduces cellular GSH (glutathione) level and induces lipid peroxidation. Insoluble Cd particles may indirectly cause oxidative damage in vivo via inflammation. Finally, Cd may inhibit DNA-ligase activity, an important mechanism of DNA repair processes [1].

The first reports on the potential carcinogenicity of cadmium to humans were published in UK after an epidemiologic study on workers at Cd-Ni battery factory. The results of this research were controversial. In 1985, the link between a long-term cadmium exposure and lung and prostate cancers was confirmed for the first time [2]. Since then, similar research was conducted in other European countries and the US. The outcomes indicated that both the types of tumour could develop in cadmium-exposed workers but lung cancer was a slightly more prevalent site. The risk of cancer significantly increased with the increasing duration of exposure and cadmium concentration in the environment. It should be noted that in combined exposures to carcinogens, e.g. to cadmium, lead and arsenic, an interaction of the chemicals, increasing cancer risk, should be considered.

In Scandinavian studies, the standardized mortality ratio (SMR) due to lung cancer amounted to 3.6 for non-smokers exposed to arsenic, 4.9 for smokers not exposed to arsenic and to as much as 14.6 for smokers exposed to arsenic [1,2].

The development of the neoplastic disease is usually a long-lasting process, and its first symptoms often appear relatively late. An early diagnosis at the pre-clinical stage of neoplastic disease, when there is a chance for treatment, constitutes the main problem in oncology. The available radiological methods are relatively simple, but their sensitivity does not allow for diagnosing tumours smaller than 1 cm (containing less than 100,000,000 cells). The immunological methods (i.e. identification of specific antigens in serum) seem to be more promising [12].

In a traditional sense, the tumour markers are biologic and immunologic macromolecules, whose concentrations are measured both in serum and tumour cells and whose elevated levels are indicative of tumour growth [12,13]. The neoplasms produce two types of antigens: specific neoantigens, which are associated with oncogenesis and the exchange of genetic information, and the accompanying antigens, related to cellular differentiation [13,14]. The etiopathogenetic neoplastic markers reflect the following three conditions:

1. Proliferation of neoplastic cells (e.g. TPS — tissue polypeptide specific antigen).
2. Differentiation of neoplastic cells (e.g. AFP — alphafetoprotein, CEA — carcinoembryonic antigen, PSA — prostate specific antigen).
3. Apoptosis of neoplastic cells (e.g. TPA, CYFRA21-1) [13–15].
The aim of the present study was to evaluate the toxicological status of workers under conditions of occupational exposure to lead and arsenic, and environmental exposure to cadmium. Moreover, we investigated the relationship between blood or urine concentration of these metals and serum level of CEA and cytokeratin cancer markers, measured in 2001 and 2003 (i.e. at the beginning and termination of the study period).

**MATERIAL AND METHODS**

**Subjects**
The subjects were 69 copper smelter workers occupationally exposed to lead and arsenic, and environmentally exposed to cadmium via tobacco smoking, who were subject to a two-year observation study (June 2001 through June 2003). They were examined twice: in 2001, at the beginning of the study period (examination I), and in 2003, at its termination (examination II). The workers enrolled in the study were employed at Furnace Charge Preparation and Metallurgy departments where arsenic exposure was at the highest level. The subjects’ characteristics, in terms of their age and mean duration of work under exposure, are presented in Table 1.

The study was performed in accordance with an agreement between Wroclaw Medical University and “Legnica” Copper Smelter (H 08/2003, AM 256, and H 01/2000, AM 282). The evaluation of the toxicological status and cancer risk was an important part of the occupational health service activity in that plant and therefore an approval of the study by local ethics committee was not necessary in that case.

The method of individual dosimetry was used to identify the hazardous agents and measure their concentration in workplace air. The results of the measurements showed the presence of arsenic and a slight excess of the maximum admissible concentration of lead.

Each subject had a routine physical examination carried out. Also, the following laboratory tests were performed:

- whole blood lead and cadmium concentrations,
- serum concentration of protective metals: copper, zinc, and selenium,
- urine arsenic concentration,
— blood free erythrocyte protoporphyrin (FEP) concentration,
— serum CEA, TPS, TPA and CYFRA 21-1 concentration.

The cigarette-year rate for each subject was calculated as well.

The blood samples for measuring lead, FEP and cadmium concentrations were collected into a 2.7 cm³ probe with EDTA. The concentrations of Zn, Cu, Se and cancer markers were determined in 3 ml of serum.

Methods

All the elements examined: zinc, copper, lead, cadmium, selenium and arsenic were determined by atomic absorption spectrometry using SOLAAR M6, ThermoElemental Co. Lead concentration in blood: graphite furnace atomic absorption spectrometry (GFAAS), absorbance measurement at 283.3 nm wave, with Zeeman background correction. The reference materials BCR-194, -195, -196 by IRMM, UE were used.

Cadmium concentration in blood: GFAAS, absorbance measurement at 228.8 nm wave, with Zeeman background correction. The reference solutions were Single-Element Zinc (Copper) Standard 1000 µg/ml certified by CPI International. Selenium concentration in serum: GFAAS, absorbance measurement at 196.0 nm wave, with Zeeman background correction and nickel nitrate modifier. Seronorm™ Trace Element Serum by Sero AS, Norway, was used as reference. Arsenic concentration in urine (acidified with concentrated HNO₃): GFAAS with hydride generation (PU 9360 Philips), absorbance measurement at 193.7 nm wave, deuterium background correction, and electrically heated quartz tube atomizer. Single-Element Arsenic Standard 1000 µg/ml certified by CPI International was used as reference.

The reference curves were plotted for certified reference materials separately for each element. Validation was performed occasionally, on a random basis. The performing laboratory which analysed the concentration in biological material, has also been monitoring the constant absorbance values for a given chemical. In the case of a 5% deviation from the constant absorbance, the reference model was used to control the measurement error. The deviation from the expected value was the sign for checking the functions of the apparatus and calling the service.

The results of laboratory tests and medical examinations were handed out to the workers. In order to evaluate their general health condition and toxicological status, calcium and magnesium concentrations in serum were measured. The findings of our toxicological measurements were compared with reference values reported in available bibliography [16,17].

The concentration of free erythrocyte protoporphyrin (FEP) was determined after Piomelli [18]. TPS, TPA and CEA concentrations in serum were determined by ELISA method, and the concentration of CYFRA 21-1 by RIE method, using commercial tests. The cigarette-year was calculated by multiplying the average number of cigarettes smoked daily by the number of years of smoking.

The mean values of the parameters examined in 2001 and 2003 were compared. To set the general trends, the results were compared with the reference values (not included in the statistical analysis).

The following methods of statistical analysis were applied:
— Wilcoxon pair test for comparison of the mean values of the parameters examined within a two years’ period,
— linear correlation coefficients for the study parameters,
— multiple regression coefficients for the study groups examined twice.

RESULTS

No symptoms of lead burden (lead concentration in blood) or the features of excessive load of this metal (FEP concentration) in smelter workers could be found either at examination I or II. The mean cadmium concentration
at both examinations exceeded the biological exposure index (BEI) for non-occupationally exposed populations (0.5 µg/l), but was lower than that for the occupationally exposed population (5.0 µg/l). However, the mean arsenic concentration in urine exceeded BEI for occupationally exposed populations in both tests. The mean concentrations of the other metals (Cu, Zn, Se), despite the statistically significant differences noted between them, were within the medium normal limit values, and higher values were observed only at examination II.

The program entitled “Reduction of blood lead concentration in copper smelter workers” was introduced in 2002. This project was focused not only on technological improvement but also on a widerange of prophylactic activities, including the promotion of health-oriented behaviour and proper diet. In our opinion, these measures have largely contributed to changing the toxicological profile of the examined workers. The mean values of the tested cancer markers were within their normal limits, except for serum TPA, which slightly exceeded the upper limit at both the examinations.

Comparing the results obtained at examination I and II, significantly lower blood lead concentrations were detected at examination II. However, the other parameters of body burden with heavy metals (FEP concentration, arsenic in urine, and cadmium in blood) were not significantly different. Likewise, tumour marker concentrations were varying, and did not show any consistent upward or downward tendency. Only at examination II, a significant increase in TPA and a significant decrease in CYFRA 21 concentrations were recorded.

As regards individual analysis, at examination I, lead concentrations exceeding BEI for occupationally exposed populations were found in 18 workers (26.1%), elevated FEP concentration in 16 (23.2%), and increased urinary excretion of arsenic in as many as 68 people (98.5%). At examination II, blood lead levels exceeding BEI were recorded in 7 workers (10.14%), elevated FEP concentration in 20 (28.9%), and urine arsenic concentration in excess of BEI in 52 workers (75.4%). Increased serum CEA concentration was found in 16 workers (23.2%) at examination I and in 12 (17.39%) at examination II. Increased

**Fig. 1.** Linear correlation between serum CEA and blood Cd concentrations.

TPS concentrations were noted in 13 workers (18.8%) at examination I and in 15 (21.7%) at examination II. The most numerous group were the workers with elevated serum TPA concentration — 19 people (27.5%) at examination I and 27 people (39.32%) at examination II. The mean TPA values slightly exceeded the upper normal

**Table 2.** Linear correlation between study parameters examined in 2001 and 2003

<table>
<thead>
<tr>
<th></th>
<th>2001</th>
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<th>2003</th>
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<tbody>
<tr>
<td>Cd–cigarette-year</td>
<td>+0.338</td>
<td>Cd–cigarette-year</td>
<td>+0.485</td>
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<tr>
<td>Cd–FEP</td>
<td>+0.275</td>
<td>Cd–Cu</td>
<td>+0.215</td>
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<tr>
<td>Cd–Cu</td>
<td>+0.215</td>
<td>Pb–FEP</td>
<td>+0.431</td>
</tr>
<tr>
<td>Cd–As</td>
<td>+0.301</td>
<td>Pb–Se</td>
<td>+0.379</td>
</tr>
<tr>
<td>Cd–Se</td>
<td>+0.432</td>
<td>As–FEP</td>
<td>+0.579</td>
</tr>
<tr>
<td>Cd–TPA</td>
<td>+0.603</td>
<td>CEA–work-period</td>
<td>−0.238</td>
</tr>
<tr>
<td>Cd–TPS</td>
<td>+0.989</td>
<td>TPS–TPA</td>
<td>−0.280</td>
</tr>
<tr>
<td>Cd–TPS–TPA</td>
<td></td>
<td>TPS–Se</td>
<td>−0.250</td>
</tr>
<tr>
<td>Cd–TPS–CYFRA</td>
<td></td>
<td>TPS–TPA</td>
<td>+0.603</td>
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TPS–TPA = +0.603
TPS–CYFRA = +0.379
The relationship between the concentration of selected cancer markers and the duration of work, and thereby the total exposure to heavy metals (Pb, As connected with work environment, or Cd related to lifestyle) was also confirmed by multiple regression tests, especially in optimal models. In these models, TPA and TPS concentrations at both the examinations were significantly related to the duration of work. This relationship repeated in all the four optimal models (Figs. 2–5).

DISCUSSION

Molecular epidemiology studies have lately been focused on occupational cancer, which may develop in workers exposed to chemical carcinogens in work environment. The measurement of serum levels of tumour markers i.e. the substances produced in transformed cells, is one of the modern methods used in the prevention or early detection of cancer [19].

The application of cancer markers in routine laboratory diagnostics seems to be helpful in detecting early cell damage, assessment of monoclonal an polyclonal antibodies inhibiting cancer growth, prognosis, choice of optimal treatment, or defining the genetic predisposition to cancer. Markers detectable in serum may also reflect the potential risk of cancer development from benign tumours [13,15].

Cancer markers can be detected in human serum several months or even years before the first symptoms of the disease appear. This information is essential for determining cancer risk in workers occupationally exposed to chemical or physical carcinogens.
There are two criteria of the usefulness of cancer markers for detecting the disease, namely sensitivity and specificity. Sensitivity is defined as the ability of a laboratory test to correctly identify individuals who have a given disease or disorder, whereas specificity refers to the ability of a test to correctly exclude individuals who do not have a given disease or disorder. Unfortunately, most of the tests employing cancer markers are not sufficiently specific and or sensitive. The probability of detecting cancer increases significantly when several markers, with similar sensitivity, are applied at the same time [12,14].

Lately, intensive research has been undertaken to develop an optimum set of markers that would be useful for evaluating not only the cancer risk, but also the course of disease. For this purpose, the derivatives of cytokeratins (CYFRA 21-1, TPS, and particularly TPA) are promising. The cytokeratins (CK) are parts of the cytoskeleton distinguishing the neoplasm from the normal tissue. The mechanism leading to their liberation from tumour is not well recognised; however, it is known to be characteristic for cellular apoptosis in which caspases are involved [20]. The complex of CK8 and CK18 cytokeratins is the main element of cytoskeleton in the epithelium (intestine, liver or mammary duct). Proper expression of cytokeratins determines the stability of the cellular structure and regulates apoptosis by modulating membrane receptor density for pro-apoptotic factors (e.g. Ras or Fas protein) and anti-apoptotic factors (ERK kinase). Cytokeratin 19 usually forms heterodimERIC complexes with cytokeratin 8 subunits [20–22].

The cytokeratin subunits are identified as CYFRA, TPA and TPS. The TPA (tissue polypeptide antigen) is a keratin-like substance, containing CK 8,18 and 19 subunits. TPS (tissue polypeptide specific antigen) is recognized by monoclonal antibody for CK18, but it is not recognized by CK19 subunits. TPA can be detected both in serum and tissue extracts. The results of Dittadi et al. [1998] study indicate that high TPA concentration in tumour cells and low TPA serum concentration in breast cancer patients positively correlate with long-lasting remission or survival. This rather surprising finding might be explained by the fact that during mitosis, the stem cells of aggressive neoplasm excrete cytokeratins in a degraded form into the extracellular space. Their rapid growth is associated with poor prognosis.

The filaments of cytokeratin are poorly soluble or insoluble in water, but their fragments, emerging after enzymatic dissociation by caspases, are soluble in water. Fragmentation of cytokeratin filaments might follow accidental cell necrosis, characteristic of the rapidly growing tumours, as during the apoptosis which is the basic control mechanism of tumour growth. The soluble fragments of cytokeratins, generated during necrosis, are immediately eliminated from blood, and those resulting from apoptosis remain in the dead cells. In both cases, the soluble fragments cannot be detected in serum. Thus, cellular TPA might be considered an important, but not specific, cellular apoptotic marker [23].

The highest sensitivity of RIA test detecting CK19 subunit, called CYFRA 21-1, was found for the squamous cell cancer, and the lowest for small cell lung cancer. However, the highest concentration of the marker was confirmed in advanced stages of cancer in patients with metastases to regional lymph nodes, and in bad general condition. Pujol et al. [1993], who applied Cox analysis in their study, found that only three factors, i.e. the patient’s general condition, the stage of cancer, and CYFRA 21-1 concentration had a significant impact on the prognosis in squamous cell cancer [24].

The research on the usefulness of TPA, CEA and CYFRA 21-1 in the diagnostics and monitoring of lung cancer was undertaken by Huang et al. [1997]. The findings revealed that determining the concentration of CYFRA 21-1 and at least one of the other markers was a more sensitive method than determining the concentration of CK 19 subunits separately. The strongest correlation was observed between CYFRA 21-1 and TPA concentrations [25]. Buccheri et al. [2003], who conducted comparative studies on the diagnostic value of TPA and CYFRA 21-1 in a group of 180 patients with non-small cell lung cancer, have come to a similar conclusion. Very similar correlations for both the markers were observed with regard to response to anticancer therapy, patient’s general health condition, stage of disease, measured by the number of distant metastases,
and prognosis. The authors recommend assessment of TPA or CYFRA 21-1 concentrations in patients with non-small cell lung cancer as the optimum diagnostic test. The choice of the test depends on other (non-clinical) factors, e.g. the experience of the laboratory or the test price [26].

Kulpa et al. [2002] observed a high frequency of abnormal CYFRA 21-1 concentrations in operable and inoperable non-small cell lung cancer patients. As in the studies reported above, CYFRA 21-1 concentration markedly correlated with the stage of the neoplasm: elevated concentration of the marker was noted in 75% of patients who died within one year. Moreover, it was the only marker that demonstrated a statistically significant correlation between CYFRA 21-1 concentration and tumour growth. It was assumed that high CYFRA 21-1 concentration might be the factor that helps distinguish between operable and inoperable patients [27].

In 2004, Kramer et al. [2004] undertook research on the method of TPS evaluation using soluble fragments of cytokeratin 18 liberated from neoplastic cells. They concluded that cytosol-soluble CK18 complex is secreted due to neoplastic cell necrosis, and apoptosis is connected with the dissociation of CK 18 filaments by caspases. Therefore, the differentiation of both the cytokeratin forms may lead to defining the mode of tumour cell necrosis. What is more, the fragment of CK18 dissociated by kaspases constitutes the smallest subunit of the total serum CK18. This would mean that apoptosis is not the main mechanism leading to the release of serum TPS. Contrary to TPA, TPS is the marker of cancer cell necrosis, which occurs under conditions of a considerable deficit of mitochondrial ATP, e.g. in hypoxia. According to the authors [28], further research should focus on the relationship between the intensity of apoptosis, measured by the concentration of dissociated CK18 subunits, and tissue hypoxia.

Many reports indicate CEA sensitivity amounting to 50–60%. It is the marker of choice for lung cancer (adenocarcinoma) with distant metastases. The physician should not rely on only one marker in the diagnosis and monitoring of neoplasm since the level of expression of these proteins differs in different cells. Many authors underline the value of simultaneous measurement of CEA and CYFRA 21-1 in the diagnosis of non-small cell lung cancer (NSCLC), squamous cell cancers of the head and neck, and cervical or bladder cancer [27]. Salama et al. [1998] reported on high pleural concentration of both the markers in patients with mesothelioma [29].

CK 19 subunits may show some characteristic morphological features of cancer cells or other diseases. In the study by Nakayama et al. [2003], the presence of these markers was shown in the course of idiopathic and systemic lung fibrosis [21].

Contrary to many reports regarding the role of cytokeratin markers in cancer diagnosis, monitoring and treatment, few papers focused on their role in the assessment of occupational cancer risk. Kulpa et al. [2000] noted elevated serum TPA concentration in over 14% of the population under long-term exposure to chromium who did not develop any cancer symptoms [30].

In our study, a strong correlation was observed between blood cadmium concentration and CEA concentration (cell differentiation marker). This finding may point to disorders in the maturation and differentiation of respiratory epithelium under the influence of cigarette smoke, and it may reflect an initial stage of cellular metaplasis. The dependence between the concentration of the TPA and TPS and the duration of exposure to carcinogenic metals, that was noted in our study, might be an indirect evidence for adverse effects of that exposure on the cell structure, mainly epithelium, leading to its gradual necrosis. All the metals considered in this study were found to have influence on the concentration of the evaluated markers, except for CYFRA 21-1, but the direction of this influence varied. The relationship between TPS concentration and lead or arsenic intoxication was confirmed only at examination II conducted in 2003, after a longer period of exposure. The positive multiple regression ratio for these correlations seems to confirm the necrotic or carcinogenic activity of these metals at concentrations not significantly exceeding the MAC value in workers under conditions of long-term exposure. The protective and anticarcinogenic role of selenium could be observed: selenium concentration increased with the increasing serum TPS concentration.
Lead (especially FEP level) and cadmium were also found to belong to the group of metals having influence on TPA concentration, although the multiple regression ratio for Cd-TPA correlation was negative. This was a rather unexpected finding, taking into account the direct proportion between cigarette-year and TPA concentration. The impact of lead was revealed at examination II, like in the case of TPS.

A direct proportion was found between arsenic or lead intoxication and serum TPS concentration, referring to a longer exposure period (examination II conducted in 2003) that could reflect exposure-related necrosis of normal epithelial cells. The negative multiple regression ratio for the correlation between serum TPA concentration and blood cadmium concentration may indicate a higher influence of cadmium on the neoplastic transformation of normal epithelial cells (strong positive Cd-CEA dependence) than on cellular apoptosis.

Zinc, copper and selenium are the microelements and protective metals. It has been shown that selenium supplementation (at concentrations ranging from 1 to 6 mg/kg of food or drinking water) has a preventive effect on chemically-induced and spontaneous or virally-induced cancers. What is more, there is some indirect epidemiological evidence that zinc deficiency may increase susceptibility to some cancers. Zinc at low concentrations antagonized the carcinogenicity of cadmium and some organic compounds [2].

In our study, the mean serum concentrations of selenium, zinc and copper significantly increased during the two years of the observation. The finding that lead concentration decreased significantly over this period of time, provides evidence for the hypothesis that the toxicological status and the dietary habits of people exposed to heavy metals play a role in determining the amount of protective metals in the organism.

CONCLUSIONS

1. The significant positive correlation between blood lead concentration or free erythrocyte protoporphyrin (FEP) level and urine concentration of arsenic in the examined workers may point to a higher risk of the late effects of intoxication with these metals under conditions of combined exposure.

4. A strong positive correlation was found between blood cadmium concentration and the concentration of CEA, the marker of abnormal cell differentiation. This relationship might reflect a neoplastic transformation of normal cells stimulated by some carcinogens (e.g. cadmium).

5. All the examined carcinogenic or potentially carcinogenic metals (As, Cd, Pb) belong to the group of factors that have influence on serum TPS and/or TPA concentrations in smelter workers.

6. No correlation was found between CYFRA 21-1 and any metal considered in the present study. However, it is plausible that changes in the concentration level of this marker might be revealed after a longer observation period.

7. Long-term combined exposure to heavy metals and arsenic in work environment is a potential risk factor for the development of occupational cancer. Supportive to this assumption is the correlation between the period of work and serum TPA and TPS concentrations as shown in our study.

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