Molecular Markers of Acute Upper Airway Inflammation in Workers Exposed to Fuel-Oil Ash

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Biomarkers in nasal lavage (NL) fluid may be useful in determining the presence and severity of upper airway inflammation. We studied 18 boilermakers overhauling a large, oil-fired boiler and 11 utility workers who served as controls for 6 wk. NL was performed before (NL1), during (NL2), and after (NL3) the overhaul. We measured nasal fluid levels of interleukins 6 (IL-6) and 8 (IL-8), eosinophilic cationic protein (ECP), and myeloperoxidase (MPO) as markers of response to fuel-oil ash exposure. In boilermakers, MPO was elevated during boiler work versus preboiler work (mean = 33.8 versus 22.7 ng/ml, $p < 0.05$), and at the 2-wk postexposure lavage (NL3) it had declined to 24.2 ng/ml ($p = 0.08$). Mean IL-8 levels increased in boilermakers between NL1 and NL2 (mean = 83.8 versus 134.8 pg/ml, $p < 0.05$), then decreased at NL3 (mean = 134.8 versus 89.0 pg/ml, $p < 0.05$). Nasal fluid vanadium increased in boilermakers between NL1 and NL2 (median < 1.0 versus 4.7 ppb, respectively, $p < 0.05$), then decreased at NL3 (median, 4.7 versus < 1.0 ppb, respectively, $p < 0.05$). Levels of IL-6 and ECP did not change significantly during the study. Utility workers showed no significant change in any marker during the study period. Particulate matter < 10 μm (PM_{10}) levels were higher for boilermakers than for utility workers before boiler work (geometric mean (GM) = 0.40 versus 0.10 mg/m^3, $p < 0.05$). This difference was more significant during boiler work (GM = 0.47 versus 0.13 mg/m^3, $p < 0.001$). Ozone levels were low during the study. These data suggest that exposure to fuel-oil ash results in acute upper airway inflammation, potentially mediated by increased IL-8 levels and the recruitment and activation of polymorphonuclear leukocytes. These changes were associated with significantly increased PM_{10} levels and concentrations of upper airway vanadium. Woodin MA, Hauser R, Liu Y, Smith TJ, Siegel PD, Lewis DM, Tollerud DJ, Christiani DC. Molecular markers of acute upper airway inflammation in workers exposed to fuel-oil ash.

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Exposure to substances found in fuel-oil ash has been shown to cause adverse respiratory effects (1–3). Vanadium, in the form of vanadium oxides, is abundant in fuel-oil ash and has been previously identified as a potent inflammatory agent in the respiratory system (3, 4–7). Many previous studies, however, have had limited exposure and/or outcome end points and consequently have been able to identify only overt respiratory disease. Also, the causal pathway leading to clinically apparent disease has remained unclear.

In this study, we evaluated nasal lavage (NL) as a possible technique to both identify and quantify biomarkers of response and exposure to fuel-oil ash. It has been suggested that the nasal cavity is useful for assessing the levels of biomarkers that may affect the respiratory system (8). Early inflammatory reactions that occur in the nasal cavity may reflect similar processes occurring in the lower airways. Early detection may allow for interventions that mitigate or eliminate exposure before chronic pulmonary conditions arise. Additionally, in contrast to examining the lung, the nasal cavity is easier to access and the nasal lavage procedure is noninvasive and easily performed under field research conditions.

The utility of nasal lavage as a tool in identifying and quantifying biomarkers in the upper airway has been demonstrated previously (8–10). Hauser and colleagues (8) have shown that when analyzing levels of polymorphonuclear leukocytes (PMNs), within-person variability is less than between-person variability. A study by Linder and colleagues (9) examined myeloperoxidase (MPO) and eosinophilic cationic protein (ECP) levels in NL samples. Frischer and colleagues (10) used nasal lavage to examine the relationship between ozone exposure and upper airway PMN levels in 44 children.

The purpose of this study was to examine the relationship

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between exposure to fuel-oil ash and changes in nasal fluid cytokines thought to play a role in cellular inflammatory reactions to pollutants. Specifically, interleukins 6 and 8 (IL-6 and IL-8), as well as two cellular proteins also involved in the inflammatory response, MPO and ECP, were measured. We also measured the concentrations of six metals (vanadium, arsenic, lead, nickel, manganese, and chromium) in the lavagate. We examined the hypothesis that exposure to fuel-oil ash and, in particular, vanadium oxides causes an increase in the concentration of certain inflammatory-response mediators in the upper airway.

METHODS

Subjects

The study was approved by the Institutional Review Board of the Harvard School of Public Health. The study population consisted of 18 boilermakers and 11 utility workers who volunteered for the study. All subjects were involved in the overhaul of a large, oil-fired boiler. No subject from either group had active allergies during the overhaul or during the 2 wk prior to the start of the overhaul. Subjects were questioned about respiratory infections at each of the nasal lavage tests, and one boilermaker was excluded at NL2 because of acute coryza. The boilermakers worked inside the boiler, where particulate exposures were highest. Utility workers served as controls since they did not work inside the boiler. Study subjects were followed for 6 wk, from mid-May 1995 to late-June 1995.

Industrial Work Process

The job entailed the repair or replacement of several large pieces of the interior wall of the boiler, which required the use of acetylene torch and carbon-arc cutting, electric-arc welding, and the removal and replacement of portions of the tubing used to circulate steam and water. The ash pit, where bottom ash collects, was repaired. In addition, repairs to the muddrum (where water returning from the condenser is held before being pumped back into the boiler tubing) and steam drum (where steam is held before entering the turbines to generate electricity) were also performed.

Exposure Measurements

Particulate matter < 10 μm (PM_{10}). Gillian and GilAir5 (Gillian, West Caldwell, NJ) personal sampling pumps were used to collect PM_{10}. A PTFE filter was used because preliminary laboratory testing indicated it had a high particle loading capacity, comparatively low background metal levels, and was easy to digest with acid. The pump flow rate was 4 L/min. Subjects who volunteered to wear a pump were instructed on how the pump worked and were told to report any problems. Each pump was logged in when the pump was started and logged out when the pump was stopped so that a time-weighted average (TWA) for PM_{10} exposure could be calculated.

Ozone (O_3). An O_3 personal sampler using a small pump, recently developed by Geyh (11), was worn by boilermakers and utility workers. Compared with passive badge collectors, the active samplers were more sensitive and relatively unaffected by wind velocity, O_3 concentration, total O_3 exposure, and relative humidity. The limit of detection (LOD) was 6 ppb over an 8-h sampling period. The laboratory analysis followed a protocol developed by Geyh (11).

Nasal lavage. Prior to undergoing nasal lavage (NL), all subjects were instructed on how to keep the nasopharyngeal cavity closed to avoid swallowing the lavage fluid. While seated with the head tilted back at approximately a 45 degree angle, subjects had 5 ml of sterile warm buffered acetate solution (Normasol; Abbott Laboratories, Chicago, IL) introduced into one nostril using a small, sterile syringe without needle. After 10 s, the subjects tilted forward and allowed the lavage fluid to drain into a sterile collecting cup. The procedure was then repeated on the other nostril, and all recovered fluid (normally 6 to 8 ml) was decanted into a sterile 15-ml test tube, which was sealed and immediately put on ice. The lavage samples were centrifuged at 1,000 *g* for 10 min. The supernatant fluid was then separated from the cell pellet and frozen at $-80°$ C until analysis.

ICP-MS analysis for metals. Nasal lavage samples were thawed and 1 g of supernatant was put into a sterile test tube to which 1.5 g of concentrated nitric acid were added to digest proteins. An internal standard of several rare earth elements (1 g) was added to aid in machine calibration. The solution was brought to 25 g using deionized water. The ICP-MS was a Hewlett-Packard 4500, equipped with an ultrasonic nebulizer and membrane desolvator made by Cetac Technologies (Omaha, NE).

The combination nebulizer/desolvator removes virtually all NaCl and water from samples, which often combine with atomic species in the ICP-MS to form polyatomic ions that greatly reduce the reliability of ICP-MS results on human samples (12, 13). By using the nebulizer/desolvator, we achieved a reliable detection limit for metals of 1 ppb. The machine was recalibrated at fixed intervals throughout sample testing to ensure continued measurement accuracy, and several duplicate samples were analyzed to ensure reproducibility.

Assays for Eosinophilic Cationic Protein and Myeloperoxidase

ECP and MPO were measured using commercially available immunoassays. After thawing, the samples were mixed with a vortex and pipetted into radioimmunoassay (RIA) tubes (ECP) or a 96-well plate (MPO). The ECP RIA (Pharmacia, Columbus, OH) was performed according to the manufacturer's recommendations. An enzyme-linked immunoabsorbent assay (ELISA) (Cayman Chemical, Ann Arbor, MI) was used to determine the concentration of MPO following manufacturer's recommendations (14).

Chemiluminescence ELISA for IL-6 and IL-8

Opaque microtiter plates (Microlite 1; Dynatech, Alexander, VA) were coated with 100 μl of diluted purified anticytokine monoclonal antibody in TRIS-coating buffer. The plates were incubated overnight at 4° C, then washed with 300 μl of PBS-Tween and blotted dry. Recombinant standards and samples (diluted in 0.5 to 1.0% PBS-Tween) were added and incubated overnight at 4° C.

Plates were then washed with PBS-Tween, and 100 μl biotinylated anticytokine monoclonal antibody were added. After incubation at room temperature for 1 h with gentle shaking, plates were washed with PBS-Tween. Enzyme-labeled avidin or streptavidin in 0.5 to 1% BSA/PBS-Tween was added and incubated at room temperature for 1 h. Plates were then washed and 100 μl/ml Lumiphos 530 (Lumigen) were added. After a 30-min incubation at room temperature, light emission was read using microtiter plate luminometer ML 1000 and BioCalc Data Analysis Software (Dynatech).

Statistical Analysis

Boilermaker and utility worker data were analyzed separately. Repeated-measures analysis of variance (RM ANOVA) was used for the IL-8 and MPO data as these measures did not deviate significantly from normality. The sign test was used to analyze the vanadium, IL-6, and ECP data since significant deviations from normality were noted. The use of the sign test resulted in multiple comparisons within these analyses, which were adjusted for using the Bonferroni method.

Because of the difficulty in interpreting a single NL sample, it was decided *a priori* not to include such subjects in any analysis. This exclusion criterion resulted in one boilermaker being excluded from the MPO, ECP, and vanadium analysis, six excluded from the IL-8 analysis, and three excluded from the IL-6 results. Four utility workers were excluded from the IL-6 and ECP analyses, one from the IL-8 data, and two from the MPO results. When all subjects were included in each analysis, there was no significant change in the results and, when only those subjects who participated in all three nasal lavages were examined, the results were not significantly changed for any of the biomarkers of interest.

To allow for computation of the median, 25th percentile, and 75th percentile, all boilermakers and utility workers with levels of vanadium below the detection limit of 1.0 ppb were assigned a value of 0.50 ppb.

Spearman's Rank Order Correlation was used to measure the strength of the association of the six metals with levels of IL8 and MPO at NL1, NL2, and NL3.

TABLE 1

DESCRIPTION OF STUDY POPULATION

	Boilermakers	Utility Workers
Number	18	11
Mean age	37	35
Age range	26–61	30–55
Smokers, %	39	17
Participated in NL1, %	83*	83[†]
Participated in NL2, %	78[‡]	83
Participated in NL3, %	39[§]	75[‖]

* Of the three subjects missing, one was unable to avoid swallowing all of the lavage fluid, one refused to participate, and one was working off-site on day of NL1.

[†] Of the two subjects missing, one refused to participate, and one was not at work on day of NL1.

[‡] In addition to the three subjects missing from NL1, one additional subject was excluded because of acute coryza at time of NL2.

[§] Approximately one-half of the boilermakers had a driving time to the union hall (where NL3 was conducted) of 2 h or greater. Many of those who did not participate cited the long drive as the reason for noncompliance.

[‖] For utility workers, participation in NL3 was done on-site on a normal work day.

RESULTS

All participants were white men, 26 to 61 yr of age for the boilermakers and 30 to 55 yr of age for the utility workers. Seven boilermakers smoked cigarettes (39%) compared with two utility workers (18%, $p > 0.10$). For boilermakers, participation in NL1 = 83%, NL2 = 78%, and NL3 = 39%. For utility workers, 83% participated in NL1 and NL2, 75% in NL3 (Table 1). All dropouts cited time as the only reason for not continuing in the study.

Environmental Exposure

Ozone levels were uniformly low. The preoverhaul geometric mean (GM) for boilermakers was 4.5 ppb (geometric standard deviation [GSD] = 1.2). Utility workers had a pre-overhaul GM of 1.6 ppb (GSD = 1.5). The difference between groups was significant ($p < 0.05$). When boilermakers were working inside the boiler, ozone levels were lower than when extensive outside work was being done early in the overhaul (GM = 3.7 ppb, GSD = 2.2). Utility workers showed a small increase in ozone exposure during the overhaul (GM = 4.8 ppb, GSD = 2.2). The difference between the groups was not significant.

A total of 20 personal air samples for boilermakers and 15 for utility workers were collected and analyzed for PM_{10} levels. All areas inside and most outside the boiler were represented in the samples. Prior to boiler work, boilermakers had significantly higher PM_{10} exposure than did utility workers (GM = 0.40 mg/m^3, GSD = 1.6 versus GM = 0.10 mg/m^3, GSD = 2.7, respectively, $p < 0.05$). During boiler work, the difference was more significant (GM = 0.47 mg/m^3, GSD = 1.9, versus GM = 0.13 mg/m^3, GSD = 2.4, respectively, $p < 0.001$).

Nasal Lavage Fluid Cytokine and Enzyme Levels

In boilermakers, changes in MPO levels over the study period are shown in Figure 1. MPO increased during boiler work (mean, 22.7 ng/ml at NL1 versus 33.9 ng/ml at NL2, $p < 0.05$). There was a trend toward NL1 levels at NL3 (mean, 33.9 ng/ml at NL2 versus 24.2 ng/ml at NL3, $p = 0.08$) (Table 2). All boilermakers exhibited similar IL-8 changes over the study (Figure 2). Mean IL-8 levels at NL2 were significantly higher than both NL1 and NL3 levels (mean, 140.9 pg/ml at NL2 versus 93.7 pg/ml at NL1 and 89.0 pg/ml at NL3, $p < 0.05$ for both comparisons) (Table 2). Nasal fluid levels of ECP and IL-6 did not change significantly ($p > 0.10$ in all comparisons) during the study period.

In utility workers, mean MPO levels were essentially identical at all three nasal lavage periods. Mean IL-8 levels declined during the study (NL1 = 69.2 pg/ml, NL2 = 58.5 pg/ml, NL3 = 47.5 pg/ml), but this finding was not significant ($p >$

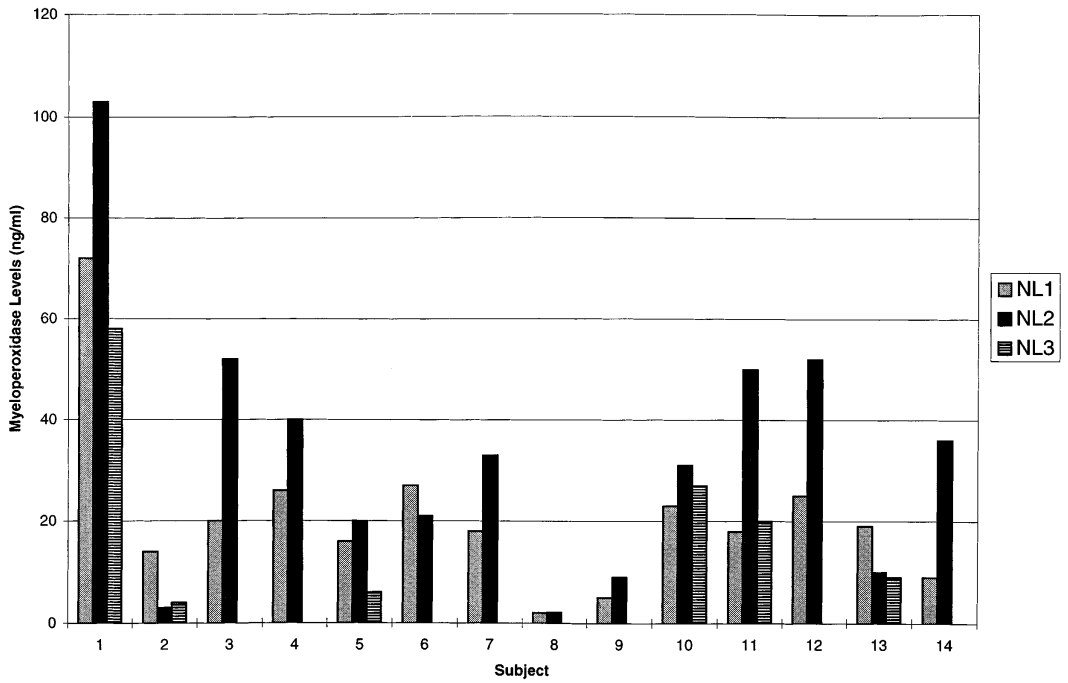

Figure 1. Nasal fluid myeloperoxidase levels in boilermakers. $p < 0.05$, NL2 versus NL1; $p = 0.08$, NL2 versus NL3.

TABLE 2

IL-8 (pg/ml) AND MPO LEVELS (ng/ml) AT NL1, NL2, AND NL3

	Nasal Lavage One		Nasal Lavage Two		Nasal Lavage Three	
	Mean	Min–Max	Mean	Min–Max	Mean	Min–Max
Interleukin-8						
Boilermakers	93.7	22.6–235.0	140.9*	32.4–307.0	89.0†	20.3–75.0
Utility workers	69.2	24.6–104.5	58.5	14.3–108.4	47.5	12.8–74.7
Myeloperoxidase						
Boilermakers	22.7	2.0–72.8	33.9*	2.0–103.0	24.2	3.9–58.1
Utility workers	25.6	10.1–47.6	27.2	4.9–66.2	25.6	4.9–51.7

Definition of abbreviations: NL1 = preexposure to work inside boiler (IL-8, n = 8; MPO, n = 15); NL2 = during interior boiler work (IL-8, n = 8; MPO, n = 14); NL3 = two weeks postexposure (IL-8, n = 4; MPO, n = 6).

*$p < 0.05$ (NL2 versus NL1).

†$p < 0.05$ (NL2 versus NL3).

TABLE 3

NASAL FLUID VANADIUM CONCENTRATIONS (ppb)

Boilermakers	NL1 (*n = 15*)	NL2 (*n = 15*)	NL3 (*n = 6*)
25%	0.5	2.8	0.5
Median	0.5	4.7*	0.5
75%	3.2	23.4	0.5
Min–Max	0.5–9.7	0.5–102.9	0.5–2.7
Utility workers	NL1 (*n = 8*)	NL2 (*n = 8*)	NL3 (*n = 6*)†
25%	0.5	0.5	
Median	0.5	0.5	
75%	0.5	1.7	
Min–Max	0.5–3.8	0.5–8.0	

* $p < 0.05$ (NL2 versus NL1); $p < 0.05$ (NL2 versus NL3).

† No utility workers had vanadium concentrations at or above the 1.0 ppb detection limit at NL3.

0.10 for all comparisons). Similar to boilermakers, mean IL-6 and ECP levels were essentially unchanged during the study period.

For boilermakers and utility workers, no significant association was found between smoking and cytokine/enzyme levels. Age also was not associated with any of the examined biomarkers.

Nasal Lavage Metal Concentrations

Analysis on nasal fluid vanadium concentrations for boilermakers revealed a large number of samples with concentrations < 1.0 ppb at NL3. To allow computation of the median, these samples were assigned a value of 0.5 ppb. Boilermakers had significantly higher nasal fluid vanadium concentrations comparing NL2 with both NL1 and NL3 (Table 3).

The situation for utility workers was very different, with only two subjects having detectable vanadium at NL1, four at NL2, and none at NL3. To compute the median, all utility workers with levels of vanadium below the detection limit were assigned a value of 0.5 ppb. Utility workers' vanadium levels remained essentially unchanged from NL1 to NL3.

Chromium, manganese, lead, arsenic, and nickel concentrations did not show any significant temporal change in either boilermakers or utility workers. With the exception of lead, which showed a few isolated high concentrations, none of the metals besides vanadium had levels in excess of 10 ppb at any of the NL sampling times.

Spearman's Rank Order Correlation test did not show a significant association between vanadium concentrations and levels of MPO or IL-8.

DISCUSSION

The results showed that boilermakers exhibited an upper airway inflammatory response while working inside the boiler where fuel-oil ash exposure was highest. The elevation in IL-8 and MPO in the nasal lavagate at NL2 is evidence that such a response was occurring. MPO is a product of neutrophils (15,

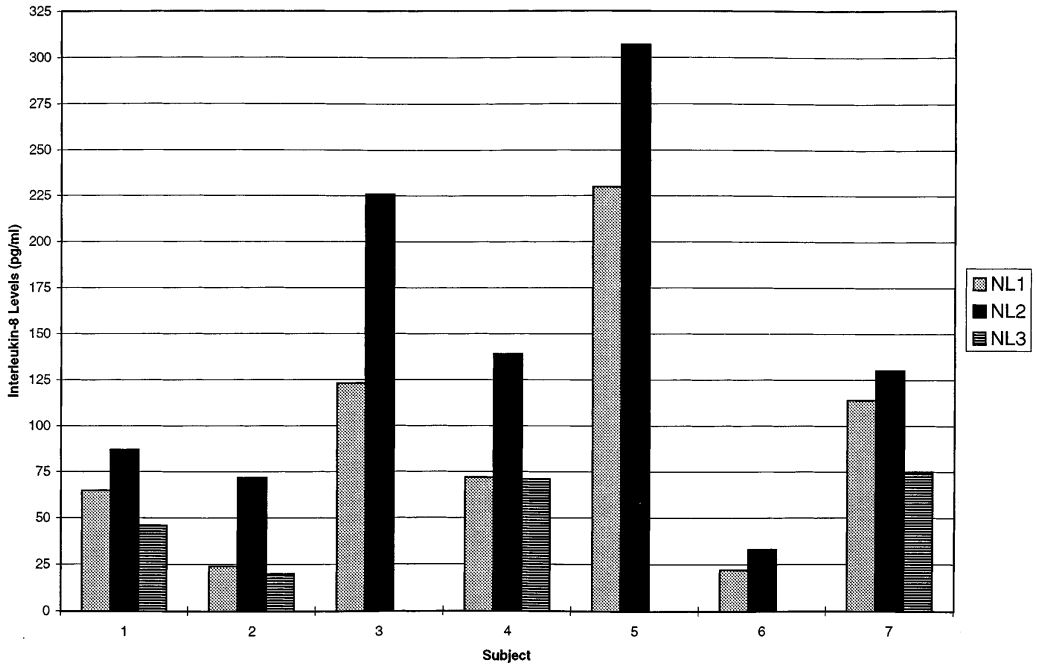

Figure 2. Nasal fluid interleukin-8 levels in boilermakers. $p < 0.05$, NL2 versus NL1; $p < 0.05$, NL2 versus NL3.

16), which are known to be recruited in high numbers in inflammatory processes (15, 17). Studies on the cytokines have shown that IL-8 levels are consistently elevated when inflammation is occurring (15, 18–21). Both biomarkers were elevated in boilermakers during the period of peak exposure.

In order to further distinguish an allergic versus inflammatory response, we included ECP as one of the biomarkers. This protein, released by activated eosinophils, has been shown to be involved in allergic processes (9, 17). Our expectation was that, if a subject's allergy was contributing to any observed upper airway inflammation, ECP would be significantly elevated at one or more of the NL periods. The results show that this was not the case. Neither subject group showed any significant change in ECP throughout the study, and the levels of ECP generally were low. We conclude that allergy is not a likely explanation for the observed inflammatory reaction.

A second possibility is that viral and/or bacterial infection(s) caused the upper airway inflammation. It is difficult to rule out this possibility completely. In order to minimize the likelihood that infection would be responsible for our results, we carefully questioned each subject about whether they currently had, or had in the previous 2 wk, any upper respiratory infection (e.g., "cold" or "flu"). One subject had active coryza at NL2 and was eliminated from the analysis. Overall, it does not seem likely, especially given the strength of the results, that an undiagnosed infectious illness was the principal cause of the observed inflammatory reaction, as this would require that almost all boilermakers, but virtually no utility workers, to have been infected with an asymptomatic upper airway infection between NL1 and NL2.

Moreover, IL-6 is normally active during the immunological reaction initiated by such infections, and may act to potentiate the actions of other cytokines (17). If a respiratory infection was the cause of our results, we would anticipate a greater change in IL-6 levels than were observed. It should be noted, however, that the study of cytokines in nasal lavage fluid is in its infancy, and it is not possible to completely separate the roles of IL-8 and IL-6, or the levels at which these roles are accomplished, at this time.

It is possible that an exposure other than the vanadium was responsible for the observed inflammatory reaction. Fuel-oil ash is a complex mixture of substances, several of which could cause or contribute to upper airway inflammation. Particulate matter less than 10 μm in aerodynamic diameter (PM_{10}), other metals (e.g., nickel), and ozone (from welding) are all potential confounding variables in this study. However, the comparatively low levels of other metals, the very low levels of ozone, and the lack of significant increase of any of these agents between NL1 and NL2, suggest that their contributory role in causing the observed inflammatory reactions is probably small.

Boilermakers did have significantly higher exposure to PM_{10} than utility workers, and this difference was greatest when they were working inside the boiler. A significant obstacle to assessing fully the role of PM_{10} exposure on the concentration of MPO and IL-8 in the nasal lavage fluid is the lack of personal air samples on the days that NL1 and NL2 were performed. The sampling pumps are not comfortable to wear, especially in confined spaces, and worker compliance with the use of the devices was low. Typically, five of the 10 available personal sampling pumps were in use on each day during the overhaul and, of these, only two or three were worn by boilermakers. This problem was especially evident when boilermakers were scheduled to work in confined spaces within the boiler. In such areas, boilermakers frequently had to work in the prone position, and the presence of the pump on their hip or lower back was uncomfortable. It would be in these areas, however, that PM_{10} exposure would likely be highest.

Hence, the PM_{10} exposure of the boilermakers was likely underestimated. However, it is not possible to conclude with certainty that PM_{10} per se caused the observed increase in inflammatory mediators seen during the course of the overhaul. As markers of acute inflammation, MPO and IL-8 levels reflect recent exposure to inflammatory agents. The nasal lavages were performed early in the morning, prior to any work being done. The most relevant PM_{10} exposure would have occurred during the previous 24 h. However, on the day prior to the collection of NL2 samples, only one boilermaker wore a personal air sampling pump. Such minimal exposure information does not allow for a meaningful analysis of the effect of recent PM_{10} exposure on MPO and IL-8 levels in NL fluid.

To assess whether vanadium concentrations in the nasal fluid were correlated with levels of IL-8 or MPO, Spearman's Rank Order Correlation test was used. No significant associations were found. This lack of correlation between vanadium concentrations and the levels of IL-8 and MPO may be attributed to the difficulties in finding the best time to perform the nasal lavages. As mentioned above, the lavages were done in the morning before any work was begun. This resulted in all subjects having at least 12 h from last exposure to interior boiler work (end-of-shift was typically 5:30 P.M.) and the nasal lavage. Performing the lavages at end-of-shift may be a better strategy for accurately characterizing an individual's metal exposure. One problem with this end-of-shift strategy is the kinetics of production of markers of inflammation such as MPO and IL-8. Several hours are necessary to allow for the recruitment of these cells to the site of inflammation and the subsequent release of their products (17). Thus, end-of-shift testing would likely have resulted in low yields of the response markers. Performing a nasal lavage at both times (i.e., end-of shift and start-of-shift) is a possibility, and may be considered in future studies.

Other studies have used NL as a tool for assessing biomarkers in the airway. Frischer and colleagues (10) used NL on 44 children during periods of high and low ozone exposure. Over the course of this 6-mo study, 148 lavages were performed during periods of high ozone exposure, whereas during low ozone periods, 106 NLs were done. These investigators found that PMNs, ECP, and MPO all increased significantly during high ozone days compared with low ozone days. They point out in their discussion that allergic children had the highest ECP counts.

Ahman and colleagues (22) used NL to assess whether working as an industrial arts (IA) teacher (in which exposure to wood dust was high) resulted in elevated levels of ECP and neutrophils (which are the source of MPO) when compared with other teachers. A significant relationship was found between number of IA classes taught per week and elevated levels of neutrophils (but not ECP). The investigators concluded that this could indicate that an inflammatory reaction was occurring in IA teachers. In their discussion, they state that no single measure of exposure correlated significantly with the noted increase in neutrophils. They hypothesize several such factors, acting together, as a possible explanation.

Linder and colleagues (9) analyzed the NL results of 20 subjects with diagnosed allergy to birch pollen. These patients underwent antigen challenge, and it was found that ECP, but not MPO, was significantly elevated on challenge tests, the magnitude of the increase appearing to be commensurate with the strength of the subject's reaction to the challenging dose. This study supports in part our assumption that ECP would become elevated if any allergic condition in our subjects was exacerbated during the study period.

The rise in nasal fluid vanadium levels noted in the boilermakers supports the hypothesis that exposure to increasing amounts of this metal may cause an acute inflammatory reaction in the upper airway. Because the precise mechanism and timing by which vanadium would cause an acute reaction is not known, it may be that the nasal lavage timing used in this study was not the best for demonstrating a dose-response between vanadium and the inflammatory markers. Further studies are needed of exposure to vanadium-containing particulates and upper respiratory inflammatory responses.

In conclusion, we found nasal lavage fluid to be a useful medium for the quantification of cytokines, enzymes, and metals. The workers exposed to fuel-oil ash experienced upper airway inflammation, characterized by significant increases in MPO and IL-8, but not ECP or IL-6. Nasal fluid vanadium levels in boilermakers also increased significantly during the course of the overhaul, but its contribution to the observed inflammatory reaction is not yet clear. Further research is needed to refine exposure assessment techniques for metals, PM_{10}, and total particulate matter.

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References

1. Williams, N. 1952. Vanadium poisoning from cleaning oil-fired burners. *Br. J. Ind. Med*. 9:50–55.
2. Hauser, R., S. Elreedy, J. Hoppin, and D. C. Christiani. 1995. Airway obstruction in boilermakers exposed to fuel-oil ash. *Am. J. Respir. Crit. Care Med*. 152:1478–1484.
3. Lees, R. E. M. 1980. Changes in lung function after exposure to vanadium compounds in fuel-oil ash. *Br. J. Ind. Med*. 37:253–256.
4. Winder, C. 1993. Toxicity of metals. *In* N. H. Stacey, editor. Occupational Toxicology. Taylor & Francis, London. 165–175.
5. Sjoberg, S. G. 1955. Vanadium bronchitis from cleaning oil-fired boilers. *Arch. Ind. Health* 11:505–512.
6. Zenz, C., and B. A. Berg. 1967. Human responses to controlled vanadium pentoxide exposure. *Arch. Environ. Health* 14:709–712.
7. Kiviluto, M. 1980. Observations on the lungs of vanadium workers. *Br. J. Ind. Med*. 37:363–366.
8. Hauser, R., M. Garcia-Closas, K. T. Kelsey, and D. C. Christiani. 1994. Variability of nasal lavage polymorphonuclear lymphocyte counts in unexposed subjects: its potential utility for epidemiology. *Arch. Environ. Health* 49:267–272.
9. Linder, A., P. Venge, and H. Deuschl. 1987. Eosinophil cationic protein and myeloperoxidase in nasal secretion as markers of inflammation in allergic rhinitis. *Allergy* 42:583–590.
10. Frischer, T. M., J. Kuehr, A. Pullwitt, R. Meinert, J. Forster, M. Studnicka, and H. Koren. 1993. Ambient ozone causes upper airways inflammation in children. *Am. Rev. Respir. Dis*. 148:961–964.
11. Geyh, A. S. 1994. The development of an active personal ozone sampler using a hollow tube diffusion denuder. *In* Proceedings of the EPA/AWMA Conference on Measurement of Toxic Air Pollutants, Raleigh, NC.
12. Houk, R. S. 1994. Elemental and isotopic analysis by inductively coupled plasma mass spectrometry. *Accounts Chem. Res*. 27:333–339.
13. Plantz, M. R., J. S. Fritz, F. G. Smith, and R. S. Houk. 1989. Separation of trace metal complexes for analysis of samples with high salt content by inductively coupled plasma mass spectrometry. *Anal. Chem*. 61: 149–153.
14. Oxis International. 1995. Immunoassay of Human Myeloperoxidase by the MPO-EIA Method. Oxis International S.A.
15. Parslow, T. G. 1994. The phagocytes: neutrophils & macrophages. *In* D. P. Stites, A. I. Terr, and T. G. Parslow, editors. Basic & Clinical Immunology, 8th ed. Appleton & Lange, Norwalk, CT. 9–21.
16. Harada, A., N. Mukaida, and K. Matsushima. 1996. The role of chemokines in ischemia and reperfusion injury. *In* A. E. Koch and R. M. Streiter, editors. Chemokines in Disease. RG Landes, Austin, TX. 179–193.
17. Oppenheim, J. J., F. W. Ruscetti, and C. Faltynek. 1994. Cytokines. *In* D. P. Stites, A. I. Terr, and T. G. Parslow, editors. Basic & Clinical Immunology, 8th ed. Appleton & Lange, Norwalk, CT. 105–123.
18. Fantone, J. C. 1997. Cytokines and neutrophils: neutrophil-derived cytokines and the inflammatory response. *In* D. G. Remick and J. S. Friedland, editors. Cytokines in Health and Disease, 2nd ed. Marcel-Dekker, New York, NY. 373–380.
19. Broaddus, V. C., A. M. Boylan, J. M. Hoeffel, K. J. Kim, M. Sadick, A. Chuntharapai, and C. A. Hebert. 1994. Neutralization of IL-8 neutrophil influx in a rabbit model of endotoxin-induced pleurisy. *J. Immunol*. 152:2960–2967.
20. Kunkel, S. L., N. W. Lukacs, S. W. Chensue, and R. M. Streiter. 1997. Chemokines and the inflammatory response. *In* D. G. Remick and J. S. Friedland, editors. Cytokines in Health and Disease, 2nd ed. Marcel Dekker, New York. 121–131.
21. Chollet-Martin, S., P. Montravers, C. Gibert, C. Elbim, J. M. Desmonts, J. Y. Fagon, and M. A. Gougerot-Pocidalo. 1994. Relationships between polymorphonuclear neutrophils and cytokines in patients with adult respiratory distress syndrome. *In* M. Chignard, M. Pretolani, P. Renesto, and B. B. Vargaftig, editors. Cells and Cytokines in Lung Inflammation. *Ann. N.Y. Acad. Sci*. 725:354–366.
22. Ahman, M., M. Holmstrom, and H. Ingelman-Sundberg. 1995. Inflammatory markers in nasal lavage fluid from industrial arts teachers. *Am. J. Ind. Med*. 28:541–550.