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ORIGINAL ARTICLE

A study of atherothrombotic biomarkers in welders

Dag G. Ellingsen[1] · Maxim Chashchin[2,3,6] · Ingebjørg Seljeflot[4,5] · Balazs Berlinger[1] · Valery Chashchin[2,6] · Leo Stockfelt[7] · Yngvar Thomassen[1]

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Abstract

Introduction Studies have shown that welders have increased cardiovascular mortality. This may be due to airborne particulate matter (PM) exposure. Elevated levels of PM in polluted urban air have been associated with increased cardiovascular mortality and morbidity. This study seeks to explore potential mechanisms for the increased cardiovascular mortality in welders.

Methods Seventy welders were compared to 74 referents. Exposure to PM was assessed by personal full-shift sampling of work room air the last 2 days before collection of blood samples. Selected biomarkers of pro-coagulant activity, endothelial/platelet activation and systemic inflammation were determined in the samples.

Results The welders had been occupationally exposed to PM for 15 years on average. The geometric mean current exposure to PM was 8.1 mg/m^3. They had statistically significantly higher concentrations of TNF-α, P-selectin, CD40L, prothrombin fragment 1 + 2 and D-dimer than the referents. Increasing concentrations of D-dimer and CD40L were observed by increasing current exposure to PM.

Discussion The study shows that welders highly exposed to welding PM were in a pro-thrombotic state with increased thrombin generation and consequently higher D-dimer concentrations. The welders had also increased endothelial/platelet activation as compared to the referents. These alterations are compatible with increased cardiovascular mortality as previously reported among welders.

Keywords Welders · Biomarkers · Thrombosis · Endothelial activation · Cardiovascular disease

Introduction

Epidemiological studies show that welders have increased cardiovascular mortality. One study reported a relative mortality risk of 1.66 (95% CI 1.01–2.68) due to ischemic heart disease compared to referents (Moulin et al. 1993). A standardized mortality rate of 1.35 (95% CI 1.1–1.6) due to ischemic heart disease was observed in a Swedish cohort (Sjögren et al. 2002). A study from Denmark reported a standardized incidence rate of 1.12 (95% CI 1.01–1.24) for acute myocardial infarction and 1.17 (95% CI 1.05–1.31) for chronic ischemic heart disease (Ibfelt et al. 2010). A recent meta-analysis suggested an increased relative risk for acute myocardial infarction of 1.69 (95% CI 1.18–2.42) (Mocevic et al. 2015).

Welders are exposed to aerosols containing a mixture of metal components and gases such as ozone and nitrogen oxides (Antonini et al. 2003). The polydispersive welding particulate matter (WPM) is formed mainly by evaporation

✉ Dag G. Ellingsen
dag.ellingsen@stami.no

1 National Institute of Occupational Health, P.O. Box 5330 Dep, 0304 Oslo, Norway

2 Northwest Public Health Research Centre, Saint Petersburg, Russia

3 Present Address: City Centre of Occupational Health, Saint Petersburg, Russia

4 Department of Cardiology, Center for Clinical Heart Research, Oslo University Hospital, Ullevål, Norway

5 Faculty of Medicine, University of Oslo, Oslo, Norway

6 North-Western State Medical University Named After I.I. Mechnikov, Saint Petersburg, Russia

7 Department of Occupational and Environmental Medicine, Sahlgrenska University Hospital, University of Gothenburg, Gothenburg, Sweden

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of the consumable electrode and wire, followed by rapid condensation of the vapor on ions and further particle growth under supersaturation conditions (Berlinger et al. 2011). Measurements of particle number concentrations have indicated that welding processes result in a log-normal distribution of the particle sizes with a median aerodynamic diameter of 120 nm (Stephenson et al. 2003), but particle number size distributions vary depending on the welding method (Berlinger et al. 2011). The WPM is present as agglomerates with primary spherical particles typically in the 5–40 nm range (Berlinger et al. 2011). Typically, the major mass of WPM is found between 0.2 and 1 µm aerodynamic diameter (Berlinger et al. 2011). The particles have different chemical compositions depending on the base material that is welded and the composition of the welding electrodes (Spiegel-Ciobanu 2003). Particles generated during manual metal arc (MMA) welding are partly soluble (Berlinger et al. 2008).

Exposure to elevated levels of ambient airborne particulate matter (PM) has been associated with increased cardiovascular mortality and morbidity (Brook et al. 2010). Biological mechanisms for the increase of cardiovascular diseases due to PM exposure are not fully understood. Potential mechanisms have been linked to increased systemic inflammation which is regarded as an independent risk factor for sudden cardiovascular death, imbalances in coagulation favoring the propagation of thrombi, and that inflammation could affect the autonomic nervous system resulting in altered heart rhythm control (Donaldson et al. 2005).

Particles depositing in the alveoli are, depending on size, phagocytized by macrophages (Geiser and Kreyling 2010). Alveolar macrophages express pro-inflammatory cytokines interacting with airway epithelial cells (Hiraiwa and van Eeden 2013). Also interleukin (IL)-6 is released, inducing the synthesis of the acute phase reactant C-reactive protein (CRP) in hepatocytes (Hiraiwa and van Eeden 2013). Cytokines like IL-1β and tumor necrosis factor-α (TNF-α) may activate a number of downstream pro-inflammatory genes in different cell types, leading to increased expression of adhesion molecules in endothelial cells and further induction of cytokines and chemokines (Kuldo et al. 2005). Also tissue factor (TF) expression is induced (Lwaleed et al. 2007). TF is the single most important inducer of the extrinsic pathway of the blood coagulation system and may contribute to a pro-coagulant state with increased thrombin generation which also is known to be induced during inflammation (Kuldo et al. 2005). Mechanisms explored in this study are the induction of systemic inflammation, endothelial/platelet activation and the presence of hypercoagulability. The subjects investigated are shipyard welders and referents employed as turners/fitters in St. Petersburg (Russia). The aim of this study was to assess selected biomarkers of pro-coagulant activity, endothelial/platelet activation and systemic inflammation in welders as compared to a reference population. The pneumoproteins club cell protein 16 (CC-16) and surfactant protein D (SP-D) were also measured to study pulmonary alterations. A further aim was to assess WPM exposure among the welders and study the impact of individual exposure on the measured biomarker concentrations.

Materials and methods

Study design and subjects

The main criteria for inclusion into this cross-sectional study were at least 1/2 year of employment as full-time male welder or fitter/assembler (as referent) in two shipyards or a heavy machinery construction plant in St. Petersburg at the time of the examination. Included welders had to be exposed at the time of the examination. Thus, subjects who were on sick leave or otherwise absent from work at the time of the examination were not considered for inclusion.

Seventy-four welders working in two different shipyards were identified as eligible and invited to participate in the study. These MMA and gas metal arc (GMA) welders were organized in groups (brigades). Welders from seven groups were asked to participate. Among the 74 eligible welders, three were on sick leave when the examination took place. One welder refused to participate. Thus, their participation rate was 94.6%.

The referents were also organized in brigades. They worked as fitters/assemblers in a heavy machinery construction plant. Eighty potential referents working in two brigades were eligible for inclusion into the study. Two subjects were on sick leave on the day of the examination and four subjects refused to participate. Thus, their participation rate was 92.5%.

Subjects, who according to the medical records of the occupational health services, had been diagnosed with, e.g., cancer, rheumatoid diseases, diabetes, chronic inflammatory disease, acute inflammations (e.g., sinusitis, pneumonia), known alcoholism or drug abuse were not considered for inclusion. However, the participants were generally in good health, and no such cases were known.

The study was approved by the Norwegian Regional Ethical Committee for Medical Research (REK2) and the Ethics Committee of the Northwest Public Health Research Centre (NWPHRC) in St. Petersburg (Russia). Participation in the study was strictly voluntary. An informed written consent was obtained from the participants.

Examinations

The examinations took place at the occupational health clinic of the respective industrial facilities. All participants underwent a structured interview, including information on smoking and alcohol habits. Blood pressure (BP) was measured in both arms while sitting in a chair with support in the back in accordance with standardized methods used in Russia. If a significant asymmetry of more than 10 mmHg for systolic and 5 mmHg diastolic BP was measured, measurements were repeated. When the measurements differed by less than 5 mmHg, the measurements were stopped and the mean value of the two arms was reported as the BP. Blood samples were collected between 8:30 and 9:30 a.m. Qualified medical personnel from the NWPHRC conducted the examinations. Table 1 shows background data of the welders and referents.

Collection of blood samples

The skin was cleaned with ethanol before sampling of blood from the cubital vein with Vacuette® vacutainer tubes (Greiner Bio-One GmbH, Kremsműnster, Austria). Serum was harvested from blood collected in tubes without additives that rested for 30 min before centrifugation for 15 min at 2000*g* for determination of TNF-α, IL-6, CRP, intercellular adhesion molecule-1 (ICAM-1), SP-D and CC-16. Citrate blood was collected for determination of TF, D-dimer, prothrombin fragment 1 + 2 (F1 + 2) and P-selectin. EDTA-blood was collected for determination of soluble(s) CD40L. The samples were immediately frozen to − 20 °C in 1.0 mL Sarstedt® cryotubes (Sarstedt AG, Nümbrecht, Germany). After shipment, the samples were stored at the National Institute of Occupational Health (Oslo, Norway) at − 80 °C until analysis.

Measurements of biomarkers

The following analyses were performed by ELISA: CRP with kits from DRG Instruments (Marburg/Lahn, Germany) [detection limit (DL) 0.1 mg/L], high sensitivity IL-6, TNF-α, ICAM-1, sCD40L and P-selectin (R&D Systems Europe, Abingdon, Oxon, UK), sTF (Imubind TF, American Diagnostic Inc., Greenwich, Conn., USA), F1 + 2 (Enzygnost® F1 + 2, Siemens, Marburg, Germany), D-dimer (Asserachrom® D-dimer, Stago Diagnostica, Asniere, France), CC-16 and SP-D (Biovendor Laboratory Medicine Inc., Brno, Czech Republic). The respective coefficients of variation (CV) were < 5%, 10.5%, 8.5%, 4.8%, 9.5%, 5.2%, 7.9%, 4.5%, 6.6%, 5.7% and 9.4%. All samples were analyzed in the same series to avoid bias due to assay variability.

Welding aerosol exposure assessment

Exposure to WPM was assessed by personal full-shift sampling of work room air the last two consecutive days before collection of biological samples by use of 25 mm "total dust" closed-face aerosol cassettes (M000025Ao, Millipore, Bedford, MA, USA) equipped with 5 µm pore-size polyvinyl chloride membrane filters (Millipore PVC502500). The cassettes were mounted in the breathing zone of the welders underneath the welding helmet. SKC Sidekick pumps (SKC Ltd., Dorset, UK) operated at an initial air flow rate of 2.0 L/min were used. A reduced air flow rate through the filters was occasionally observed due to collection of high particle masses. These cassettes were substituted with unexposed filter cassettes for further exposure measurements. Thus, two air samples were collected during the work shift for some welders. The reduced air flow rate was considered in the final air volume calculations. The impact on the uncertainty of the air volumes was estimated to be less than 20%.

Table 1 Background data of welders and referents

	Welders ($N = 70$)		Referents ($N = 74$)		
	AM[c]	Min–max	AM	Min–max	*p* value
Age (years)	37.1	20–70	37.3	19–58	0.94
Education (years)	11.7	9–17	12.8	10–17	0.002
Current smokers (%)[d]	57	–	51	–	0.49
Number of cigarettes[a]	16.9	5–30	13.9	3.5–25	0.07
Current alcohol users (%)[d]	70	–	47	–	0.005
BMI (kg/m^2)[b]	25.5	18.4–31.3	25.0	17.6–40.1	0.37
Blood $pressure_{systolic}$ (mmHg)	129.4	100–196	123.9	100–140	0.01
Blood $pressure_{diastolic}$ (mmHg)	80.2	60–116	76.5	50–90	0.03

[a]Daily use among current smokers

[b]Body mass index

[c]Arithmetic mean

[d]Prevalence

Gravimetric measurements of welding aerosol particulate matter

The filters were measured gravimetrically before and after air sampling after acclimatization of the filters for at least 1 week before weighing. Static charge was eliminated using a ^{210}Po Staticmaster® static eliminator (NRD, LLC, Grand Island, USA). A Sartorius Micro model MC5 balance (Sartorius AG, Göttingen, Germany), placed in a climate-controlled room with continuous monitoring of temperature (20 ± 1 °C) and relative humidity (40 ± 2%), was used. The DL of WPM was 0.002 mg (3 × standard deviation of blank filters).

Measurements of selected elements in welding fume particulate matter

Hatch's solution, an artificial lung lining fluid simulant (Hatch 1992), was used for leaching the air filters as previously described (Ellingsen et al. 2013). The contents of the Hatch soluble ($Hatch_{sol}$) and Hatch non-soluble ($Hatch_{non\text{-}sol}$) fractions of iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu), which by mass were the most abundant of the measured elements, were either determined by an element 2 (Thermo Fisher Scientific Inc., Bremen, Germany) inductively coupled plasma sector field mass spectrometer (ICP-SF-MS) (soluble) or a Perkin Elmer Optima 7300 (Perkin Elmer Inc., Waltham, MA, USA) inductively coupled plasma optical emission spectrometer (ICP-OES) (non-soluble). Both instruments were calibrated with matrix matched (Hatch's solution and/or acids) solutions. The accuracy was assessed by comparing analytical data obtained by analyzing realistically exposed welding aerosol filters by a previously validated method (Thomassen et al. 1999) to the total amounts of elements ($Hatch_{sol} + Hatch_{non\text{-}sol}$) obtained using ICP-SF-MS and ICP-OES procedures. DLs were calculated for each analytical run ($3 \times SD_{Blanks}$) based on blank sample measurements ($N = 8$–12) run on specific days. The highest of these daily DLs was used. The DLs (in µg/filter) were: Fe_{sol} 0.73, Mn_{sol} 0.011, Zn_{sol} 0.17, Cu_{sol} 0.70, $Fe_{non\text{-}sol}$ 2.5, $Mn_{non\text{-}sol}$ 0.12, $Zn_{non\text{-}sol}$ 4.4 and $Cu_{non\text{-}sol}$ 2.0.

Statistics

The distribution of the variables was visually assessed and their skewness calculated. Variables were log-transformed when skewness of a distribution exceeded 2.0. Thus, the variables TNF-α, IL-6, D-dimer, CRP and all welding fume components were log-transformed. The geometric means (GM) and ranges are presented for these variables. Otherwise, arithmetic means (AM) and ranges are given. Differences between groups were assessed using Student's *T* test. A general linear model was used to adjust for relevant covariates between groups. Multiple linear regression analysis (backward procedure) was carried out with effect biomarkers in blood as dependent variables. All variables with p value < 0.10 were entered into the final statistical model. Independent variables were exposure category (1/0), age (years), current smoking (1/0), body mass index (BMI) (kg/m^2), the presence of known chronic diseases (1/0), use of alcohol (1/0) and storage time (lg) (days) between blood collection and analysis. A two-tailed p value < 0.05 was considered to be of statistical significance. The statistical data package SPSS®, version 25.0 (IBM Corp., Armunk, NY, USA), was used for the statistical calculations.

Results

The welders and the referents were of similar age (Table 1). Also, current smoking prevalence and BMI were similar, while the welders reported to have slightly lower education than the referents. The prevalence of ex-smokers was 17.8 and 20.0% among the referents and welders, respectively. Systolic and diastolic BP was higher in the welders.

The exposed subjects had worked as welders for 15.0 years on average (Table 2). Their current GM exposure to WPM was 8.1 mg/m^3 based on the mean individual air concentration of the 2 days of personal sampling. The main WPM metal components were by weight Fe, followed by Mn, Zn and Cu. The metal components were only slightly soluble in Hatch solution, e.g., the GM concentration of $Hatch_{sol}$ Fe was 12 µg/m^3 compared to 2323 µg/m^3 of $Hatch_{non\text{-}sol}$ Fe.

The mean concentration of TNF-α, but not IL-6 and CRP, was significantly higher among welders than among

Table 2 Geometric mean (GM) concentrations of welding particulate matter (WPM) and selected welding components from two sampling days according to solubility in the lung lining fluid simulant Hatch solution in 70 welders

	GM	Min–max
Years of welding	15.0[a]	1.5–53.0
WPM (mg/m^3)	8.1	1.4–154
Non-soluble components		
Fe (µg/m^3)	2323	250–51,000
Mn (µg/m^3)	286	10–3290
Zn (µg/m^3)	75	< DL–832
Cu (µg/m^3)	34	< DL–314
Soluble components		
Fe (µg/m^3)	12	< DL–98
Mn (µg/m^3)	29	1–466
Zn (µg/m^3)	17	3–89
Cu (µg/m^3)	5	< DL–62

[a]Arithmetic mean

referents (Table 3). The welders had also statistically significantly higher concentrations of P-selectin, F1 + 2 and D-dimer. The difference in sCD40L between the two groups did not quite attain statistical significance. However, after adjusting for differences between the two groups in the storage time of the samples before analysis, the welders had also significantly higher sCD40L concentrations than the referents. The TF concentrations were similar in the two groups.

Results from multiple linear regression analysis showed that the concentrations of TNF-α, P-selectin, F1 + 2 and D-dimer were positively associated with being a welder when taking into account a number of potential confounders, whereas TF and sCD40L were of borderline significance ($p < 0.10$) (Table 4). Age and BMI were important contributors to the measured concentrations of particularly CRP and IL-6. Time of storage before analysis had a statistically significant impact on the concentrations of TF, D-dimer and sCD40L, and was therefore used to adjust for these concentrations as presented in Table 3.

There were no statistically significant linear associations between the concentrations of WPM and any of the effect biomarkers among the welders except for sCD40L and exposure 2 days before ($r = 0.28$; $p = 0.02$) and mean exposure of the 2 days before blood sampling ($r = 0.30$; $p = 0.01$). When stratifying the welders into tertiles of high, medium and low WPM exposure according to the mean air concentrations of the 2 days of sampling, the concentrations measured the day before and 2 days before blood sampling, respectively, a tendency toward higher concentrations of particularly D-dimer and sCD40L among the welders most highly exposed 2 days before blood sampling was observed. D-Dimer concentrations were statistically significantly higher among the highest ($p = 0.003$) and medium ($p = 0.001$) exposed welders, while the D-dimer concentrations among the low exposed welders did not differ significantly ($p = 0.15$) when compared to the referents (Fig. 1). The concentrations of sCD40L were significantly higher ($p < 0.001$) among the most highly exposed welders, but not in those with medium ($p = 0.10$) or low ($p = 0.52$) exposure when compared to the referents (Fig. 2).

The concentrations of the serum pneumoproteins SP-D and CC-16 did not differ statistically between the welders and the referents (Table 5). Further, no association

Table 3 Arithmetic mean (AM) concentrations of biomarkers of coagulation, systemic inflammation and endothelial/platelet activation in blood of welders and referents

	Welders ($N = 70$)		Referents ($N = 74$)		
	AM	Min–max	AM	Min–max	p value
IL-6[a] (pg/mL)	1.26[b]	0.3–15.9	1.16	0.3–10.6	0.51
TNF-α[a] (pg/mL)	1.84[b]	0.8–10.6	1.11	0.6–9.4	< 0.001
CRP[a] (mg/L)	1.01[b]	0.1–12.8	0.92	0.1–12.0	0.60
P-selectin (ng/mL)	82[c]	16–188	68[b]	26–169	0.007
sCD40L (pg/mL)	1386[c]	180–5230	1099[b]	122–4396	0.11
$sCD40L^{e}_{Adj}$	1413[c]	–	1021[b]	–	0.03
ICAM (ng/mL)	256[b]	90–512	252[b]	87–477	0.78
TF (pg/mL)	794[d]	224–2097	795[b]	174–2774	0.99
TF^{e}_{Adj}	855[d]	–	734[b]	–	0.15
F1 + 2 (pmol/L)	1383[d]	242–1578	835[b]	146–1414	< 0.001
D-Dimer[a] (ng/mL)	462[c]	35–13,999	233[b]	54–2983	0.001
$D\text{-Dimer}^{a}{}^{e}_{Adj}$	495[c]	–	218[b]	–	< 0.001

[a]Geometric mean

[b,c,d]Number of subjects missing

[e]Adjusted for storage time

Table 4 Results from multiple linear regression analysis (backward procedure) including 70 welders and 74 referents. The following variables were included in the model; exposure (1/0), age (years), current smoking (1/0), current alcohol use (1/0), storage of samples (days), body mass index (BMI) (kg/m^2) and diseases

	β-Coefficients							
	Exposure	Age	Smoking	Storage	Alcohol	BMI	Diseases	R^2
IL-6 (lg) (pg/mL)	–	0.01****	0.09*	–	–	0.02**	–	0.27****
TNF-α (lg) (pg/mL)	0.24****	0.004**	–	–	–	–	–	0.27****
CRP (lg) (mg/L)	–	0.02****	–	–	–	0.04****	–	0.25****
P-selectin (ng/mL)	14***	–	–	–	–	–	–	0.05***
sCD40L (pg/mL)	347*	23***	–	−1278**	–	–	–	0.13****
ICAM (ng/mL)	–	–	22*	–	–	4.6**	−52**	0.09***
TF (pg/mL)	143*	–	–	−1258****	–	–	−300*	0.16****
F1 + 2 (pmol/L)	549****	–	–	–	–	–	–	0.37****
D-Dimer (lg) (ng/mL)	0.33****	–	–	−0.75**	0.24***	−0.02*	–	0.17****
CC-16 (µg/L)	–	–	−0.8*	–	–	−0.13**	–	0.04**
SP-D (µg/L)	–	–	–	62**	–	−3.5***	–	0.08***

****$p < 0.001$; ***$p < 0.01$; **$p < 0.05$; *$p < 0.10$

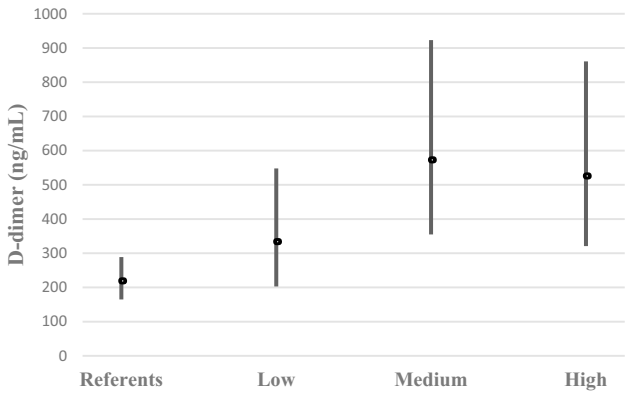

Fig. 1 The geometric mean (GM) concentrations and 95% CI of D-dimer adjusted for body mass index, days of storage and alcohol drinking in referents and in welders stratified into three equally large groups according to low (< 5.32 mg/m^3; GM = 2.5), medium (5.32–13.1 mg/m^3; GM = 8.3) and high (> 13.1 mg/m^3; GM = 22.9) welding particulate matter exposure 2 days before blood sampling

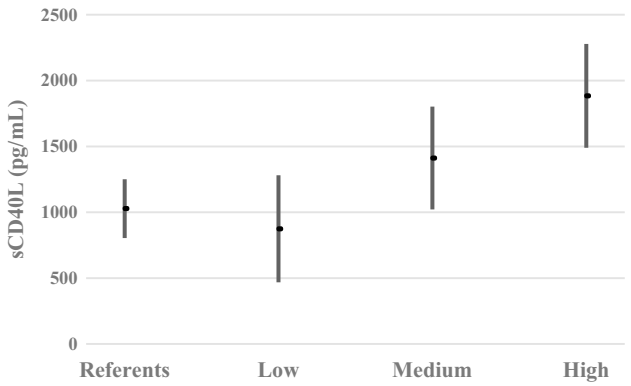

Fig. 2 The arithmetic mean concentrations and 95% CI of sCD40L adjusted for age and time of storage in referents and in welders stratified into three groups according to low [< 5.32 mg/m^3; geometric mean (GM) = 2.5], medium (5.32–13.1 mg/m^3; GM = 8.3) and high (> 13.1 mg/m^3; GM = 22.9) welding particulate matter exposure 2 days before blood sampling

between WPM and CC-16 or SP-D was observed (results not tabulated).

Discussion

This study shows significantly higher concentrations of biomarkers associated with endothelial/platelet activation and coagulation among welders as compared to referents. Higher levels of TNF-α, but not CRP and IL-6, all reflecting systemic inflammation, were also shown. This is to our knowledge the first study of such biomarkers among welders. The welders were generally in good health, but highly exposed to WPM, the average concentration being 8.1 mg/m^3.

Table 5 Arithmetic mean (AM) serum concentrations of pneumoproteins in welders and referents

	Welders ($N = 70$)		Referents[a] ($N = 74$)		
	AM	Min–max	AM	Min–max	*p* value
CC-16 (μg/L)	4.6	< DL–14	4.8	< DL–12	0.74
SP-D (μg/L)	130	44–314	138	42–493	0.44
CC-16 (μg/L)$_{adj1}$	4.7	4.1–5.3[b]	4.7	4.1–5.3[b]	0.88
SP-D (μg/L)$_{adj2}$	128	115–141[b]	135	122–148[b]	0.43

Adj1 smoking and body mass index, *Adj2* body mass index and storage

[a]Two referents missing

[b]95% confidence interval

The D-dimer concentrations were significantly higher among the welders. The plasma half-life of this fibrin degradation product has been reported to be between 3 and 48 h (van der Putten et al. 2006). Thus, it is plausible that the D-dimer concentrations reflect occurrences shortly before blood sampling, e.g., exposure to WPM. The welders had also higher concentrations of F1 + 2, a cleavage product of prothrombin which is generated during its conversion to thrombin. The significantly higher concentrations of these biomarkers among the welders indicate that they are in a state of increased pro-coagulant activity and thrombus formation. However, the concentrations of TF, the most potent initiator of the intrinsic coagulation system, were similar in the two groups. It should be emphasized that total TF antigen and not TF activity was measured. As the half-life of TF mRNA is short, around 0.75–1.5 h (Ahern et al. 1993), a potential increase in TF may also be difficult to demonstrate since the participants were examined at least 12 h after the last WPM exposure. This may also be the case for the other measured biomarkers, e.g., IL-6. There is, to our knowledge, no established association between alcohol consumption and D-dimer, which may be a random finding in the present study.

The higher P-selectin concentrations among the welders indicate ongoing platelet activation (van der Putten et al. 2006). Binding of platelets to other cell types, such as endothelial cells or leukocytes, is primarily mediated by P-selectin (Yun et al. 2016). sCD40L also differed between the groups after adjusting for different storage times of the blood samples. CD40L enhances platelet activation, aggregation and platelet–leucocyte conjugation on the endothelial cells, which are important steps for the induction of atherothrombosis (Pamukcu et al. 2011). More than 95% of circulating CD40L is derived from platelets (André et al. 2002). The TNF-α concentrations were also higher among the welders. It is of interest that TNF-α has been shown to induce CD40 expression (Pamukcu et al. 2011) and also activate a number of pro-inflammatory genes in endothelial cells

related to the expression of adhesion molecules, cytokines and chemokines (Kuldo et al. 2005). It has been shown that sCD40L was increased simultaneously with F1 + 2 and P-selectin in hypercholesterolemic patients (Cipollone et al. 2002). No dose–response relationships other than for D-dimer and sCD40L were observed.

The welders had low serum CRP concentrations and the levels were similar to that of the referents, although WPM exposure was very high. Animal studies have shown elevated serum CRP as a response to inhalation of carbon black and ambient air particles (Niwa et al. 2008; Rohr et al. 2010). However, a recent review did not find clear epidemiological evidence that CRP levels are increased in relation to PM exposure in healthy adults (Li et al. 2012). It is also noteworthy that the concentrations of SP-D and CC-16, intended as markers of airway effects, were similar in the two groups. Previous studies of CC-16 concentrations in welders have shown conflicting results (Halatek et al. 2000; Halatek et al. 2004).

The applied cardiovascular biomarkers have not been reported on in welders, but in other PM-exposed human populations. An experimental study of men with coronary heart disease showed reduced release of tissue plasminogen activator, indicative of impaired fibrinolytic capacity upon exposure to diesel exhaust (Mills et al. 2007). Coagulation factors were not affected in another experimental study of 13 subjects exposed to 200 μg/m^3 diesel exhaust particles (DEP) (Carlsten et al. 2007) or in tunnel construction workers exposed to 51 μg/m^3 of elemental carbon as a marker of DEP exposure (Ellingsen et al. 2017a). Healthy volunteers exposed to wood smoke had increased levels of factor VIII of the coagulation system (Barregård et al. 2006). Men with ischemic heart disease exposed to ambient urban air had increased level of F1 + 2, without increased D-dimer concentrations (Rűckerl et al. 2006). Increased levels of plasma fibrinogen and plasminogen activator inhibitor (PAI) were associated with the level of the particulate air pollution in 76 students (Chuang et al. 2007). Increased D-dimer concentrations were shown in urban air-exposed patients with multiple cardiovascular risk factors (Chen et al. 2017). Humans exposed to low levels of urban air pollution had, in contrast, lower serum concentrations of sCD40L (Arden Pope et al. 2014).

Animal studies have reported increased vascular thrombosis after PM exposure. Intratracheally instilled 60 nm polystyrene particles or crystalline silica induced increased thrombosis in hamsters and guinea pigs, respectively (Nemmar et al. 2003, 2005). A pro-thrombotic status was reported in two studies of mice intratracheally instilled with PM collected from urban air and in one study of carbon nanotubes (Cascio et al. 2007; Cozzi et al. 2007; Nemmar et al. 2007). Also P-selectin was increased in mice intratracheally instilled with urban air particles or carbon nanotubes (Cascio et al. 2007; Cozzi et al. 2007; Nemmar et al. 2007). Thus, there is evidence from human and animal studies that PM exposure other than WPM exposure may cause a procoagulant status.

The highest air concentrations of the metallic elements in the WPM were measured for Fe, Mn, Zn and Cu. With few exceptions, e.g., hexavalent chromium (Cr), WPM components are mostly non-soluble. A minor fraction is, however, soluble in lung lining fluid simulants (Berlinger et al. 2008). Thus, welders have a systemic uptake of metals, e.g., Cr, vanadium, molybdenum, tungsten and Mn (Ellingsen et al. 2013; Ellingsen et al. 2017b). Welders are mostly exposed to agglomerates. These chain-like structures often have an aerodynamic diameter ranging from around 100 nm to 1 μm (Berlinger et al. 2011). The agglomerates consist of primary particles, typically in the size of around 5–40 nm (Berlinger et al. 2011). Whether a de-agglomeration occurs in the bronchoalveolar fluid is not known. The amount of primary single nanoparticles in welding aerosols is low (Berlinger et al. 2011). A small fraction of nanoparticles may enter the blood circulation and reach secondary organs in animals, as shown for 15 nm iridium, 5–8 and 20 nm gold and fullerene particles (Semmler et al. 2004; Takenaka et al. 2006; Naota et al. 2009; Schleh et al. 2013). No studies have, to our knowledge, studied translocation of WPM across the alveolar membrane, but it cannot be excluded that a small fraction may be translocated.

Although studied limitedly, there are indications that elevated concentrations of D-dimer may be a risk factor for future cardiovascular death among apparently healthy men, elderly patients and the general population (Ridker et al. 1994; Alehagen et al. 2004; Willeit et al. 2013). Thus, the higher D-dimer concentrations among the most highly exposed welders are compatible with epidemiologic literature showing increased cardiovascular morbidity and mortality among welders (Moulin et al. 1993; Sjögren et al. 2002; Ibfelt et al. 2010). It is, however, well known that D-dimer is increased in venous thromboembolism. Welders may also be exposed to hand–arm vibrations to a certain extent. The hand–arm vibration syndrome has a vascular component, and cases of vascular thrombosis of the hands have been reported (Thompson and House 2006). Welders may also work in confined spaces in awkward working positions. We have not found any literature addressing thrombosis in subjects working in such positions. However, long-haul air plane passengers are at increased risk for venous thrombosis, but increased D-dimer concentrations were not measured among 1260 healthy subjects during a simulated 8-h flight (Kuipers et al. 2007; Ideal Cabin Environment (ICE) Research Consortium 2017). Nitrous oxides and ozone may also be generated during welding, and such exposures may induce pulmonary inflammation, but to what extent these components may cause the alterations measured in this study

is poorly understood (Hiraiwa and van Eeden 2013). The longer storage time of the blood samples among the welders compared to the referents may have resulted in an underestimation of the differences in the biomarker concentrations between the groups. The two groups were blue collar workers living in the same area. Age and BMI were also comparable. Thus, we assume that different nutritional status do not contribute to the differences in the biomarker concentrations observed between the groups. The higher BP measured in the welders than in the referents was not hypothesized a priori, but generalized endothelial dysfunction predisposes to vasoconstriction and the development of hypertension (Brandes 2014; Touyz et al. 2018).

This study shows that welders highly exposed to WPM (GM 8.1 mg/m^3) are in a pro-thrombotic state with increased thrombin generation as indicated by the significantly higher F1 + 2 and D-dimer concentrations. They also have increased endothelial/platelet activation. The study cannot determine whether the alterations are related to pulmonary inflammation with subsequent systemic inflammation due to WPM exposure, translocation of non-soluble nanosized particles into the systemic circulation or the release of soluble WPM metal components into the blood circulation. The mechanisms should be explored further.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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