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Cross-sectional Study of Workers Employed at a Copper Smelter—Effects of Long-term Exposures to Copper on Lung Function and Chronic Inflammation

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Objective: The aim of the study was to assess the effect of exposure to copper-containing dust on lung function and inflammatory endpoints among workers of a German copper plant, effects rarely studied before. **Methods:** One hundred four copper-exposed smelter workers and 70 referent workers from the precious metal and lead facilities were included, with different metal exposures in both groups due to the different process materials. Body plethysmography, exhaled nitric oxide (FeNO) measurements, and blood sampling were conducted in all workers. Smoking status and the use of respiratory protective equipment were considered. In a subgroup of 40 nonsmoking volunteers (28 copper-exposed and 12 referents), sputum biomarkers were assessed. **Results:** Median lung function values of both copper-exposed and the referent groups were within reference ranges of "healthy" individuals, and statistical differences between the groups were mostly not evident. Similarly, differences in blood and sputum biomarkers were too small to be biologically relevant. **Conclusion:** The results suggest the absence of the detectable effects of copper-containing dust exposure on lung function or chronic inflammation within the investigated cohort.

Keywords: copper, cross-sectional study, induced sputum, inflammation, lung function, occupational exposures

Copper and its alloys possess unique conductive characteristics and are therefore produced and used on a large scale in the production of various electrical devices such as wire, cables, transformers, and generators.[1–3] Owing to the large-scale production and use of copper, occupational exposure to copper-containing dusts and fumes may occur during the mining, smelting, and refining of copper-containing ores as well as possible downstream uses of copper. Although copper is an essential element for human health,[4] the reactivity of its ions means that excess exposure could result in oxidative stress, inflammation, and potential disease.[1] A limited number of animal studies have demonstrated local effects of high doses of copper on the respiratory tract, including immune suppression and alterations of the immune function. Those findings were one of the rationales for the European Commission's (EC's) Scientific Committee on Occupational Exposure Limits (SCOEL) to recommend an occupational exposure limit for copper and its inorganic compounds of 0.01 mg/m^3 (8-hour time-weighted average, respirable fraction) in 2014.[1] The second pillar of this recommendation was a study reported by Gleason[5] in 1968. Mild symptoms suggesting metal fume fever, an acute self-limiting systemic inflammatory response to metal fume,[6] were observed among three male workers exposed to copper-containing dust levels of 0.120 mg/m^3 after lapping of copper plates.[5] The German Senate Commission for the investigation of Health Hazards of Chemical Compounds (MAK) derived a similar value to the SCOEL level in 2013, based on the same endpoints.[3]

In general, inhalation of a variety of compounds, especially particulates, is a critical pathway for the development of perturbation of lung function and, possibly, several occupational diseases. For example, long-term occupational exposure to such substances can lead to chronic inflammation of the lung and, subsequently, obstructive or restrictive impairment of lung function. Interestingly, there is little published or reported evidence in the occupational health literature of copper-related exposure leading to adverse health effects, which is noteworthy in the light of the long and extensive industrial uses of copper.[7] A recent historical health surveillance study published by some of the authors addressed cumulative exposure to copper-containing dust among smelter workers. At an average of 4.61 mg/m^3-years of inhalable copper dust over an average of ~22 years of employment, no adverse effects on lung function measured as the decline in the forced expiratory volume per 1 second (FEV_1) were found.[8] The process of copper smelting involves the enrichment and refinement of copper from a concentrated ore to a high-purity commercial product. The copper smelter at the study facility has been in operation since 1972, and today, approximately 200 employees work in three shifts per day throughout the year. At the facility, copper concentrates with a copper content of 30% are used in the production of copper anodes (99.5% Cu) by stepwise refinement. All stages of the process are located in the same large hall.[8]

The present study investigates a cross section of workers employed at the same copper plant (Aurubis in Hamburg, Germany), who underwent a series of examinations in 2019. The study cohort included a group of workers occupationally exposed to copper-containing dust in a copper smelter as well as a comparative referent group not exposed to copper, but exposed to similar conditions (eg, physical exertion, heat) and mixed dust exposures.

Spirometry metrics, including FEV_1 and forced vital capacity (FVC), are traditionally used as indices of chronic lung function impairment[3,9,10] and have been described as the criterion standard in

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Ethical Considerations and Disclosures: The study methods were reviewed and approved by the Ramboll US Corporation Institutional Review Board (IRB00001256) before the data collection and the project updates were filed with the IRB annually. The IRB consists of six members with various occupational backgrounds and expertise, who evaluated the study under ethical considerations as an independent party. The study subjects gave their informed, written consent to participation in the study and to the publication of (aggregated) data obtained from the examinations.

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evaluating lung function.[11] However, whole-body plethysmography provides additional information on early obstructive or restrictive impairments, which might not be expressed as clinically measurable alterations in FEV_1 or FVC.[11,12] In the current study, emphasis was placed on FEV_1, FVC, transfer factor (or diffusion capacity) of the lung for carbon monoxide (DL_{CO} or TL_{CO}), airway resistance (R_{tot}), and expiratory flow volumes, as these represent some of the most sensitive and commonly reported spirometric and plethysmographic parameters.

Airway resistance represents the ratio of the driving pressure to the rate of airflow. An increased value of R_{tot} may indicate airway obstruction, especially when observed together with a decrease in the Tiffeneau-Pinelli index (FEV_1/FVC ratio).[11,13] DL_{CO} or TL_{CO} is a measure of the gas transfer through the alveolar lung tissue and may be influenced by lung vasculature and other factors. A reduced value, however, may indicate a range of conditions such as emphysema or pulmonary fibrosis.[14–16] Expiratory flow volume was reported as maximum expiratory flow at a volume of 25% (MEF_{25}), 50% (MEF_{50}), and 75% (MEF_{75}) of the FVC.[17] It is worth noting that MEF_{25}, MEF_{50}, and MEF_{75} are complementarily related to the forced expiratory flow (FEF) at exhaled percentages of 25% (FEF_{25}), 50% (FEF_{50}), and 75% (FEF_{75}), respectively.[18] Peak expiratory flow (PEF) values were also assessed.

In addition to direct measures of respiratory impairment, sampling of inflammatory biomarkers from blood and sputum, as well as measurement of exhaled nitric oxide (FeNO), was conducted. This was because occupational inhalation exposure to particles or gases can trigger inflammation within the airways, and chronic inflammation is a key driver of respiratory disease.[19] Biomarkers of inflammatory and immunological response have been used widely in studies investigating the effects of occupational exposure to inorganic dusts, many focusing on markers associated with chronic obstructive pulmonary disease.[19] As such, to determine if airway inflammation was present in workers occupationally exposed to copper-containing smelter dusts (including arsenic and sulfur dioxide), analysis of a range of inflammatory biomarkers was measured in sputum. In addition, FeNO was chosen as an indirect, noninvasive marker for atopy-associated airway inflammation.[20]

The primary study objective was to determine whether differences in effects on lung function and biomarkers of inflammation could be detected between workers exposed to copper-containing smelter dusts and a referent group of workers at the same site whose smelter-dust exposure did not contain significant amounts of copper. For both endpoints (lung function and inflammation), the influence of possible confounders, such as age, smoking status, and anthropometric data (ie, height and weight), was considered. Further, usage of respiratory protective equipment (RPE) was examined. Copper was measured in serum to assess possible elevation in body copper levels, which may result in toxicity.

METHODS

Study Population

The study cohort consisted of 104 copper smelter workers (copper-exposed group) and 70 workers from the precious metal and lead facility (referent group), all males, employed during the study period of January through July 2019. Except for five subjects, the investigated workers were also part of the previous historical cohort study.[8] All workers who volunteered to participate in the study were included, which resulted in a participation rate of approximately 55% for the copper smelter workers and approximately 50% for the precious metal and lead facility workers. The copper smelter and the precious metal and lead facility are part of the same plant but are located in separate buildings (the precious metal and lead facility being divided into two separated working areas) and use different ores, resulting in differences in metal dust exposures. Because the nature of the work in terms of physical demands was similar for both study groups, comparable sociodemographic and geographical conditions were assumed and verified. Process conditions in both facilities and copper dust exposure levels in the smelter between 1982 and 2018 were described previously.[8] The inhalable copper dust exposure level during the study period was approximately 0.120 mg/m^3 (based on n = 12 measurements in 2018) and similar to earlier years.[8]

Participants employed at the copper smelter (ie, copper-exposed study subjects) were categorized into constant RPE users, occasional RPE users, and never RPE users. Respiratory protective equipment use (full-face mask with air supply) became mandatory in the copper facility on February 1, 2019, to provide greater protection from exposure to arsenic and cadmium. Therefore, all smelter workers who were examined on or after February 1, 2019 (44 of 104 study subjects), were constant RPE users. For smelter workers who were examined before that date, information on RPE usage was collected as part of a survey questionnaire. In total, 66 smelter workers were constant RPE users on their examination day, whereas the remaining 36 workers were never or occasional users. For two copper-exposed subjects, data on RPE usage were not available.

Referent subjects working in the precious metal area were considered unexposed to copper and other compounds because silver was the main material (98%) processed. Levels of inorganic silver compounds were known to be below the maximum allowed concentrations according to the German TRGS 900 (0.01 mg/m^3).[21] Referent subjects working in the lead area were consistent RPE users because RPE use had been mandatory since 2003. Therefore, all 70 referent subjects were classified as unexposed or as constant RPE users.

Medical Examination

On the examination day during the regular work shift (ie, between 8:00 AM and 2:00 PM), trained personnel from the medical department of the copper plant used a standardized questionnaire to assess demographic parameters, tobacco consumption, and RPE usage. Basic information about current and past illness of the respiratory organs (nose, throat, bronchi, and lungs) was also obtained. Blood samples were collected, and whole-body plethysmography and FeNO measurements were performed. Given the focus on chronic lung disease, biomarkers more specific to acute effects, such as asthma attacks, were not included.

Participants were asked about their current and former smoking status and the number of cigarettes smoked per day (CPD and categorized into current, former, and never smokers). The self-reported information was compared with information about tobacco consumption recorded in the medical files. If the survey-based smoking status differed from the information in the medical files, the most conservative category was chosen. For example, if a participant reported being a never smoker but tobacco consumption was recorded in the medical file, the person was classified as a former smoker. All participants who provided sputum samples were never smokers or former smokers with <5 pack-years.

Body Plethysmography and FeNO

Lung function parameters were measured using the plethysmograph PowerCube Body (Ganshorn Medizin Electronic, Niederlauer, Germany) according to the criteria of the Deutsche Atemwegsliga.[18] Predicted values were calculated using an equation derived by the Global Lung Function Initiative (GLI-2012, GLI-2017) that takes into account height, age, and ethnicity of the subject.[20,22] For R_{tot}, PEF, and MEF_{25}, MEF_{50}, and MEF_{75}, the predicted values were based on the reference equations derived by the European Community for Steel and Coal in 1993 (EGKS values), which were incorporated into the plethysmograph's software.[23] Although GLI values are preferred for the evaluation of spirometry data, equations for those variables are not available yet.[24] The FeNO value was recorded after 10 seconds of exhalation using the Vivatmo pro device (Bosch Healthcare Solutions, Waiblingen, Germany) according to the instructions provided by the manufacturer.

Blood Sampling

One 4-mL EDTA Monovette, one 4-mL whole blood tube, one 9.6-mL serum Monovette, and one 5-mL EDTA Monovette were collected. The whole blood and 4-mL EDTA sample were directly sent to a contract laboratory to determine the differential blood count and copper serum levels. The remaining serum sample was left for 30 to 60 minutes for coagulation, centrifuged (2000 × g for 10 minutes at room temperature), and aliquoted. The 5-mL EDTA Monovette was immediately stored on ice and was centrifuged (1500 × g for 10 minutes at 4°C) within 45 minutes and aliquoted. All aliquots were stored at −20°C for a maximum of 2 weeks and subsequently stored at −80°C until analysis. The inflammatory biomarkers myeloperoxidase (MPO), eosinophilic cationic protein (ECP), club cell protein (CC16), serum amyloid A protein (SAA), neutrophil elastase (NE), E-selectin (ESel), C-reactive protein (CRP) were analyzed via enzyme-linked immunosorbent assay; interleukin 6 (IL-6), IL-8, tumor necrosis factor α, monocyte-chemotactic protein (MCP), and intercellular adhesion molecule-1 (ICAM1) were determined using an ECL multiarray technology (Meso Scale Discovery, Rockville, MD). Assay details are provided in Supplementary Table 1, http://links.lww.com/JOM/B100. Atomic absorption spectrometry was used after digestion of the material to assess the copper concentration in serum.

Sputum Analysis

All sputum inductions were performed after March 7, 2019, when RPE equipment was already mandatory. Sputum samples were obtained from a subgroup of 40 nonsmoking volunteers (28 from the copper-exposed group and 12 from the referent group) by experts from the Fraunhofer Institute for Toxicology and Experimental Medicine, Hanover, Germany. The sputum samples were collected between March and July 2019 from two to four volunteers per day either before the start or following a regular work shift between 10:00 AM and 2:00 PM.

Sputum induction and analysis were performed as described by Janssen and colleagues[25] in 2013. Briefly, all workers inhaled 200 µg salbutamol followed by increasing concentrations of nebulized hypertonic saline (OMRON NE-U17; OMRON Medizintechnik Handelsgesellschaft, Mannheim, Germany) at 3%, 4%, and 5% for 10 minutes each. Every 5 minutes, the FEV_1 was checked, and the subjects tried to cough up sputum. Sputum "plugs" were immediately selected from saliva by microscopic examination to ensure good separation from squamous cells and processed in the Aurubis site. Total cell number and cell viability were determined with a Neubauer hemocytometer. Sputum supernatant was frozen at −20°C for a maximum of 14 days before shipment to Hannover and storage at −80°C until analysis. Cytospots were prepared (Cytospin; Shandon, Pittsburgh, PA) and stained with Diff-Quik (Medion Diagnostics, Düdingen, Switzerland). Differential cell counts were performed by two experienced, independent observers from 400 nonsquamous cells, and the results were averaged. Cytokines and inflammatory mediators were analyzed as described previously for serum samples.

Data Analysis

Lung Function

The data for most lung function parameters were nonnormally distributed. Therefore, differences between copper-exposed and referent subjects, stratified by smoking status, were assessed via the nonparametric Wilcoxon rank sum test. $P < 0.05$ indicates a statistically significant effect. The prevalence of obstructive and restrictive patterns in lung function was estimated among copper-exposed and referent subjects for different definitions published in the literature (Table 1).

Because the spirometry and body plethysmography parameters reflect chronic effects and are unlikely to have been affected by exposure on the examination date, analyses of lung function were not stratified by RPE usage.

TABLE 1. Definitions for the Classification of Restrictive and Obstructive Patterns

Disorder Pattern	Def.	Def. No.	Source of Def.
Restrictive	FEV_1/FVC > LLN and FVC < LLN	1	26,27
	FEV_1/FVC ≥ LLN and FVC < 80%	2	28
Obstructive	FEV_1/FVC < 70%	3	9,29
	FEV_1/FVC < LLN	4	27
	FEV_1/FVC < LLN and FVC > LLN and FEV_1 < LLN	5	26
	FEV_1/VC < 70% and R_{tot} > 0.3	6	13

Def., definition derived from literature; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; LLN, lower limit of normal; VC, vital capacity.

Inflammatory Biomarkers

Data for most inflammatory biomarkers were nonnormally distributed, and differences between copper-exposed and referent subjects, stratified by smoking status, were assessed via the nonparametric Wilcoxon rank sum test. $P < 0.05$ indicates a statistically significant effect. Potential correlations between each biomarker and differential cell counts in blood and sputum were explored using nonparametric Spearman correlation coefficients. For blood biomarkers, copper-exposed subjects were stratified into constant RPE users and never/occasional users of RPE in sensitivity analyses. Furthermore, Wilcoxon rank sum tests were performed to assess the influence of smoking status, workplace (copper-exposed, referent group), and RPE usage on the blood cell and biomarker levels.

RESULTS

Study Population

Approximately half of the currently employed worker force volunteered to participate in the study, resulting in a study population of 174 workers. Nonparticipating copper smelter workers had a 5-year higher median age and a 1-year longer median employment duration, whereas nonparticipants working in the precious metal and lead facility had a 7-year higher median age and a 7-year longer median employment duration, compared with the participating groups. Length of exposure was equally high among copper-exposed participants and nonparticipants.

Demographic and Anthropometric Data

Age at hire was comparable between the copper-exposed and referent subjects, with medians of 25 and 27 years, respectively (Table 2). However, the median age at examination was 6.5 years higher among the copper-exposed subjects, and the median employment duration, defined as time between the date of hire and the examination date, was approximately 9 years longer for the copper-exposed than the referent subjects. The median height was similar in the two groups; however, the median body mass index was higher among the copper-exposed workers (Table 2).

Tobacco Consumption

The proportion of current smokers was approximately 7.5 percentage points higher among referent than among copper-exposed subjects, whereas former and never smokers were more common among the copper-exposed workers (Table 3). Current and former smokers in the copper-exposed group smoked more cigarettes per day than current and former smokers in the referent group. There were three copper-exposed subjects whose smoking history was not recorded in the medical files and who also did not answer the smoking-related questions in the survey. The three smoking strata

TABLE 2. Descriptive Statistics of Demographic and Anthropometric Data of the Exposed and Referent Groups

	Copper Exposed		Referent	
	n = 104		n = 70	
	Median	IQR	Median	IQR
Age at hire, y	24.9	11.9	27.0	14.7
Age at examination, y	43.0	16.5	36.5	19.0
Employment duration, y	18.5	18.7	9.6	13.4
Weight, kg	93.0	19.0	84.0	15.0
Height, cm	179.0	9.5	178.0	6.0
BMI, kg/m^2	28.7	4.2	26.6	4.0

BMI, body mass index; IQR, interquartile range.

showed differences in hematological parameters. Smoking statistically significantly increased the blood levels of leukocytes, neutrophils, and immunoglobulin E and statistically significantly decreased the percentage of blood monocytes. Statistically significantly increases in the blood concentrations of ICAM1 and MPO and a statistically significant decline in blood CC16 were observed as well. These differences were also observed when only referent workers were included in the analysis; however, statistical significance was not always reached, most likely because of the lower numbers of subjects.

Lung Function

Box-and-whisker plots showed that FEV_1/FVC (Fig. 1A), R_{tot} (Fig. 1B), DL_{CO} or TL_{CO} (percentage of the predicted value for healthy nonsmokers [%pred]) (Fig. 1C), and PEF (Fig. 1D) were distributed similarly among referent and copper-exposed subjects. Wilcoxon rank sum tests showed that FEV_1, FVC, and FEV_1/FVC (absolute and % pred values) were not statistically significantly different between the copper-exposed and referent groups, even after stratification by smoking status. This was also the case for R_{tot} and for absolute and %pred values of MEF_{25}, MEF_{50}, and MEF_{75}. Absolute and %pred values of PEF were statistically significantly higher for copper-exposed than referent subjects only among never smokers.

Restrictive patterns were observed in more than 10% of copper-exposed subjects but in fewer than 9% of referent subjects based on either definition (Table 4). Obstructive patterns were more common among referent subjects or similar among copper-exposed and referent subjects, depending on the definition used. However, the prevalence of obstructive patterns among referent subjects did not exceed 7.1% based on any of the four definitions.

Additional data on lung function are reported in Supplementary Table 2, http://links.lww.com/JOM/B101.

Inflammatory Parameters

Blood Biomarkers

Differences in blood cell counts, blood biomarkers, and copper levels in serum between copper-exposed and referent subjects, stratified by smoking status, are provided in Supplementary Table 3, http://links.lww.com/JOM/B102. Supplementary Table 4, http://links.lww.com/JOM/B103, provides the respective data for the impact of RPE usage.

Statistically significantly higher values were observed for IL-6, lymphocyte cell counts, and NE among copper-exposed never smokers and for SAA and NE among copper-exposed former smokers compared with the respective referent subjects. Blood neutrophil percentages, monocyte cell counts, ICAM1 (among current smoker), and CRP, IL-6, leukocytes, and neutrophil cell counts (among current and former smoker) were statistically significantly higher among non–RPE-wearing subjects of the copper smelter compared with RPE wearing subjects of the copper smelter and the referent group. Overall, there was no difference in the level of Cu in serum between groups; however, Cu levels in serum were statistically significantly higher among former smokers not wearing RPE.

Blood leukocyte levels, the absolute number of blood neutrophils, and the concentration of IL-6 were significantly affected by both smoking status and workplace exposure, with the highest levels observed in smokers (Fig. 2 and Supplementary Table 5, http://links.lww.com/JOM/B104).

There were differences among workers of the copper smelter with respect to the extent of RPE usage. In a further analysis, only the copper smelter workers were included, and the effect of exposure (RPE usage) and smoking status tested. In workers not wearing RPE, we found significantly higher levels of blood neutrophils, leukocytes, and CRP. However, these effects were smaller than the increase caused by smoking (Fig. 3 and Supplementary Table 5, http://links.lww.com/JOM/B104).

Sputum Biomarkers

Sputum analyses were limited to a subset of 40 nonsmokers or former smokers (<5 pack-years), 28 workers from the copper smelter and 12 referents. Two copper-exposed volunteers and one referent were excluded from the analysis because of a suspected ongoing infection during the time of sputum collection, which was later confirmed. This left 26 workers from the copper smelter (7 former smokers and 19 never smokers) and 11 referent subjects (3 former smokers and 8 never smokers) for analysis. The mean ages of the 26 copper-exposed workers and referents were 44 and 47 years, respectively.

In one case, the sputum quality of a copper-exposed worker was so low that no differential cell count could be performed. Sputum eosinophils above the generally accepted normal value of 3%[31,32] were observed in three referents (5.1%, 7.0%, and 14.8%) and four workers from the copper smelter (4.0%, 4.0%, 11.0%, and 21.5%). The increased sputum eosinophil levels were reflected by higher concentrations of ECP in blood and sputum, blood eosinophil counts, and FeNO values. In most of these cases, the workers indicated acute allergic symptoms or known underlying atopic diseases. Despite this and based on the nearly equal distribution between groups, we included all subjects into the analysis, but carefully tested any potential bias on the results.

Supplementary Table 6, http://links.lww.com/JOM/B105, lists the demographics and the results of the sputum cell and supernatant analysis separately for copper-exposed and referent workers. The percentage of sputum neutrophils was statistically significantly higher in referent workers. Because we observed a small but significant difference in ciliated epithelia cell counts (data not shown), we also analyzed the sputum neutrophils percentage relative to sputum white blood cells. The percentage of sputum neutrophils remained statistically significantly higher in referent workers (Fig. 4).

TABLE 3. Descriptive Statistics of Smoking Status and CPD of the Copper-Exposed and Referent Groups

		Copper Exposed			Referent		
		n (%)			n (%)		
Smoking category	Current smoker	37 (36.6)			31 (44.3)		
	Former smoker	38 (37.6)			24 (34.3)		
	Never smoker	26 (25.7)			15 (21.4)		
		n	Median	IQR	n	Median	IQR
CPD	Current smoker	34	20.0	8.0	30	15.0	10.0
	Former smoker	32	16.0	14.0	15	15.0	14.0

Abbr.: CPD = cigarettes smoked per day; IQR = interquartile range.

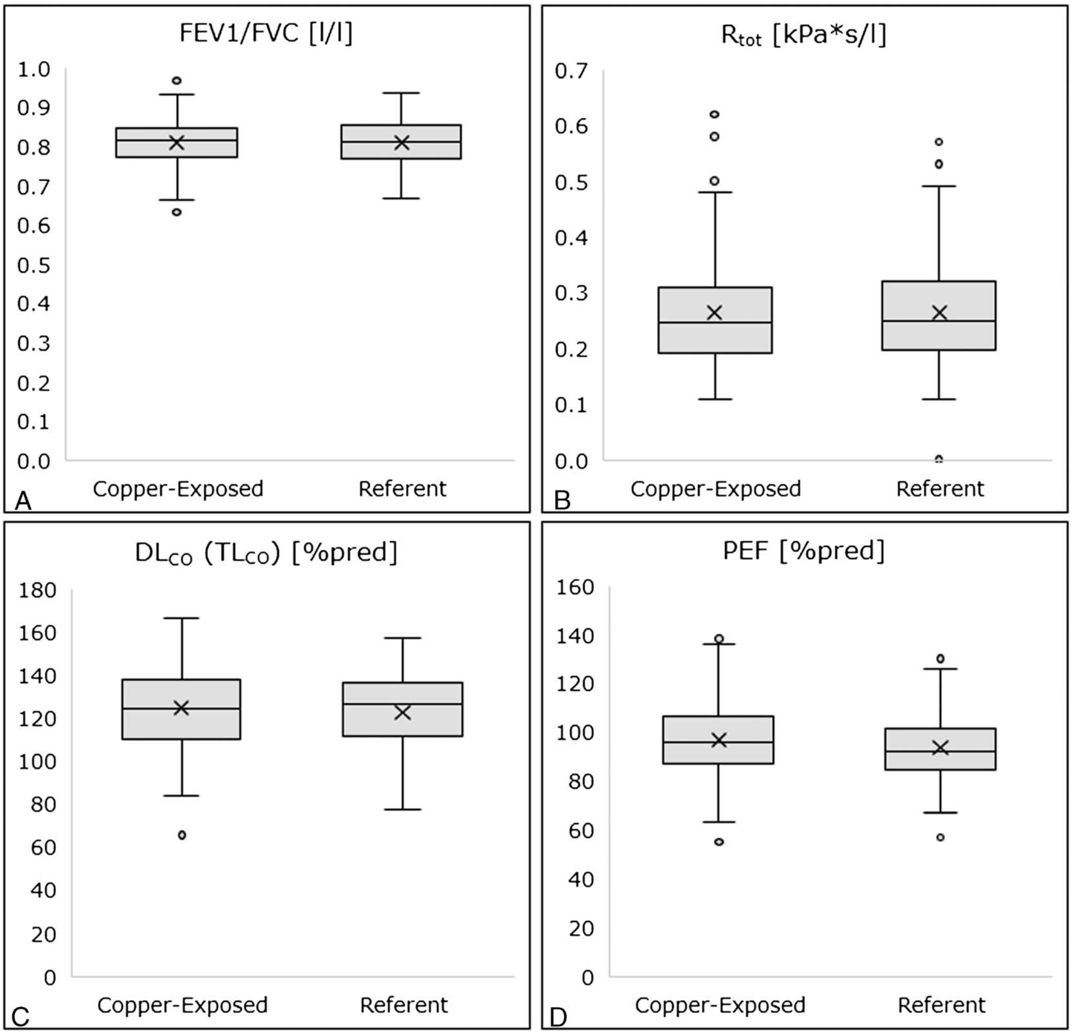

FIGURE 1. Box-and-whisker plots of the unstratified data of (A) FEV_1/FVC; (B) R_{tot}; (C) DL_{CO} or TL_{CO} %pred and (D) PEF by exposure group. Box is reflecting the IQR with the median (line) and mean value (*x*) of the data. Upper and lower whiskers indicating the data variability: 25th percentile – 1.5 *IQR; 75th percentile + 1.5 *IQR. Empty circles represent outliers. %pred, percentage of the predicted value for healthy nonsmokers; DL_{CO} or TL_{CO}, transfer factor of the lung for carbon monoxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; IQR, interquartile range; PEF, peak expiratory flow; R_{tot}, airway resistance.

FeNO

One referent subject did not participate in the FeNO examination. For the remaining study subjects, no statistically significant differences were detected between the copper-exposed and referent groups overall and after stratification by smoking status (Supplementary Table 3, http://links.lww.com/JOM/B102) and/or RPE use

TABLE 4. Number (n) and Prevalence (%) of Restrictive/Obstructive Disorder Patterns Among the Copper-Exposed and Referent Groups

		Copper-Exposed (n = 104)		Referent (n = 70)		
Disorder Pattern	**Def. No.**	**No. Cases (n)**	**Prevalence (%)**	**No. Cases (n)**	**Prevalence (%)**	**Source of Def.***
Restrictive	1	11	10.6	6	8.6	27,28
	2	13	12.5	6	8.6	29
Obstructive	3	6	5.8	5	7.1	9,32
	4	5	4.8	5	7.1	28
	5	3	2.9	2	2.9	27
	6	6	5.8	4	5.7	13

Def., Definition derived from literature.
*Refer to definitions in Table 1.

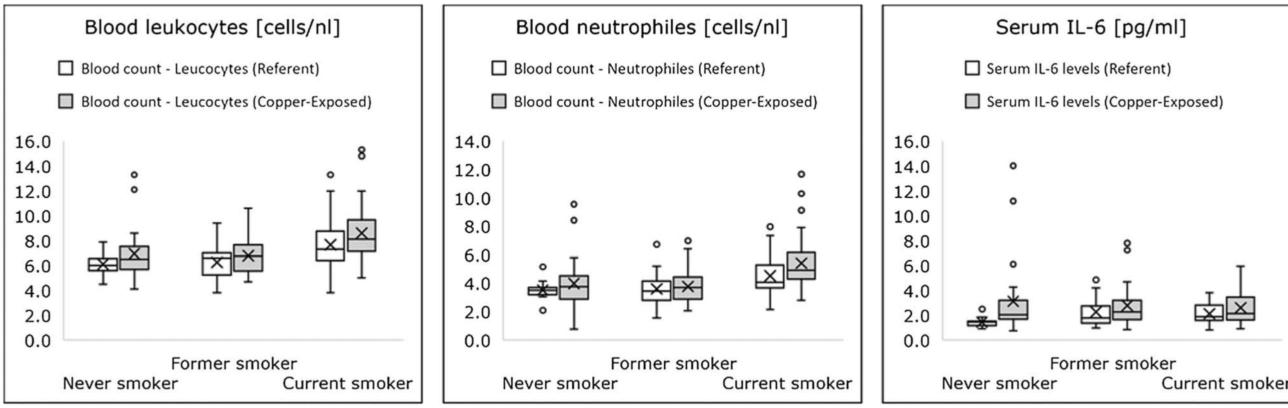

FIGURE 2. Box-and-whisker plots of the absolute number of blood leukocytes, blood neutrophils, and serum IL-6 levels in copper-exposed workers and referents, separately for smoking status. Box is reflecting the IQR with the median (line) and mean value (*x*) of the data. Upper and lower whiskers indicating the data variability: 25th percentile – 1.5 *IQR; 75th percentile + 1.5 *IQR. Empty circles represent outliers. IL, interleukin; IQR, interquartile range.

(Supplementary Table 4, http://links.lww.com/JOM/B103). However, as expected, lower FeNO values were observed in current smokers compared with former or never smokers.

DISCUSSION

The present study was a cross-sectional study of lung function and inflammatory biomarkers data in long-term smelter workers exposed to dusts containing copper. Owing to the specific working conditions of smelting operations such as physical exertion, heat, and mixed dust exposures, comparisons to normative values may have been affected by confounding. To limit confounding, an internal reference group was chosen working at the same plant but in a precious metal and lead facility. These workers have similar working conditions, although because of the different ores and production materials, the dust to which they were exposed did not contain significant amounts of copper. Although a completely nonexposed referent group may have been preferable, most workplace epidemiologic studies cannot obtain truly unexposed subjects. Copper-exposed and referent subjects were similar in terms of demographic and anthropometric characteristics, and all analyses were adjusted for smoking and RPE usage.

Assessment of the lung function parameters revealed no biologically relevant differences between the copper-exposed and referent groups. The median FEV_1/FVC ratio was well above 0.7 among copper-exposed and referent subjects with ratios below this cutoff point indicate a persistent airflow limitation.[9] Median %pred values of the assessed lung function parameters, which take into account the age and height of the examined person, exceeded 90% for most parameters in both groups. Furthermore, median levels of R_{tot}, DL_{CO} or TL_{CO}, and PEF of both groups fell within typical ranges among never smokers (R_{tot}: <0.30 kPa · s/L[33]; DL_{CO} or TL_{CO}: >80%[16]; PEF: 6.7 to 11.7 L/s[34]), and there were no meaningful differences in the prevalence of obstructive impairments between both of these groups. In light of these observations, the results do not indicate any exposure-dependent impairment of the lung function in relation to copper exposure, and this is consistent with previous findings.[8]

As expected, the effect of smoking was evident in the study population. In line with literature data, we observed a significant increase in blood leukocytes, neutrophils,[35] and immunoglobulin E[36] and a decrease in the percentage of blood monocytes in the current smokers. An increase in the blood concentration of ICAM1 and MPO and a clear decline in blood CC16 were also observed.[37,38] Furthermore, FeNO values were lower among current smokers, a finding consistent with existing literature.[39] However, all FeNO values fell well below 25 ppb, which is the threshold indicating airway inflammation.[40]

Some systemic biomarkers were increased in copper-exposed workers compared with referents and among copper-exposed workers in non-RPE compared with RPE-wearing workers. However, as the

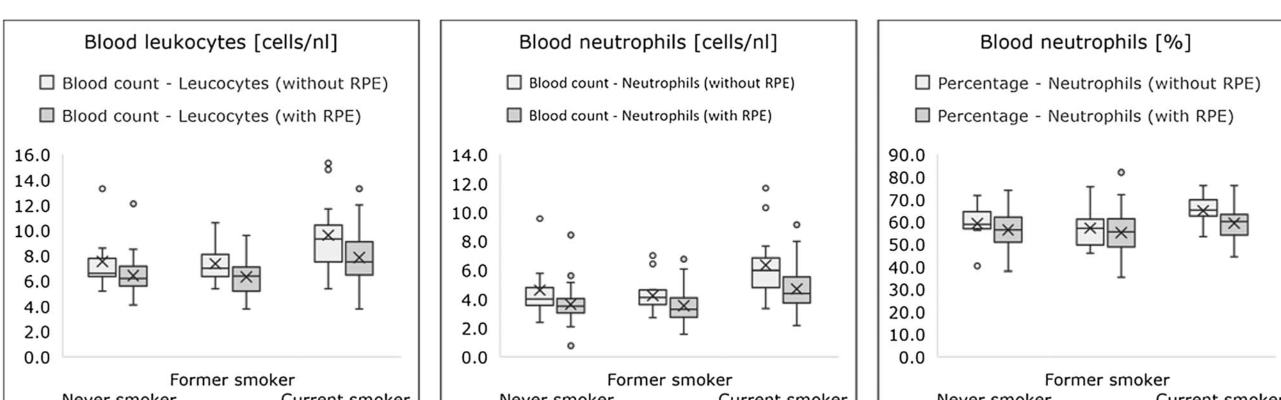

FIGURE 3. Box-and-whisker plots of the absolute number of blood leukocytes, blood neutrophils, and the percentage of blood neutrophils in copper-exposed workers with and without RPE, separately for smoking status. Box is reflecting the IQR with the median (line) and mean value (*x*) of the data. Upper and lower whiskers indicating the data variability: 25th percentile – 1.5 *IQR; 75th percentile + 1.5 *IQR. Empty circles represent outliers. IQR, interquartile range; RPE, respiratory protective equipment.

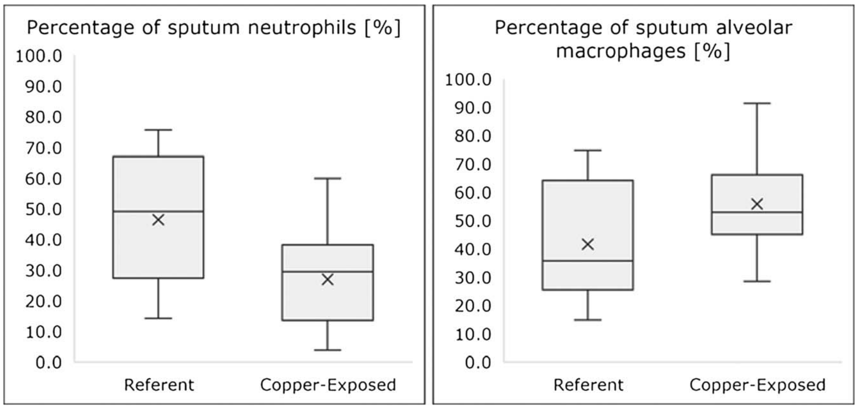

FIGURE 4. Median and IQR of the percentage of sputum neutrophils (left) and macrophages (right) in 25 nonsmoking copper-exposed workers and 11 nonsmoking referent workers. IQR, interquartile range.

multivariate analysis showed, the effect of smoking generally exceeded the effects of workplace dust exposures, and the data also provided evidence that the introduction of RPE had a beneficial effect. Slightly lower total copper blood levels ($P = 0.002$) of RPE-wearing workers (n = 66, median [interquartile range] 14 [5] μmol/L) compared with non-RPE users (n = 36, 16 [6] μmol/L) are compatible with this interpretation. It needs to be noted that despite differences between groups, blood neutrophils, leukocytes, and Cu levels were within the standard reference ranges of the contract laboratory suggesting that the observed differences were not adverse.

Sputum induction was performed in nonsmokers or former smokers at a time when wearing RPE for the copper-exposed group was already mandatory. The median quality score was 1.3 (on a 5-point scale 0 to 2[41]), and all samples except one could be evaluated. In line with this, the median squamous cell contamination was 5.5%, indicating good to very good quality of the samples. There was a significant correlation between the number of sputum neutrophils per milliliter of sputum and the concentration of IL-8 in sputum supernatant ($r = 0.70$, $P < 0.001$), which also suggests plausible sputum data. Some subjects reported allergies or had an atopic background, which was in most cases confirmed by increased sputum eosinophils and/or higher FeNO values. As these subjects were evenly distributed across groups, we decided to include them into the analysis.

There were significantly higher percentages of neutrophils in the referent group, which was supported by higher levels of IL-8 and MPO in the sputum supernatant; however, differences in these two parameters did not reach statistical significance. The reason for higher sputum neutrophils in the referent subjects is not clear but may reflect a common attribute of the smelting environment (eg, dust). Indeed, the referent subjects working at the precious metal facility were not required to wear RPE. As such, their exposure to ambient non–copper-containing dust may have been higher, leading to low-grade cellular response (eg, dust response) to increase lung clearance capacity. However, despite these differences between groups, most workers were within the range of normal subjects.[30,31] As the second major cell type in sputum, macrophage percentages were higher in the copper-exposed workers, although this difference was only borderline significant ($P = 0.06$). In conclusion, only small differences were observed between groups in blood and sputum, which did not lead to detectable functional changes, for example, in lung function.

When comparing the median values of the blood biomarkers to those of "healthy subjects" reported in literature, median MCP, MPO, ICAM-1, and ECP levels exceeded the upper boundaries of the respective reported ranges (MCP: 20.1 to 78.9 pg/mL[42]; MPO: 49.7 ± 27.6 ng/mL[43]; ICAM-1: 128.9 to 347.48 ng/mL[44]; ECP: 33 ± 37 ng/mL[43]). This was . equally observed among copper-exposed and referent subjects and so may reflect a common component of smelter working conditions. All other median blood biomarker levels fell within the respective reported ranges (CRP: <0.2 to 10.5 mg/L,[46] IL-6: 1 to 13.1 pg/mL,[47] IL-8: 24.4 to 35.9 pg/mL,[42] tumor necrosis factor α: 7.91 to 12.73 pg/mL,[48] CC16: 14.4 ± 4.6 ng/mL,[49] ESel: 9.15 to 65.19 ng/mL,[44] NE: 123.5 ± 24.3 ng/mL,[50] and SAA: <11.0 μg/mL[51]). However, there are limitations to comparing "healthy" subject value ranges between studies such as population demographics and experimental differences in the use of commercial analytical assays that hamper meaningful comparisons.

Our observations of a lack of copper exposure–related effects on lung function are consistent with a historical health surveillance study performed at the same copper plant that focused on the decline in FEV_1 over a mean exposure duration of approximately 22 years. Declines in FEV_1 among copper-exposed and referent never smokers were similar to the typical age-dependent decline in a healthy nonsmoking reference population.[8,52] The current study included a more sensitive assessment of lung function measures as well as inflammatory biomarkers to elucidate possible subtle effects, which might have been missed in the historical health surveillance study. However, such effects were not detected.

There are several other biomarkers that could have been included (such as IL-4, IL-5, IL-13), some of which are associated with asthma, and these might be considered in subsequent investigations. However, given the lack of any meaningful association observed between copper exposure and any spirometry measure or biomarker, the broad set of sensitive outcome measures we used provided no indication that workers occupationally exposed to high concentrations of copper-containing dust were different from the unexposed comparison group. The same is true for additional measures from the body plethysmograph that might have been used.

A general feature of the smelting environment is the elevated levels of dust involved in the refinement of the base ore. This mixed exposure may include other metals as well as "granular biopersistent particles without known specific toxicity" (GBSs). Such dusts are not biologically inert but are lower in toxicity than other, more bioactive components such as copper and other metals. This is reflected in the much higher occupational exposure limits for GBS,[53] as compared with silver and copper. Indeed, repeated exposures to GBS during smelting may also result in adaptive effects, such as increased alveolar macrophage recruitment (and associated cytokine signaling such as MCP). This reflects the increased particle load in the lungs and is designed to rid the airspaces of particles as part of a homeostatic response rather than part of a process that leads to pathological change. Coexposures to other inorganic dust particles including metals occurring in the refinement process of copper, lead, and precious metals were discussed in detail in the health surveillance study.[8] Respiratory protective equipment use became mandatory in the lead area in 2003, and therefore, an impact of mixed metal/GBS exposure on current workers in the lead area examined in 2019 was unlikely. Processing of precious metals was generally performed without RPE, and inhalation exposure to particulates (eg, silver and GBS) was likely. The base material consisted of up to 98% silver, and exposures to inorganic silver compounds were below the maximum allowed concentrations according to the German TRGS 900 (0.01 mg/m^3).[21] In relation to the coexposures at the copper smelter, pulmonary responses may also be triggered by substances occurring as part of the copper-refining process such as cadmium, sulfur dioxide, or antimony.[54–57] Assuming that none of the coexposures had a protective effect on lung function or inflammation, it is highly unlikely that coexposures to other metals masked a true effect of copper exposure.

It is important to note that this study was not able to identify potential acute effects of copper-containing dust exposure as two-thirds of the copper-exposed group (and the entire subgroup participating in the sputum biomarker assessment) used RPE during their shift on the day of sampling. By comparing the “RPE” and “non-RPE” groups for the blood biomarkers and FeNO, this limitation was partly diminished, although the sample size of the “non-RPE” group was relatively small. Nevertheless, near-term exposure (or reduction of exposure due to RPE use) would not be expected to affect the results of the chronic pathologies resulting in changes to lung function. Similarly, when inflammation is chronic in nature (ie, nonresolving), elevated levels of serum biomarkers such as IL-6, CRP, and IL-8 would be expected as noted in chronic inflammatory diseases such as chronic obstructive pulmonary disease[58] and cardiovascular disease.[59] Furthermore, CC16 is a lung-derived biomarker, secreted by epithelial cells found predominantly in the respiratory bronchioles, as well the large and small airways.[58] It serves as a specific marker for epithelial cell dysfunction, and serum levels rise following acute exposure to inflammatory stimulus such as cigarette smoke and lipopolysaccharide.[61] However, it is also an ideal candidate for use in longitudinal studies for lung disease of the lower respiratory tract.[62] This is because, over time, inflammation-related damage to these epithelial cells and remodeling leads to a reduction CC16 secretion and subsequent lower serum levels than in healthy controls. As such, copper-induced effects may be characterized by elevation in CC16 levels after acute exposure (acute inflammation), whereas the more chronic effects of copper-containing dust exposure such as epithelial dysfunction would be characterized by a reduction in CC16 serum levels. Neither response was observed in this study.

We relied on volunteers employed at a single, albeit large, copper smelter, and only ~50% of the workforce participated in the current study. This resulted in a relatively small sample of 174 workers. Although a selection bias in the copper-exposed group cannot be entirely excluded, its effect on the results is minor, considering the comparable length of exposure that was equally high among copper-exposed participants and nonparticipants. Furthermore, the selection of a sample of the referent group of younger age also had minor impact on the study findings, because any exposure-dependent effects would have been more pronounced in comparison with the copper-exposed participants.

Workers in the current study demonstrated no biologically relevant reductions in any of the sensitive lung function parameters used in the study or evidence for chronic inflammation, despite working for over 18 years (median of the copper-exposed group), in a smelter with inhalable copper dust levels of approximately 0.120 mg/m^3 (in 2018) and likely higher levels in previous years. If long-term exposure to copper can potentially lead to inflammation of the airways, as suggested in the SCOEL and MAK recommendations,[1,3] such an effect was not evident in this study.

CONCLUSION

This cross-sectional study investigated whether copper-containing dust exposures could affect lung function and associated inflammatory parameters. Based on the present findings, there is no indication of an alteration of the lung function of copper-exposed workers. This observation is supported by the absence of biologically relevant inflammatory responses measured in blood, sputum, and exhaled nitric oxide.

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REFERENCES

1. European Commission. Recommendation from the Scientific Committee on Occupational Exposure Limits for Copper and Its Inorganic Compounds (SCOEL/SUM/171). 2014;1–24. Available at: https://ec.europa.eu/social/BlobServlet?langId=en&docId=11815&. Accessed November 11, 2020.
2. Services H. Toxicological profile for copper. *ATSDR Toxicol Profiles*. 2004.
3. Forschungsgemeinschaft D. Copper and its inorganic compounds [MAK value documentation, 2014]. *MAK Collection Occup Health Saf*. 2015;1–18.
4. Stern BR. Essentiality and toxicity in copper health risk assessment: overview, update and regulatory considerations. *J Toxicol Environ Health A*. 2010;73: 114–127.
5. Gleason RP. Exposure to copper dust. *Am Ind Hyg Assoc J*. 1968;29:461–462.
6. Wardhana, Datau EA. Metal fume fever among galvanized welders. *Acta Med Indones*. 2014;46:256–262.
7. Borak J, Cohen H, Hethmon TA. Copper exposure and metal fume fever: lack of evidence for a causal relationship. *AIHAJ*. 2000;61:832–6.
8. Haase LM, Birk T, Bachand AM, Mundt KA. A health surveillance study of workers employed at a copper smelter—effects of long-term exposure to copper on lung function using spirometric data. *J Occup Environ Med*. 2021;63:e480–e489.
9. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis management, and prevention of chronic obstructive pulmonary disease (2019 report). 2019. Available at:https://goldcopd.org/wp-content/uploads/2018/11/GOLD-2019-v1.7-FINAL-14Nov2018-WMS.pdf. Accessed January 21, 2022.
10. Gibson GJ. Spirometry: then and now. *Breathe*. 2005;1:206–216.
11. Criée CP, Sorichter S, Smith HJ, et al, Working Group for Body Plethysmography of the German Society for Pneumology and Respiratory Care. Body plethysmography—its principles and clinical use. *Respir Med*. 2011;105:959–971.
12. Bösch D, Criée CP. *Bodyplethysmographie. Lungenfunktionsprüfung Durchführung—Interpret—Befundung*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013:49–88.
13. Meyer FJ, Ewert R, Hoeper MM, et al, German PPH Study Group. Peripheral airway obstruction in primary pulmonary hypertension. *Thorax*. 2002;57:473–476.
14. MacIntyre N, Crapo RO, Viegi G, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J*. 2005;26: 720–735.
15. Modi P, Cascella M. Diffusing capacity of the lungs for carbon monoxide. In: *StatPearls [Internet]*. Treasure Island, FL: StatPearls Publishing; 2022.
16. British Lung Foundation. Gas transfer (or TLco) test [Internet]. 2020. Available at: https://www.blf.org.uk/support-for-you/breathing-tests/gas-transfer-tlco. Accessed August 3, 2021.
17. Güder G, Brenner S, Störk S, et al. Diagnostic and prognostic utility of mid-expiratory flow rate in older community-dwelling persons with respiratory symptoms, but without chronic obstructive pulmonary disease. *BMC Pulm Med [Internet]*. 2015;15:83.
18. Criée C-P, Baur X, Berdel D, et al. Deutsche Atemwegsliga, Deutsche Gesellschaft für Pneumologie und Beatmungsmedizin und Deutsche Gesellschaft für Arbeitsmedizin und Umweltmedizin. München: Dustri-Verlag Dr. Karl Feistle; 2015.
19. Kamaludin NH, Jalaludin J, Tamrin SBM, Akim AM. Biomarker of occupational airways inflammation for exposure to inorganic dust. *Futur Food J Food Agric Soc*. 2018;6:23–28.
20. Dweik RA, Boggs PB, Erzurum SC, et al, American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med*. 2011;184:602–615.
21. Pflaumbaum W. Neue technische regeln für gefahrstoffe TRGS 900 “arbeitsplatzgrenzwerte”. *Gefahrstoorig Reinhalt Luft*. 2007;67:126.

22. Global Lung Function Initiative. Spirometry Tools, Online Calculator [Internet]. Cited April 1, 2019. Available at: https://www.ers-education.org/guidelines/global-lung-function-initiative/spirometry-tools/online-calculator.aspx. Accessed January 20, 2022.
23. Cooper BG, Stocks J, Hall GL, et al. The Global Lung Function Initiative (GLI) Network: bringing the world's respiratory reference values together. *Breathe (Sheff)*. 2017;13:e56–e64.
24. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests, European community for steel and coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl*. 1993;16:5–40.
25. Baur X. [Recommendation of new reference values for spirometry and body plethysmography.] *Pneumologie*. 2013;67:401–405.
26. Janssen O, Schaumann F, Holz O, et al. Low-dose endotoxin inhalation in healthy volunteers—a challenge model for early clinical drug development. *BMC Pulm Med*. 2013;13:19.
27. Wang ML, Storey E, Cassidy LD, et al. Longitudinal and cross-sectional analyses of lung function in toluene diisocyanate production workers. *J Occup Environ Med*. 2017;59 suppl 12(suppl 12):S28–S35.
28. Quanjer PH, Weiner DJ, Pretto JJ, Brazzale DJ, Boros PW. Measurement of FEF25–75% and FEF75% does not contribute to clinical decision making. *Eur Respir J*. 2014;43:1051–1058.
29. Carsin AE, Fuertes E, Schaffner E, et al. Restrictive spirometry pattern is associated with low physical activity levels. A population based international study. *Respir Med*. 2019;146:116–123.
30. Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, Hargreave FE. Induced sputum cell counts in healthy adults. *Am J Respir Crit Care Med*. 2000;161(2 pt 1):475–478.
31. Spanevello A, Confalonieri M, Sulotto F, et al. Induced sputum cellularity. Reference values and distribution in normal volunteers. *Am J Respir Crit Care Med*. 2000;162(3 pt 1):1172–1174.
32. Devasahayam J, LaFreniere K, Naik R. Chronic Emphysema. [Updated 2020 Aug 8]. In: *StatPearls [Internet]*. Treasure Island, FL: StatPearls Publishing; 2020.
33. Goldman MD, Smith HJ, Ulmer WT. Whole-body plethysmography. *Eur Respir Mon*. 2005;31:15–43.
34. British Lung Foundation. Peak flow test [Internet]. 2020. Available at: https://www.blf.org.uk/support-for-you/breathing-tests/peak-flow. Accessed August 4, 2021.
35. Schwartz J, Weiss ST. Cigarette smoking and peripheral blood leukocyte differentials. *Ann Epidemiol*. 1994;4:236–242.
36. Kim YS, Kim HY, Ahn HS, et al. The association between tobacco smoke and serum immunoglobulin E levels in Korean adults. *Intern Med*. 2017;56:2571–2577.
37. Khan NA, Lawyer G, McDonough S, et al. Systemic biomarkers of inflammation, oxidative stress and tissue injury and repair among waterpipe, cigarette and dual tobacco smokers. *Tob Control*. 2019;29(suppl 2):s102–s109.
38. Andelid K, Bake B, Rak S, Lindén A, Rosengren A, Ekberg-Jansson A. Myeloperoxidase as a marker of increasing systemic inflammation in smokers without severe airway symptoms. *Respir Med*. 2007;101:888–895.
39. Högman M, Thornadtsson A, Bröms K, et al. Different relationships between F_ENO and COPD characteristics in smokers and ex-smokers. *COPD*. 2019;16:227–233.
40. Backé E, Lotz G, Tittelbach U, Plitzko S, Gierke E, Schneider WD. Immunological biomarkers in salt miners exposed to salt dust, diesel exhaust and nitrogen oxides. *Int Arch Occup Environ Health*. 2004;77:319–327.
41. Pedersen F, Zissler UM, Watz H, Rabe KF, Hohlfeld JM, Holz O. Rating sputum cell quality in clinical trials for asthma and COPD treatment. *Int J Chron Obstruct Pulmon Dis [Internet] Dove Medical Press*. 2019;14:195–198. Available at: https://pubmed.ncbi.nlm.nih.gov/30662260. Accessed July 29, 2021.
42. Kleiner G, Marcuzzi A, Zanin V, Monasta L, Zauli G. Cytokine levels in the serum of healthy subjects. *Mediators Inflamm*. 2013;2013:434010.
43. Hoy A, Trégouët D, Leininger-Muller B, et al. Serum myeloperoxidase concentration in a healthy population: biological variations, familial resemblance and new genetic polymorphisms. *Eur J Hum Genet*. 2001;9:780–786.
44. Deneva-Koycheva TI, Vladimirova-Kitova LG, Angelova EA, Tsvetkova TZ. Serum levels of siCAM-1, sVCAM-1, sE-selectin, sP-selectin in healthy Bulgarian people. *Folia Med (Plovdiv)*. 2011;53:22–28.
45. Giusti D, Gatouillat G, Le Jan S, et al. Eosinophil cationic protein (ECP), a predictive marker of bullous pemphigoid severity and outcome. *Sci Rep*. 2017; 7:4833.
46. Erlandsen EJ, Randers E. Reference interval for serum C-reactive protein in healthy blood donors using the Dade Behring N latex CRP mono assay. *Scand J Clin Lab Invest*. 2000;60:37–43.
47. Fernandez-Real JM, Vayreda M, Richart C, et al. Circulating interleukin 6 levels, blood pressure, and insulin sensitivity in apparently healthy men and women. *J Clin Endocrinol Metab [Internet]*. 2001;86:1154–1159.
48. Himmerich H, Fulda S, Linseisen J, et al. TNF-alpha, soluble TNF receptor and interleukin-6 plasma levels in the general population. *Eur Cytokine Netw*. 2006; 17:196–201.
49. Hermans C, Petrek M, Kolek V, et al. Serum Clara cell protein (CC16), a marker of the integrity of the air-blood barrier in sarcoidosis. *Eur Respir J*. 2001;18: 507–514.
50. Sun G, Ota C, Kitaoka S, et al. Elevated serum levels of neutrophil elastase in patients with influenza virus–associated encephalopathy. *J Neurol Sci*. 2015; 349:190–195.
51. Liu Q, Li Y, Yang F, et al. Distribution of serum amyloid a and establishment of reference intervals in healthy adults. *J Clin Lab Anal*. 2020;34:e23120.
52. Redlich CA, Tarlo SM, Hankinson JL, et al, American Thoracic Society Committee on Spirometry in the Occupational Setting. Official American Thoracic Society technical standards: spirometry in the occupational setting. *Am J Respir Crit Care Med*. 2014;189:983–993.
53. Morfeld P, Bruch J, Levy L, et al. Translational toxicology in setting occupational exposure limits for dusts and hazard classification—a critical evaluation of a recent approach to translate dust overload findings from rats to humans. *Part Fibre Toxicol*. 2015;12:3.
54. Chai R, Xie H, Zhang J, Ma Z. Sulfur dioxide exposure reduces the quantity of $CD19^+$ cells and causes nasal epithelial injury in rats. *J Occup Med Toxicol*. 2018;13:22.
55. Charan NB, Myers CG, Lakshminarayan S, Spencer TM. Pulmonary injuries associated with acute sulfur dioxide inhalation. *Am Rev Respir Dis*. 1979;119: 555–560.
56. Sundar S, Chakravarty J. Antimony toxicity. *Int J Environ Res Public Health*. 2010;7:4267–4277.
57. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for cadmium [Internet]. 2012. Available at: https://www.atsdr.cdc.gov/toxprofiles/tp5.pdf. Accessed November 11, 2020.
58. Faner R, Tal-Singer R, Riley JH, et al, ECLIPSE Study Investigators. Lessons from ECLIPSE: a review of COPD biomarkers. *Thorax*. 2014;69: 666–672.
59. Luan YY, Yao YM. The clinical significance and potential role of C-reactive protein in chronic inflammatory and neurodegenerative diseases. *Front Immunol*. 2018;9:1302.
60. Lomas DA, Silverman EK, Edwards LD, Miller BE, Coxson HO, Tal-Singer R, Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) investigators. Evaluation of serum CC-16 as a biomarker for COPD in the ECLIPSE cohort. *Thorax*. 2008;63:1058–1063.
61. Lakind JS, Holgate ST, Ownby DR, et al. A critical review of the use of Clara cell secretory protein (CC16) as a biomarker of acute or chronic pulmonary effects. *Biomarkers*. 2007;12:445–467.
62. MacNee W. Systemic inflammatory biomarkers and co-morbidities of chronic obstructive pulmonary disease. *Ann Med*. 2013;45:291–300.

