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NEUROLOGICAL AND RESPIRATORY SYMPTOMS IN SHIPYARD WELDERS EXPOSED TO MANGANESE

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Abstract
Objective: The nervous system is the major target of the toxic effect of manganese (Mn) and its compounds in welding fumes. In humans, inhalation is the most frequent route of Mn access, therefore, the respiratory tract and lungs are usually involved in the process of translocation of inhaled noxius agent by blood to the brain. This study was performed to assess whether it is possible to use neurophysiological tests for the detection of early effects of exposure to low Mn concentrations. It is also known that irritating welding fumes affect distal bronchioles of nonciliated, epithelial Clara cells, which secret anti-inflammatory and immunossupresive Clara cell protein (CC16) into the respiratory tract. The examination of usefulness of CC16 as early pulmonary biomarker for neurophysiological abnormal results of welding fumes exposure was performed. **Materials and Methods:** The study group comprised 59 welders employed at different workposts in a shipyard, matched for age and smoking habits with the control group composed of 23 mechanicians and electricians not exposed to welding fumes. Subjective neurological symptops (CNS), visual evoked potentials (VEP) and electroencephalography (EEG) were examined in welders and the relationships between Mn concentrations in the air, blood and urine as well as between cummulative exposure index (CEI) (Mn mg/m^3 • years of exposure) were investigated. Effects of exposure were expressed in the form of biomarkers of the body burden, and CC16 as early pulmonary biomarker in welding exposure was examined by sensitive latex-immunoassay. **Results:** Abnormal results of VEP and EEG and the lowest CC16 levels were found in the youngest welders exposed to welding fumes. Those changes were related to the highest Mn airborne levels (x_g > 0.3 mg/m^3) and high blood Mn concentrations (~14.0 µg/dL). The highest values of correlation coefficients were found only in welders characterized by abnormal neurophysiological results, VEP (r = 0.83) and VEP and VEP+EEG (r = 0.82). The multiple linear regression analysis from all analyzed subgroups, indicated that those with only abnormal neurophysiological tests, VEP and EEG, showed the highest values of partial correlation. It also revealed partial correlation cofficiants between Mn in the air, CEI (Mn mg/m^3• years) and CC16, Mn-B and Mn-U in VEP and VEP+EEG groups. It was found that the highest partial correlations were between the magnitude of exposure – Mn mg/m^3, CEI and Mn-B concentration (R^2 = 0.72, R^2 = 0.66) as well as between CC16 pulmonary biomarker effects and Mn-B concentration (R^2 = 0.51). **Conclusions:** The subclinical effects revealed in neurological endpoints and abnormal results of neurophysiological tests, VEP and EEG, confirmed that those sensitive tests could be used for the detection of early effect of exposure to low manganese concentration. Inhibition of Clara cell protein secretion in younger welders not adapted to the Mn environment suppresses anti-inflammatory effect in the respiratory tract and probably enhances the absorption and thus the incidence of subclinical neurotoxic symptoms related to airborne Mn and Mn-B levels.

Key words:
Clara cells protein, Welding fumes, Manganse, Neurotoxicity

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INTRODUCTION

Manganese (Mn) is an essential metal of great toxicological concern, primarily because its exposure is via inhalation. It is estimated that more than 1 million workers worldwide perform some type of welding as part of their work duties. The nervous system is the major target of the toxic effect of Mn and its compounds [1]. It has been recognized that chronic Mn poisoning assumes a particular form of Parkinson's syndrome. However, this applies only to exposures in extremely poor working conditions (e.g., Moroccan Mines) [2]. Later observations of Mn poisoning revealed its multifocal process. The pyramidal and cerebellar symptoms as well as mental disorders in the form of psychoorganic syndrome of different intensity seem to justify their denomination as toxic encephalopathy [3–7]. Among other manifestations, the retrobulbar damage of the optic nerve and polyneuropathic symptoms have been reported in the literature [6,7]. Mn accumulates selectively in the globus pallidus of basal ganglia, where it can produce hyperintensitive signals in the brain magnetic resonance imaging (MRI) [8,9]. Nowadays, due to improved hygiene at work and decreased Mn concentrations at workposts, serious cases of poisoning by this metal are no longer reported, and the neurological diagnosis is directed towards detecting early symptoms of poisoning and abortive forms whose diagnosis was not feasible in the past. The introduction of neurophysiological examinations into neurotoxicology has rendered it possible to study the nervous system thoroughly and consequently to detect subclinical neurological pathologies in persons occupationally exposed to manganese.

Growing evidence suggest that in exposure to welding fumes a significant role is played by nanoparticles uptake [10] by cells of the respiratory epithelium [11]. Nanoparticles reach the blood and many other target sites, e.g., the liver, heart and blood cells [12,13] or may be translocated into the brain [14,15]. It is well established that occupational exposure to welding fumes contributes to an increased incidence of respiratory diseases in the exposed population [16–19]. It has been demonstrated that impairment of airway epithelium and/or Clara cells in bronchioles may also lead to illnesses of the respiratory system [20]. In our previous studies, adverse effects of welding fumes on Clara cells were found [21,22]. Clara cell protein (CC16), a peripheral marker of the respiratory epithelial injury, was proved to be a sensitive pulmonary marker of early symptoms in workers occupationally exposed to asbestos or silica and in smokers [23–25]. CC16 is supposed to be a suitable biomarker of adverse effects of pneumotoxicants on airway epithelium, which could predict the disease outcome [26,27].

The aim of this study was to assess whether it is possible to find any relationship between disturbances in the function of the nervous system and concentrations of Mn in the air or between its cummulative exposure index (CEI) (Mn mg/m^3 • years of exposure). To this end, we examined subjective neurological symptops (CNS), visual evoked potentials (VEP) and electroencephalographic (EEG) recordings in welders. The magnitude of exposure was evaluated by individual air sampling at shipyard workplaces and by assessing biomarkers of exposure, concentrations of Mn in blood (Mn-B) and urine (Mn-U). Spirometry and vital capacity (VC) of lung was also performed. In addition, the usefulness of CC16 as an early pulmonary biomarker of effects in such exposures was analyzed.

In this study it was assumed that the exposure to welding fumes produced early neurological symptoms revealed in VP and EEG, depending on Mn concentration, and may alter distal airspace epithelial cells and/or Clara cells and provoke impaired integrity of the bronchoalveolar/blood barrier.

MATERIALS AND METHODS

The study design was approved by the local Ethics Committee and complied with the current laws in Poland. The examined subjects were informed about the range and purpose of the study and their informed consent was obtained.

Study population

The study group comprised 59 welders employed at different workposts in a shipyard, matched for age and smoking habits with the control group composed of 23 mechani-

cians and electricians not exposed to welding fumes. Samples of blood and urine as well as welding fumes from the breathing zone of the welders involved in manual metal arc, metal active gas/mild steel (MMA, MAG/MS) and oxy-acetylene gas cutters and fitters were collected. MMA welders used coated electrodes (EB 150, EB 246, ER 146). The metal welded was mild steel (structural, usual-grade, ST 4, ST 6).

Neurological and neurophysiological examinations

Neurological examinations performed under ambulatory conditions covered both subjective and objective status of the nervous system. Medical history was extended to include questions about symptoms possibly attributable to Mn exposure. In the objective examination, the condition of the cranial nerves and muscular system, the presence of limb tremor and its nature, the movement coordination and diadochokinese were assessed. The data obtained were documented in a form of neurological examination standardized for all study subjects, developed especially for this purpose. EEG was taken on an 8-channel Beckman unit in the international system of 10–20 electrodes. The speed of paper transport was 30 mm/sec, gain 5 mm = 50 μV, time constant 0.3 sec. After EEG recording at rest, and checking the reaction retention, a 3-min hyperventilation was performed, followed by rhythmical flash activation. During the final trial lasting 30 min, a 10-sec flash series at the frequency of 1–30 Hz was applied. EEG recordings were assessed on the basis of the generally recognized criteria, including frequency, amplitude, morphology, localization, rhythms and their reaction to stimuli. The results were divided into two groups: normal and abnormal, according to the classification of Majkowski [28].

The EEG recordings with one of the basic rhythms of the brain bioelectric functions and those with about 5% of symmetric slow theta waves of 6–7 Hz in temporal-occipital leads were classified in the normal group. Abnormal recordings were classified according to the nature of pathologies and their intensity. The following four subgroups were distinguished: generalized changes, focal changes, paroxysmal changes, and asymmetrical recordings. Visual evoked potentials were recorded using a Dantec 2000 C Neuromatic unit with stimulation of each eye by the checkerboard reversal pattern. The responses were received from the active electrode placed at the cranial midline, 3 cm above the inion. A total of 200 evoked responses were averaged over 300 ms analysis time. Latency of Nl, P100, N2, amplitude of Nl P100, P100 N2 and VEP configuration were evaluated. Taking an ophthalmologic history and checking visual acuity preceded the examination.

Exposure assessment

Individual dosimetry method, as described in PN-Z-04008-7:2002 [29], was used to assess occupational exposure to manganese present in the ambient air at workposts of welders and fitters employed in the ship industry and in the production of batteries. The welders and fitters welded by hand mainly low-alloy steel type ST-3 (MS-mild steel), using low-hydrogen coated electrodes EB-146 (manual metal arc welding), or solid wire type SPG 3S in CO_2 shielding (metal active gas welding). They also used acetylene-oxygen flame for cutting steel grades. The welders worked on ships and in production rooms, also in closed spaces (double bottom of ships). The welding dust comprised mainly iron, manganese, silicon, fluorides, calcium, sodium (MMA method), and iron, manganese, silicon, and copper (MAG method). The welding fumes comprised mainly carbon oxides (CO, CO_2) and nitrogen oxides (NO, NO_2). Manganese dioxide, ammonium chloride, zinc oxide, and electrolyte, containing ammonium chloride and zinc chloride, were used to manufacture batteries. The air was constantly sampled at the breathing zone of the subjects exposed during the effective working hours (6–7 h).

Flame atomic absorption spectrophotometry was used to analyze Mn concentration in the samples. Manganese was determined using a Varian Spectr AA 250 atomic absorption spectrophotometer with wave length of 279.5 nm in the air-acetylene flame.

In the ambient air at workposts of welders and fitters, Mn concentrations varied from 0.004 to 2.667 mg/m^3 (arithmetic mean, 0.399 mg/m^3; geometric mean, 0.154 mg/m^3; standard deviation, 0.586). Of the 59 persons exposed, 30

worked in Mn concentrations exceeding MAC values. The cumulative exposure index (CEI) (a product of total Mn exposure index and exposure duration in years) was calculated for each worker exposed. In the whole group of workers, CEI values varied from 0.008 to 35.52 (arithmetic mean, 8.045; geometric mean, 4.615; standard deviation, 6.562).

Manganese determination

Personal air sampling at breathing zone was applied (Casell AFC-123, flow rate 2 l/min). Total dust was collected (over a period of about 7 h/shift) on membrane filter (Sartorius 111304, 0.8 μm, ∅32 mm). In the sample collected on the filter, Mn was determined by flame atomic absorption spectrometry using Varian SpectorAA 250 atomic spectrometer.

Manganese in blood was determined by graphite furnace atomic absorption spectrophotometry [30]. Blood samples were collected by veinpuncture using Vacutainers (Bencton-Dickinson) and stored at +4° C. Each sample was distributed in the laboratory into two polyethylene tubes and stored at -20° C until determination. Blood was diluted six times by distilled water and Mn modifier was added. For internal quality control, Seronorms No. 205052, 205053 and 203056 and AMI B No. 1001, 1002, 1003, 1004, 1005 lyophylized blood samples were used.

Manganese in urine was determined by graphite furnace atomic absorption spectrophotometry according to the method of Perkin Elmer for manganese [31]. Urine was diluted two times by distilled water and Mn-pallad modifier was added. For internal quality control, Seronorm No.403125 was used. The solution obtained was analyzed using Perkin-Elmer model 4100 ZL Zeeman with an autosampler AS-70 and computer data station PE 6200.

Biochemical determination

Clara cell protein was determined by a latex immunoassay [32]. Specific rabbit antibody against CC16/Protein 1 from Dako A/S, Denmark was used. To eliminate possible inteferences (complement, chylomicrons), the serum samples were pretreated by heating at 56°C for 30 min. and by the addition of polyethylene glycol 600 (16%, v/v 1/1) and trichloroacetic acid (10%, v/v 1/40). After overnight sedimentation, the samples were centrifuged and CC16 was determined in supernatants.

Spirometry

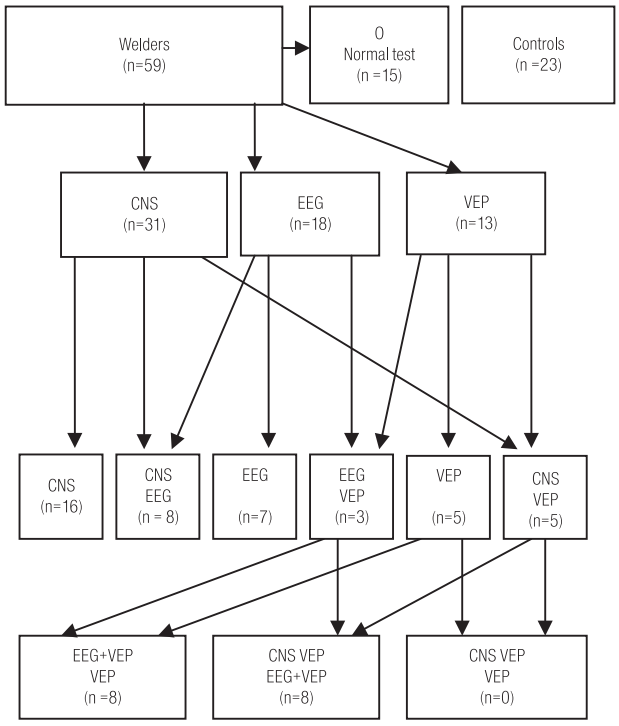
The respiratory function measurements, vital capacity (VC), were taken during the routine medical examination.

Statistical analysis

Statistical analysis of the results involved the analyses of r-Pearson correlation coefficients and multiple linear regression analysis with partial correlation coefficients. The relationship between continuous variables was examined by linear regression.

RESULTS

Figure 1 shows the flowchart of neurological and neurophysiological examinations. The prevalence of subjective neurological symptoms and neurophysiological performances (EEG and VEP) were studied. In neurological examinations, functional disorders of the nervous system, complaints most frequently reported by the workers were as follows: increased emotional irritability (49.3%), headache (29.3%), sleepiness (14.7%), vertigo (12.0%), dysmnesia (12,0%), mood swings (12.0%), concentration difficulties (12.0%), anxiety and fear (9.3%), pains (13.3%) and limb paresthesia (13.3%). Any specific features did not characterize headaches reported by workers. Their location, duration and time varied. In a large group of workers, headache occurred at work and subsided after leaving the production premises. However, in many cases, headache persisted even at rest and assumed the form of vasomotor pains. Headaches were often accompanied by vertigo, frequently intensified with the changed position of the body, but in general, they were not so acute as to lead to difficulties in walking or collapse. EEG yielded 30.5% abnormal and 69.5% normal recordings in the study group. Normal EEG recordings were characterized by the presence of alpha and beta rhythms as well as by the alpha rhythm blockade. The alpha rhythm frequency in those exposed was 9–11 Hz. The alpha wave amplitude varied,



CNS – subjective neurological symptoms;
VEP – visual evoked potentials.

Fig. 1. Flowchart of neurological and neurophysiological examination of shipyard welders.

and it accounted most frequently for about 40–60 μV. Beta activity of the frequency between 14 and 30 Hz and amplitude below 25 μV was another background rhythm. Recordings with 5% of symmetric slow free theta waves, frequency of 6–7 Hz in parietal leads, frequency of 4–6 Hz in anterior-temporal leads or deep structures and amplitude below 40 μV were classified as normal EEG recordings. In abnormal EEG recordings, those with generalized changes predominated.

The individual assessment of the VEP results showed abnormalities in 22.0% of welders. In 13 persons, prolonged latency of the study components, and P100 component in particular (144 ms) along with interocular asymmetry in the latency of this component, were observed. In 3 cases with abnormal VEP, asymmetries in interocular P100 latency exceeding 6 ms and decreased amplitude in single tests were found.

Table 1 summarizes the baseline characteristics of examined groups of the study population. Respiratory symptoms, VC, serum CC16 and Mn-B and Mn-U concentrations were examined in the study group of shipyard welders exposed to Mn-containing welding fumes and in the control group. Both groups did not differ with respect to age and duration of employment. The integrated cigarette smoking index (cigarette pack per year) was higher in the control group than in welders. Mn concentration in the ambient air ranged from 0.001 mg/m^3 to 0.17 mg/m^3 in the control group and from 0.003 mg/m^3 to 3.37 mg/m^3 in the study group. The amount of Mn excreted with urine of subjects exposed to welding fumes did not significantly differ from that in the control group. Mn-B concentration in the exposed workers ranged from 1.5 μg/dm^3 to 46 μg/dm^3 and was higher than in non-exposed subjects. Smoking habits did not influence Mn-B and Mn-U concentrations in both groups.

The exposed welders were divided in seven subgroups according to the results of neurophysiological examinations (Table 2). The workers with the longest employment duration who reported subjective neurological symptoms were characterized by the lowest VC and high Mn-B levels (Fig. 2). Abnormal results of neurophysiological testes (EEG and VEP) were found in a very small group of the youngest welders with statistically shortest employment duration who showed the lowest CC16, the highest Mn-B levels and the highest Mn air geometric mean levels x_g >0.3 mg/m^3, exceeding Polish TLV.

Table 3 shows the results of the analyzed r-Pearson correlation coefficients of CC16 with markers of exposure to Mn in the air and cumulative exposure index (Mn mg/m^3 • years of exposure) in shipyard welders with sub-

Table 1. Age, duration of employment and exposure indices in the study group exposed to welding fumes and controls

Group	n	Age	Duration of employment	Index of smoking (cigarette pack-years)	Mn in the air (mg/m^3)	Mn-B (μg/dL)	Mn-U (μg/g creatinine)
Study group	59	40.6 ± 9.9	19.7 ± 9.9	6.9 ± 9.1	0.59 ± 0.85*	11.42 ± 8.37	0.41 ± 0.36
Controls	23	41.5 ± 10.7	23.2 ± 11.4	13.9 ± 20.2	0.002 ± 0.002	6.07 ± 2.3	0.39 ± 0.30

Mean values ± SD; * P < 0.05 vs. controls; Mn-B – blood Mn concentration; Mn-U – urine Mn concentration.

Table 2. Characteristics of the study group by the type of nervous system disorders compared with controls

Neurological subjective symptoms and abnormal results of neuropysiological tests	Study group							Controls (N = 23)
	CNS (N = 16)	CNS, EEG (N = 8)	EEG (N = 7)	VEP, EEG (N = 3)	VEP (N = 5)	CNS,VEP (N = 5)	O – normal (N = 15)	
Age (years)	43.3 ± 8.2	43.6 ± 9.4	36.1 ± 7.8	26.7 ± 0.6*	35.4 ± 10.4	40.0 ± 13.9	39.0 ± 10.8	41.5 ±10.7
Employment duration (years)	21.9 ± 11.3	22.1 ± 12.4	15.4 ± 6.1	6.0 ± 3.5*	14.0 ± 10.8	18.2 ± 15.7	17.6 ± 11.9	23.2 ± 11.4
Exposure to Mn (mg/m^3)	0.23 ± 0.47	0.56 ± 0.81	0.55 ± 0.94	0.40 ± 0.17	0.71 ± 1.11	0.33 ± 0.44	0.54 ± 1.01	0.02 ± 0.02
Smokers (pack-years)	7.9 ± 9.2	5.9 ± 8.8	4.8 ± 7.2	2.0 ± 3.5	10.2 ± 19.6	4.0 ± 8.9	11.3 ± 9.9	13.9 ± 20.2
Spirometric parameter:								
VC	82.6 ± 15.5*	81.4 ± 8.7*	87.6 ± 14.4	88.4 ± 7.2	91.0 ± 14.3	83.0 ± 4.2	96.5 ± 8.8	100.5 ± 13.7
Biomarkers:								
CC16, μg L-1	14.7 ± 7.3	15.1 ± 6.3	14.6 ± 5.9	9.9 ± 1.7	12.1 ± 4.7	17.8 ± 4.3	15.1 ± 7.0	15.6 ± 5.3
Mn-B, μg dL-1	12.8 ± 12.3	10.6 ± 2.8*	10.6 ± 4.9	13.7 ± 12.6	8.4 ± 5.5	9.0 ± 1.6*	9.2 ± 4.7	6.1 ± 2.3
Mn-U, μg g creatinine	0.46 ± 0.42	0.30 ± 0.14	0.30 ± 0.12	0.23 ± 0.06	0.22 ± 0.03*	1.08 ± 1.03	0.44 ± 0.40	0.38 ± 0.3

Mean values ± SD; * P < 0.05 vs. controls; VC – vital capacity; other abbreviations as in Fig. 1 and Table 1.

Table 3. Correlation coefficients of CC16 with markers for manganese exposure, Mn mg/m^3, and cumulative exposure index (CEI, Mn mg/m^3 • years)

Correlation coefficient	Study subgroups								Pooled subgroups with abnormal VEP + EEG + CNS		
	Controls (N = 23)	CNS (N = 16)	CNS, EEG (N = 8)	EEG (N = 7)	VEP, EEG (N = 3)	VEP (N = 5)	CNS,VEP (N = 5)	Normal test (N = 15)	(VEP, EEG) + VEP (N = 8)	(VEP, EEG) + (CNS,VEP) (N = 8)	(CNS,VEP) + VEP (N = 10)
CC16 (Mn mg/m^3)	-0.02 (0.99)	0.01 (0.96)	0.05 (0.96)	0.31 (0.49)	0.64 (0.56)	0.83 (0.08)	0.12 (0.85)	-0.99 (0.74)	0.82 (0.015)	0.02 (0.96)	0.33 (0.35)
CC16 (Mn mg/m^3 • year)	-0.11	-0.05	-0.26	0.35	-0.53	0.85	0.38	0.15	0.83	0.37	0.38
	(0.63)	(0.86)	(0.53)	(0.45)	(0.65)	(0.07)	(0.53)	(0.61)	(0.01)	(0.36)	(0.28)

Percentage vs. respective control values. Statistically different from the control group, p < 0.05. Abbreviations as in Fig. 1.

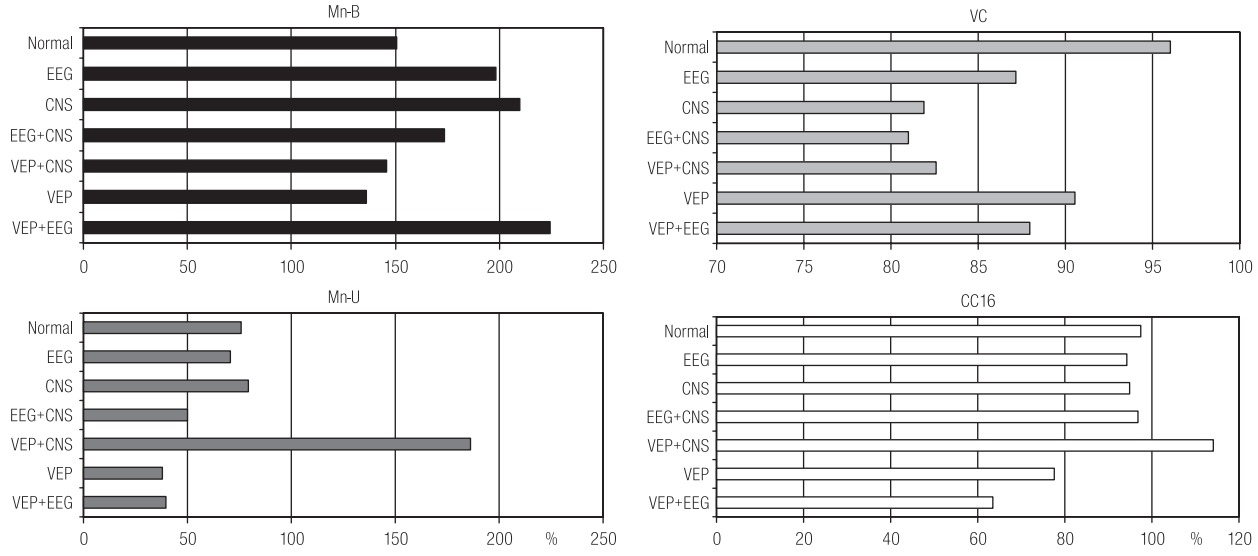


Fig. 2. Comparative study of biomarkers for Mn in blood (Mn-B), Mn in urine (Mn-U), vitalcapacity (VC), and CC16 in workers with neurological subjective symptoms (CNS), abnormal neurophysiological EEG and visual ewoked potential (VEP) results. Abbreviations as in Fig. 1.

Table 4. Multiple linear regression of Mn in the air, cumulative exposure index (CEI) – (Mn mg/m^3 • year), CC16 with Mn-B and Mn-U in welders with abnormal results of neurophysiological tests, VEP and (VEP+EEG)

	(VEP+EEG) + VEP (n=8)		
	Partial correlation	t-student	P
Mn mg/m^3 (Mn-B)	0.72	5.81	0.03
Mn mg/m^3 (Mn-U)	0.30	2.48	0.13
CEI, Mn mg/m^3 • y (Mn-B)	0.66	7.93	0.01
CEI, Mn mg/m^3 • y (Mn-U)	0.32	3.85	0.61
CC16 (Mn-B)	0.51	1.69	0.23
CC16 (Mn-U)	0.06	0.22	0.85

Mn mg/m^3 – R^2 = 0.97; CEI, Mn mg/m^3 • years – R^2 = 0.99; CC16 – R^2 = 0.82. Abbreviations as in Fig. 1 and Table 1.

jective symptoms, and divided into subgroups according to neurophysiological examination results. To improve the statistical power of r-Pearson analysis we also examined the group of welders pooled by neurophysiological and other results. The highest values of correlation coefficients were found only in welders characterized by abnormal neurophysiological tests, VEP (r = 0.83) and VEP and VEP+EEG (r = 0.82). In multiple linear regression analysis from all analyzed subgroups, the pooled subgroup with only abnormal neurophysiological tests, VEP and EEG, also showed the highest values of partial correlation (Table 4). This table also shows partial correlation cofficients between Mn in the air, CEI (Mn mg/m^3• years) and CC16, Mn-B and Mn-U in VEP and VEP+EEG groups. It was found that the highest partial correlations were between the magnitude of exposure – Mn mg/m^3, CEI and Mn-B concentration (R^2 = 0.72, R^2 = 0.66) as well as between CC16 pulmonary biomarker effects and Mn-B concentration (R^2 = 0.51).

DISCUSSION

It is well documented that the central nervous system is the critical target organ of chronic exposure to manganese, which is manifested by neurobehavioral symptoms and neurological signs characteristic of an extrapyramidal syndrome, which has several similarities to Parkinson's disease [33–37]. Biomonitoring of Mn exposure is still a crucial problem in toxicology [38–41]. Furthermore, no biochemical indicator is available for the detection of early neurotoxic Mn effects. Nowadays, neurofunctional examinations (e.g., measurements of visual reaction time, eye-hand coordination, hand steadiness) represent the most sensitive approach to this issue [33,34,37]. In our previous study of neurotoxic effect of exposure to aluminum foundry fumes [42], we found in the youngest workers early abnormal changes in neurophysiological test (VEP), the highest levels of serum aluminum levels and the lowest CC16 and Fe concentrations. In the literature, there is lack of information on the significance of Clara cells protein in Mn exposure and neurotoxicity.

The present study carried out in the group of welders pointed to changes in neurophysiological test – VEP. In subgroups selected according symptoms and neurophysiological abnormal results (Fig. 1), a statistically significant association was found between CC16 and current Mn concentrations in the air and even more significant association between CC16 and CEI in the VEP and pooled (VEP,VEP+EEG) groups (Table 3). In a small group of welders with VEP and EEG, these changes depended on blood concentration of 12.7 μg Mn/dL, which corresponded with Mn concentration in the air x_g = 0.35 mg Mn/m^3. It should be added that TLV value in Poland accounts for 0.3 mg Mn/m^3. The results confirm the potential risk of neurological changes in workers occupationally exposed to manganese. However, in these subjects, the lowest serum CC16 concentration (Table 2) as well as positive correlation between CC16 and the level of Mn exposure were observed (Table 3). It was concluded that lung injury induces a decrease in 16 kDa Clara cell protein in bronchoalveolar lavage fluid owing to a reduced protein production by damaged Clara cells, and an increase in serum protein levels, resulting from its enhanced leakage across the infracted bronchoalveolar/blood barrier in high lung irritant exposure [43]. This observation was confirmed in our previous study of shipyard welders [21].

It should be noted that although exposure to welding fumes is associated with exposure to nanoparticles, it is not sufficiently documented in the literature [10], but it may suggest the relationship between the respiratory and neurologal systems. Nanoparticles may enter cells of re-

spiratory epithelium and thus also Clara cells [11], translocate through cell membranes and pass the blood brain barrier. In recent years, the classical tests for lung injury have changed the endpoint testing for induction of oxidative stress, cell activation and signaling, and release of inflammatory mediators [15]. The consideration of dose--response relationship in the toxicology of nanoparticles is a significant problem. Concentration of nanoparticles and resulting total an extensive surface area determine their interactions with biological systems [44,45].

In the reviev of manganese neurotoxicity, Verity [36] proposes a multifactor hypothesis on coupling of Mn^{2+} uptake with coincident transport of aluminum and iron. Selectivity of dopaminergic neurons is dependent upon interactions of Mn^{2+} with dopamine transport and the role of Mn^{2+} as a pro-oxidative toxicant in conjunction with changes in Fe concentration. The assumption that manganese at low exposure levels is one of possible mechanisms, by which certain protective lung functions could be disturbed and thus making the organism more susceptible to infections is supported by toxicological studies [46].

The effects of long-term Mn exposure on the respiratory system in workers have been discussed in numerous studies [16,19,33,47].

The respiratory effects found in Mn-exposed workers, probably caused by magnesium present at workplaces and smoking are not synergistic, but the longer duration of employment is linked to abnormal subjective symptoms (Table 2). In our study, it was confirmed that Mn exposure increased Mn-B concentration (Tables 1 and 2, Fig. 2). It should be emphasized, however, that in the study carried out by Roels et al. [34] it was not yet possible to identify a biological marker for assessing the intensity of exposure to Mn or its concentration in the target organ. Evaluation of individual exposure to Mn is thus best carried out by its concentration monitoring in total and respirable dust in the breathing zone of the workers. This fact has been explained by very efficient homeostatic mechanisms that prevent large fluctuations of Mn concentration in whole blood since Mn is mainly excreted by the biliary route.

Clewell et al. [37] after reviewing the scientific evidence on Mn toxicity, determined the most appropriate measure of exposure to airborne Mn that induces subclinical effects, which are more related to the most recent exposure and respirable particulates, and calculated benchmark concentrations for eight endpoints ranging from 0.09 to 0.27 mg Mn/m^3.

In our study, multiregression analysis revealed strong partial correlation between airborne Mn slightly exceeding 0.3 mg Mn/m^3, CEI, CC16 and Mn-B concentrations, evoked subtle, subclinical symptoms manifested by neurological endpoints tested by VEP and EEG (Table 4).

Those facts support our opinion that in young welders, not yet adapted to work in exposure, VEP+EEG abnormal tests correlated with Mn-B and serum CC16 levels, which may explain suppressing anti-inflammatory effects of CC16 in the respiratory tract in these conditions.

Increased Mn-B levels may indicate carrier-mediated brain entry. Considering carrier-mediated brain influx, repeated excessive Mn exposure should produce higher Mn accumulation in the brain demonstrated by subclinical symptoms [48–51].

CONCLUSIONS

The subclinical effects revealed in neurological endpoints and abnormal results of neurophysiological tests, VEP and EEG, confirmed that those sensitive tests could be used for the detection of early effect of exposure to low manganese concentration.

Inhibition of Clara cell protein secretion in younger welders not adapted to the Mn environment suppresses anti-inflammatory effect in the respiratory tract and probably enhances the absorption and thus the incidence of subclinical neurotoxic symptoms related to airborne Mn and Mn-B levels.

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