Lung function, inflammatory markers, and occupational exposure in cement production workers

A thesis by
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Oslo 2010

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo
No. 1164

ISBN 978-82-8264-156-2

Cover: Inger Sandved Anfinsen.
Printed in Norway: AIT Oslo AS.

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Table of contents

PREFACE

1.1. ACKNOWLEDGEMENTS

First, I would like to thank the employees of the cement production plants, who by their participation made this study possible.

I am deeply grateful to my main supervisor professor Johny Kongerud who contributed with his extensive knowledge, excellent guidance, constructive criticism, patience and support throughout the years of my work.

I would also like to warmly thank my supervisors Marit Skogstad MD, Phd and Karl-Christian Nordby MD, Phd, for collaboration, for sharing their knowledge, for their kind support, and friendship. Thanks also to Helge Kjuus MD, Phd for supervision during the first year.

My sincere thanks to Professor Wijnand Eduard, and Martin V. Svendsen, MSc, for kind support and patient explanation of epidemiological and statistical issues.

My special thanks to co-author Liv Ingunn B. Sikkeland, MSc, Phd for collaboration, constructive criticism and advice.

Thanks to occupational hygienists Hilde Notø MSc and Harald Evenseth MSc for collection and analysis of the exposure measurements as well as for friendship and support.

My warm thanks to my colleges at the department of occupational and environmental medicine, Telemark Hospital for support and encouragement, especially to Trude Fossum MD for ensuring enough time to perform the research activities during my Phd-period.

I would especially also like to thank the late Knut Erik Andersen MD, for his enthusiasm, encouraging spirit and kind support in the early phases of this study.

Thanks to co-writers and fellow researchers not yet mentioned professor Petter Kristensen, Thore Egeland MSc, Phd, Reidun Øvstebø Phd and Anne Marie Siebke Trøseid MSc.

My sincere thanks to Thomas R. Thomassen MD who as a company physician at Norcem Inc, initiated this work. I am grateful to the company nurses Ellen H. Irgens and Ellen V. Pedersen at Norcem Inc who made the necessary arrangements at the plants, for us to carry out the study.

The study was supported by grants from the South-Eastern Norway Regional Health Authority, from the Confederation of Norwegian Enterprises (CNE) Working Environmental Found and from the European cement industry; CEMBUREAU.

I wish also to thank my dear father and sister and my beloved late mother for always believing in me, and for their never failing encouragement and love.

Finally, I want to thank my husband and best friend Jörg, and our children Tobias, Sebastian and Julia for always standing behind me and for their encouragement, patience and love.

1.2. SUMMARY

Background

The evidence of an association between exposure to cement dust during cement production and airway effects has been contradictory. Limited data on exposure and the presence of selection bias in many studies have made dose–response evaluations difficult. According to a systematic literature search (PubMed, EMBASE, ISI Web of Knowledge), no previous studies have reported the use of biological materials (e.g., biopsies, bronchial lavage, sputum) to detect possible airway inflammation among cement production workers, or have addressed the effects of exposure to cement dust on their gas diffusion capacities or fractional exhaled nitric oxide levels. Information on the possible systemic effects of exposure in this industry is also scarce.

Aims

The overall aims of the study were: i) to explore the association between occupational exposure to dust and the development of airway symptoms and changes in lung function in cement production workers; and ii) to study the signs of early airway and systemic inflammation, and their possible association with present exposure to cement production dust.

Material and methods

Four different designs were applied.

(I) A retrospective cohort study. All former and present employees at a cement plant in southern Norway, born between 1918 and 1938, who were still alive and who could attend assessment sessions or receive home visits in 1989 and 1999, were included. A system for weighting their previous exposure was used, based on interviews with a group consisting of 18 long-term workers (focus group). One hundred nineteen workers and 50 controls underwent spirometric testing and completed a questionnaire on their respiratory symptoms.

(II) An induced sputum study. Thirty-five healthy dust-exposed nonsmoking workers were assessed with induced sputum and spirometry tests, and completed a self-reported questionnaire on their respiratory symptoms after a period of exposure and again after five days without work or exposure. An internal control group consisting of nonexposed workers

and an external control group were established, consisting of 15 and 29 workers, respectively. The inflammatory cell counts and marker levels of all subjects were assessed.

(III) A cross-shift study. Ninety-five workers employed in the two existing cement plants in Norway were assessed with spirometry, gas diffusion, FeNO measurements, and blood sampling at baseline ("preshift"), after the shift ("postshift"), and again 32 h after the baseline measurements. Inflammatory markers and markers of coagulation were measured in their blood samples.

(IV) A multinational prospective study. The first cross-sectional inclusion study, comprising 4,265 workers from 24 plants in eight European countries, was conducted in 2007. The workers underwent spirometry assessment and filled in self-reported questionnaires on their respiratory symptoms and exposure to cement dust. Personal exposure measurements were made on the day of the health examinations. The levels of forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), and FEV_1/FVC (FEV_1%) were recorded, the prevalence of symptoms and airway limitation (defined as $FEV_1/FVC < 0.7$) were calculated, and their associations with exposure were analyzed.

Main results

In the retrospective cohort study, the prevalence of symptoms and the mean pulmonary function indices were similar in the exposed workers and the controls. The prevalence of chronic obstructive airway disease (COPD) in the two groups was similar: 14.3% in the exposed group and 14.0% in the controls. The high FVC levels among the exposed workers indicated the presence of healthy-worker effects. There was a slight tendency toward lower FEV_1% in the most exposed group, with a regression coefficient β of –0.03 and a 95% confidence interval (CI) of –0.07–0.01.

In the induced sputum study, a significantly higher percentage of neutrophils was observed in samples from the cement production workers collected during the exposure period compared with those of the nonexposed workers and those from the external reference group. This elevated percentage of neutrophils corresponded to an increased level of interleukin 1β (IL1β) in their sputum. No associations between the exposure measurements and inflammatory cells or markers were detected.

In the cross-shift study, reductions in the forced expiratory volume in 1 s (FEV_1), forced expiratory flow ($FEF_{25–75\%}$), diffusion capacity (DL_{CO}), and FeNO levels, corresponding to increased numbers of leucocytes, elevated levels of fibrinogen, and tumor necrosis factor α (TNFα), and reduced levels of IL10, were observed. No associations between the exposure measurements and outcome variables were detected, but the baseline level of fibrinogen was associated with the highest respirable aerosol level (> 0.4 mg/m^3) and was elevated by 0.39 g/L (95% CI, 0.06–0.72).

The cross-sectional analysis of the prospective cohort showed elevated odds ratios for symptoms and airflow limitation (with a range of 1.2–2.6 in the highest quartile), when the lowest quartile of exposure was used as the reference, except for chronic bronchitis. FEV_1 showed an exposure–response relationship, with a 250 mL lower levels of FEV_1 (95% CI, 190–300 mL) estimated for workers with the highest exposure levels compared with those workers with the lowest exposure levels. The association between FEV_1 and exposure was stronger than its association with FVC, when both job types and job exposure matrix (JEM) values were used.

Conclusions

We observed changes in the prevalence of respiratory symptoms, in dynamic lung volumes, and in the occurrence of possible early signs of airway and systemic inflammation among cement production workers compared with those in periods of nonexposure or those in the controls. In a multinational study that included 4,265 workers, associations were demonstrated between airway symptoms, reduced lung function, and exposure to dust in cement production plants. Because any possible selection bias would tend to weaken the association between exposure and health outcomes, I do not think that our findings are overestimated. However, the limitations of the studies are recognized, and to my opinion a prospective study is required to test the study hypotheses further.

1.3. SELECTED ABBREVIATIONS

AM	Arithmetic Mean
ATS	American Thoracic Society
BMRC	British Medical Research Council
CI	Confidence Interval
COPD	Chronic Obstructive Lung Disease
CRP	C-Reactive Protein
DL_{CO}	Diffusion Lung capacity for Carbon Monoxide
ERG	External Reference Group
ERS	European Respiratory Society
$FEF_{25-75\%}$	Forced Expiratory Flow between 25 and 75% of FVC
FeNO	Fractional Exhaled Nitric Oxide
FEV_1	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity
GM	Geometric Mean
GSD	Geometric Standard Deviation
GOLD	Global Initiative for Chronic Obstructive Lung Disease
IL	Interleukin
IUATLD	International Union against Tuberculosis and Lung Diseases
JEM	Job Exposure Matrix
OEL	Occupational Exposure Limit
OR	Odds Ratio
PEF	Peak Expiratory Flow
ROS	Reactive Oxygen Species
SD	Standard Deviation
SEM	Standard Error of the Mean
SMR	Standard Mortality Rates
TAC	Total Antioxidant Capacity
TNF-α	Tumor Necrosis Factor alpha
TTM	Total Thiol Molecules

1.4. LIST OF PAPERS

I. Fell AK, Thomassen TR, Kristensen P, Egeland T, Kongerud J. Respiratory symptoms and ventilatory function in workers exposed to Portland cement dust. J Occup Environ Med 2003;45:1008–14.

II. Fell AK, Sikkeland LI, Svendsen MV, Kongerud J. Airway inflammation in cement production workers. Occup Environ Med 2010;67:395-400 Epub 2009 Oct 22.

III. Fell AK, Notø H, Skogstad S, Nordby KC, Eduard W, Svendsen MV, Øvstebø R, Trøseid AM, Kongerud J. A cross-shift study of lung function, exhaled nitric oxide, and inflammatory markers in blood in Norwegian cement production workers. Submitted 2010.

IV. Nordby KC, Fell AK, Notø H, Skogstad M, Thomassen Y, Kongerud J. Kjuus H. Exposure to thoracic cement dust, airway symptoms and lung function in cement production workers. Submitted 2010.

2. INTRODUCTION

'There is a powder that by its own natures works in mysterious and wonderful ways. It is found in the areas around Vesuvius. If this powder is mixed with lime and ground stone, the result is not only a construction of great strength, but the masonry standing in the sea will stand against both storms and waves.' Vitruvius Pollio.

The powder described by the famous Roman architect Vitruvius Pollio in his 'De Architura Libri X', as early as 25 years BC, was cement. Pollio had identified the reasons for the success of cement as the world's most widely used building material: its great strength and water resistance.

The first known reports of the health effects associated with exposure to cement described dermatitis among bricklayers, published in Bernardino Ramazzini's book 'De Morbis Artificum Diatriba' in 1700. Evidence for an association between the chromate sensitivity induced by cement exposure and dermatitis was reported by Jaeger and Pelloni in 1950 (1). Since then, a considerable number of studies have reported an increased prevalence of respiratory symptoms, impaired lung function, chronic bronchitis, emphysema, asthma, and radiographic abnormalities (2–15), as well as cancer of the larynx and ventricle in cement production workers. However, many of these studies are hampered by limitations such as selection bias, lack of adjustment for possible confounding variables, and scarce information on exposure, so the evaluation of dose–response relationships is difficult (16).

The content of chromates and silicates in cement, and its alkalinity when it comes into contact with moist mucus membranes (pH ~12), have been suggested as possible inducers of airway inflammation, but little is known about the mechanisms underlying the reported airway effects. A review of the literature on the association between aerosol exposure during cement production and its health effects, conducted in 2005, concluded that regardless of the contradictory evidence for impaired lung function in cement-aerosol-exposed populations, there is reason to believe that dust exposure during cement production is associated with declining lung function, and that a dose–response relationship probably exists (17). However, the matter has been the subject of controversy and further research is required.

Norwegian legislation requires that workers be monitored when exposed to potentially harmful agents. The Norwegian cement industry initiated the present study, to examine the possible association between exposure to cement dust and the effects on the airways of their workers, to comply with legislative requirements. The European association of cement producers (CEMBUREAU) has initiated a longitudinal study among their workers exposed to cement aerosols, to be completed in 2012, and several subset studies, of which this work is part.

The overall aims of these studies were: i) to explore the association between occupational exposure to cement dust and the development of airway symptoms and obstructive airway disease in cement production workers; and ii) to study the signs of early airway and systemic inflammation in cement production workers and their possible associations with current exposure.

2.1. Health effects

The occupational contribution to the burden of airway disease in the European population is estimated to be at least 15%, and obstructive airway diseases are the most prevalent category of occupational respiratory disorders (18;19). Respiratory diseases rank as the third most prevalent occupational disease category (after ergonomic and stress-related diseases), according to a survey of occupational diseases in the European Union (20). In Europe, 52,700 work-related deaths from respiratory disease (chronic obstructive airway disease [COPD], 39,300; pneumoconioses, 7,200; asthma, 6,200) were estimated in the year 2000 (21). Because of shortcomings in the data, these are probably underestimations of the true numbers of deaths. In Norway, respiratory disorders are the third most important cause of sick leave and exclusion from the workplace (22).

2.1.1. Airway inflammation

Inflammation is a non-specific immune response typically initiated by tissue damage from endogenous factors or exogenous factors such as exposure to dust in the workplace. The complex process of airway inflammation is still only partly understood, and it is unclear when an acute inflammatory response becomes a chronic state or which factors induce the development of respiratory disease. However, it is known that persistent inflammation may lead to the remodeling of the airways and the development of COPD, as is seen in asthma patients (23). It has also been established that occupational exposure to particles and fumes

may cause asthma as well as COPD, even in nonsmokers (24). Furthermore, it has been demonstrated that exposure to occupational airborne particulates can trigger inflammatory changes in the airway, which may occur before the onset of clinical symptoms (25). Therefore, the investigation of early inflammatory changes in healthy, occupationally exposed workers is important and may extend our knowledge of the mechanisms underlying the development of respiratory disease.

The inflammatory cells that infiltrate the airway in response to exposure to particles, gases, or fumes are dominated by neutrophils and lymphocytes, and are orchestrated by a variety of markers (signal molecules) that coordinate the inflammatory responses (25;26). Tumor necrosis factor α (TNFα) and interleukin 1β (IL1β) are known to be early players in the inflammatory process (25;27;28). IL1β is a proinflammatory cytokine that upregulates the adhesion molecules on endothelial cells and induces cytokine production by many cells, stimulates hematopoesis, and, together with TNFα and IL6, is responsible for the activation of the acute phase response. The plasma concentration of TNFα increases markedly 30–45 min after an inflammatory stimulus in healthy subjects and reaches its maximum after 60–90 min, whereas the increase in the level of IL6 occurs 15 min after the increase in TNFα, with maximum values at 120 min (27). IL1β has been difficult to detect in plasma, so the response pattern of this cytokine in plasma has not been described well hitherto (27;29). This response then generates increased levels of C-reactive protein (CRP), fibrinogen, and other coagulation factors (25;28). IL8 is produced by a number of cell populations and is a potent chemoattractant of neutrophil cells (30). In contrast, IL10, together with IL4 and IL13, is involved in the downregulation of inflammation through the inhibition of proinflammatory cytokines and is inhibited in subjects exposed to cigarette smoke (31;32).

2.1.2. Systemic inflammation

Several studies have shown that a systemic inflammatory response is detectable in subjects with airway inflammation (25;28;33) and that the exposure of humans to particle inhalation is associated with a systemic inflammatory response (34–36). Therefore, the measurement of inflammatory markers in the blood of workers exposed to airborne particles and gases is relevant.

In addition to the link between airborne particle exposure and systemic inflammation, an association between such exposure and vascular effects has been demonstrated in both

humans and animals (35–37). It has been hypothesized that the deposition of particles in the lungs leads to low-level alveolar inflammation, which may exacerbate COPD and asthma and also increase blood coagulation activity, resulting in possible cardiovascular death in susceptible individuals (35;37). Therefore, the identification of early inflammatory changes among exposed workers might be important in screening for, and preventing, both respiratory and cardiovascular diseases.

2.1.3. Detection of airway and systemic inflammation

Traditionally, respiratory symptoms and spirometric changes have been used to detect the effects on the airways of exposure to cement dust. Because changes in these measures only occur when inflammation has already caused structural changes in the lungs, as seen in COPD, methods that can detect earlier changes have been sought. Bronchial lavage and biopsies were initially used, but they are invasive methods, unsuitable for large samples or fieldwork. Consequently, the development of the induced sputum technique to obtain samples for the investigation of airway inflammation in occupationally exposed workers has been welcomed (38). The methods used for the measurement of inflammatory cells and levels of inflammatory markers in the blood have also developed rapidly over recent decades and are now increasingly used to study systemic responses to inhalable substances in occupational settings.

2.1.4. Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is defined by the Global Initiative for Chronic Obstructive Lung Disease as follows: COPD is a preventable and treatable disease, with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation in COPD is associated with an abnormal inflammatory response to noxious particles or gasses (39) and may also be induced by such exposure in the occupational setting (34;40;41). Reduced lung function and changes in the levels of inflammatory cells and markers may be detected in sputum and serum samples from these patients (25;26;33).

2.2. Previous studies of airway inflammation or obstructive airway disease in cement production workers

2.2.1. Cross-sectional studies

A number of cross-sectional studies have demonstrated an increased prevalence of respiratory symptoms and/or a reduction in lung function measures in cement production workers compared with those of controls (Table 1). However, because contradictory studies have also been reported (2;11), these findings are controversial.

In 1960, Jenny et al. reported that 41% of workers from three Swiss cement production plants complained of chronic cough, and that doctor-diagnosed asthma or chronic bronchitis was detected in 7% of these workers (42). In subsequent years, a variable prevalence of bronchitis, asthma, or emphysema (ranging from 5.7% to 11.2%) was reported in various studies of workers in the cement production industry (16). However, these early studies did not include lung function measurements or control groups, and consequently, no firm conclusions about the role of occupational exposure to cement dust could be drawn.

In 1973, Kalacic (8;9) reported a significantly higher prevalence of respiratory symptoms, chronic bronchitis, and airflow obstruction in Yugoslavian cement workers than in controls. However, because there was no standardization for age, age could possibly have explained the observed relationship. In subsequent years, several studies have demonstrated an impairment of lung function in cement production workers (5;10;15;43-45). In some of these studies, no control workers were examined or the authors failed to consider smoking as a possible confounding variable, or both (Table 1). In other studies, adjustments for age and smoking were made, but none of these studies included former workers, and most studies presented no or little information on personal exposure levels. Two cross-sectional studies with relevant reference populations could not demonstrate any differences in the spirometric measurements of workers and controls (2;11). Mean dust levels have been reported in studies from Malaysia (10), Jordan (4), Taiwan (15), Tanzania (43;44), and Iran (45), and these studies have shown levels twofold (or more) higher than the levels reported in cement production plants in the USA (2).

In two recent Tanzanian studies, the prevalence of airway symptoms was higher in exposed workers than in the controls, and lower forced vital capacity (FVC), FEV_1, FEV_1/FVC, and peak expiratory flow (PEF) values were demonstrated among cement production workers

(43;46). Cumulative total dust exposure of more than 300 mg/m^3 years (versus 100 mg/m^3 years) was significantly associated with an increased risk of developing airflow limitation (43). A study undertaken in Saudi Arabia also reported that levels of wheezing and shortness of breath were related to dust exposure (47).

Table 1. Overview of selected cross-sectional studies

Country	Exposed Workers N	Blue-collar worker controls	Adjustments for age and smoking	Outcome variables	Conclusion S/LFT	Reference
Egypt	223	x	x	S*	+***	(7)
Yugoslavia	847			S	+	(8)
Yugoslavia	290			LFT**	+	(9)
Denmark	301	x	x	LFT	─	(11)
Libya	110		x	LFT	+	(13)
USA	2736	x	x	S/LFT	─/─	(2)
Denmark†	546	x	x	H‡	─	(48)
Taiwan	591		x	S/LFT	+/+	(15)
Jordan	348			S/LFT	+/─	(4)
Mexico §	425			S	+	(14)
Malaysia	62		x	S	+	(10)
UAE#	67		x	S/LFT	+/+	(5)
Saudi Arabia	150	x	x	S	+	(47)
Tanzania	126	x	x	LFT	+	(43)
Tanzania	120	x	x	S	+	(46)
Iran	80			LFT	+	(45)

*Symptoms; **lung function test (spirometry); ***+, higher prevalence of symptoms or dynamic lung volumes in cement workers; ─, no increase in symptom prevalence or difference in dynamic lung volume in cement workers.

† Inclusion of former workers.

‡ Hospitalization study.

§ Use of a exposure weighting system.

United Arab Emirates

2.2.2. Cross-shift studies

Three previous studies have reported the acute effects of cement dust exposure on lung function. Ali et al. investigated the changes in pulmonary function during the work shift in workers from three Saudi Arabian Portland cement factories (6). The mean reductions in FEV_1, the FEV_1/FVC ratio, and the forced expiratory flow $FEF_{25–75\%}$ were significantly greater in the high-exposure workers than in the controls. However, the readings were not adjusted for height, and the groups were unlikely to have been equivalent in terms of socioeconomic status, a possible confounding factor. Two studies have shown a cross-shift reduction in PEF, which was most pronounced among high-exposure workers (44;49). In the latter studies, a high prevalence of respiratory symptoms (stuffy nose 85%, shortness of breath 45%, and sneezing 47%) was reported among workers exposed to high levels of

cement dust during production (the geometric means [GMs] for total dust exposure ranged from 18.5 to 38.6 mg/m^3).

2.2.3. Longitudinal studies

In a longitudinal study that followed 68 cement workers from 1973 to 1984, small, nonsignificant reductions in FVC and FEV_1 were observed for all workers over the follow-up period (50). In two longitudinal studies, cement workers showed an increased rate of bronchitis and also a larger reduction in lung function than expected when compared with the normal population (12;51).

In a study of long-term exposure to cement dust and later hospitalization with respiratory disease, cement workers were compared with other blue-collar workers and with the general population (48). In that study, the cement workers had no increased rate of hospitalization during the 10-year follow-up period compared with those of the controls. However, a tendency toward increasing rates of hospitalization with COPD was observed with increasing duration of exposure to cement dust for up to 30 years. Thereafter, there was a decline in the rate of hospitalization. The authors suggested that this might have been attributable to the healthy-worker effect.

2.2.4. Studies of mortality from respiratory diseases

Few studies have assessed the mortality attributable to respiratory diseases in cement production workers, and even fewer have reported respiratory diseases other than cancer. In a 32-year follow-up of 607 cement workers, 419 of the subjects died, and 27 of these deaths were from bronchitis, emphysema, or asthma (52). The mortality rate from respiratory disease (Standardized Mortality Ratio (SMR): 0.78) did not exceed the national rate in England (52).

No increased risk of overall or respiratory cancer was detected in a Danish cohort sampled in 1973 and containing 546 cement production workers aged 46–69 years, compared with the national rates, when men with documented asbestos exposure were excluded (53). Another study of 2,392 men in Sweden, employed for at least 12 months in two Swedish cement plants, similarly reported no excess of respiratory cancer (54). These findings were confirmed in a recent study reporting a 15-year follow-up of 9,118 French cement production workers (55). In contrast, a study from Lithuania reported an increased risk of lung cancer among cement production workers, but no individual smoking data were

available in that study (56). None of these studies reported respiratory diseases other than cancer.

In addition to mortality studies, several studies have shown that aerosol exposure during cement production and/or handling (construction work) is associated with increased morbidity, involving airway or cardiovascular diseases, among workers in these industries (57-59).

2.2.5. Other studies

The biological effects of cement dust were investigated in three early animal studies (60–62). In the first study, which involved the intraperitoneal administration of cement dust containing 5% free silica to rats, fibrotic and collagenic changes in the lungs were demonstrated (60). In the second study, atrophy of the elastic fibers and focal pulmonary emphysema were observed in the pulmonary tissues of rats exposed to inhalations of cement dust containing up to 30% silica (62). In the third study, cement containing 1.3% free silica was injected into the peritoneum of mice, and initially caused necrotic and exudative changes, and then granulomas were formed around the particles (61).

One recent animal study reported the cytotoxic and proinflammatory effects of cement dust exposure and signs of oxidative stress when the NR8383 rat alveolar macrophage cell line and primary rat alveolar macrophages were tested (63). None of the cement dust samples was found to cause toxicity to the macrophages or notable glutathione depletion compared with the positive control (quartz dust (DQ12)). The cement dust samples also failed to activate the generation of reactive oxygen species by macrophages or the production of the inflammatory cytokines IL1β and macrophage inflammatory protein 2 (MIP2). However, in contrast, most of the cement dust samples activated macrophage TNFα production, which was significantly associated with the CaO content of the dust samples.

In a study of the oxidative stress status of cement plant workers, the total antioxidant capacity (TAC) and total thiol molecules (TTM) in their sera were significantly lower in directly exposed workers compared with those of indirectly exposed workers (64). This finding was interpreted as indicating reduced protection against oxidative stress in these workers, and correlations were demonstrated between serum levels of chromium, TAC, and platelets in the directly and indirectly exposed groups, and between serum levels of chromium and the levels of TTM and platelets.

Hematological changes were studied in a group of 50 healthy cement mill workers aged 20–60 years in Pakistan and were compared with those of matched controls (65). The study demonstrated increased leukocyte counts ([mean ± SEM] 6,587 ± 235/μL versus 7,527 ± 265/μL, respectively; $P < 0.02$) and an increased erythrocyte sedimentation rate (mean 10.3 ±1.21mm/hr versus 15.4 ± 2.30mm/hr; $P<0.05$, respectively) among exposed workers. However, no information on the levels of exposure was included, and the changes were not related to the duration of exposure in the cement mill.

In a study of the respiratory muscles, the dose–response effects of cement dust on the ventilatory muscle functions were examined in 50 nonsmoking cement mill workers, using electromyography with surface electrodes (66). Reductions in the number of peaks ($P < 0.0005$), the maximum peak amplitude ($P < 0.0005$), the peak-to-peak amplitude ($P < 0.0005$), and the duration of the response ($P < 0.0005$) were shown in the cement mill workers compared with those of their matched controls. In a later study, the author reported impaired phagocytic function in the polynuclear neutrophils in the blood of healthy, nonsmoking cement mill workers compared with that in the nonexposed controls (67). However, because no information on the levels of exposure or on the content of free silica in the cement dust was available, these results are difficult to interpret.

In summary, at the time of writing, the evidence for an association between cement dust exposure and the prevalence of respiratory symptoms and lung function impairment is contradictory. Information on exposure is limited, and no studies have reported the use of biological materials (e.g., bronchoalveolar lavage, biopsies, or sputum) from cement-dust-exposed workers to detect signs of possible early inflammation. However, the available data do raise concerns that chronic respiratory deficits may develop with long-term exposure to cement dust.

3. OBJECTIVES

The objective of this study was to examine the association between systemic and respiratory health outcomes (respiratory symptoms, lung function, and inflammatory markers in the sputum and blood) and occupational exposure to dust in cement production workers.

To achieve the overall objective, the following aims were established:

- to examine the effects of exposure to dust on respiratory symptoms and ventilatory function, and on the prevalence of COPD among cement production workers;
- to examine possible differences in levels of inflammatory cells and soluble markers in the induced sputum samples from healthy Norwegian cement production workers between periods of exposure and periods of nonexposure;
- to investigate possible cross-shift changes in lung function variables, exhaled fractional nitric oxide (FeNO), and inflammatory markers in the blood of Norwegian cement production workers, and the possible association between these changes and exposure; and
- to investigate the associations between exposure to dust in the cement production industry and respiratory effects in a multinational, cross-sectional study.

4. THE CEMENT PRODUCTION INDUSTRY

4.1. The history of cement production

The first known constructions made with concrete date from 5,000 years BC. Earlier still, the Assyrians and Babylonians had used a binding agent consisting of bitumen and clay when building stone constructions. The Egyptians used a mixture of lime, water, and burned gypsum to build the pyramids. However, it was not until Roman engineers developed pozzolan cement that the term “cement” was introduced (68).

With the fall of the Western Roman Empire in 474 AD, the knowledge of cement disappeared, and it was not rediscovered until around 1750, when the French, English, and Germans started to use a mixture of burned clay and lime sand, which they called “Roman cement”. However, the quality of this mixture was inadequate and it was only produced on a small scale. At that time, the English engineer John Smeaton experimented with different methods and succeeded in developing hydraulic cement, using a mixture of burned limestone containing a considerable proportion of clay.

Finally, in 1824, Joseph Aspdin, a mason and bricklayer from Leeds, England, took out a patent for cement, which he named ‘Portland cement’, because it resembled the stone quarried on the Isle of Portland (69;70). Adjustments have since been made to the production process of Portland cement, to meet particular specifications related to its strength and resistance to corrosive conditions and chemicals, and to increase the capacity and cost effectiveness of production facilities.

4.2. The process of cement production

Portland cement, which is the most commonly used cement worldwide, is a mixture of calcium oxide (60%–67%), silicon dioxide (17%–25%), aluminum trioxide (3%–8%), and ferric oxide (0%–5%), and it also contains low levels of chromium (71). The main raw material of Portland cement is limestone, which is usually mined on the production site. The first step in the modern manufacturing process is crushing and grinding the limestone, together with quartz (or another source of silica) and iron ore, as a wet slurry or in a dry state. This mixture is burned in a tilted, rotating kiln.

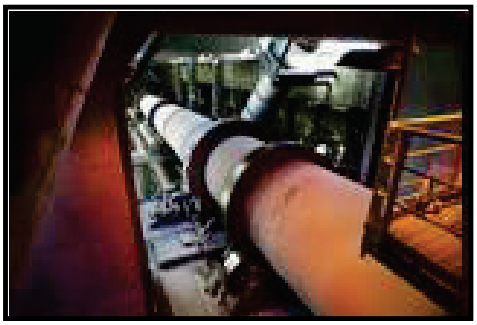

Photo of rotating kiln (Kiln VI, Brevik plant).

Burning fuel, consisting of coal, natural gas, oil, and/or alternative fuels (waste), is fed into the kiln. Inside the kiln, where the temperature rises to approximately 1450 °C, a series of chemical reactions causes the materials to fuse (sintering) and form grey marble-sized pellets called “cement clinker”. The clinker is mixed with gypsum (retarder) and other additives, and ground to a very fine particulate powder to yield cement.

The basic chemical reactions of the process are the evaporation of all moisture, the calcinations of the limestone to produce free calcium oxide, and reactions between the calcium oxide and sand, clay, and iron.

Different types of cement can be produced with different proportions of the raw materials or with additives, fillers, or puzzolana materials (material of volcanic origin), depending on the desired properties of the concrete.

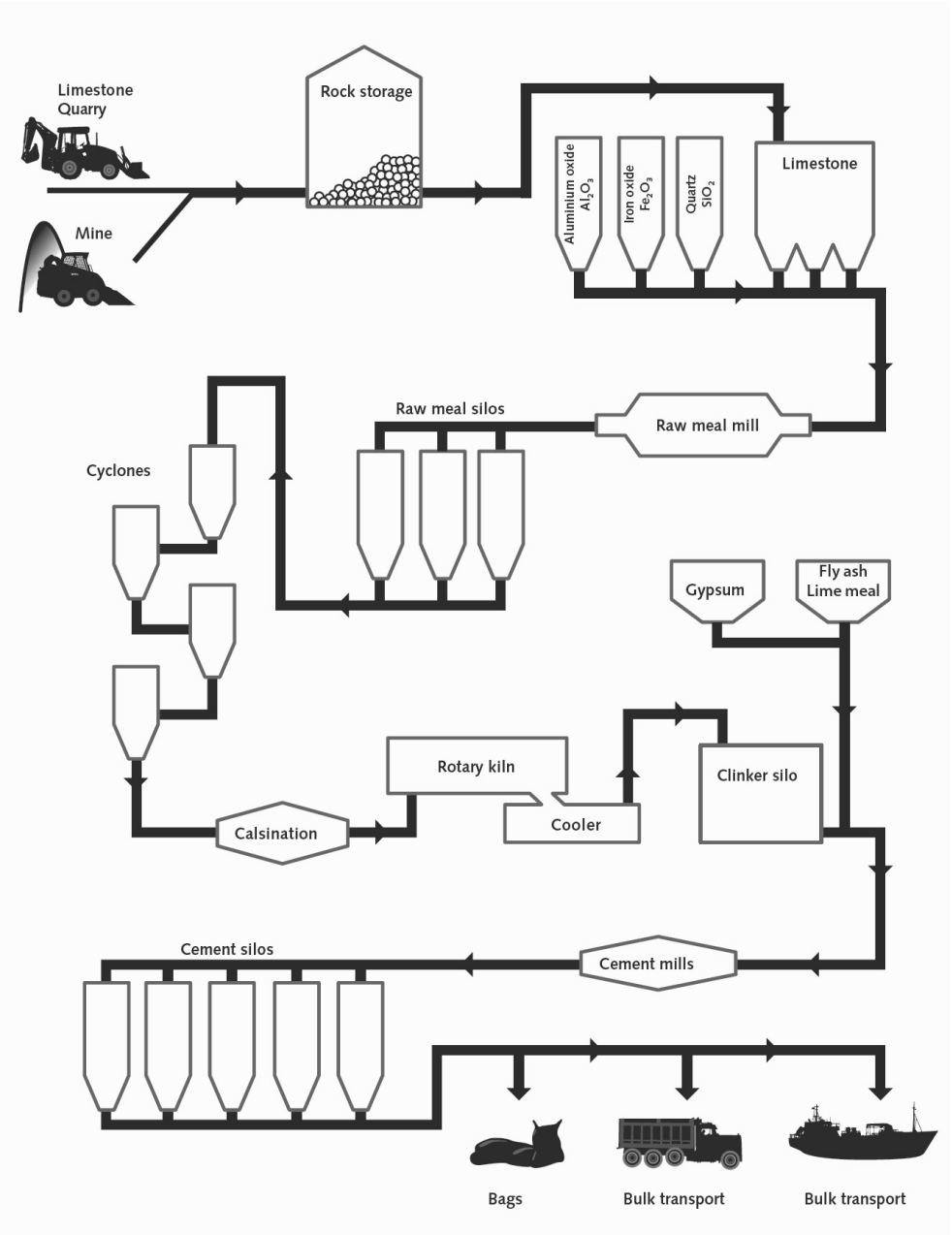

Fig. 1. Illustration of the cement production process.

4.3. Production processes in Norway

The Brevik plant in southern Norway began production in 1919, and the Kjøpsvik plant in northern Norway has been producing cement since 1920. The Kjøpsvik plant used the wet process of cement production until 1992, when it changed to the dry process, whereas Brevik has used the dry process from its initial production in 1920. Therefore, exposure to the wet process is still relevant in the analysis of chronic effects. The production facilities in the two plants have been similar since 1992, and therefore the exposure experienced in both plants is comparable in terms of evaluating its acute effects. The Brevik plant produces 1.2 million tons of cement per year and employs 180 workers (production and administration), whereas the smaller Kjøpsvik plant produces 0.5 million tons and has a total of 150 employees.

Photo of the Kjøpsvik plant.

Photo of the Brevik plant.

4.4. Dust generated during cement production

Cement processing generates dust during quarrying and the preparation of the raw materials, from the additives used to supplement the raw materials, during the burning of fuels and the calcination and grinding of the clinker, and when the finished cement is blended, packed, and shipped. Cement production workers are exposed to aerosols with a wide particle-size distribution, including particles as small as 0.05 μm in aerodynamic diameter (dae). Exposure varies across locations and work tasks, and with the additives and alternative fuels used in the kilns.

Portland cement dust is a mineral dust with a respirable fraction of approximately 50% of the total dust (71). The deposition of the particles in the respiratory tract depends on both the physical and chemical properties of the dust. Physical properties, such as the particle size, surface area, and strong alkalinity of cement dust (pH ~12) in contact with water are important, as are the chemical components known to be sensitizing or irritating, such as chromium and silica. The possibility of overloading the clearance mechanisms in the alveolar regions of the lung, which is suggested to have pulmonary effects even for low-toxicity dusts, must also be considered (72;73).

Small amounts of hexavalent chromium (Cr[VI]) are present in cement (soluble chromium, range 0.036–0.225 mg/kg; aggregate chromium, range < 0.002–0.083 mg/kg) (74). Historical measurements at a Swedish plant in 1980 showed levels of chromium of 49–389 mg/kg (median, 58 mg/kg) in the production departments and 40 mg/kg in the finished cement (54). The sources of chromium are the raw materials, but it also derives from chromium steel grinders and refractory bricks in the kiln. To reduce skin sensitization, ferrous sulfate, which transforms Cr(VI) to Cr(III), is added to the cement.

The raw materials entering the cement production process also contain various concentrations of quartz (free crystalline silica). However, the finished cement usually contains undetectable or low levels of quartz because it is converted into amorphous silicate during heating. Data on the free silica content of cement production dust are scarce, although levels varied between 1% and 30% in early studies (60–62). Our measurements of quartz (study I) show that the levels were below the Norwegian occupational exposure limit (OEL; respirable dust 0.1 mg/m^3).

Few previous studies of the health effects of dust inhalation in cement production workers have provided sufficient exposure information to allow comparisons to be made with other studies or dose–response evaluations. The assessment of exposure is crucial in epidemiological studies, to allow the evaluation of the association between the health risk and the degree of exposure, and the implementation of exposure reduction measures. Individual exposure measurements also better reduce the probability of misclassifying the workers than does the alternative use of tenure or job categories only. Specific exposure information, such as lifetime exposure and the flexibility of tasks within jobs, may also be important (75).

Another problem that arises when the health effects of cement dust are considered is the lack of historical quantitative exposure measurements of airborne dust in a cement plant. Alvear-Galindo et al. developed a method for estimating particle exposure based on interviews with a group of former workers and showed that dust exposure is likely to vary among different groups of workers in the cement industry (14). This finding was supported by Mwaiselage et al., who showed considerable variability in the levels of total personal dust exposure between groups of cement production workers; the highest level of exposure occurred in crane workers: GM ± geometric standard deviation (GSD) = 38.64 ± 2.51 mg/m^3; and the lowest levels occurred in maintenance workers: 1.16 ± 3.10 mg/m^3 (76). Peters et al. (2008) also demonstrated in a study of construction workers that high concentrations of dust (inhalable dust GM = 55 mg/m^3) and cement dust (inhalable cement dust GM = 33 mg/m^3) can occur, especially during cleaning tasks (77). In that study, the variability within the job groups and the temporal variability in the exposure concentrations generally outweighed the differences in the average concentrations between workers. "Using a broom", "outdoor wind speed", and "presence of rain" were the most influential factors affecting exposure to inhalable cement dust.

5. MATERIALS AND METHODS

5.1. Study population and design

The two first studies (papers I and II) were undertaken at the largest cement production plant in Norway, located in the southern part of Norway. Paper III also includes subjects from this plant, together with workers from the second Norwegian plant, located in northern Norway. The fourth study (paper IV) is the baseline registration of a multinational four-year follow-up study conducted at 24 plants in eight different countries. In all four studies, never-smokers and ex-smokers who had stopped smoking at least three years before their examination were categorized as nonsmokers. In the fourth study, a category for those with uncertain smoking histories was used, because the smoking status of some participants was unclear. In study I, the life dose of tobacco was estimated as grams of tobacco smoked per day (one cigarette = 1 g of tobacco) multiplied by the length of time the subject had been smoking (rounded to the nearest month).

In the first study, all men born between January 1, 1918 and December 31, 1938, and with one year or more of employment in this particular cement plant, were selected from the employee list. This sample comprised 226 men. All office workers were excluded. We were able to identify the workers who had died before January 1, 1998, from the Norwegian Cause of Death Registry. We made home visits to the former workers who were unable to come to an interview or a testing session. Three patients with pulmonary cancer were excluded because of their poor general clinical condition. Three spray painters and one full-time welder were also excluded from the exposed group because their occupational exposure to other aerosols was likely to have had a significant effect on their respiratory systems.

The control plant produced ammonia and was located 10 km from the cement plant. All men born between January 1, 1918 and December 31, 1938, and who had been employed at the control plant for at least one year, were selected. Ninety individuals were identified from the employee register. We also visited former workers among the controls who were unable to come to an interview or a testing session. No subject in the control group was excluded for poor health or senile dementia, or for a history of full-time welding or spray painting.

At the time of our second study (2007), the plant in southern Norway employed 78 production workers (from the furnace and maintenance departments) and 23 office workers.

Only male workers were employed in these departments at the time of recruitment. All nonsmoking, dust-exposed workers from the group of production workers were invited to participate. In total, 45 exposed, nonsmoking men were eligible. Four cement workers were excluded because they resigned from their jobs during the study period (change of employer), two workers were excluded because they were unable to produce a sufficient quantity of sputum, and four workers did not wish to participate, so 35 workers were ultimately included in the analyses. Of the workers who did not participate in the study, none reported respiratory disease, but one worker who left the company during the study period reported occasional wheezing and coughing during the night.

The male office workers, who spent less than 10% of their working time in areas with dust exposure, were invited to participate as an internal reference group (office workers). All 15 nonsmoking, healthy, male office workers were included. The external reference group comprised 39 nonsmoking, nonexposed healthy students and hospital workers from a regional hospital.

For the third study, eligible workers were identified from the company's register, and 144 workers (5% female) from the production and maintenance departments were invited to participate. Ninety-five subjects (7% female) were included in the study, and 29 workers did not wish to participate. Among the workers who did not wish to participate in the study, two had a known diagnosis of COPD and one of asthma. In the same group, eight workers had administrative jobs and were expected to have had very low exposure or none at all. The nonparticipating smokers tended to be more heavily exposed to tobacco smoke than those included in the study.

In the multinational study (IV), all workers employed in the administration or production departments of 24 cement plants in Turkey, Greece, Italy, Spain, Switzerland, Lithuania, Sweden, and Norway were invited to participate. Workers employed in the quarries and those employed in external companies providing outsourced services to the cement plants were excluded, because of their predicted higher exposure to crystalline silica in the former workers and the problem of tracking the latter individuals throughout the study. Two hundred ninety-two participants (7%) did not meet the American Thoracic Society (ATS)/European Respiratory Society (ERS) criteria for valid spirometry tests (78) of FEV_1. For FVC, we only considered measurements to be valid that met both the repeatability and end-of-test (EOT) criteria. From a total of 4,265 (100%) spirometry tests, we obtained 3,332

(78%), 3,966 (93%), and 3,206 (75%) valid tests for FVC, FEV_1, and FEV_1/FVC, respectively. Unfortunately, no information regarding the nonparticipants was available.

5.2. Study variables

5.2.1. Questionnaires (studies I, II, III, and IV)

Questionnaires and interviews were used to collect information on the possible effects of exposure to cement dust, the covariates involved, and the exposure of the subjects. The use of reliable and validated questionnaires in epidemiology is considered to be essential in determining the risk factors for disease, and to reduce the probability of information bias. The reliability of a questionnaire can be tested by the administration of the same questionnaire two or more times to the same individuals, as has been shown for the British Medical Research Council (BMRC) questionnaire by Kongerud et al. (1989) (79). A valid questionnaire must have sufficient specificity and sensitivity. Sensitivity can be described as the fraction of truly diseased subjects found to be diseased when the questionnaire is used, and specificity can be defined as the fraction of truly healthy subjects found to be healthy. Validity can be tested by comparing the findings of a clinical physiological investigation with those of the questionnaire in question. Comparisons with the histamine bronchial challenge test have been made to test the validity of the International Union against Tuberculosis and Lung Disease (IUATLD) questionnaire (80–83), which we used in studies II and III.

In studies I and II, we chose to use a Norwegian modification of the BMRC questionnaire for respiratory symptoms (84). This questionnaire was developed in 1960 for the identification of chronic bronchitis. It was expanded in 1966 and 1986 to include questions dealing with asthma and asthma-like symptoms. This choice seemed justified because the primary focus of study I was the identification of chronic respiratory symptoms and disease in a cohort of workers, most of whom were no longer exposed. In retrospect, this questionnaire may have been less suited to identifying acute respiratory symptoms or asthma in study II, in which the cohort consisted of young subjects with ongoing exposure.

The questionnaire developed in 1984 by the IUATLD and extended in 1986 with a question regarding doctor-diagnosed asthma, to determine the most effective combination of symptom-based items for the valid identification of asthma (83), was used in studies III and IV. To adapt the questionnaires to the different countries evaluated in study IV, all the

questionnaires were translated into the nine languages spoken in the different cement plants. The translations were validated by independent translators, who checked all the questions using the English version as the gold standard (no workers answered the questions in English).

In study IV, all participants also completed a questionnaire on their historical exposure, focusing on the workers' occupational histories (lifetime exposure) and the time spent in different jobs and tasks during the two-year period before spirometry testing. The workers were divided into seven job-type categories: administration, production, plant cleaning, maintenance, foreman, laboratory, and other/unspecified, based on information given in this questionnaire. The participants who indicated one job-type category only were allocated to one of the seven categories, whereas those indicating more than one job type were allocated to an eighth category for workers involved in several job types. A questionnaire based on the same questions as the historical exposure questionnaire was developed and completed on the day of the whole-shift sampling, to obtain information concerning the work situations on the day of sampling.

5.2.2. Lung function testing (studies I, II, III, and IV)

In study I, spirometry was performed between 0830 and 1430, and consisted of at least three forced expirations that met the ATS guidelines (85), using a Vitalograph S spirometer (Vitalograph Ltd, Buckingham, England). The spirometry measurements were made with the subject sitting without a nose clip. The temperature was measured and the spirometer was calibrated using a 1 L syringe each day before the first spirometry session, and again before each examination when home visits were made. The subjects were asked to exhale until completely empty. Prediction equations from a Norwegian population were used (86). Airflow limitation was defined as $FEV_1/FVC < 0.7$ and $FEV_1 < 80\%$ predicted in study IV. COPD was diagnosed in subjects with a history of chronic cough, phlegm when coughing, breathlessness, and/or wheezing in addition to a FEV_1/FVC value of < 0.7. No reversibility testing was performed.

The lung function tests in study II were performed under the same conditions as described for study I. For shift workers, spirometry measurements were made during their work shift. In these cases, the second measurements were made at the same time of the day as the first, to avoid any influence of diurnal variation (87).

The lung function testing in studies III and IV was performed in accordance with the ATS/ERS guidelines (78). In study III, the Jaeger MasterScreen PFT (Erich Jaeger GmbH & Co. KG, Würzburg, Germany) was used, which has a program that measures the validity of the tests. Reversibility testing was not considered feasible and was therefore not performed. In study IV, all charts from the Vitalograph 2160 spirometers (Vitalograph, Buckingham, England) were read manually to ensure that only valid measurements were included in the study. To meet the repeatability and EOT criteria, differences of 150 mL between the best and second-best tests of FVC and FEV_1 and an increase in volume of 100 mL during the last 2 s of the FVC maneuver were chosen, respectively. The spirometry tests were considered to be valid when these criteria were met. We calculated FEV_1/FVC and the percentage of the predicted values for FVC (FVC% predicted) and FEV_1 (FEV_1% predicted), using published reference values for Europeans (88). Reversibility testing was not feasible in study IV. Airflow limitation was defined as $FEV_1/FVC < 0.7$.

5.2.3. Sputum induction

Sputum was induced and processed as described by Sikkeland et al. (89), using an ultrasonic nebulizer (DeVilbis 2000, DeVilbis Co., Somerset, PA, USA) with an output of 1.5 mL/min. Aerosols of hypertonic saline solutions at concentrations of 3%, 4%, and 5% were inhaled, each for 7 min, unless FEV_1 declined by more than 10% between inhalations. In subjects who exhibited a decline in FEV_1 of more than 10%, the saline concentration was not increased further for subsequent inhalations. If FEV_1 declined by more than 20% from the baseline value, the procedure was discontinued. The subjects were asked to expectorate sputum every 7 min. No bronchodilator was given before the inhalation of the hypertonic saline solutions. The subjects were advised to blow their noses and rinse their mouths with water before coughing the sputum sample. The whole sample was processed within 2 h. Dithiothreitol was used to dissolve the sputum plugs. At least 300 nonsquamous cells were counted, and the cytospin slides were stained with the May–Grünewald–Giemsa method (Diff-Quik, Medion Diagnostics GmbH, Düdingen, Germany). The supernatant was frozen at –80 °C. The differential cell counts were made by two blinded readers, and the average of the two observations was calculated and used as the result for each subject. The differential cell counts are presented as percentages of the total nonsquamous cell counts. The mean difference in the percentage of neutrophils (exposed period) between readers was 3 ± 8%. All sputum samples showed a cell viability of > 50% and a percentage of squamous cell

contamination of < 40%. One of the exposed workers was unable to produce a sputum sample at T_1 but managed to produce one at T_2.

5.2.4. Blood samples and biomarkers

In study II, blood samples were collected before sputum induction on both occasions. The samples were analyzed consecutively using standard procedures at the Fürst Medical Laboratory, Oslo, Norway (ISO/IEC 17025 certified). The concentrations of white blood cells, immunoglobulin E, CRP, and eosinophil cationic protein were measured. The concentrations of the cytokines IL1β, IL6, and IL8 in the sputum supernatant were measured in the same batch using enzyme-linked immunosorbent assays (DuoSet ELISA Kits from R&D Systems, Minneapolis, MN, USA). The analyses were performed according to the manufacturer's instructions.

In study III, the blood samples were collected at 0, 8, and 32 h in vacuum tubes containing citrate or ethylenediaminetetraacetic acid (EDTA) as anticoagulants, or containing no additives (serum). The citrate and serum tubes were centrifuged at 1400 × *g* for 10 min. The plasma or serum was then aspirated and aliquoted into 1.5 mL polypropylene Eppendorf cryotubes within 1 h. The plasma and serum tubes were stored at –80 °C until analysis. The leucocytes were analyzed in the EDTA blood samples within 48 h, using the Sysmex hematology system (Sysmex Europe GmbH; Hamburg, Germany) at the Oslo University Hospital, Ullevål, Oslo, Norway.

High sensitivity CRP (hsCRP) from serum samples was quantitated with a high-sensitivity immunoturbidimetric assay on a Hitachi 917 Automatic Analyzer (Roche® Diagnostics, Penzberg, Germany). The interassay variation (coefficient of variation, CV) was 5%. The fibrinogen concentrations in the citrate plasma samples were analyzed with a clotting test on the STA-R Evolution (Diagnostica Stago, Asnières-sur-Seine, France). The CV was 4%. The D-dimer citrate plasma samples were analyzed with an immunoturbidimetric method on the STA-R Evolution. The CV was 3%.

The serum samples were analyzed for cytokines using a microsphere-based multiplexing bioassay system with xMAP technology (Luminex Corporation, Austin, USA). TNFα, IL1β, IL6, IL8, and IL10 were analyzed with the Bio-Plex Human Group 1 assay 6-plex (Bio-Rad, Hercules, USA), according to the manufacturer's instructions. The CVs were calculated from supernatant aliquots (n = 8) of lipopolysaccharide-exposed human

monocytes, stored at –80 °C, and were as follows: TNFα, 12%; IL1β, 8%; IL6, 12%; IL8, 16%; and IL10, 15%. The detection limits were set as the lowest standard in each assay: TNFα, 0.16 pg/mL; IL1β, 0.06 pg/mL; IL6, 0.18 pg/mL; IL8, 0.04 pg/mL; and IL10, 0.16 pg/mL.

5.2.5. Fractional exhaled nitric oxide (III)

The nitric oxide (NO) levels in the exhaled air were measured according to the ATS/ERS criteria (90), using the NIOX MINO (Aerocrine AB, Solna, Sweden). This device measures fractional exhaled nitric oxide (FeNO) at an exhalation flow rate of 50 mL/s, expressed in parts per billion (ppb), using an electrochemical sensor. The accuracy range of the NIOX MINO device is ± 3 ppb for measured values < 30 ppb and 10% of the measured value for values > 30 ppb, expressed as the standard deviations of 10 consecutive measurements. The measurements were made before and after the work shift and again 32 h after baseline. The subjects were advised not to consume food or beverages 1 h before the measurements. Only nonsmokers were selected for FeNO testing, and the measurements were made before the lung function measurements.

5.3. Exposure measurements

In general, previous occupational exposure is difficult to estimate with reasonable accuracy because the data are usually incomplete. Therefore, in the first study, we used a two-phase method to estimate particle exposure, similar to the one suggested and evaluated by Alvear-Galindo and coworkers (14). The first phase uses a homogeneous group (focus group) technique to reconstruct the production process and to estimate the level of dust exposure in each working position. For this purpose, we interviewed 18 long-term workers. Historical and technical information was available to document the exact times of changes in exposure. Four levels of exposure to cement dust were defined and given an exposure number ($E_1 = 1$, $E_2 = 2$, $E_3 = 4$, and $E_4 = 10$). The focus group agreed on the number 10 for two jobs where the exposure was particularly high. In the second phase, all the cement workers were interviewed. The index determined by the focus group was used to calculate the individual worker's exposure to cement dust. The number obtained at each position was multiplied by the time spent in that position (exposure = $(E_1 \times T_1) + (E_2 \times T_2) + \ldots + (E_n \times T_n)$. Ea = exposure level in the first job, T_1 = years of work in the first position, and so on). The index numbers were also used for the evaluation of the dose–response relationship.

The contemporary cement dust levels were also measured to compare them with the dust levels reported in former studies. Personal dust samples were collected over 8 h on three days of work. Asbestos exposure was considered a possible confounding factor, and the exposure weighting technique described above for cement dust exposure was used to estimate individual asbestos exposure. The level of α-quartz was measured in one of every three of the worst-case personal dust samples that were collected in the area where quartz is added to the raw material.

Photo. Exposure measurement equipment used in study III.

In studies II and III, no historical exposure information was collected because the workers were their own controls, and only acute effects were studied. Therefore, contemporary dust levels were considered to correlate best with possible effects. For study II, the respirable aerosol concentrations were measured in 2005, simultaneously with the sampling of sputum from furnace department workers, using an SKC 225-69 cyclone operated with SKC 224-PCEx7 pumps (SKC Ltd, Dorset, UK), with airflow at 2.2 L/min. The personal thoracic aerosol concentration of exposure was measured for maintenance workers in 2007, using a BGI 2.69 cyclone (BGI, Waltham, Massachusetts, USA), operated with SKC 224-PCTXR8 pumps (SKC Ltd), with airflow at 1.6 L/min, during the period of sputum induction. Thoracic aerosol samples were collected in 2007, also from the furnace department workers

who had undergone sputum induction in 2005. All aerosol measurements were made on 8 h shifts.

In study III, the inhalable aerosol fraction that contained the particles that enter the nose and mouth (aerodynamic diameter [dae] < 100 μm) was collected with an IOM Inhalable Dust Sampler (SKC Ltd) equipped with a 25 mm cellulose-ester membrane filter with a pore size of 5 μm (Millipore, Billerica, USA), at a flow rate of 2.0 L/min. The thoracic fraction contains particles that pass the larynx (50% cutoff at dae = 10 μm) and was collected with the BGI GK 2.69 respirable/thoracic sampler with 37 mm polyvinyl chloride (PVC) filters with a pore size of 5 μm (filters were from Millipore, SKC or Pall Inc Port Washington NY, USA), at a flow rate of 1.6 L/min. The respirable fraction (50% cutoff at dae = 4 μm) that enters the alveoli (91) was collected with the respirable cyclone (Cassella Inc., Amherst, USA), with 37 mm PVC filters with a pore size of 5 μm, at a flow rate of 2.2 L/min. Each worker carried several samplers at the same time, and if the results for one of the samplers were missing, the regression equations for the other samplers were used to calculate the expected level for the missing fraction. If the aerosol levels were negative after corrections were made for blind filters, this value was substituted with 50% of the lowest positive measured value for the same job type.

In study IV, which is part of a four-year prospective study, exposure measurements were scheduled for collection every two years, together with the health measurements. In connection with spirometry all participants filled out a questionnaire on personal historical occupational exposure developed by the National Institute of Occupational Health in Norway and the National Coordinators of the study. Another questionnaire describing job types and work conditions on the day of exposure sampling was completed after each full-shift sampling. The same questions and categories as in the historical exposure questionnaire were used, with added information about the sampling (time, flow, equipment). Workers were selected for sampling once or several times using a group-based strategy excluding workers not entering the cement production areas. The workplace aerosol measurements were collected with GK 2.69 cyclones (BGI Instruments, Waltham, USA), to sample the thoracic fraction of the aerosol at 1.6 L/min, using portable pumps and 37 mm PVC filters. The airflow at the end of sampling was accepted when values were between 1.28 and 1.92 L/min, otherwise the measurement was considered non-valid. The dust mass on the filters was determined by gravimetry, according to a standard procedure,

using a Sartorius MC Micro Balance (Sartorius AG, Goettingen, Germany). The use of personal respiratory protection was reported in the questionnaire, but was not considered in the exposure assessment.

5.4. Statistical analyses

5.4.1. Study I

The lung function variables FVC, FEV_1, and FEV_1/FVC were analyzed as the response variables by multiple linear regression, with adjustments made for the predictors: age, height, life dose of tobacco, and asbestos exposure. The cement exposure index value was included in the analyses as a continuous variable. The logistic regression analyses were performed with symptom indicators as the dependent variables. The cement-exposed group was divided into two subgroups—workers with high- and low-level exposure—for further analysis based on the individual dust exposure levels. Adjustments were made for age and lifetime dose of tobacco (independent variables). Occurrences are given as prevalence, and exposure associations are given as adjusted odds ratios (ORs). Comparisons of the lung function variables were made for exposure (exposed = 1, controls = 0) and for different levels of cement dust exposure. The cement-exposed group was divided into quartiles to evaluate the dose–response relationship. Each subgroup of cement-exposed workers was compared with the least-exposed group.

The estimated power required to detect a true difference of 0.3 L between FEV_1 in the two groups was 0.90, assuming a 95% significance level.

5.4.2. Study II

Because the sample size was limited, the two groups of exposed workers (from the kiln and maintenance departments) were combined into one group for the analysis of the exposed production workers. The Wilcoxon signed-rank test was used to compare the differential cell counts and cytokine concentrations from before (T_1) and after exposure (T_2) within the same group. The Mann–Whitney test was used to compare the sputum cell counts and cytokine concentrations between groups. The percentage of neutrophils in sputum increases with increasing age (92). Therefore, to adjust for age, a multiple linear regression analysis was performed. In the regression model, exposure and age were used as the independent variables, and the two following categorical variables for exposure were included: first variable, exposed = 1 and nonexposed = 0; second variable, internal controls = 1 and the

exposed and external reference groups = 0). We used the percentage of neutrophils at T_2 as the dependent variable for the exposed workers and office workers, and at T_1 for the external reference group (the only test available for this group).

The study was designed to include at least 15 participants in each group, based on a power estimate of 0.80 which assumed a 95% significance level, to detect a true difference in the percentages of neutrophils of at least 16% between the two tests (before and after five days without exposure).

5.4.3. Study III

Student's paired *t* test was used to compare normally distributed continuous outcomes (the cross-shift differences in dynamic lung volumes). The Wilcoxon signed-rank test was used to compare the FeNO levels and inflammatory markers across shifts because these data were not normally distributed. The Spearman rank test was used to evaluate correlations. Independent variables considered to be biologically important cofactors were included in the linear regression models. We also included the job task and location (plant 1 or 2) in the linear regression models, based on assumptions of differences in exposure levels. The cross-shift difference and the preshift level (8 h) of the health outcomes were analyzed as dependent variables. Sex, age, height, body mass index, location (plant 1 or 2), report of doctor-diagnosed asthma, upper respiratory infection during the preceding three weeks, work tasks, life dose of tobacco (in kg), and the tertile of exposure levels (low, medium, and high levels as the dummy variables compared with the lowest level) were included as independent variables for the lung function analysis. The above-mentioned independent variables (except for height and reported doctor-diagnosed asthma) were used in the regression model for the inflammatory markers. Skewed variables were log transformed to generate acceptable linear regression models.

The study was designed with a power of 0.80 to detect a true difference of 1.5% between FEV_1/FVC measured at two time points and a change in cytokine levels of 0.8 ng/L at a 95% significance level. At least 90 subjects were required.

5.4.4. Study IV

The exposure measurements were grouped by plant and job type in a job exposure matrix (JEM) based on the group median exposure for each job type and plant combination. "Job types" was defined using the seven job-type categories cited in the questionnaires;

administration, production, cleaning, maintenance, foreman, laboratory, and other. An eighth category of workers was created for those reporting tasks in several job types. Exposure from the JEM was allocated to each employee belonging to the job type of the respective plant.

Associations between exposure and airway symptoms, airflow limitation, and lung function were investigated using exposure estimated by two alternative strategies; 1) job types using the administration as reference, and 2) exposure estimated using the JEM. As estimates obtained for administration personnel entering production areas were not representative of the majority of the administration employees not doing so, administration was excluded from analysis using the JEM. The JEM exposure value was either used in models assuming a linear relationship with outcomes, or categorised in quartiles and used as dummy variables in models not assuming linear relationships.

Lung function was analysed using the observed values of FVC, FEV1 and FEV1/FVC in models adjusted for gender, age, standing height and smoking by multiple regression. Participants reporting doctor-diagnosed asthma were excluded from the main analysis. The potential confounding factors were assessed, and we adjusted for them if they altered the estimates of exposure effects by 15% or more. The following age intervals were used to adjust for age: 17–29, 30–39, 40–49, 50–59, and > 60 years.

The statistical analyses (study I, II, III and IV) were performed using SPSS versions 9.0, 15.0, 18.0 (SPSS Inc., Chicago, IL, USA).

6. ETHICS

We considered that the purpose of the present studies and the methods used do not violate generally accepted ethical values. We aimed to include men and women, smokers and nonsmokers, all age groups, and every nationality or ethnic group in the studies. In occupational settings, workers may feel a greater obligation to participate than in other surveys, so the voluntary nature of their participation must be clearly communicated. Therefore, we stressed this issue in both the written and the oral information given to all the participants. The biological material and information were collected with the participants' consent. All the participants signed consent forms after receiving written information regarding the study. The participants were also informed that they could withdraw from the study at any time, without giving a reason for their withdrawal.

All study protocols were approved by the regional ethics committee and by the data inspectorate. If any further medical evaluation or follow-up of the participants in the project was required, this was provided by the local occupational health service of each plant or their health affiliates.

Because the cement industry contributed financial support to studies III and IV, we ensured the right to publish freely the results of the studies.

7. RESULTS

7.1. Paper I

Study I was a cross-sectional study of respiratory symptoms and lung function, with a retrospective exposure weighting. Twenty person-related dust measurements were also made, showing a mean total dust level of 7.4 ± 12.9 mg/m^3 and mean respirable dust of 0.91 ± 0.55 mg/m^3. The median levels (range) were 3.1 mg/m^3 (0.4–53.7) and 0.82 (0.0–2.3), respectively. Fifteen percent of the samples had concentrations exceeding the Norwegian OEL. This was not the case for any of the respirable dust concentration measurements (respirable dust OEL: 5 mg/m^3; total dust OEL: 10 mg/m^3). The workers exposed to the highest dust levels, with a total dust level over 50 mg/m^3, were those responsible for removing spilled cement dust from different areas of the plant. Personal samples of α-quartz levels, obtained from three worst-case situations, showed 0.06 mg/m^3 for one sample (Norwegian OEL: 0.1 mg/m^3) and undetectable levels (detection limit, 0.01 mg/m^3) for the other two samples.

We observed no significant differences in symptoms between the cement workers and the controls. The mean pulmonary function indices were similar for the exposed workers and the controls. Three respiratory symptoms (cough during the day, breathlessness when resting, and symptoms during work) were positively associated with exposure, but all the confidence intervals for the association measures included unity. There was a slight tendency for a better performance on all measures among cement production workers compared with controls, but this was significant only for FVC, with a regression coefficient β of 0.0027 and a 95% CI of 0.00–0.005, indicating the presence of selection bias because of a healthy-worker effects. Adjustments were made for age, height, and tobacco and asbestos exposure. There was a tendency toward lower FEV_1% in the most-exposed group, with a regression coefficient of β of –0.03 and a 95% CI of –0.07–0.01, relative to that in the least-exposed group.

Spirometric airflow limitation ($FEV_1/FVC < 0.7$ and $FEV_1 < 80\%$ of the predicted value) was observed in 17.6% of the exposed population and in 20.0% of the controls. The prevalence of COPD in the two groups was 14.3% and 14.0%, respectively.

7.2. Paper II

Study II was a cohort study of inflammatory cells and markers in induced sputum samples from exposed workers, compared with those of an internal (reference) group of unexposed/low-exposed subjects and an external reference group of healthy, unexposed office workers.

The median thoracic aerosol concentration was measured in a subgroup of the included workers during one work shift (8 h) and showed levels of 0.6 mg/m^3 (range, 0.2–8.1) in maintenance workers and 1.75 mg/m^3 (range, 0.2–15.5) in kiln department workers. In cement production workers, the percentage of neutrophils was significantly higher during the exposure period than during the nonexposure period ($P = 0.04$). Both the numbers and percentages of neutrophils and lymphocytes were higher in the exposed workers than in the external reference group, but these values did not differ between the exposed workers and the office workers. In the multiple linear regression model, exposure to cement aerosol (yes/no) was significantly associated with the percentage of neutrophils measured during the exposure period, when adjusted for age. The mean percentage of neutrophils increased by 16.7% from the nonexposed to the exposed period (β coefficient, 16.7; $P < 0.001$).

The median IL1β concentration was 28 pg/mL (range, 21–36) in the exposed workers, 17 pg/mL (range, 13–20) in the office workers, and 17 pg/mL (range, 13–21) in the workers in the external reference group. The cross-shift differences in the IL6 and IL8 concentrations were similar between the groups. In the cement-aerosol-exposed workers, the concentrations of IL1β, IL6, and IL8 were also similar in the nonexposure and exposure periods.

7.3. Paper III

In study III, dynamic lung volumes, gas diffusion capacity, FeNO, and inflammatory markers in the blood were measured preshift (baseline), postshift (8 h), and again 32 h after the baseline measurements. The workers were also exposed between 24 and 32 h. The median respirable aerosol level was 0.3 mg/m^3 (range, 0.02–6.2). The thoracic aerosol fraction was significantly higher at plant 2 (GM ± GSD = 2.5 ± 3.8 mg/m^3) than at plant 1 (3.0 ± 4.7 mg/m^3), whereas there was no difference in the inhalable or respiratory fractions. At plant 1, 46% of the workers had used respirators (all the time or for part of the day), whereas at plant 2, only 77% had done so.

We detected reductions of 37 mL in FEV_1 ($P = 0.04$) and 170 mL/s in $FEF_{25–75\%}$ ($P < 0.001$) during the shift. There was reduction of 0.17 mmol/min/kPa ($P = 0.02$) in the gas diffusion capacity during the shift. No associations between exposure and changes in the lung function variables were observed. This was the case for the whole group of workers and also when those who did not use respiratory protection were analyzed separately.

There was a correlation between FEV_1% of predicted and the years of employment in the cement production industry (Fig. 2).

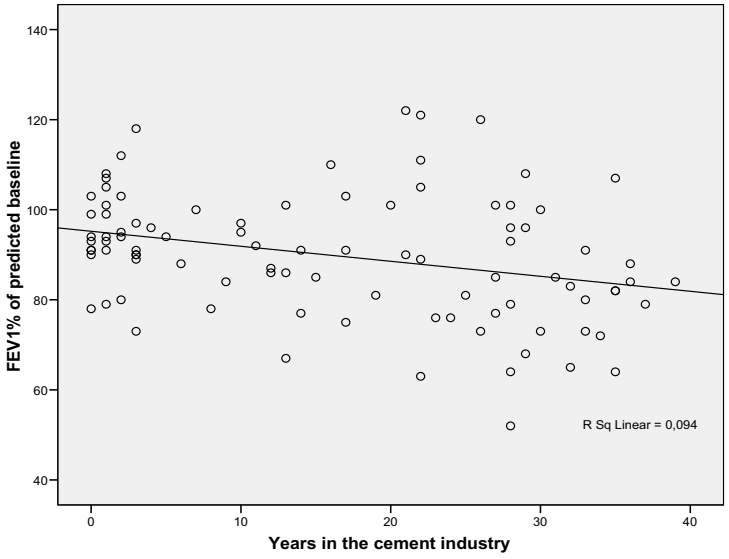

Fig. 2. FEV_1% of predicted according to years of employment in the cement production industry.

A reduction of 2 ppm ($P = 0.008$) in FeNO between the baseline values and those at 32 h after baseline was observed in study III. Furthermore, a cross-shift increase in white blood cells of 0.6×10^9 cells/L ($P < 0.001$) was detected, and fibrinogen levels increased by 0.02 g/L ($P < 0.001$) from baseline to 32 h. The TNFα levels increased, whereas the IL10 levels decreased during the shift. Thereafter, there was a reduction in all inflammatory markers, except IL10.

There was a positive correlation between the differences (0–32 h) in fibrinogen and hsCRP ($r = 0.48$, $P < 0.001$). In a multiple linear regression model, the 0 h level of fibrinogen was associated with the highest respirable aerosol level (> 0.4 mg/m^3), and this increased by 0.39 g/L (95% CI, 0.06–0.72). There were no associations between the cross-shift changes in the inflammatory markers and the exposure variables either for the whole group of workers or for those who did not use respirators, when analyzed separately.

7.4. Paper IV

In study IV we reported the baseline cross-sectional data from a multinational prospective study of lung function and respiratory symptoms in cement production workers. A total of 4,265 participants from Estonia, Greece, Italy, Norway, Spain, Sweden, Switzerland, and Turkey completed questionnaires and underwent spirometry testing. The prevalence of symptoms was higher in the production, maintenance, "other", and "several" job-type groups than in the administrative workers and was also higher among those reporting previous occupational exposure to dust or fumes. The odds ratios for symptoms (wheezing, dyspnea and coughing) ranged from 1.0 to 2.8 according to quartiles of exposure, and ranging from 1.2 to 5.5 according to job types. We obtained 3,332 (78%), 3,966 (93%), and 3,206 (75%) valid tests for FVC, FEV_1, and FEV_1/FVC, respectively. Reduced dynamic lung volumes were found in most of the exposed groups compared with the white-collar controls (administration). FEV_1 levels were affected more strongly than FVC values. Dynamic lung volumes did not differ between those who reported previous occupational exposure to dust or gases and those who did not. FEV_1/FVC decreased with age, and so did the prevalence of airflow limitation (FEV_1/FVC < 0.7). Substantial differences in the prevalence of asthma and allergy, and in smoking status and pack-years were observed between countries.

The geometric mean of 2670 exposure samples was 0.85 mg/m^3 (geometric standard deviation 4.6 mg/m^3). The group median values for thoracic dust levels in the JEM ranged from 0.07 mg/m^3 to 36 mg/m^3, with a mean of 1.8 ± 4.4 mg/m^3 and a GM of 0.7 ± 3.2 mg/m^3. The exposure variable was divided into quartiles, resulting in delimiting values of 0.49, 1.08, and 1.73 mg/m^3 between the quartiles. One JEM group of 37 employees with an exposure level of 36 mg/m^3 was excluded as outliers from the JEM. Forty percent of the participants reported using personal respiratory protection most of the time, 37% part of the time, and 17% reported no use of respiratory protection. Among workers with the highest

level of median exposure (highest quartile) 65% reported the use of respirators most of the time, whereas 54%, 31% and 35% did so in the groups with lower levels of exposure (second, third and forth quartile).

The adjusted odds ratios (ORs) of symptom prevalence were higher in the production, maintenance, foreman, laboratory, and several job type categories compared to the administration, while airflow limitation was not associated with job type. Exposure was associated with symptom prevalence both using the quartiles and the linear value of group median exposure. Reduced dynamic lung volumes were found in the production-related departments compared to the administration. Exposure was associated with reduced dynamic lung volumes both using the quartiles and the nominal value, showing a definite dose - response relationship for FEV_1 (Table 2).

Table 2. Lung function differences according to dust exposure levels in cement production workers.[†]

	FVC (mL)	**FEV_1 (mL)**	**FEV_1/FVC (%)**
	N = 2,696	**N = 3,244**	**N = 2,599**
		Coefficient (95% CI)[2]	
Exposure (quartiles)			
Exposure < 0.49 mg/m^3	Reference = 0	Reference = 0	Reference = 0
Exposure 0.49–1.08 mg/m^3	–170 (–240; –99)	–130 (–190; –79)	–0.4 (–1.0; +0.2)
Exposure 1.09–1.73 mg/m^3	–140 (–210; –65)	–180 (–230; –130)	–0.8 (–1.4; –0.2)
Exposure > 1.74 mg/m^3	–230 (–300; –160)	–250 (–300; –190)	–0.9 (–1.5; –0.3)
Exposure (linear)[§]			
Exposure effect per mg/m^3	–24 (–38; –11)	–33 (–43; –22)	–0.17 (–0.28; –0.06)

[†]Workers reporting doctor-diagnosed asthma (n = 116) and employees in administration (n = 629) were excluded from the analysis.

[‡]Linear regression coefficients interpreted as mL differences compared with the reference category (FVC, FEV_1) and percentage points of change compared with the reference category (FEV_1/FVC), with 95% confidence intervals.

Adjustments were made for sex, age (years), height (cm), and smoking.

[§]Exposure effect per mg/m^3 (odds ratio), adjusted for sex, age (years), height (cm), and smoking, assuming linear associations between exposure taken from the exposure matrix and outcomes.

Previous occupational exposure to dust and gases was associated with symptoms, but not with lung function. Among those workers with a doctor-diagnosed asthma, who were

excluded from the primary analysis, both symptoms and dynamic lung volumes were associated with job type, but the associations were weaker than those for workers without this condition (results not presented in paper IV).

The set of dummy variables for "plant" confounded the estimates of the effect of "cleaning" on symptoms, of "foreman" on chronic bronchitis, and of the "several" job types on airflow limitation. For the lung volume analyses, adjustments for "plant" were made for all strata and job types. The coefficients for the set of dummy variables were highly significant, in both the models of symptoms and those for lung function, indicating that there were differences in the effects of job type according to the plant at which the workers were employed (results not presented in paper IV).

We analyzed the influence of different countries and age strata on the associations and found that the associations between exposure and lung function and exposure and symptoms were stronger among the non-Turkish participants, with the exception of chronic bronchitis, which showed a stronger association in the Turkish participants. The mean values of dynamic lung volumes did not change considerably when participants were excluded country by country. When the cohort was restricted to those aged < 45 years, the associations between job types, and symptoms, chronic bronchitis, and airflow limitation increased, with the exception of the "other" and "several" job-type groups. When the JEM values for exposure were assessed in workers less than 45 years old, the associations between exposure and symptoms were stronger, except for chronic bronchitis, which showed the same degree of association, and airflow limitation, which showed a reduced degree of association.

8. DISCUSSION

8.1. Methodological considerations

8.1.1. Study designs and sample size

This work consists of four studies, with different designs. The first study (I) was a retrospective cohort study that included former cement production workers. The induced sputum study was a short-term follow-up study (II), in which comparisons were made with an internal and an external reference group. In the third study (III), we applied a cross-shift design, with a comprehensive exposure assessment, and the last study (IV) was the first cross-sectional (baseline) report of a four-year multinational prospective study that also included comprehensive exposure assessments.

In cohort studies, the hypotheses in question can be tested given that the outcomes are relatively common and involve sufficiently large numbers of workers during the observational period (93;94). Study I, II and III all had relatively small sample sizes. In study I, all the available workers were included, resulting in an estimated power of 0.90 to detect a true difference of 0.3 L in FEV_1 between the two groups, assuming a 95% significance level. The study was nonpositive, and therefore the null hypothesis (that there is no association between exposure to cement production dust and respiratory symptoms or impaired lung function) could not be rejected, leaving us with unanswered questions regarding the association between cement dust exposure and respiratory health outcomes. In studies II and III which are cohort studies, we ensured sufficient power to allow the detection of changes in the outcomes of interest. However, it is still possible that greater changes in the outcome variables would have been observed if the period between the two measurements, during which exposure was avoided, had been longer.

In study IV, we assumed that the size of the study was sufficient to detect interesting differences in health outcomes from both the epidemiological and the clinical perspectives, and to detect dose–response relationships between the exposure to cement dust and respiratory health outcomes.

A study that includes all persons at the time of analysis or a representative sample of such persons, selected without regard to exposure or disease status, is usually referred to as a "cross-sectional study" (93). Cross-sectional studies often have limited validity or precision

and can be described as “hypothesis-testing studies” (95). This was the case for studies I and IV.

8.1.2. Validity

The overall objective of an epidemiological study is to obtain a valid and precise estimate of the frequency of a disease or of the effect of an exposure on the occurrence of disease in the source population of the study (95). An estimate that has little systematic error may be described as “valid” (95). Systematic errors are commonly referred to as biases, and the opposite of bias is validity. An estimate with little random error can be described as “precise”. The large number of possible biases in observational studies can lead to considerable variation in the findings of similar studies of the same phenomenon (93). This may be one explanation of the differences observed in the outcomes of two of our studies (I and IV) and may also have contributed to the conflicting results of previous studies of the association between cement dust exposure and respiratory effects. The validity of a study can be divided into two components: its internal validity (violated for instance by confounding factors, selection bias, or information bias) and its external validity, which can be described as its generalizability (95).

Selection bias is a systematic difference in the association between exposure and outcome in those who participate and those who do not participate in a study (95), which will restrict the generalizability of the findings of the study. It is important to consider the likelihood of selection bias, in both cross-sectional and cohort studies, because subjects with respiratory symptoms or diseases are less likely to enter the workforce of an industry known for its dusty environment (the healthy-hire effect) (96–97). The selection of the group of workers could not be evaluated in any of the present studies. The healthy-worker effect (whereby those who experience symptoms or effects are more likely to quit their jobs) and the healthy-hire effect might explain the higher FVC levels in the cement workers compared with those in the controls (study I) and the low prevalence of airway limitation in study IV. The exclusion from study I of workers with less than one year of employment could also have increased the healthy-worker effect, given that workers with a short employment history were more likely to have experienced respiratory effects. However, five of the nine former workers (those who were employed for more than one year but had left their job after a few years) were diagnosed with COPD, indicating that their inclusion may have reduced the selection bias. Similarly, if the proportion of subjects who died of respiratory

disease was higher among cement plant workers than among the controls, this could have led to selection bias. The similar percentage of deaths observed among the controls and the cement plant workers suggests that this bias was not important (study I). However, we cannot exclude the possibility that some exposure among the controls could have led to the similar proportion of deaths from respiratory disease.

The selection of controls in the first study was performed on the assumption that these workers had similar background exposure. The controls lived in the same geographic area and had education and environmental exposure (to aerosols other than cement) in their earlier occupations that was similar to those of the cement production workers. We specifically addressed exposure to occupational airway irritants in thorough interviews with the controls. However, it is possible that unrecognized occupational or environmental exposure among the control workers could have led to changes in lung function, and that the use of a control group that was not representative of the general population could have produced a biased estimate.

In the studies in which the workers were used as their own controls, the design resembles the experimental situation more closely, as in cross-sectional studies, allowing a better control of selection bias. However, the possibility that a healthy-hire effect caused less-susceptible subjects to be evaluated should also be considered in these studies. In this thesis overall, I consider that the presence of selection bias probably would have caused an underestimation of the association between exposure to cement dust and respiratory health outcomes, rather than to an overestimation of these effects.

Information bias

Bias when estimating an effect can be introduced by measurement error in the required information, and this is often referred to as “information bias”. Information bias can be defined as the result of the misclassification of the study participants with respect to their disease or exposure status, which can be divided into differential and nondifferential misclassification (94). The differential misclassification of exposure occurs when the probability of misclassifying the exposure differs in diseased and nondiseased persons. Analogously, the differential misclassification of a disease may occur when the probability of misclassifying the disease differs in the exposed and nonexposed subjects. In study I, the subjects with respiratory symptoms could have recalled their previous exposure better than those without symptoms or disease (recall bias). This is a common problem when

qualitative exposure data are used to construct categories of exposure and may lead to the differential misclassification of the subjects' exposure, thereby contributing to either an overestimation or underestimation of its effects.

Nondifferential misclassification occurs when the probability of misclassification is the same for the two groups being compared. The nondifferential misclassification of exposure typically leads to a dilution of the estimated effect and therefore is of greater concern in the interpretation of studies that indicate the absence of an effect (95). This kind of misclassification may have occurred in study I, if the exposed workers were assigned to an incorrect exposure category based on the historical exposure weighting system. In study II, nondifferential misclassification might have occurred if the workers or the controls were exposed to respiratory irritants during the periods they spent away from work.

In study II, the researchers were blinded to the subjects' levels of exposure while they performed the sputum cell counts, to reduce the misclassification error. In study III, the collection of individual exposure measurement for all participants was considered to reduce the probability of misclassification of their exposure. In study IV, the measurements of the personal exposure of all 4,265 workers included would have required several measurements for each subject and was therefore not considered cost effective or feasible, so a group-based strategy was used to collect the exposure measurements. Because their exposure to cement production dust may have varied from day to day and between the individuals in a single job category, we could not be sure that the workers were assigned to the correct levels of dust exposure. In this case, nondifferential misclassification could have occurred, leading to an underestimation of the effect. The thoracic aerosol fraction was considered the most relevant to the workers' bronchial effects and was therefore chosen for sampling (studies II, III, and IV), to reduce the misclassification of their exposure more than would be possible if the total dust or respirable dust fractions were used.

Bias in estimating an effect can be caused by measurement errors during the collection of the required information. Such bias during the lung function measurements would probably have affected all subjects equally, causing a dilution of the effects. To increase the precision of the studies, we trained the staff well and ensured that all data were collected according to standard methods and up-to-date guidelines. In studies I, II, and III, the same investigator (AKMF) performed all lung function measurements.

The internal validity of study IV was ensured by having the first and second authors read all the spirometry charts carefully and by classifying the data for each individual test in compliance with the ATS/ERS criteria. This classification made it possible to choose different criteria for FVC and FEV_1 repeatability, and for the EOT criterion. However, the possible removal of individuals with respiratory disease from the population because they did not meet the validity criteria for lung function testing, might have introduced underestimation of the effects (99).

The use of validated questionnaires in all four studies is regarded as having reduced the probability of information errors. Information about airway symptoms was collected on the day of the spirometry testing, with a questionnaire based on the BMRC questionnaire (studies I and II) or the IUATLD questionnaire (studies III and IV). Although these questionnaires are widely used and have been validated for the detection of respiratory disease (83), they have not been validated for the detection of early or acute respiratory effects, which were the outcomes of studies II and III. Therefore, the specificity and sensitivity of the questionnaires for these effects are not known, possibly introducing information bias as to detection of acute respiratory symptoms.

Confounding factors

Confounders are factors (exposures, interventions, treatments, etc.) that explain or produce all or part of the difference between the measure of association and the measure of effect that is obtained by a counterfactual ideal (95). In this context, confounding occurs when the exposed and nonexposed subpopulations have different background disease risks (94).

In all four studies, stratification according to possible confounders was performed, and adjustments were made for the factors considered to alter the effect estimates. In addition to smoking, asbestos exposure was considered to be a possible confounder in study I. Therefore, the weighting for asbestos exposure and an adjustment to the regression analysis were made. Because older workers could have experienced greater exposure to occupational aerosols in the past leading to changes in their sputum cells and markers, and because increasing age enhances neutrophil counts, the age of the workers could have biased the comparison of the exposed workers and the internal controls in study II. However, when we compared the results for the exposed and nonexposed periods (T_1 and T_2) in study II and study III (0 h and 8 h), the probability that age was a confounding factor was almost

certainly reduced because the workers were used as their own controls and the time between the two measurements was short.

In study IV, potential confounders were assessed and adjusted for in the statistical analysis if they altered the estimated effects of exposure by 15% or more. We excluded quarry workers to minimize the distortion of the results caused by exposure to crystalline silica. However, there may have been traces of crystalline silica in the raw materials and the final product, so some exposure of the participants to free crystalline silica cannot be ruled out.

Selection of the control groups

In study I, the controls were carefully chosen and presumably had socioeconomic backgrounds similar to those of the exposed workers, but there was limited information about their lifetime (previous) exposure to cement dust. Unrecognized exposure of the controls that induced a reduction in their dynamic lung volumes also may have been a confounding factor to study I. If so, this could also have led to an underestimation of the effects.

In study II, the socioeconomic status of the external controls probably differed from that of the workers, entailing better health. This could have led to an overestimation of the effects of exposure to cement dust. However, internal controls with a similar socioeconomic status to that of the workers were also included, and differences in health outcomes of the cement production workers and this group were also demonstrated. In study IV, both white-collar controls (administration) and blue-collar workers with low exposure levels were used as references, allowing comparisons to be made with groups with either similar or presumably different socioeconomic backgrounds. Nevertheless, there is also evidence of an association between respiratory disease and the aerosol exposure encountered during office work, such as the dust from carbonless copy paper and from photocopiers and printers (100;101). If present, such effects may have led us to underestimate the differences in the prevalence of respiratory symptoms between the cement production workers and the office-worker controls (studies II and IV).

8.2. Discussion of exposure measurements and results

Both qualitative and quantitative exposure data were collected in all four studies, but an extensive collection of quantitative data, with records made of the inhalable, thoracic, and respirable fractions of dust, was not feasible in study I.

However, the retrospective exposure weighting performed in study I is considered to represent exposure better than does tenure only, and it made possible the comparison of the groups based on qualitative exposure data. The dust samples collected in study I showed that the contemporary dust levels did not exceed the levels measured in a large study in the USA (2). The exposure levels in the cement production industry were higher in the past because of the older technology, so an exposure weighting system was applied to deal with the lack of past dust measurements. Although the estimates of individual occupational exposure were not exact in any way, we are confident that we registered the differences in exposure more accurately than had we used job titles or tenure only. In study I, we demonstrated a tendency toward lower FEV_1% in the most-exposed group compared with that in the least-exposed group, with a regression coefficient β of –0.03 and a 95% CI of –0.07–0.01.

In study II, the levels of inflammatory cells and markers in the samples from the workers in their exposed periods are thought to reflect recent exposure. The mean respirable aerosol level among the exposed workers was well below the Norwegian OEL for respirable aerosols (5 mg/m^3). The thoracic fraction was also below the OEL. However, the workers reported day-to-day variations in exposure, and high peaks associated with special tasks. Therefore, monitoring exposure each day over the whole two-week period would have allowed us to correlate the workers' exposure with their health outcomes. Unfortunately, this was not feasible in the sputum study. Nevertheless, we identified a group of eight subjects in whom the increase in the percentage of neutrophils was > 20% (high responders, n = 8). Six of these workers (75%) regularly performed inspection rounds, which may have included frequent peak exposures. Of the other exposed workers, only seven reported this kind of exposure (26%). This suggests that regular peak exposure is related to an increased percentage of sputum neutrophils.

As well as measuring the respirable aerosol concentrations, we also measured the thoracic fractions of airborne cement particles in the exposed workers in studies II, III, and IV. The thoracic fraction represents the aerosols that reach the airway below the larynx and includes the respirable fraction, which contains particles small enough to reach the alveoli. Induced sputum contains cells and fluid from the large central respiratory sections below the larynx (102). Consequently, both these fractions of aerosols are relevant when studying the changes in induced sputum samples. Similarly, measurements of the thoracic fraction are

considered to reflect better the dust that reaches the site of the effects measured by spirometry, whereas the respirable fraction is probably an appropriate measure of particles that reach the area in which gas diffusion occurs.

Surprisingly, no correlations between exposure and cross-shift changes in the outcome variables were observed in study III. In this study, the respirable aerosol levels among the exposed workers were well below the Norwegian OEL (respirable aerosol, 5 mg/m^3), as were the levels of the thoracic aerosols. The exposure measurements also showed that the particle size distributions of the aerosols measured at both plants were predominantly inhalable and will therefore be primarily deposited in the upper respiratory system. Therefore, other descriptors of exposure, such as the chemical composition of the aerosols at different locations in the plant and peak exposures, could be important and should be considered for inclusion in future studies. Furthermore, the regression analysis in study III showed that the preshift level of fibrinogen was associated with exposure in the workers with the highest levels of exposure, which may indicate that exposure in the period before our measurements were made was also important.

In the multinational study (IV), the prevalence of symptoms after adjustments were made for sex, age, smoking status, plant, and self-reported allergies showed weakly increased ORs for the production, maintenance, foreman, laboratory, and "other" job categories, and a more pronounced increase in the OR for those who reported working in several job categories. Previous occupational exposure to dust and gases was also weakly associated with symptoms. Furthermore, the dynamic lung volumes among workers in production-related departments were significantly lower than those of administrative employees.

The use of exposure measurements (studies I, II, III, and IV) and the registration of work tasks and previous exposure (IV) were expected to reduce the probability of bias related to the misclassification of exposure, and they also allowed correlation and linear regression analyses between health outcomes and exposure to be performed. However, we had no information on complete lifetime exposures, the increased flexibility in the tasks within jobs and industries, the chemical composition of the dust in the different areas of the plants, or the peak exposure patterns, which may have made the detection of an association between the respiratory health outcomes and exposure to cement production dust more difficult.

In study IV, we used the median exposure level of job groups as exposure estimates to minimize the influence of outliers. However the arithmetic mean exposure levels are likely to be higher. The levels of exposure varied substantially between plants, although all plants used a similar dry production process, except the Estonian plant, which used the wet process. Differences in dust control measures, such as ventilation and filtration technologies, and in how the plants were cleaned (manually with a broom or with machines) may partly explain such differences in exposure within plants using similar production technologies. The differences in use of personal protection among workers with different levels of exposure (more frequent use among workers with high levels of exposure than among those with lower levels), is considered to potentially dilute the associations between exposure and outcomes in study IV.

Inaccuracies in the estimation of exposure tend to mask any exposure–response relationships present. This could have been the case in studies I, II, and III. The methods used to estimate exposure varied throughout the period in which the present studies were performed. Improved exposure estimates, in addition to the improvement of their validity and power in study IV, were probably necessary to allow the detection of associations between outcomes and exposure in workers exposed to levels of cement production dust below the Norwegian OEL (respirable dust, 5 mg/m^3; total dust, 10 mg/m^3).

Photo. Removal of cement production dust.

8.3. Discussion of the results

8.3.1. Study I

In study I, the advanced age of the workers and the high prevalence of smoking probably explain the high crude prevalence of symptoms in the cement plant workers. Because the prevalence of smoking was similar in the exposed cement production workers and the controls, background exposure or previous exposure before entering the workforce at the cement plant or the control plant also may have been important. Similar findings were reported in a large American study, from which the authors concluded that the exposed workers did not have a higher prevalence of symptoms than the reference population, except that 5.4% of the cement workers had dyspnea compared with 2.7% of the controls (2). The prevalence of COPD among exposed workers (14%) in our study was similar to that of other Norwegian blue-collar workers (13.5%) (103). The high prevalence of symptoms, but a prevalence of COPD similar to that in the other blue-collar workers, is consistent with the finding that chronic cough and sputum production precede the development of COPD by many years (39) and could indicate that this group of cement production workers will present with an elevated prevalence of COPD some years hence. The predictive value of respiratory symptoms for the later development of COPD was demonstrated in an earlier study of cement production workers (104). In that study, respiratory symptoms, especially breathlessness, were associated with both COPD and overall mortality. These findings emphasize the importance of studies that demonstrate an increased prevalence of respiratory symptoms among exposed workers.

In study I, the mean pulmonary function indices were similar in exposed workers and controls. Our results are consistent with the findings of a large cross-sectional study that reported similar dust levels (2) and with the results of a Danish study (11). In both studies, no significant differences in lung function was detected between cement workers and blue-collar workers with similar smoking habits, but selection bias resulting from the healthy worker-effect was considered to be important. In contrast to earlier studies, we tried to trace former workers to evaluate the selection of the sample of workers from the population. This is believed to result in less selection bias from the nonrepresentativeness of the presently employed workers compared with the real population of exposed workers, which should

also include those who have left the occupation in question. Although the sample size of former workers was small (n = 9), the prevalence of 56% of doctor-diagnosed COPD in this group may indicate that the healthy-worker effect was substantial.

8.3.2. Study II

In this study, we found a higher percentage of sputum neutrophils in cement production workers during the exposure period than during the nonexposure period and also in comparison with those of an external reference group. The concentration of IL1β in the sputum was higher in the workers during the exposure period than in the office workers or in the external reference group. These findings support the study hypothesis that there is an association between cement production aerosols and inflammation in the airways of otherwise healthy workers. The difference in the percentages of neutrophils in the two observation periods was only observed in the cement production workers and not in the office workers. The lack of difference between the two sampling times in the office workers indicates that physical activity, climatic conditions, or other unknown factors probably do not explain the observed differences in the levels of inflammatory markers between the exposed workers and the internal controls. Consequently, there is reason to suspect that these changes reflect their exposure to cement production aerosols. This is consistent with the findings of previous studies that have reported an association between neutrophilic inflammation and exposure to particles derived from tobacco smoke, air pollution, or occupational exposure (25;26).

The percentage of neutrophils was higher in the exposed period (T_2) than in the nonexposure period (T_1), and the percentages of neutrophils and lymphocytes were also higher in the cement production workers than in the external reference group. However, the percentages of these cells did not differ between the exposed workers and office workers at T_2. In contrtast, after adjustments were made for age, the analysis showed that the percentage of neutrophils was related to exposure. Because we did not have exposure measurements for all the participants in the study and because of interindividual day-to-day variations in the tasks and exposure of the workers, we used categorical variables to evaluate exposure. The percentage of neutrophils increased with age, after adjustment was made for other covariates. This is consistent with reports of increasing neutrophil levels in sputum with increasing age (92). It is possible that some of the office workers were exposed to low levels of cement production aerosols in their working environments. One of the

office workers reported previous exposure to cement production dust. Therefore, the inclusion of an external reference group with a similar mean age was considered to improve the comparison, although these workers may have differed from the workers in factors such as their socioeconomic status.

In exposed workers, the total cell counts and absolute numbers of inflammatory cells were similar in both periods and similar to the values for office workers. This may reflect greater variance in these variables than in the percentage of neutrophils. Nevertheless, the absolute numbers of neutrophils and lymphocytes (in both the T_1 and T_2 measurements) were significantly higher in the cement production workers than in the external controls. This may indicate that exposure to cement aerosols must be avoided for a longer period to normalize the absolute number of neutrophils.

The elevated concentration of the cytokine IL1β in the sputum of exposed workers compared with that of both office workers and the external reference group indicates a cytokine response to exposure among the cement production workers. This is consistent with the elevated serum levels of IL1β found in American cement masons, whose major exposure is to cement aerosols (105). IL1β upregulates the adhesion molecules on endothelial cells and neutrophils, and contributes to the accumulation of neutrophils in the airway (25). Although the neutrophil counts among the exposed workers decreased after five days with no exposure, the IL1β concentration did not decline during this time. This suggests that a longer period of avoidance of cement aerosol exposure is required to normalize the sputum concentration of IL1β. The observed decline in neutrophils without a parallel decline of IL1β remains unexplained, but a negative feedback signal for the production of IL1β might be generated not only by airway neutrophil numbers but also by other inflammatory mechanisms or markers.

The similar concentrations of IL6 and IL8 in the three groups suggest a low response of these inflammatory markers. IL6 is a common inflammatory marker that can be activated by several stimuli. IL6 increases during the exacerbation of COPD and appears to be useful in evaluating the intensity of the disease (30). A low level of this cytokine is expected in healthy subjects. IL8 has a chemotactic effect on neutrophils and correlates negatively with lung function in patients with COPD (106). Accordingly, the low IL8 concentrations

detected in our study probably reflect low-grade neutrophilic activation in these healthy cement production workers who have normal spirometric values.

8.3.3. Study III

In the cross-shift study, we observed a significant reduction in FEV_1, $FEF_{25–75\%}$, DL_{CO}, and FeNO levels, and an increase in white blood cells and fibrinogen levels, together with elevated TNFα levels and reduced IL10 across shifts in the cement production workers with low-level exposure. Our finding of a cross-shift reduction in dynamic lung volume is consistent with the results of two earlier cross-shift studies among cement production workers exposed to higher levels of dust, which showed reductions in FEV_1, FEV_1/FVC, $FEF_{25–75\%}$, and/or PEF (6;44;49). Cross-shift studies are of particular interest because in occupationally exposed groups, a cross-shift reduction in lung function seems to be associated with a longitudinal reduction in these values (107;108).

We did not detect an association between the cross-shift changes in the spirometric indices and the individual exposures measured in this study. This suggests that exposure to cement production dust during a work shift does not result in a decline in lung function, or that these declines also occur if the effects are induced by exposure variables other than those measured (e.g., peak exposures). However, the possibility cannot be excluded that exposure does not cause a decline in lung function, when the pre- and postshift levels are similar, because an increase during the first 6–8 h of time awake is expected in healthy, nonexposed subjects, as normal diurnal variations in the lung function indices (86). The effects of exposure in the period before the cross-shift measurements (e.g., in the previous two weeks) also may have influenced our findings. However, when the analysis of workers was restricted to those who were not exposed during the preceding two weeks, no associations were detected between the changes in dynamic lung volumes and exposure.

A cross-shift reduction in the gas diffusion capacity (assessed as DL_{CO}) was observed. No previous study has examined the gas diffusion capacities of workers in this industry, but reduced gas diffusion capacities have been demonstrated in other industries with dusty environments (109). A possible mechanism underlying this decrease in DL_{CO} could involve a fraction of the aerosol small enough to reach the alveoli, affecting the alveolocapillary function and thereby reducing the gas diffusion capacity. Another possibility is that exposure to substances other than cement production dust affects gas diffusion. For instance, unknown (unreported) smoking less than one hour before the measurements were

made or the inhalation of carbon monoxide from vehicles or machines could block the hemoglobin molecules in the blood and thereby lower the gas diffusion capacity. However, until other studies of gas diffusion capacity have been undertaken in these workers, this issue remains unresolved.

The changes in lung function were not accompanied by cross-shift changes in FeNO levels, but a small significant reduction was observed when the baseline values were compared with those measured at 32 h. Reduced FeNO levels are observed in smokers (110;111). Our finding possibly indicates a similar response, but because the observed changes were small and we did not take measurements at 24 h, this finding must be confirmed in future studies.

The numbers of blood leucocytes increased across the shift. In light of this observation, we suspect that exposure to cement dust causes an increase in neutrophil activity. There is a known diurnal variation in leucocyte numbers, with increasing values during the day, a zenith in the afternoon, declining levels throughout the evening and night, and a nadir in the morning (112;113). The increase in levels of leukocytes observed during a work shift (8 h), with normalization on the following day, is therefore probably attributable to diurnal rhythmicity, but a correlation with exposure or other work conditions cannot be ruled out. If measurements were made also during the shift and in the hours thereafter, and if the study group was restricted to day-shift workers only, it might be possible to test whether significant changes occurred in these workers over and above those attributable to normal diurnal variation. However, the increased levels of circulating leucocytes observed during the exposure period at work are consistent with our previous finding of an increased proportion of neutrophils and elevated levels of IL1β in the induced sputum of cement production workers during periods of exposure (study II).

The observed increases in fibrinogen and hsCRP levels at 32 h, together with the positive association between the changes in fibrinogen and hsCRP, are consistent with studies that have shown that the inhalation of very fine dust from air pollution and from occupational exposure can influence blood coagulation (28;34;35;114). This increase in fibrinogen levels could theoretically have been induced by workplace exposure or may be the result of diurnal variations (115). However, an argument against diurnal variations is that we included workers from day, afternoon, and night shifts. Nevertheless, no changes in the levels of D-dimer were detected, and the observed differences in fibrinogen were small. Therefore, this finding should be interpreted with caution.

We observed an increase in TNFα and a reduction in IL10 levels across the shifts. The diurnal rhythmicity in the production of the proinflammatory cytokine TNFα has a peak in the early morning, with a subsequent fall during the day (116), whereas the anti-inflammatory cytokine IL10 peaks during the daytime (117). Our finding is consistent with the results of studies of cytokine levels in bronchial epithelial cells after exposure to cigarette smoke (31;32). However, the changes were small, and because no associations with exposure were detected, it remains unclear whether this response is related to the inhalation of cement dust.

Our data show that the levels of the proinflammatory cytokines IL1β, IL6, and IL8 decreased during the observation period. These findings, together with the FeNO results, are probably indicative of low or no inflammatory activity. A reduction in cytokine levels could also occur if the workers experienced higher levels of physical activity during the work shift than during the preceding period of rest, as shown in studies of healthy, nonexposed subjects (118;119). However, the analysis of FeNO and inflammatory markers in our study was based on the examination of the effects at only three different time points, and it is possible that other or additional time points (e.g., 4 h and 24 h) would have revealed different patterns of responses and made the interpretation of these findings less challenging.

8.3.4. Study IV

In the multinational cross-sectional study of respiratory function and symptoms, we demonstrated associations between exposure to dust in cement production plants and airway symptoms and reduced lung function. These findings are consistent with the results of several previous, cross-sectional studies and with the results of three prospective studies of cement production workers (12;50;51). In addition, we demonstrated a dose-response relationship for FEV_1 with 250 ml lower levels estimated for workers with the highest exposure level compared with those with the lowest level of exposure.

The odds ratios for symptoms (wheezing, dyspnea and coughing) ranged from 1.0 to 2.8 according to quartiles of exposure, and ranging from 1.2 to 5.5 according to job types. These results were comparable to findings in some earlier studies (10;46;47;49). The association between exposure and chronic bronchitis in our study was weaker than the association between exposure and symptoms. In a study from Taiwan with mean exposure levels of 3.6

mg/m^3 respirable dust, odds ratios for symptoms ranged from 1.2 to 1.5, but were close to unity for chronic bronchitis (15), while in an earlier study symptom prevalence was similar in workers exposed to 0.22, 0.55 and 1.2 mg respirable dust/m^3 (120). The geometric mean exposure level of 0.85 mg thoracic dust/m^3 in our study (IV) indicates lower respirable dust levels (91), probably similar to the later study from Taiwan (15).

The association between exposure and FEV_1 was stronger than between exposure and FVC, when both job types and JEM values as alternative strategies for comparison were used. These results support the hypothesis that the inhalation of cement production dust may lead to airflow limitation, and are consistent with the results of several studies including exposure measurements (10;15;43;45). Other studies have also identified demonstrating associations between exposure and reductions in dynamic lung volumes using job titles as indices of exposure (5;12;13;66).

The prevalence of airflow limitation was not significantly increased among exposed workers compared with that in controls (administration), with the exception of the foreman group. Possible interpretations of this finding are that the most serious cases of airflow limitation do not occur at these levels of exposure, or that our results have been distorted by selection bias, such as the healthy-worker effect. The presence of the healthy-worker effect was also suspected in a Danish study of median exposure levels to total dust of 3.3 mg/m^3, which showed an increased rate of COPD-related hospitalization only among workers exposed to cement production dust up to 30 years with a subsequent hospitalization rate thereafter (48).

In our first study we used the criteria of $FEV_1/FVC < 0.7$ and $FEV_1<80$ % predicted, to define airflow limitation. These criteria were commonly used at that time and allowed comparison with other studies. Since then the GOLD guidelines, using $FEV_1/FVC < 0.7$ to classify airway obstruction, have been introduced and are frequently used. A third option for classification of airway obstruction, suggested to reduce misclassification of patients, is using the 5^{th} percentile of FEV_1/FVC as the lower limit of normal (LLN5%) (121-123). We performed sensitivity analysis to test if such misclassification might occur in study IV. The exclusion of participants with age ≥45 years demonstrated that the OR of airflow limitation in the two highest exposure quartiles was reduced by half and thereby showed that such misclassification occurred among the older workers. This is in accordance with studies showing that the use of GOLD guidelines may give false positive rates of airflow limitation

among older patients (121-123). Otherwise, the sensitivity analysis in restricted age intervals demonstrated stable associations between exposure and health outcomes across strata of age. Therefore, and because the mean age of the participants in our study was relatively low (39.9 years, SD 10), we chose to use the GOLD-criteria to define airway limitation in study IV.

We also excluded participants who reported doctor-diagnosed asthma, to improve the comparability of this study to other COPD studies that included reversibility testing (122), although it was unlikely that some of these subjects would achieve normal lung function after bronchodilator use and still meet the COPD criteria. Therefore, the misclassification of airflow limitation in the study would probably dilute the effect estimate (123). The prevalence of doctor-diagnosed asthma seemed to be low (2.7%), with an expected gradient between the eastern and western parts of Europe, as demonstrated in studies of general populations (124;125). We detected a lower prevalence of asthma than expected in several of the countries included, possibly indicating the selective removal of employees with known asthma or allergy.

9. CONCLUSIONS

The prevalence of symptoms and the mean lung function indices in study I were similar in the exposed workers and the controls. No dose–response-related increase in symptoms or reduction in dynamic lung volumes was observed in this study. The prevalence of COPD was 14.3% in the exposed group and 14.0% in the control group. These findings do not support the hypothesis that exposure to cement production dust induces a decline in dynamic lung volume or an increase in respiratory symptoms. However, we noted that the workers reported a high prevalence of respiratory symptoms, and that the healthy-worker effect could have led to an underestimation of effects. Furthermore, possible unrecognized exposure that induced airway symptoms or impaired lung function among the controls may also have led to an underestimation of the effects.

The findings of study II showed a higher percentage of sputum neutrophils in cement production workers during the exposure period than during the nonexposure period and compared with those in the external reference group. The concentration of IL1β was higher in the sputum of workers during the exposure period than in that of the office workers or the external reference controls. This supports our hypothesis that cement production aerosols stimulate the inflammatory mechanisms in the airways of otherwise healthy workers.

In study III, minor cross-shift reductions in FEV_1, $FEF_{25–75\%}$, DL_{CO}, and FeNO levels were observed, corresponding to increased numbers of circulating leucocytes, elevated levels of fibrinogen and TNFα, and reduced levels of IL10 in the serum. No correlations were found between exposure to cement dust and the cross-shift changes in the outcome variables. Nevertheless, a regression analysis showed that among the workers with the highest levels of exposure, the preshift level of fibrinogen was associated with exposure, indicating that exposure before the study period could also have been an important factor. This may indicate that the "washout" period before the baseline measurements were made could have been too short to detect the full range of changes attributable to cement production aerosol exposure.

In the multinational cross-sectional study of respiratory function and symptoms (IV), we demonstrated associations between the outcomes of the study and exposure to dust in the cement plants. These findings are consistent with results from of several cross-sectional studies. In addition, we demonstrated a dose-response relationship for FEV_1 with 250 ml

lower levels estimated for workers with the highest exposure levels as compared to reference workers.

The findings of studies II, III, and IV are consistent in indicating possible effects of exposure to dust on respiratory symptoms, lung function, and on levels of inflammatory markers in healthy, cement production workers compared with those in periods of no exposure or in the controls groups. Associations between exposure to cement production dust and respiratory health outcomes were also demonstrated in the multinational study, indicating a dose–response relationship between the thoracic fraction of the dust and FEV_1 levels. However, limitations of these studies are recognized, so prospective studies are required to test the study hypothesis further.

10. FUTURE RESEARCH AND RECOMMENDATIONS

Our findings indicate that subclinical airway inflammation may occur in cement production workers exposed to levels of cement production dust that are considered low on a global scale. Although it is unclear whether the acute changes observed in dynamic lung volume and inflammatory markers represent the early stages of respiratory disease or an appropriate immune response without clinical consequences, the observed dose–response relationship for dynamic lung volumes in the multinational study indicates that further measures should be taken to reduce the exposure levels of workers to cement production dust, especially in those workers with the greatest exposure.

Based on the results of our studies, we recommend that future studies of the effects of exposure to cement dust on airway inflammation should consider the levels of physical activity of the workers during the periods of exposure and no exposure, and the influence of shift work on the outcome variables. Measurements of CRP and fibrinogen levels in the blood should also be considered, to explore possible effects of exposure to cement dust on blood coagulation and the risk of cardiovascular disease. Longitudinal studies of dynamic lung volume and changes in inflammatory markers in the sputum and blood are required to evaluate further the association between exposure to cement production dust and the development of airway disease.

Further research is also required that characterizes the physical and chemical composition of the dust to which workers are exposed in different areas of the production plants, and the identification of possible peak exposure situations, to allow the analysis of associations between these variables and respiratory health effects.

Reduced exposure to cement dust is considered the primary aim in the prevention of airflow limitation and inflammatory changes in workers in the cement production industry. I recommend the increased use of respiratory equipment in the areas of plants in which it is difficult to reduce exposure.

Our findings demonstrate that the prevalence of smoking is still high among these workers and represents a threat to workers' health in this industry. Therefore, the implementation of smoking cessation programs is recommended as an important measure for the primary prevention of respiratory disease.

The secondary prevention of respiratory and inflammatory diseases in the cement production industry could be achieved with surveillance programs and early detection. I recommend that surveillance programs that include lung function testing, monitoring the markers of inflammation, and personal exposure measurements be undertaken at regular intervals. It is also critical that the results be used for the prevention of disease and made available for further research.

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CORRECTIONS IN THE THESIS

Place	Text	Changed to
p. 43, last sentence	46%	54%
	77%	23%

12. APPENDICES

Appendix I Questionnaires

Appendix II Papers I-IV

Questionnaire used in studies I and II

PLAGER FRA LUFTVEIENE (MRC-skjema)

1.Hoster eller harker (kremter)du vanligvis om morgenen? ❑ Ja ❑ Nei

2.Hoster du vanligvis ellers om dagen? ❑ Ja ❑ Nei

3.Har du vanligvis oppspytt når du hoster eller harker? ❑ Ja ❑ Nei

4.Hoster du daglig til sammen 3 måneder eller lenger i løpet av et år? ❑ Ja ❑ Nei

5.Har du i løpet av de siste par årene i forbindelse med fokjølelse hatt hoste og eller oppspytt som har vart mer enn 3 uker? ❑ Ja ❑ Nei ❑ En gang ❑ Flere

6.Blir du mer tungpusten enn jevnaldrende når du går i motbakker? ❑ Ja ❑ Nei

7.Blir du tungpusten når du går opp 2 etasjer i vanlig fart? ❑ Ja ❑ Nei

8.Blir du tungpusten når du går i vanlig fart på flat mark? ❑ Ja ❑ Nei

9.Blir du tungpusten når du sitter i ro? ❑ Ja ❑ Nei

10.Hender det at du får anfall av tung pust? ❑ Ja ❑ Nei

11.Har du noen gang hatt piping (pipelyd) i brystet? ❑ Ja ❑ Nei

12.Har du hatt øvre luftveisinfeksjon (forkjølelse, tett nese, sår hals) de siste 3 ukene? ❑ Ja ❑ Nei

13.Har du noen gang hatt luftveisplager (hoste oppspytt, tung pust, pipelyd) i forbindelse med arbeidet? ❑ Ja ❑ Nei

14.Hvis svaret var ja på forrige spørsmål, besvar da: Hadde du bedring av symptonene ved fravær fra arbeidsstedet (f.eks. i helger, ferier etc.)? ❑ Ja ❑ Nei

Exposure weighting used in study I

EKSPONERINGSVEKTING STØV

Sted	arbeidstittel	-46	46-56	57-61	62-66	67- 76	77- 80	81-88	89-90	91-
Råmelsmølleri	aerofall-møllekjører									
	rørmølle-kjører									
	silopasser									
	rundemann									
Ovnsavdelingen	brenner ovn 1 og 2									
	brenner ovn 3									
	brenner ovn 4									
	brenner ovn 5									
	brenner ovn 6									
	grannulerings mann									
	rennepasser									
	tårnmenn									
Sementmølleri	krankjører									
	møller CM 1-2-3									
	møller CM 4-5-6									
	silopasser									
	rundemann									
Pakkeriet										
Tomta										
Verksted	innefolk									
	utefolk									
Gruppeverksted										
	sementmølleriet									
Filterarbeidere										
Arbeidsledere										

Appendix 2, Health questionnaire, p1-2 Revised 06 Jan 2009

Prospective monitoring of exposure and lung function among cement workers

ID Code ☐-☐☐☐ Date (dd.mm): ☐☐.☐☐ 2009

HISTORY, present and former

Have you been treated by a doctor or in hospital for pneumonia or bronchitis?	Yes	No
As a child, 0-14 years old	☐	☐
As an adolescent, 15-20 year old	☐	☐
As an adult	☐	☐

Have you been treated by a doctor or in hospital for any of the following diseases?	Please mark
Eye or nasal allergies, including hay fever	☐
Eczema, including eczema as a child	☐
Tuberculosis	☐
Plevritis	☐
Sarcoidosis	☐
Pneumoconiosis, e.g. silicosis, asbestoses	☐
Fibrosis of the lung	☐
Emphysema or COPD (Chronic obstructive pulmonary disease)	☐
Coronary heart disease (infarcts or angina)	☐
Other diseases of the heart, please state:	☐

.....

ALLERGY

	Yes	No
Have you ever had allergy against e.g. grass, animals?	☐	☐

If "yes", please state what sort of allergy:

.....

If "yes", what sort of symptoms do/did you have?	Please mark
Symptoms from the eyes, running	☐
Symptoms from the nose, sneezing, runny or blocked nose	☐
Asthma	☐
Skin symptoms, eczema, and itchy rash	☐

	Yes	No
Did a doctor confirm the diagnosis of allergy?	☐	☐

Has any in your family ever had allergy?	Yes	No
Mother, father or siblings	☐	☐
Children	☐	☐
Spouse	☐	☐

ASTHMA

	Yes	No
Have you ever had asthma?	☐	☐

If "yes",	Please mark
Yes, during childhood or as an adolescent	☐
Yes, as an adult	☐
Yes, presently	☐

	Yes	No
Have you had an attack of asthma at any time in the last 12 months?	☐	☐
Are you currently taking any medicines, (including inhalers, aerosols or tablets) for asthma?	☐	☐
Did the asthma (for the first time) start during the last ten years?	☐	☐
Did a doctor confirm the diagnosis of asthma?	☐	☐

Has any in your family ever had asthma?		
Mother, father or siblings	☐	☐
Children	☐	☐
Spouse	☐	☐

BRONCHIAL SYMPTOMS, present and former

	Yes	No
Have you had wheezing or whistling in your chest, at any time in the last 12 months?	☐	☐
Have you been woken up with a feeling of tightness in your chest first thing in the morning at any time in the last 12 months?	☐	☐
Have you at any time in the last 12 months had an attack of shortness of breath that came on during the day when you were not doing anything strenuous?	☐	☐
Have you had an attack of shortness of breath that came on after you stopped exercise at any time in the last 12 months?	☐	☐
Have you at any time in the last 12 months been woken at night by an attack of shortness of breath?	☐	☐
Have you at any time in the last 12 months been woken at night by an attack of coughing?	☐	☐
Do you usually cough first thing in the morning?	☐	☐
Do you usually bring up phlegm from your chest first thing in the morning?	☐	☐
Have you brought up phlegm from your chest like this most mornings for at least 3 months each year?	☐	☐

Which of the following statements best describes your breathing?	Please mark
a.) I never or only rarely get trouble with my breathing	☐
b.) I get regular trouble with my breathing, but it always gets completely better	☐
c.) My breathing is never quite right	☐

When you are in a dusty part of the house or with animals (for instance dogs, cats or horses) or near feathers (including pillows, quilts and eiderdown) do you ever:	Yes	No
a) Get a feeling of tightness in your chest?	☐	☐
b) Start to feel short of breath?	☐	☐

SMOKING

	Yes	No
Do you now smoke, as of one month ago?	☐	☐

If you do not smoke each day now, please answer:	Yes	No
Have you ever smoked for as long as a year? (At least one cigarette per day for one year)	☐	☐

	Please mark
Did you quit smoking less than a year ago?	☐
Did you quit smoking more than a year ago?	☐

If you smoke or have smoked, please answer:	No. of years
How old were you when you started smoking?	☐☐
How many years have you smoked altogether?	☐☐

How much do/did you smoke on average?	No. of cigarettes
Number of cigarettes per day	☐☐

Subjects' characteristics and lung function	ID Code □-□□□	Date (dd.mm): □□.□□ 2009

Age at examination (years) □□

Height (cm) □□□

Weight (kg) □□□

Gender: female □ male □

The id-code as well as the date & time of spirometry should be written in the top left field of the spirometry chart.
Room temperature: □□,□ (please read from the extra supplied thermometer).
Vitalograph display temperature setting: □□ (it should be **adjusted to the room temperature**, rounded to the nearest degree).
Barometer reading: □□□□

	Pred	Measured. Best of three manoeuvres	% of pred
FVC (l)	□.□□	□.□□	□□□
FEV_1 (l)	□.□□	□.□□	□□□
FEV_1 %	□□□	□□□	□□□
$FEF_{25-75\%}$ (l/s)	□.□□	□.□□	□□□
$FEF_{75\%}$ (l/s)	□.□□	□.□□	□□□

Spirometry operator (initials) .....

Prospective monitoring of exposure and lung function among cement workers

Family name		Date of birth
Given name		

Questionnaire to be completed in connection with spirometry	ID-code ☐-☐☐☐

Revised Jan 6, 2009

1 Today's date (dd.mm): ☐☐ - ☐☐ - 2009

Male ☐ Female ☐ Daytime work ☐ Shift work including night-time ☐

When were you employed at the company where you work at present?	Month ☐☐ Year ☐☐☐☐
Job type	Production ☐ Plant cleaning and yard ☐ Maintenance ☐ Foreman ☐ Management and Administration ☐ Laboratory ☐ Other ☐(please specify.....)

Note: Production including package/shipping, Management and administration including engineers.

	Completed Years	
2 How many years have you been employed in **any** cement company (including the present):	☐☐	Please include vacations and leaves

3 Did you use respiratory protective equipment (a respirator) at work?	No ☐
	Yes, Occasionally ☐
	Yes, Most of the time ☐

4 Have you worked in any of the following activities during a time period of **one year or more** in this or any other company?	Tick those relevant	
Asbestos cement production	☐	Consider all years of your employment career, the present company included
Other work with asbestos products	☐	
Mining industry	☐	
Road or tunnel construction	☐	
Foundry or metal production	☐	
Wood industry	☐	
Agricultural production (farming)	☐	
Painting	☐	
Welding or sheet metal work	☐	
Industries using solvents or polymers	☐	
Other industries with high dustiness	☐	

5 During the **last two** years, did you

Have pet animals in your home? ☐ Live on a farm? ☐

Perform dust/gas producing activities regularly during leasure time? ☐

6 If you have been working **only** with **control room or administrative tasks** (without tasks in areas of raw material, clinker or cement handling), please tick this box:	☐

7 During the **last two years**, have you spent your working hours/had tasks within these categories? (please indicate time spent, if any)	Time (years) in each category			
	Yes, but, **less than** ½ year	½ - 1	More than 1, less than 1.5	1.5 - 2
Quarry	☐	☐	☐	☐
Production (mobile sites, not in control room)	☐	☐	☐	☐
Control room	☐	☐	☐	☐
Packing & Shipping	☐	☐	☐	☐
Control Laboratory	☐	☐	☐	☐
Electrical Maintenance	☐	☐	☐	☐
Mechanical Maintenance	☐	☐	☐	☐
Storehouse	☐	☐	☐	☐
Safety & Environment	☐	☐	☐	☐
Oiling or greasing	☐	☐	☐	☐
Other production-related tasks	☐	☐	☐	☐
Administration	☐	☐	☐	☐
Time spent absent from work (sick leave, other leaves)	☐	☐	☐	☐

8 Time per week on an average spent on **each of these special tasks** during the **last two years**. (Cleaning includes brooming/vacuum cleaning of dust related to the production machinery or premises and handling of the removed dust) (please, tick one box per line, tick "0" if no time spent)	Time (days) per week on average				
	0	½ Day or less	½-1 Day	More than 1 Day, but less 2 days	2 days or more
Cleaning, raw-meal preparation (mills included)	☐	☐	☐	☐	☐
Cleaning, clinker production	☐	☐	☐	☐	☐
Cleaning, cement or final product (mills included)	☐	☐	☐	☐	☐
Cleaning of ventilation/filters in the production	☐	☐	☐	☐	☐
Cleaning of Lepol grates	☐	☐	☐	☐	☐
Cleaning of by-pass filter in precalciner	☐	☐	☐	☐	☐
Cyclone clogging removal	☐	☐	☐	☐	☐
Dismantling before re-lining cyclone/kiln/cooler	☐	☐	☐	☐	☐
Dismantling before repair work	☐	☐	☐	☐	☐
Handling of alternative solid fuels	☐	☐	☐	☐	☐
Handling of alternative liquid fuels	☐	☐	☐	☐	☐
Welding or sheet metal work	☐	☐	☐	☐	☐
Other repair work (not included cleaning)	☐	☐	☐	☐	☐

Please check that all relevant questions have been answered. Thank you for your kind cooperation!

Appendix 1: Exposure sampling log, p 1-2.

Prospective monitoring of exposure and lung function among cement workers

Family name		Date of birth
Given name		
Exposure Sampling Log – To be completed for each measurement		ID-code □-□□□

Revision Jan 6, 2009

1 Sampling data Male □ Female □

Today's Date (dd.mm): □□ - □□ - 2009

Name of the shift (if applicable)		Filter cassette S09-	□□□□
Rotameter reading at start sampling (thickest part of floater)	□□ . □	Cyclone #	□□□
Time at start sampling (hh-mm)	□□ : □□	Rotameter #	□□□
Time display at start sampling (on SKC-pumps only)	□□□	Pump #	□□□
Rotameter reading at end sampling (thickest part of floater)	□□ . □	Battery #	□□□
		Pump type	
Time at stop sampling (hh-mm)	□□ : □□	Time is recorded when the person leaves for the shift and upon return to give back the equipment, using both point of time (reading the clock) and the display reading of the pump	
Time display at stop sampling (on SKC-pumps only)	□□□		

For the PS-101 pump, which has no display, just disregard the space for display readings.

2 **Today's work** – to be completed at end of each work shift when the worker is carrying measurement equipment

Job type	Production □ Plant cleaning and yard □ Maintenance □ Foreman □ Management and Administration □ Laboratory □ Other □(please specify.....)

Note: Production including package/shipping, Management and administration including engineers.

	hour - minute
3 Today's work shift normally starts	□□ - □□
4 Today's work shift normally ends	□□ - □□

5 How were the conditions regarding **dustiness** of your work today:	As usual □	Better than usual □	Worse than usual □

6 Have you used respiratory protective equipment (a respirator) at work **today**?	No □
	Yes, Occasionally □
	Yes, Most of the time □
	Yes, All the time □

7 Plant operations today:

Normal □ Problems affecting exposure measurements □

8 During **today's work,** please indicate the time spent working with each task category (Please, indicate time spent today, if any)	Time spent in each category in Minutes **or** Hours					
	Time spent, but < 15 minutes	16-29 minutes	30-59 minutes	1-2 hours	Between 2 and 5 hours	5 or more hours
Quarry	☐	☐	☐	☐	☐	☐
Production (mobile sites, not in control room)	☐	☐	☐	☐	☐	☐
Control room	☐	☐	☐	☐	☐	☐
Packing & Shipping	☐	☐	☐	☐	☐	☐
Control Laboratory	☐	☐	☐	☐	☐	☐
Electrical Maintenance	☐	☐	☐	☐	☐	☐
Mechanical Maintenance	☐	☐	☐	☐	☐	☐
Storehouse	☐	☐	☐	☐	☐	☐
Safety & Environment	☐	☐	☐	☐	☐	☐
Oiling or greasing	☐	☐	☐	☐	☐	☐
Other production-related tasks	☐	☐	☐	☐	☐	☐
Administration	☐	☐	☐	☐	☐	☐
Time spent absent from work (sick leave, other leaves, waiting time)	☐	☐	☐	☐	☐	☐

9 Time spent **today** on **indicated tasks**. Give approximate time in hours and/or minutes in the boxes of tasks that you did today. Leave other boxes empty. (Cleaning includes brooming and vacuum cleaning of dust related to the production machinery or premises and handling of the removed dust)	Hours-minutes
Cleaning, raw-meal preparation (mills included)	☐☐ - ☐☐
Cleaning, clinker production	☐☐ - ☐☐
Cleaning, cement or final product (mills included)	☐☐ - ☐☐
Cleaning of ventilation/filters related to the production	☐☐ - ☐☐
Cleaning of Lepol grates	☐☐ - ☐☐
Cleaning of by-pass filter in precalciner	☐☐ - ☐☐
Cyclone clogging removal	☐☐ - ☐☐
Dismantling before re-lining cyclone/kiln/cooler	☐☐ - ☐☐
Dismantling before repair work	☐☐ - ☐☐
Handling of alternative solid fuels	☐☐ - ☐☐
Handling of alternative liquid fuels	☐☐ - ☐☐
Welding or sheet metal work	☐☐ - ☐☐
Other repair work (not including cleaning)	☐☐ - ☐☐

Please check that all relevant questions have been answered. Thank you for your kind cooperation!

Operator filling in the exposure log data (initials).....

I

II

III

A cross-shift study of lung function, exhaled nitric oxide, and inflammatory markers in blood in Norwegian cement production workers

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Word count 4474

Word count abstract 280

Number of figures and tables one figure, four tables

ABSTRACT

Objectives: To study possible effects of aerosol exposure on lung function, fractional exhaled nitric oxide (FeNO), and inflammatory markers in blood from Norwegian cement production workers across one work shift (from 0 to 8 hours (h)), and again 32 h after the non-exposed baseline registration.

Methods: Ninety-five workers from two cement plants in Norway were included. Assessment of lung function included spirometry and gas diffusion pre- and post-shift (0 and 8 h). FeNO concentrations were measured and blood samples collected at 0, 8, and 32 h. The blood analysis included cell counts of leucocytes and mediators of inflammation

Results: The median respirable aerosol level was 0.3 mg/m^3 (range 0.02–6.2 mg/m^3). FEV_1, $FEF_{25–75\%}$ and DL_{CO} decreased by 37 mL ($p = 0.04$), 170 mL/s ($p < 0.001$), 0.17 mmol/min/kPa ($p = 0.02$), respectively across the shift. A reduction of FeNO between 0 and 32 h of 2 ppm ($p = 0.01$) was detected. The number of leucocytes increased by 0.6×10^9 cells/L ($p < 0.001$) across the shift while fibrinogen levels increased by 0.02 g/L ($p < 0.001$) from 0 to 32 h. The tumour necrosis factor-α level increased, and interleukin-10 decreased across the shift. The baseline levels of fibrinogen were associated with the highest level of respirable dust, and increased by 0.39 g/L (95% CI 0.06–0.72).

Conclusions: We observed small cross-shift changes in lung function and inflammatory markers among cement production workers indicating that inflammatory effects could possibly occur at exposure levels well below 1 mg/m^3. However, because the associations between these acute changes and personal exposure measurements were weak and while the long-term consequences are unknown the findings should be tested in a follow-up study.

INTRODUCTION

The raw materials needed for the production of cement are mainly limestone and sources of silica, aluminium, and iron. These are quarried, crushed, and milled to a raw meal, which is heated in a kiln to approximately 1450 °C to form clinker (cement base). The clinker is milled together with calcium sulphate and other additives to produce cement of different qualities.

A substantial number of cross-sectional studies have found associations between exposure and adverse respiratory health effects in cement production workers.[1-7] However, other studies do not show such associations.[8, 9] Most studies have limitations because of their cross-sectional design, selection bias, and sparse quantitative exposure data.

In addition to the cross-sectional studies, there are two cross-shift studies that show acute effects among cement production workers. Reductions in forced expiratory volume in 1 s (FEV_1), FEV_1/forced vital capacity (FVC), and forced mid-expiratory flow rate $(FEF)_{25–75\%}$ across a shift were observed among Saudi Arabian workers [10] and a cross-shift decrease in peak expiratory flow (PEF) was demonstrated in a Tanzanian study.[11] Both studies were conducted in workers exposed to respirable aerosol levels between 7 and 15 mg/m^3, which are well above the present occupational exposure limit (OEL) of 5 mg/m^3 in most European countries. Hence, they do not provide information about health effects at lower levels of exposure.

Few studies contribute to the elucidation of the underlying physiological mechanisms involved in cement-induced respiratory effects. Irritation of mucus membranes because of the alkaline properties of cement dust (wet cement has a pH of about 12) and the possibility that other content particles (quartz, chromium) cause inflammation have been suggested. In a recent experimental study, cement dust was found to activate macrophage tumour necrosis factor (TNF)-α production in rat alveolar macrophages.[12] We have previously observed an increase

in the proportion of neutrophils and levels of interleukin (IL)-1β in induced sputum samples from cement production workers.[13] However, information on the effects of assessed exposure on gas diffusion capacity (DL_{CO}), fractional exhaled nitric oxide (FeNO), or inflammatory markers in blood is lacking.

To study further the acute effects associated with cement dust exposure, we aimed to investigate possible cross-shift changes in lung function variables, FeNO, and inflammatory markers in peripheral blood. Personal aerosol levels were obtained in order to analyse associations between exposure and effect.

MATERIALS AND METHODS

Design

Workers from two cement production plants in Norway were examined before and after a shift of exposed work, during winter conditions in 2008 and 2009. At baseline, each worker was required to be off work for at least two days. The workers were exposed to cement production dust between 0 and 8 h, and again between 24 and 32 h. The participants underwent spirometry, gas diffusion, FeNO, and blood sampling at baseline (0 h) and after a work shift (8 h). In addition, a third examination was performed consisting of FeNO measurements and blood sampling 32 h after baseline in order to study possible delayed effects. Only non-smokers (defined as never-smokers or ex-smokers who had stopped smoking at least one year before the examination) underwent FeNO sampling.

After completion of the health examinations at baseline, each worker carried a back-pack containing equipment for personal exposure measurements. The sampling cassettes collected respirable, thoracic, and inhalable aerosol fractions and were mounted on the shoulder

straps as close to the mouth as possible. To minimise bias caused by their position, samplers were carried in front of either the right or the left shoulder in a random pattern. At the end of the shift, the equipment was removed and a questionnaire on work tasks performed was completed before the workers underwent the second health examination. In this study, it was not feasible to collect aerosol measurements on the second day (between 24 and 32 h).

Subjects

Exposed workers from the production and maintenance departments were identified from the company's register and invited to participate in the study. The eligible workers comprising of 144 subjects (5% females) were offered appointments for health examinations and exposure measurements. Of the 124 workers present on examination days 95 (7% females) were willing to participate and included in the study. The participation rate was 66%. The inclusion and exclusion of workers are shown in figure 1 and the population characteristics are given in table 1.

Table 1 Population characteristics given as mean (standard deviation), duration of exposure and smoking status of cement production workers

n	95
Age, yr	41 (13)
Height, cm	179 (7)
BMI*	28 (4)
Duration of cement aerosol exposure, yr	16 (13)
Smoking Status (%)	
Never-smokers	36
Ex-smokers	23
Smokers**	41

*BMI, Body mass index; **Including current smokers (n=37) and ex-smokers who stopped less than one year before the examinations (n=2).

The eligible workers received verbal and written information and informed consent was obtained from all the participating subjects.

All included workers completed the investigation at baseline and at 8 h, but two workers did not attend for the third investigation (32 h). Spirometry was performed for all workers, but because of technical problems, the spirometry or gas diffusion tests could not be performed for three of the workers at 8 h. One worker did not meet the American Thoracic Society/European Respiratory Society (ATS/ERS) criteria for spirometry or gas diffusion [14, 15] and was excluded from the cross-shift analysis of lung function tests. Blood samples were not obtained on every occasion from eight subjects and for one subject we did not obtain any blood sample. The workers were asked: Was your allergy confirmed by a physician? Was your asthma confirmed by a physician? If the answer was positive to one ore more of these questions they were defined as having doctor-diagnosed allergy or asthma.

Among the workers who did not want to participate in the study, two subjects had a known diagnosis of COPD and one of asthma. In the same group, eight workers had administrative jobs and were expected to have very low exposure or not to be exposed at all. Fourteen of the non-participants were non-smokers and 12 were smokers at the time of the measurements. We had no information on the smoking habits of three non-participating workers, but the smokers tended to be more heavily exposed to tobacco smoke than those included in the study.

Exposure assessment

The production of cement generates aerosols by mechanical and condensation processes and particle size ranges from ultra fine to above the inhalable. The workers in both departments reported day-to-day variation of work tasks and perceived exposure and that the use of respiratory equipment varied between individuals and tasks. Hence, the use of

respirators was registered in order to allow comparison of outcomes between those who used a respirator and those who did not. The workers had access to Airstream-, P2- and P3-respirators, but we did not have information on which of these respirators the workers selected during this particular shift. The workers used respirators made by different manufacturers. The exposure measurements were performed outside the respirators.

The inhalable aerosol fraction that contains particles that enter the nose and mouth (< 100 μm aerodynamic diameter, dae) was collected with the IOM inhalable dust sampler (SCK, Blandford Forum, Dorset, UK) equipped with a 25 mm cellulose-ester membrane filter with pore size 5 μm (SMWP02500 Millipore, Billerica, USA) at a flow rate of 2.0 L/min. The thoracic fraction contains particles that pass the larynx (50% cut-off at dae = 10 μm) and was collected with the BGI GK 2.69 respirable/thoracic sampler (BGI, Waltham, Massachusetts, USA) with 37 mm polyvinyl chloride (PVC) filters with pore size 5 μm (Millipore, SKC and Pall Inc.) at a flow rate of 1.6 L/min.

The respirable fraction (50% cut-off at dae = 4 μm) that enters the alveoli [16] was collected by the respirable cyclone (Cassella Inc., Amherst, USA) with 37 mm PVC filters with pore size 5 μm at a flow rate of 2.2 L/min.

Lung function tests

The lung function testing was performed in accordance with ATS/ERS Guidelines [14, 15] using the Jaeger Master Screen PFT (Erich Jaeger GmbH & Co. KG, Würzburg, Germany). The same investigator (AKMF) performed all lung function measurements. The workers were given standardised instructions on the forced maximal expiratory manoeuvres and the transfer factor for the carbon monoxide (DL_{CO}) test, with demonstration of the procedures. The tests were performed with the subject seated, breathing through the mouthpiece with a nose clip. The spirometer was calibrated with a 3 L syringe and test gas calibrations were performed using the

instrument's automatic calibration programme. Both calibrations were performed daily. The best result, according to ATS/ERS criteria, of at least three manoeuvres of flow-volume measurements was used in the analysis. FVC, FEV_1, $FEF_{25–75\%}$, and forced expiratory flow rates at 25%, 50%, and 75% of FVC expired ($FEF_{25\%}$, $FEF_{50\%}$, $FEF_{75\%}$) and PEF were measured.

Two measurements of DL_{CO} were taken on each occasion and the average of the two results was used in the analysis. Effective alveolar volume was measured simultaneously by helium dilution and the gas transfer per unit effective alveolar volume (K_{CO}) was calculated. The lung function measurements were performed before and after the work shift. Age, height, smoking habits, and weight were registered. The lung function testing was performed subsequent to the exposure assessments, blood sampling and FeNO measurements to allow adjustment to indoor temperatures.

Fractional exhaled nitric oxide

FeNO in exhaled air was measured according to the ATS/ERS criteria [17] using the NIOX MINO (Aerocrine AB, Solna, Sweden). This device provides FeNO measurements at 50 mL/s exhalation flow rate, expressed in parts per billion (ppb) using an electrochemical sensor. The accuracy range of the NIOX MINO device is ± 3 ppb for measured values < 30 ppb and 10% of the measured value for values > 30 ppb, expressed as standard deviation of ten consecutive measurements. The measurements were performed before and after the work shift and again 32 h after baseline. Subjects were advised not to consume food or beverages 1 h before the measurements. Only non-smokers were selected for FeNO testing and the measurements were performed before the lung function measurements.

Assessment of blood parameters

Blood samples were collected at 0, 8, and 32 h in vacuum tubes containing citrate or EDTA as anticoagulant or containing no additives (serum). The citrate and serum tubes were centrifuged at 1400 × *g* for 10 minutes. Plasma or serum was then aspirated and aliquoted into 1.5 mL Eppendorf polypropylene cryotubes within 1 h. The plasma and serum tubes were stored at –80 C° until analysed. Leucocytes were analysed in EDTA blood samples within 48 h (in accordance to the instructions from the laboratory) using the Sysmex haematology system (Sysmex Europe GmbH; Hamburg, Germany) at the Oslo University Hospital, Ullevaal, Oslo, Norway. The time period between collection of blood samples and analysis at the laboratory was approximately the same for the two plants due to similar time used for transportation.

Quantitation of human serum C-reactive protein (hsCRP) was performed using a high sensitive (hs) immunoturbidimetric assay on a Hitachi 917 Automatic Analyzer (Roche® Diagnostics, Germany). The inter-assay variation (coefficient of variation, CV) was 5%. The fibrinogen concentration in citrate plasma samples was analysed using a clotting test on the STA-R Evolution (Diagnostica Stago, Asnières-sur-Seine, France). The inter-assay variation (CV) was 4%. D-dimer citrate plasma samples were analysed using an immunoturbidimetric method on the STA-R Evolution. The inter-assay variation (CV) was 3%.

The serum samples were analysed for cytokines using a microsphere-based multiplexing bioassay system with Xmap technology (Luminex Corporation, USA). TNF-α, IL-1β, IL-6, IL-8, and IL-10 were analysed using the Bio-Plex Human Group 1 assay 6-plex (Bio-Rad, USA). Analysis was performed according to the instructions from the manufacturer. The inter-assay variations (CV %) were calculated from supernatant aliquots (n=8) of LPS exposed human monocytes, stored at -80^{o}C; TNFα 12%, IL-1β: 8%, IL-6: 12%, IL-8: 16% and IL10: 15%. The

detection limits were set as the lowest standard in each assay; TNFα: 0.16 pg/ml, IL-1β: 0.06 pg/ml, IL-6: 0.18 pg/ml, IL-8: 0.04 pg/ml and IL10: 0.16 pg/ml.

Statistical methods

Student's paired *t*-test was used to compare normally distributed continuous outcomes (the cross-shift changes in lung function indices). The Wilcoxon signed-rank test was used to compare the FeNO and inflammatory markers cross-shift. The Spearman rank test was used for correlations.

Independent variables considered to be biological important cofactors were included in the linear regression models. In addition, we included job task and location (plant 1 and 2) based on assumptions of differences in exposure levels. The cross-shift difference and the pre-shift level (8 h) of the health outcomes were analysed as dependent variables. Sex, age, height, body mass index (BMI), location (plant 1 or 2), report of doctor-diagnosed asthma, upper respiratory infection during the preceding three weeks, work tasks, life dose of tobacco (in kg) as well as the tertiles of the exposure levels (low, medium and high level as dummy variables compared to the lowest level) were included as independent variables for the lung function analysis. For the inflammatory markers, the above-mentioned independent variables, except for height and report of doctor-diagnosed asthma, were used in the regression model. Skewed variables were log-transformed in order to obtain acceptable linear regression models.

The study was designed with a power of 80% to detect a true difference of 1.5% between FEV_1/FVC measured at two time points and a change in cytokine levels of 0.8 ng/L at a 5% significance level. At least 90 subjects were needed. Statistical analysis was performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Exposure

Production workers performed inspection rounds throughout the plant, participated in solving problems which occurred during production (such as cleaning dust spills, minor mechanical work and opening up clogged production equipment) and also performed tasks in the control room. The maintenance workers maintained production equipment throughout the plant and inside their workshops. Measurements of the respirable, thoracic, and inhalable aerosol fractions from the two cement producing plants are presented in table 2.

Table 2 Exposure measurements by job category, and the values for workers using respirator compared to those without respirators

		Inhalable aerosol mg/m^3			Thoracic aerosol mg/m^3			Respirable aerosol mg/m^3		
	n	AM	GM	GSD	AM	GM	GSD	AM	GM	GSD
Job category										
Production	37	5.2	2.3	4.5	0.65	0.32	3.6	0.37	0.20	3.4
Electric. maintenance	9	5.5	2.7	3.6	0.59	0.37	2.7	0.24	0.19	2.2
Mechanic. maintenance	27	7.7	3.5	4.0	1.2	0.59	3.2	0.73	0.33	3.5
Laboratory	3	0.91	0.65	3.2	0.20	0.14	3.3	0.11	0.10	2.0
Other	19	7.8	4.0	4.3	1.1	0.52	4.2	0.46	0.31	2.6
Total	95	6.3	2.8	4.3	0.88	0.42	3.6	0.47	0.24	3.2
Plant 1	39	5.2	2.5	3.8	0.62	0.30	3.5	0.36	0.21	3.0
Without respirator	18	1.4	0.90	2.8	0.16	0.11	2.4	0.13	0.088	2.3
With respirator	21	8.4	6.2**	2.2	1.1	0.73**	2.1	0.56	0.46**	1.9
Plant 2	56	7.1	3.0	4.7	1.1	0.52†	3.5	0.55	0.27	3.2
Without respirator	43	4.6	2.5	3.7	0.60	0.40	2.8	0.32	0.21	2.5
With respirator	13	15	5.6	8.0	2.5	1.3*	4.4	1.3	0.58*	4.8

†Significantly different from plant 1 p=0.04

*Significantly different from the category above p<0.05, **p<0.001

Six inhalable, four thoracic and eight respirable samplers did not obtain valid measurements of aerosol; the most important reason was pump failure. The missing values were substituted with values predicted by linear regression models of the three aerosol fractions using the other as independent variables. The regression equation for the thoracic fraction was: Log (thoracic value) = 0.291 + 1.01 log (respirable value) - 0.12 (factor for plant1). For the inhalable fraction: log (inhalable value) = 0.82 + 0.96 log (thoracic value) and for the respirable fraction; log (respirable value) = -0.32 + 0.79 log (thoracic value). The variance explained by the regression models were 77-83%. The aerosol weight of samples was corrected with field blanks that had been weighted in the same day. When the weight was below the detection limit, the actual observed weight was used in the data analysis, and values of 0 or less were replaced by the lowest observed positive value within the job group divided by 2. Two of the samples were below the results of the field blanks.

The thoracic aerosol fraction was significantly higher in Plant 2 than in Plant 1, whereas there was no difference in the inhalable or respiratory fractions. In Plant 1, 54% of the workers had been using respirators while in Plant 2, 23% had done so. Of the thirty-four workers reporting use of respirators 13 workers reported occasional use, 12; most of the time and 9; all the time. The aerosol concentrations were higher for those using respirators (table 2). The median respirable aerosol as a fraction of the inhalable was 10% and 8%, respectively, for Plants 1 and 2. The thoracic aerosol as a fraction of inhalable was 15% for Plant 1 and 18% for Plant 2.

Health effects

Lung function

We detected a decrease of 37 mL ($p = 0.04$) and 170 mL/s ($p < 0.001$), respectively, in FEV_1 and $FEF_{25–75\%}$ during the shift. There was a decrease in the gas diffusion capacity of 0.17

mmol/min/kPa (p = 0.02) across the shift. Selected lung function and gas diffusion indices are presented in table 3.

Table 3 Selected lung function variables pre- and post shift (0 and 8 h) in cement production workers, in non-smoking workers and in those without doctor-diagnosed asthma or allergy.

Parameter	Pre-shift (n= 91) Mean (SD)	Post-shift (n= 91) Mean (SD)	Cross shift-change All workers (n=91) Mean (95% CI)	Cross-shift change No allergy/asthma (n=70) Mean (95% CI)	Cross-shift change Non-smokers (n=56) (Mean, 95% CI)
FVC (L)	5.02 (0.91)	4.99 (0.97)	- 0.032 (-0.066, 0.0016)	-0.041 (-0.080, -0.0023)*	-0.050 (-0.086, -0.015)*
FEV_1 (L)	3.92 (0.81)	3.88 (0.81)	-0.037 (-0.072, -0.0017)*	-0.046 (-0.086, -0.0063)*	-0.042 (-0.075, -0.009)*
FEV_6 (L)†	4.98 (0.93)	4.95 (0.97)	-0.034 (-0.070, 0.0088)	-0.046 (-0.087, -0.0049)*	-0.053 (-0.090, -0.017)*
FEV_{1}/FVC	78.1 (7.39)	78.0 (7.06)	-0.15 (-0.69, 0.37)	-0.20 (-0.79, 0.40)	-0.034 (-0.58, 0.51)
PEF (L/s)	9.68 (1.69)	9.73 (1.82)	0.056 (-0084, 0.20)	0.053 (-0,11, 0.21)	-0.0064 (-0.19, 0.18)
$FEF_{25-75\%}$ (L/s)	3.39 (1.31)	3.22 (1.30)	-0.17 (-0.26, -0.081)**	-0.18 (-0.28, -0.070)**	-0.15 (-0.24, -0.057)*
DLco (mmol/min/kPa)	10.7 (2.1)	10.5 (2.0)	-0.17 (-0.32, -0.023)*	-0.052 (-0.11, 0.23)	-0.25 (-0.46,- 0.04)*
Kco (mmol/min/kPa/L)	1.57 (0.26)	1.55 (0.26)	-0.02 (-0.044, -0.010)*	-0.0041(-0.027, 0.019)	-0.026 (-0.056, 0.0035)
VA (L)	6.85 (1.06)	6.85 (1.10)	0.0037 (-0.061, 0.069)	0.020 (-0.058, 0.098)	-0.015 (-0.10, 0,072)

*Significantly changed from before work shift $p < 0.05$; **$p < 0.001$

†n=89

No associations between the changes in lung function variables and exposure were observed. This was the case for the whole group of workers and also when those who did not use respiratory protection were analysed separately.

FeNO and blood parameters

There was a decrease of 2 ppm ($p = 0.008$) in FeNO between baseline values and those at 32 h after baseline. Furthermore, a significant cross-shift increase in white blood cells of 0.6×10^9 cells/L ($p < 0.001$) was detected while fibrinogen levels increased by 0.02 g/L ($p < 0.001$) from baseline to 32 h. The TNF-α level increased, whereas IL-10 decreased across the shift. Thereafter, there was a decrease in all inflammatory markers except IL-10. The levels of inflammatory markers and FeNO at 0, 8, and 32 h are shown in table 4.

Table 4 Blood parameters and FeNO levels pre-and post-shift (0 and 8 h) and at 32 h

Parameter	n	0 hours Median (Range)	n	8 hours Median (Range)	n	32 hours Median (Range)
FeNO (ppb)	58	14 (0 to 96)	58	14 (0 to 98)	55	12 (0 to 82)†
Leucocytes (10^9/L)	86	7.4 (4.8 to 13.5)	86	8.0 (4.8 to 13.6)**	83	7.2 (4.3 to 14.3)**
CRP (ng/L)	93	1.58 (0.26 to 16.38)	93	1.53 (0.19 to 12.79)	90	1.87 (0.27 to 18.94)*
Fibrinogen (g/L)	86	3.09 (1.68 to 5.13)			86	3.11 (2.26 to 5.08)**
D-dimer (ng/L)	86	0.29 (0.22 to 1.04)			86	0.29 (0.22 to 1.04)
IL-1β (ng/L)	88	0.25 (0.09 to 7.00)	88	0.23 (0.09 to 3.51)	88	0.20 (0.09 to 3.51)*
IL-6 (ng/L)	88	3.15 (0.35 to 25.40)	88	3.30 (0.32 to 13.33)	88	2.80 (0.98 to 23.94)*§
IL-8 (ng/L)	88	7.61 (3.57 to 63.23)	88	6.82 (1.70 to 59.30)	88	6.21 (1.63 to 65.10)*§
IL-10 (ng/L)	88	1.12 (0.18 to 10.78)	88	0.91 (0.09 to 6.45)**	88	0.97 (0.10 to 4.55)§
TNF-α (ng/L)	88	5.47 (0.00 to 39.50)	88	6.17 (0.47 to 69.53)*	88	5.51 (1.26 to 53.48)

*Significantly different from previous time point $p < 0.05$; **$p < 0.001$

†Significantly different from 0 h $p < 0.05$; §$p < 0.001$

The pattern of changes in inflammatory markers remained unchanged when those without doctor-diagnosed allergy or asthma were analysed separately. This was also the case for non-smokers.

There was a positive correlation between the differences (0 to 32 h) in fibrinogen and hsCRP ($r = 0.48$, $p < 0.001$). In a multiple linear regression model, the 0 h level of fibrinogen

was associated with the highest respirable aerosol level (> 0.4 mg/m^3), and increased by 0.39 g/L (95% CI 0.06–0.72). There were no associations between the cross-shift changes of the inflammatory markers and the exposure variables for either the whole group of workers or when stratified for the variable regarding use of respiratory protection.

DISCUSSION

We observed a cross-shift reduction in FEV_1, $FEF_{25–75\%}$, DL_{CO} and FeNO levels, an increase in white blood cells and fibrinogen levels, together with elevated TNF-α levels and decreased IL-10 in low level-exposed cement production workers. No positive correlations between the cross-shift changes in lung function, FeNO, or inflammatory markers and the measurements of personal exposure levels were detected.

Our finding of a cross-shift decrease in lung function indices agrees with the results of two earlier cross-shift studies among cement production workers exposed to higher levels of dust, which showed a reduction of FEV_1, FEV_1/FVC, $FEF_{25–75\%}$, and/or PEF.[10, 11] Cross-shift studies are of particular interest because in occupationally exposed groups, a longitudinal decrease in lung function seems to be associated with a cross-shift reduction in these values.[18, 19] We did not detect an association between the changes in spirometric indices and individual exposures measured in this study. Nevertheless, because diurnal variation in spirometry in healthy, non-exposed subjects indicates an increase during the first 6–8 h of time awake,[20] the observed reduction in spirometric indices could possibly be associated with job tasks, peak exposure or also with other unknown conditions not measured in our study.

A cross-shift reduction in the gas diffusion capacity (assessed as DL_{CO} and K_{CO}) was observed. There are no prior studies on gas diffusion capacity among workers in this industry, but reduced gas diffusion capacity has been shown in other industries with dusty

environments.[21] A possible mechanism of a decrease in DL_{CO} could be that a fraction of the aerosol small enough to reach the alveoli interacts with the alveolocapillary function and thereby reduces gas diffusion capacity. Another possibility is that exposures other than cement production dust, such as inhalation of carbon monoxide from vehicles or machines, could blockade the haemoglobin molecules and thereby lower the gas diffusion capacity. However, until other studies on gas diffusion capacity have been reported among these workers, this will remain speculation.

The changes in lung function were not accompanied by cross-shift changes in FeNO levels, but a small significant decrease was observed when baseline values were compared with those measured at 32 h. Reduced FeNO levels are observed in smokers.[22, 23] Our finding could possibly indicate a similar response, but because the observed changes are minor and because we did not have measurements at 24 h, this finding needs to be confirmed by others.

There was an increase in the number of leucocytes across the shift. In light of this observation, one could suspect that exposure to cement dust may cause an increase in neutrophil activity. This would be in agreement with our previous findings of an increased proportion of neutrophils and levels of IL-1β in induced sputum from these workers.[13]

It has been shown that inhalation of very fine dust from air pollution and from occupational exposures can induce release of mediators that may influence blood coagulation.[24-28] Thus, the observed increase in fibrinogen and hsCRP levels at 32 h accompanied by a positive correlation between the differences in fibrinogen and hsCRP could indicate an effect on blood coagulation among the workers. This finding could be induced by workplace exposure or perhaps more likely a result of diurnal variation. Still, fibrinogen levels show low biological variability and the highest values in healthy, non-exposed subjects are recorded in the late morning.[29] However, there was no change in the level of D-dimer and

the observed difference in fibrinogen is small. Hence, the observed increase in fibrinogen levels should be interpreted with caution.

We observed an increase in TNF-α and a decrease in IL-10 levels across the shift. The diurnal rhythmicity of the pro-inflammatory cytokine TNF-α show production peaks in the early morning with a subsequent fall during the day,[30] whereas the anti-inflammatory cytokine IL-10 peaks during daytime.[31] It is not clear if our finding of an inverse pattern, represents an inflammatory response or if it is only a marker of exposure. The finding agrees with results from studies of cytokine levels in bronchial epithelial cells after exposure to cigarette smoke. [32, 33] But, the changes are small and because no associations to exposure were detected it will remain unclear if this finding represents a true response.

Our data show that levels of the pro-inflammatory cytokines IL-1β, IL-6, and IL-8 decreased during the observation period. These findings, together with the FeNO results, are probably indicative of no or low inflammatory activity. A decrease in cytokine levels could also possibly occur if the workers had higher levels of physical activity during the work shift than during the preceding period of rest, as shown in studies of healthy, non-exposed subjects.[34, 35] However, it is noted that the analysis of FeNO and inflammatory markers in our study is based on the examination of effects at three different time points only and it seems possible that other or additional time points (e.g. 4h and 24h) could have revealed other patterns of response and made interpretation of these findings easier.

The changes in lung function indices and inflammatory markers across the shift were similar in those without doctor-diagnosed allergy or asthma as compared to the whole group of workers. In non-smokers the changes in spirometric indices were somewhat larger than in the whole group while the changes in inflammatory markers were comparable. These findings indicate that the observed effects probably can not be explained by allergy or smoking alone.

However, a decrease in the DL_{CO} level of 0.60 (95% CI 0.29, 0.91) mmol/min/kPa among those with allergy or asthma was demonstrated while there was no change among those without these conditions. This could possibly indicate that the changes in gas diffusion could be influenced by subjects with hyperresponsive airways.

The personal sampling of aerosol concentration allows correlation and linear regression analyses with exposure as a continuous variable. Surprisingly, no correlations were observed between exposure and cross-shift changes in the outcome variables. This was also the case when those using respirators and those who did not were analysed separately. The respirable aerosol level among the exposed workers in this study was well below the Norwegian OEL (respirable aerosol, 5 mg/m^3), as was the thoracic fraction. In addition, the exposure measurements showed that the particle size of the aerosols in the measured periods for both plants was mostly inhalable and therefore will deposit in the upper respiratory system. Thus, other descriptors of exposure such as the chemical composition of the aerosol at different locations of the plant and peak exposures could be of importance and should be considered for inclusion in further studies. Furthermore, regression analysis showed that in the workers with the highest levels of exposure, the pre-shift level of fibrinogen was associated with exposure, indicating that previous exposure could also be of importance.

It is possible that individuals who are susceptible to adverse effects from cement aerosols had left the cement industry, leaving only robust subjects in the work-force to be included in the study. If this were the case, we would have underestimated the inflammatory effects of exposure. However, because workers are used as their own controls in this study, selection bias is probably less important.[36] To reduce bias related to the collection of data, all lung function tests were performed by one researcher. Standard instructions were followed for spirometry, lung diffusion, and FeNO and the blood samples were analysed by individuals blinded to exposure information.

Confounders that may not be controlled adequately or adjusted for in the analysis could include unknown respiratory irritants outside the workplace, especially in the period before the first health measurements were performed. It is also possible that a greater cross-shift change in lung function, FeNO, or in levels of inflammatory markers would have occurred if the period of non-exposure before baseline had been extended.

We observed a higher prevalence of smokers among the non-participants than among the included subjects. This is not considered to be a limitation of the study because it is likely that already existing tobacco smoke-related inflammatory effects among these workers would have made detection of effects from the cement production aerosol difficult, resulting in an underestimation rather than in an overestimation of effects.

It is not clear whether the cross-shift changes in lung function indices and inflammatory markers as observed in this study represent an early stage of inflammation leading to respiratory disease or whether they represent an appropriate immune response without clinical consequences. But, until follow-up studies are completed and interpreted in relation to these questions, we recommend a reduction of exposure for the workers with the highest exposure levels and that spirometric surveillance is carried out at regular intervals.

In conclusion, we observed small but significant cross-shift reductions in FEV_1, $FEF_{25-75\%}$, DL_{CO} and FeNO levels corresponding with increased numbers of leucocytes, elevated levels of fibrinogen and TNF-α and decreased levels of IL-10 in low level-exposed cement production workers. Because the correlations to exposure were weak and while the long-term consequences of these acute changes in lung function indices and inflammatory mediators are unknown, the hypothesis that low-grade cement aerosol exposure causes airway disease should be tested in a follow-up study.

Acknowledgements We are grateful to the participants from the Norcem AS cement plants in Brevik and Kjøpsvik, Norway. We thank Harald Evenseth for help with the collection of exposure data.

Competing interests None

Funding The study was supported by a grant from the South-Eastern Norway Regional Health Authority and from The European Cement Association (Cembureau).

Ethics approval The National Committee for Medical and Health Research Ethics approved the protocol.

Figure legend

Figure 1 Flow chart for the inclusion and exclusion of cement production workers.

*Lung function testing

**Workers who did not wish to participate 32 h after baseline

What this paper adds

- There are no former studies of gas diffusion capacity, fractional exhaled nitric oxide (FeNO), or inflammatory markers in blood among cement production workers.
- We observed a cross-shift reduction in FEV_1, $FEF_{25–75\%}$, and in FeNO levels, an increase in white blood cells and fibrinogen levels, together with augmented TNF-α levels and decreased IL-10 in low level-exposed cement production workers.
- Because the correlations to personal exposure measurements were weak and while the long-term consequences of these acute changes in lung function indices and inflammatory mediators are unknown, the hypothesis that low-level cement aerosol exposure causes airway disease should be tested in a follow-up study.

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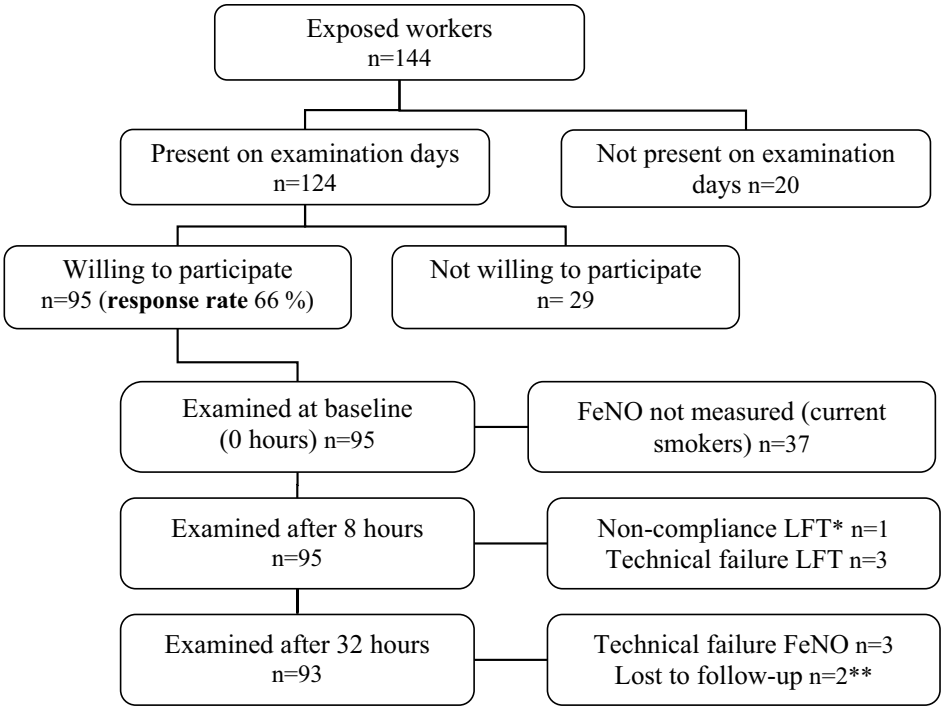
Exposed workers
n=144
Present on examination days
n=124
Not present on examination days n=20
Willing to participate
n=95 (**response rate** 66 %)
Not willing to participate
n= 29
Examined at baseline
(0 hours) n=95
FeNO not measured (current smokers) n=37
Examined after 8 hours
n=95
Non-compliance LFT* n=1
Technical failure LFT n=3
Examined after 32 hours
n=93
Technical failure FeNO n=3
Lost to follow-up n=2**

IV