

**Associations between ambient air pollutants and serum
Clara cell protein concentrations among elderly men in
Oslo, Norway**

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2. ABSTRACT

Associations between ambient air pollutants and serum Clara cell protein concentrations among elderly men in Oslo, Norway

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OBJECTIVE: This study aims to investigate associations between serum Clara cell protein concentrations, among a population of elderly men in Oslo, Norway, and exposures to ambient air pollutants.

METHODS: A variety of methods have been used to study the effects of ambient air pollution on human health. Recently, a new approach referred to as, "pneumoproteinemia" was developed to evaluate the integrity of the pulmonary epithelium by measuring the concentration of lung-specific proteins in serum. The prototypic pneumoprotein is the 16-kDa Clara cell protein (CC16), an anti-inflammatory protein secreted by Clara cells in the respiratory tract and predominantly in terminal bronchioles from where it leaks into serum. This study used an enzyme-linked immunosorbent assay (ELISA) to determine serum CC16 concentrations for 1200 blood samples that were drawn during the Oslo Study II.

RESULTS: Unadjusted serum CC16 concentrations ranged from 1.2 to 115.2 $\mu\text{g/L}$ with a geometric mean of 9.4 $\mu\text{g/L}$. Statistically significant associations were found between serum CC16 concentrations and the following: age; chronic bronchitis and/or emphysema; smoking status; number of cigarettes smoked per day; and particulate matter (PM_{10} and $\text{PM}_{2.5}$) exposures (one-week before the basic clinical examination). No associations were found between serum CC16 concentrations and the following: education level; NO_2 , PM_{10} , and $\text{PM}_{2.5}$ exposures one-year before the basic clinical examination.

There was a 0.2 percent change in serum CC16 concentrations for each one $\mu\text{g/m}^3$ change in PM_{10} exposure that occurred one-week before the basic clinical examination. In addition, there was a 0.2 percent change in serum CC16 concentrations for each one $\mu\text{g/m}^3$ change in $\text{PM}_{2.5}$ exposure that occurred one-week before the basic clinical examination. These effects remained unchanged after adjusting for age, self-reported chronic bronchitis and/or emphysema, smoking status, and number of cigarettes smoked per day.

CONCLUSIONS: The findings of this study support other studies that have found evidence of associations between serum CC16 concentrations and ambient air pollutants. In addition, results of this study are consistent with other studies that have reported small but statistically significant increases in adverse respiratory effects as a result of recent PM_{2.5} and PM₁₀ exposures. Lastly, the results indicate that effects of PM_{2.5} and PM₁₀ exposures that occurred one-week before the basic clinical examination on serum CC16 concentrations were less than that of other known factors such as cigarette smoking.

Keywords: ambient air pollution, cross-sectional study, cigarette smoking, respiratory disease, serum Clara cell protein (CC16).

3. BACKGROUND AND SIGNIFICANCE

During the first half of the 20th century, the effects of ambient air pollution on human health became apparent in Europe and the United States after thousands of people were inadvertently exposed to extremely high levels of ambient air pollutants. The most severe episodes occurred in the Meuse Valley, Belgium, in 1930; Donora, Pennsylvania, in 1948; and London, in 1952 (1-3). Dramatic increases in morbidity and mortality were observed during each of the episodes. Subsequently, clean air legislation and regulatory actions led to reductions in ambient air pollution in many regions of the world. However, recent epidemiological studies that are utilizing more sophisticated research tools such as biomarkers, geospatial technology, and advanced statistical methods have identified serious health effects of ambient air pollution even at the low concentrations that are typical of most Western industrialized cities. A number of these studies, mostly conducted in Western Europe and the United States, have documented acute and chronic effects of ambient air pollutants on the human cardiopulmonary system (4). At the same time, people living in rapidly expanding megacities in Asia, Africa, and Latin America are increasingly being exposed to rising levels of ambient air pollution that rival and often exceed those experienced in industrialized countries during the first half of the 20th century (5, 6).

Recently the World Health Organization's Global Burden of Disease Initiative has estimated that ambient air pollution causes about five percent of trachea, bronchus and lung cancer, two percent of cardiorespiratory mortality and about one percent of respiratory infections mortality globally. This amounts to about 800,000 premature deaths and 6.4 million years of life lost annually, with the burden of disease occurring predominantly in the developing countries of Asia (7, 8). It is important to understand that these estimates only consider the impact of ambient air pollution on mortality, and not morbidity, due to the dearth of epidemiological data. The current body of evidence indicates that ambient air pollution is linked to increases in illness, utilization of health care services, and premature death among exposed populations. (7-12).

The aim of this thesis project is to investigate associations between serum Clara cell protein (CC16) concentrations and exposures to ambient air pollutants. Clara cell protein is a relatively new biomarker that has the potential to detect acute and chronic lung epithelium damage. First, I present a review of the relevant methods that have been used to explore the effects of ambient air pollution on human health.

Comprehensive and Critical Review of Relevant Research Methods

Historical observations of severe ambient air pollution episodes

The earliest and most methodologically simple studies evaluated daily variation in mortality induced by extreme levels of ambient air pollution. Although there have been a number of documented events where extreme levels of ambient air pollution have led to increased morbidity and mortality rates, three severe episodes during the middle of the 20th century are among the most intensively studied; Meuse Valley, Donora, and London. The observations from these historical events were mainly used to compare death counts over periods of several days or weeks before, during, and after the episodes. Each of these episodes provided valuable information for drawing attention to the hazards of ambient air pollution and for generating hypotheses. However, information from these episodes could not be used to determine cause and effect relationships or assess confounding due to the scarcity of individual level data. Even so, observations from these episodes provided indisputable evidence that extreme levels of ambient air pollution have important acute effects on human health (13). Subsequently, national and international organizations formulated air quality guidelines and standards based on information from previous episodes of severe ambient air pollution. The initial legal and other corrective measures contributed to a decrease in ambient air pollution to moderate or low levels in many Western countries (14). However, these legal and other corrective measures mainly focused on reducing air pollution by setting emission standards for stationary sources such as power plants and steel mills. They did not take into account mobile sources of air pollution, which has increasingly become a major source of ambient air pollution.

Time-series studies

In the 1970s and 1980s, time-series studies were conducted which collected daily mortality and ambient air pollution data from a single city or community for several years and analyzed correlations in the data (15). An advantage of the time-series study design is that it does not require episodes of extreme ambient air pollution, and allows for evaluation of potential mortality effects of relatively low, more typical levels of ambient air pollution. In addition, time-series studies tend to be much less expensive than

prospective cohort studies. However, time-series studies often fail to capture the chronic health effects of long-term exposure to ambient air pollution. Correlations between daily mortality and ambient air pollution have been observed, but past time-series studies have been hampered by insufficient ambient air pollution data and inadequate statistical methods. In the 1990s, results from several time-series studies that utilized more rigorous statistical modeling techniques were published (16-24). The primary statistical approach was time-series modeling of count data using Poisson regression. The results from these studies indicated a significant association between daily mortality counts and ambient air pollution, even at levels well below the established air quality standards.

Although time-series studies have revealed significant associations between elevated mortality and short-term exposure to ambient air pollution, a recently discovered programming error has called into question the results of many of these analyses (25), leaving government regulators and others wondering about the magnitude and validity of the association (26). However, results from an extensive re-analysis of a previous time-series study corroborated with results from the original analysis (27). Even so, compared with randomized controlled experimental studies in which the investigator controls the intervention, findings from time-series analyses are susceptible to uncontrolled biases and must therefore be interpreted cautiously. There have been a lot of questions and concerns about the inherent limitations of time-series studies, including: the repeatability of results; whether the observed associations are due to biased analytic approaches or statistical modeling techniques; whether associations are due to confounding because of inadequate control of long-term time trends, seasonality, weather, or some other pollutant; whether associations are biologically significant or plausible; whether statistically significant effects of ambient air pollution on human health could be observed at pollution levels well below U.S. ambient air quality standards; and whether a threshold level of ambient air pollution exists where no health effects are observed below the threshold level.

Subsequent research efforts have partially addressed some of the above questions and concerns. The results have been largely replicated by other researchers (28), and more importantly, similar associations have been observed in many other cities with very different climates, weather conditions, and pollution mixtures, as discussed in recent reviews (4, 29-34). Furthermore, increasingly rigorous and sophisticated statistical time-series modeling techniques have also been used to try to better control for potential confounders. For example, generalized additive models that use nonparametric

smoothing have allowed for highly flexible fitting of seasonality and long-term time trends as well as nonlinear associations with weather variables such as temperature and humidity (35-37). These nonparametric smoothing approaches have allowed for modeling flexible nonlinear exposure-response relationships with air pollution to explore for a no-effects threshold. A well-defined threshold has generally not been consistently observed. The exposure-response relationship between particulate air pollution and mortality has generally been near linear. Synoptic weather modeling has also been used in some of the studies (37, 38). The air pollution effects generally persisted after controlling for weather by either nonparametric smoothing of temperature and humidity or controlling for synoptic weather patterns. Moreover, previous time-series analyses have highlighted the importance of using multiple methodological perspectives in exploring the effects of ambient air pollution on human health.

Prospective cohort studies

Over the years, scientists and policymakers have shown more interest in the health effects of long-term ambient air pollution exposure. Most of the studies completed to date have used large cohorts with mortality follow-up using Cox regression modeling. Although cohort studies are often considered the Gold Standard for assessing the health effects associated with long-term ambient air pollution (39), only a handful of cohort studies have been conducted. The cohort studies that have explored the relationship between long-term ambient air pollution exposure and mortality include: the Harvard Six City cohort, the American Cancer Society cohort, the Adventist Health Study cohort, the Netherlands Diet and Cancer cohort, the Firestone clinic study, and the more recently published Norwegian health study (40-46). Collectively, these cohort studies communicate a significant, positive association between long-term exposure to ambient air pollution and mortality. Specifically, some have found statistically significant associations between ozone (42); sulphur dioxide (43, 44); or nitrogen dioxide (45, 46); PM_{2.5} (41); PM₁₀ (40) and mortality.

The cohort study design is advantageous when evidence suggests an association of a disease with a certain exposure or exposures. In addition, the cohort study design facilitates the calculation of incidence rates, minimizes recall bias, and makes it possible to study many disease outcomes simultaneously. Although powerful, many of the cohort

studies that have studied the health effects of long-term ambient air pollution exposure have been criticized for non-representative samples (47), lack of control for ecologic confounder (48) at the correct scale (45), short follow-up for assessment of effects (30) and inadequate control for spatial autocorrelation (49, 50), which may influence the size and statistical significance of the health effects (51). In addition, cohort studies often take many years and require many resources. This may partially explain why so few cohort studies have been conducted. Nevertheless, results from cohort studies will continue to be major sources of data for refining legal and regulatory measures in an effort to account for increasing amounts of ambient air pollution from mobile sources.

Cross-sectional studies

With the immediate need for evidence on the effects of ambient air pollution on human health and the growing interest in the effects of chronic exposures, cross-sectional studies hold great potential for contributing to science and policy. In a cross-sectional study design, a sample of reference population is examined at a given point in time. This study design can be used to analyze cohort data, in that it consists of taking a "snapshot" of a cohort by recording information on health outcomes and exposures simultaneously. Cross-sectional studies are important for examining associations between exposures and outcomes. In addition, cross-sectional studies are useful for comparing the health effects of ambient air pollution across several locations at a given time. Also, cross-sectional analyses tend to be less expensive and require less time compared to cohort studies. Moreover, they have an advantage over time-series analyses in that they can capture both acute and chronic effects of ambient air pollution. However, a major constraint of cross-sectional studies is the need to account for a large number of potentially confounding factors, such as differences in age, smoking status, alcohol consumption, environmental tobacco smoke exposures, and occupational exposures. In addition, another major limitation to the cross-sectional study design is that it is not possible to establish a temporal relationship between exposure and outcome. One recent cross-sectional study that linked ambient air pollution to mortality in the counties of the US (48) demonstrated how this study design can be used to investigate the health effects of chronic exposure. The cross-sectional study design will continue to be used since it offers the opportunity to

test numerous health determinants to assess how these confound or are confounded by exposures to ambient air pollution.

Intervention studies

Population-based randomized controlled studies of the relationship between ambient air pollution and health are unethical. Instead, epidemiologists often take advantage of natural experiments or interventions, where sudden changes in exposure levels in an area could possibly influence levels of morbidity and mortality. Many intervention studies have highlighted the health benefits of improvements in air quality and provided useful information to decision-makers and policy-makers. Intervention studies have provided strong circumstantial evidence of the health gains from efforts to improve air quality. However, these studies are very rare. The few examples that exist can be categorized as describing short-term and long-term interventions.

Short-term interventions

One of the classic intervention studies was reported in 1991 (52). The temporary closing of a steel mill because of a labor dispute in the Utah Valley during the late 1980s provided researchers with a unique opportunity to demonstrate the relationship between exposure to particle air pollution and respiratory health. The study assessed the association between respiratory hospital admissions and particulate matter (PM₁₀) pollution in the Utah, Salt Lake, and Cache valleys during April 1985 through March 1989. The Utah and Salt Lake valleys had increased PM₁₀ pollution levels, due to industrial activities, which violated both the annual and 24-hour standards issued by the Environmental Protection Agency (EPA). Much lower PM₁₀ pollution levels occurred in the Cache valley. Pope studied the frequency of hospital admissions during the 13 months that the steel mill was closed and 16 months before and 19 to 35 months after the strike. Bronchitis and asthma admissions for preschool age children were compared between the valleys and were found to be approximately twice as frequent in Utah Valley when the steel mill was operating versus when it was not. During the period when the steel mill was closed for thirteen months because of a strike, differences in per capita admissions between Utah and Cache valleys narrowed considerably. During the mill's

closure the number of children with respiratory symptoms admitted into hospitals decreased substantially and then increased to pre-strike levels when the mill reopened. The results from this intervention study suggest that reductions in PM₁₀ pollution from industrial sources can improve respiratory health.

Another study took advantage of the implementation of a modified transport strategy, to reduce traffic congestion during the 1996 Summer Olympic Games in Atlanta. This intervention study investigated the health impact of a short-term change in levels of transport-related air pollution (53). For a total of more than ten weeks (4 weeks before, 17 days during and 4 weeks after the Games), data was collected for the following: the number of medical emergency visits, the number of hospitalizations for asthma and non-asthma events, air quality, weather conditions, and traffic and public transportation. The air quality data included measurements of PM₁₀, nitrogen dioxide, and ozone. The results of the analysis show a significant decrease in the number (41.6 percent) and incidence of acute care events for asthma (RR: 0.48, 95 percent CI: 0.44-0.86) during the Olympic Games. In the same period, air quality improved, with significant reductions in ozone (from 163 $\mu\text{g}/\text{m}^3$ to 117 $\mu\text{g}/\text{m}^3$ mean of one-hour daily maximum), carbon monoxide (from 1.80 mg/m^3 to 1.47 mg/m^3 , eight-hour means) and PM₁₀ (from 36.7 $\mu\text{g}/\text{m}^3$ to 30.8 $\mu\text{g}/\text{m}^3$ daily mean) concentrations. In addition, the peak weekday morning traffic counts were reduced by 22.5 percent from the baseline period, and the peak daily ozone concentration was significantly correlated with traffic counts (correlation coefficient $r = 0.36$).

The results from the Olympic Games example suggest that reductions in car emissions and the associated ozone and PM₁₀ levels, resulting from changes in city transportation systems, can prevent disease and lead to a reduced number of asthma exacerbations that require medical attention. This study, however, has some noticeable weaknesses that should be considered, including low statistical power, a limited number of air pollution monitoring sites and a poor traffic counting system. Moreover, due to the high correlation between the levels of PM₁₀ and ozone, it is impossible to determine which pollutants' reduction was responsible for the decrease in asthma events, although ozone levels were reduced further (28 percent) than PM₁₀ levels (16 percent). However, these results support the general opinion that reductions in automobile emissions can contribute to the improvement in air quality, which is not necessarily affected so much by weather conditions since they remained relatively constant during the observation period.

Despite some limitations, the immediate inferences are apparent and highlight the need to introduce changes in traffic that improve air quality so the detrimental effects of ambient air pollution, generated by mobile sources, on human health can be minimized.

Long-term interventions

Some intervention studies that assessed the health effect of long-term changes in ambient air pollution have been published. There are a variety of long-term changes that can be made to reduce the adverse effects of exposures to transport-related ambient air pollution on human health. One increasingly used option involves regulating traffic, for example, building tunnels, diverting traffic to different routes, constructing roundabouts and regulating speed. Environmental evaluation reports often document the impact of traffic regulation in terms of changes in ambient air pollution levels, living conditions and the well-being of the local residents. However, the number of published studies that specifically assess the effects of these types of interventions on human health is very limited.

Two studies have been published in connection with the construction of traffic tunnels in Norway. They were constructed to reduce the effect of traffic on the urban environment in Oslo. Bartonova et al. (54) used a dispersion model to estimate the effect of these changes on the residents' levels of exposure. After both tunnels were in use, the average exposure to nitrogen dioxide decreased from 51 $\mu\text{g}/\text{m}^3$ to 40 $\mu\text{g}/\text{m}^3$ (54). Another study investigated the effect of the tunnels on the self-reporting of symptoms of reduced health and on the well-being of adults living in Oslo (55). The decrease in the levels of nitrogen dioxide reported by Clench-Aas et al. (55) was related to a decrease of about 5 to 10 percent in the risk of being bothered by fatigue.

Improvements in technology, such as emission controls or changes in fuel composition can also serve as interventions and provide new opportunities for epidemiological research to investigate the effects of ambient air pollution on human health. In 1990, a fuel restriction was introduced in Hong Kong that required all power plants and road vehicles to use fuel oil with a sulfur content of not more than 0.5 percent by weight. One study examined the effect of this intervention on immediate and long-term health benefits. In the first year after introducing this intervention, the mean reduction in sulfur dioxide was 53 percent, and this reduction was sustained at 35 to 53

percent for five years (56). More importantly, the intervention led to significant declines in the average annual trends in deaths from all causes (2.1 percent; $p < 0.01$), respiratory causes (3.9 percent; $p < 0.01$) and cardiovascular diseases (2.0 percent; $p = 0.05$). Reductions in risks for overall mortality were greater in districts that had large reductions in sulfur dioxide than in those that did not. In addition, differences in age-specific death rates before and after the intervention suggest that it resulted in an average gain in life expectancy of 0.73 years for men aged 25 to 100 years, because of a $10 \mu\text{g}/\text{m}^3$ reduction in exposure to sulfur dioxide for 15 years (56).

More recently, Lou et al. (57) provided additional support for the beneficial effects of changes in fuel composition on levels of lead in blood in China. In 1998, gasoline stations in the city of Shantou were prohibited from selling leaded gasoline. The effects of this intervention on the levels of lead in children's blood were investigated for three consecutive years, when the average levels declined from $104 \mu\text{g}/\text{L}$ in 1999 to $94 \mu\text{g}/\text{L}$ in 2000 and $79 \mu\text{g}/\text{L}$ in 2001. These decreases were all statistically significant. The current standard elevated level of lead in blood for children, which was set by the Centers for Disease Control and Prevention in the United States, is $100 \mu\text{g}/\text{L}$. Lou et al (57) found that the percentage of children in Shantou with levels above this standard was reduced by 35.8 percent in 2000 and 23 percent in 2001. These results indicate that prohibiting the sale of leaded gasoline in the city had beneficial effects. Considering the size of the transit sector, the implementation of similar regulations in other parts of China should help to reduce these numbers even more. However, it is important to be aware of the fact that elevated levels of lead in blood can have other sources, although automobile emissions from the combustion of leaded gasoline have been recognized as one of the major sources of widespread environmental lead contamination.

Intervention studies can provide valuable information that aids in understanding the effects of ambient air pollution on human health, and in highlighting the observable health benefits of air quality legislation and regulation, and the emission sources involved. Unfortunately, intervention studies are rare. However, the use of intervention studies for elucidating the effects of ambient air pollution on human health should be encouraged (58).

The short-term intervention studies that have been performed so far suggest that reductions in ambient air pollution may have a direct health benefit by reducing acute asthma attacks and related medical care. The intervention studies of long-term changes

reported several health benefits such as decline average annual trends in deaths from all causes and from respiratory and cardiovascular diseases, and a gain in life expectancy. However, because of the limited amount of evidence, one needs to be cautious about drawing firm conclusions about the health benefits of the particular interventions.

Case-control and Case-crossover studies

The relationship between ambient air pollution and human health has also been studied in a few case-control investigations (59-61) and more recently with the use of case-crossover studies (62, 63). Case-crossover studies by Neas et al. (62) and Lee and Schwartz (63) have provided an interesting alternative approach to analyzing mortality effects of short-term exposure to air pollution. Rather than using time-series analysis to evaluate associations between daily death counts and air pollution, these two studies adapt a common case-control design. This approach matches exposures at the period of time of death (case period) with one or more periods when the death did not occur (control period) and evaluates potential excess risk using conditional logistic regression. Deceased individuals essentially serve as their own controls. By choosing control periods on the same day of the week and within one to three weeks of death, this approach restructures the analysis such that day of week, seasonality, long-term time trends, and changes in population size and composition are dealt with by design rather than by statistical modeling. Because this approach focuses on individual deaths rather than death counts, there are more opportunities to evaluate factors that may modify or influence the mortality effects of air pollution. However, the case-crossover approach has some limitations. The results can be sensitive to the selection of control periods, especially when clear time trends exist. To overcome this constraint, Neas et al. and Lee and Schwartz suggest choosing symmetric control periods both before and after the date of death. In addition, the case-crossover approach has lower statistical power due to the loss of information from control periods that cannot be included in the analysis.

The results from these case-crossover studies are in concordance with previous studies that explored the effects of ambient air pollution on human health. However, a major drawback of these two case-crossover studies is that they do not provide much information on biological plausibility or the specific pollutant or mix of pollutants

responsible for the observed mortality effects. Recent reviews (4, 29-34) of the overall epidemiologic evidence support a probable link between fine combustion-related ambient air pollution and cardiopulmonary disease and mortality. In addition, several recent studies have reported that long-term exposure to ambient air pollution that contains fine particulate matter is associated with an elevated risk of mortality (40-42). Nevertheless, there is remaining uncertainty about the role of chemistry versus size of the particles and the role of the various constituents of ambient air pollution.

It is not clear that the case-crossover design is necessarily superior or inferior to the various advanced time-series approaches. Yet, the case-crossover design does contribute further evidence that the associations between daily mortality and ambient air pollution are relatively robust and are probably not due to methodologic bias or confounding by day of week, seasonality, long-term time trends, or weather variables. It is evident that future epidemiological studies will need to focus on elucidating the interplay between ambient air pollutants and biological mechanisms that lead to adverse effects on human health.

Exposure assessment methodologies

In most epidemiological studies that investigate the effects of ambient air pollution on human health, concentrations are measured at one or a few fixed monitoring stations and the result of the measurements are used to characterize daily variation in exposure for a whole population. Such exposure measurements are suited for time series studies of short-term health effects. When it comes to studies of long-term health effects of ambient air pollution there is a need for methods that can measure or estimate long-term exposure within a population at a more individual level.

In general, there is a lack of data on ambient air pollutants. Therefore, new methods have been developed to estimate concentrations of ambient air pollutants. Information on emissions, topography, meteorology and ambient air pollution has led to the development of mathematical models for estimating ambient air pollution exposure at different locations and time intervals in a geographical area. Such models, often incorporated into geographical information systems, are now used for surveillance of ambient air pollution in many urban areas. A geographical information system is a collection of computer hardware, software, and geographic data for capturing, managing,

analyzing and displaying different forms of geographically referenced information (64). These models have the potential to estimate ambient air pollution exposure retrospectively and for studies of the relation between ambient air pollution and chronic health effects. For calculating long-term concentrations (months to years), Gaussian Diffusion Models have been used for many years. Such models use frequencies of different winds and stability, and are based upon homogeneous stationary conditions. In general, they overestimate concentrations but adjustments in the models give results surprisingly close to measurements (65).

Recently, the Norwegian Institute for Air Research (NILU) developed an air dispersion model, based on a geographical information system, for assessing ambient air pollution levels in Oslo, Norway. The model was developed to describe residential ambient air pollution levels for the population, and to enable assessment of the health effects of long-term air exposure during the same period. It has been used to estimate residential ambient air pollution for a cohort of 16,209 men living in Oslo. This information has been linked with information on cancer development from the Norwegian Cancer Register in order to estimate associations between long-term exposure to ambient air pollution and the development of lung cancer (66).

Biological methodologies

Epidemiological study designs have been used to identify biological mechanisms. However, much uncertainty exists with regard to the biologic plausibility of associations from epidemiological studies. Biologic plausibility is enhanced by the observation of a coherent cascade of cardiopulmonary health effects and by the fact that non-cardiopulmonary health end points are not typically associated with ambient air pollution. A review of the literature (4, 29-34) reveals that numerous cardiopulmonary health endpoints have been observed to be associated with day-to-day changes in ambient air pollution. In addition to cardiopulmonary mortality, some constituents of ambient air pollution have been associated with emergency room and physician's office visits for asthma and other respiratory disorders, hospital admissions for cardiopulmonary disease, increased reported respiratory symptoms, and decreased lung function. Recently, studies have attempted to assess specific physiologic endpoints, in addition to lung function, such

as plasma viscosity (67), hypoxemia and heart rate (68, 69), heart rate variability (70, 71), and acute inflammatory responses (72, 73). Nonetheless, more research on the pathophysiologic mechanisms linking cardiopulmonary mortality, morbidity, and ambient air pollution needs to be done.

Many studies investigating the effects of ambient air pollution on human health have mainly relied on spirometric tests to measure changes in lung function, self-reported symptoms, or hospital admissions or mortality due to respiratory diseases (74, 75). The effects of ambient air pollution on the respiratory system have been investigated and documented using a variety of indicators and tests, including invasive medical procedures such as bronchoalveolar lavage (BAL) and bronchial biopsy. Currently, most medical investigations of inflammation or injury to the respiratory system rely upon BAL and bronchial biopsy. However, the invasive nature of BAL and bronchial biopsy is not appropriate for monitoring populations exposed to air pollutants in the environment or workplace because they demand a lot of time and resources. Furthermore, for ethical and practical reasons, BAL and bronchial biopsy cannot be used for assessing health risks in children and patients with compromised cardiopulmonary function who are the most vulnerable to ambient air pollutants. More importantly, ambient air pollutants can produce effects on the pulmonary epithelium that are underestimated or even undetected by BAL and bronchial biopsy (76).

One of the earliest clinical manifestations of lung inflammation and injury is an increased permeability of the lung epithelium, which is the main barrier hindering the bidirectional air-blood flux of proteins (77). Until recently, this increased permeability could be confirmed only by measuring the leakage of plasma proteins into the epithelial lining fluid sampled by BAL. Recent research in the field of biomarkers offers new opportunities with the development of molecular tools to measure non-invasively the extent of lung inflammation or injury. Methods for analyzing biomarkers in exhaled air or exhaled breath condensate have recently become available to monitor airway inflammation and injury. One of the most validated tests consists of measuring nitric oxide in exhaled air (eNO), a test that has increasingly proved to be a reliable measure of inflammation and oxidative stress in the bronchial epithelium (78).

More recently, a new approach referred to as, "pneumoproteinemia" was developed to evaluate the integrity of the pulmonary epithelium by measuring the concentration of lung-specific proteins in serum. The prototypic pneumoprotein is the

16-kDa Clara cell protein (CC16), an anti-inflammatory protein secreted by Clara cells in the respiratory tract and predominately in terminal bronchioles from where it leaks into serum (79, 80). Serum CC16 concentrations for healthy nonsmokers have been measured and range from 10-15 µg/L, which is about 10,000 times lower than in epithelial lining fluid. This huge concentration gradient most likely provides the driving force for the diffusion of CC16 from the lung into the blood. Because of its short half-life of approximately two to three hours, it has been hypothesized that CC16 in serum more accurately reflects the time course of ambient air pollutant-induced permeability changes than plasma proteins in BAL (81).

In human and experimental animal studies, it has been demonstrated that the serum concentration of CC16 is a sensitive biomarker to detect an increased permeability of the bronchoalveolar-blood barrier, which is one of the earliest signs of lung injury induced by ambient air pollutants (74, 76, 82, 83). Several ambient air pollutants have been observed to be associated with either increases or decreases in serum CC16 concentrations. However, it is important to be cognizant of the fact that serum CC16 concentrations can increase by a disruption of the epithelial barrier and decrease following a destruction of Clara cells. For example, serum CC16 concentrations have been shown to increase with acute lung injury involving disruption of the bronchoalveolar-blood barrier. Increases have been associated with smoke inhalation among firefighters (75), trichloramine among regular indoor-chlorinated swimming pool attendees (82, 84), and acute exposures to ambient air pollution, including ozone, PM_{2.5}, PM₁₀, and NO₂ (76, 85, 87). Conversely, serum CC16 concentrations have been shown to decrease with chronic exposures. Decreases have been associated with smoking (85) and occupational exposures to nitric oxides (83), and silica (86).

Justification for this study

Currently, there is a paucity of studies that specifically explore associations between serum CC16 concentrations in elderly populations and exposures to ambient air pollutants. To explore associations between ambient air pollutants and serum concentrations of CC16 among elderly men in Oslo, Norway a cross-sectional study design was selected. This method was mainly chosen because statistical analyses for this thesis project focus on a sub-sample taken from a prospective cohort study. Essentially,

this study is a "snapshot" of a cohort with information on health outcomes and exposures determined simultaneously for each subject. Also, cross-sectional analyses tend to be less expensive and require less time compared to cohort studies. Moreover, they have an advantage over time-series analyses in that they can capture both acute and chronic effects of ambient air pollution.

This study used data collected during the second round of the Oslo Study (Oslo Study II), a health study of 7,157 men between the ages of 67 and 77 years of age from the city of Oslo. The data collection occurred from January to June 2000 and included questionnaires and a basic clinical examination. The basic clinical examination included measurements of blood pressure and pulse, as well as the collection of venous non-fasting blood samples.

To determine serum CC16 concentrations, the main outcome or dependent variable of this study, 1200 blood samples from the Oslo Study II were tested during March and April 2006, using an enzyme-linked immunosorbent assay from BioVender Laboratory Medicine (Brno, Czech Republic), according to the protocol provided by the manufacturer. All analyses were performed in duplicate. Calibrator samples and quality control samples were included in each assay to control for performance and interassay variability.

To assess both short-term and long-term exposures to ambient air pollution, I used estimates from the Air Quality Information System (AirQUIS-EPISODE) model developed at The Norwegian Institute of Air Research (NILU). Indicators of ambient air pollution exposure at each participant's home address included nitrogen dioxide (NO₂), particulate matter with aerodynamic diameter <2.5 µg/m³ (PM_{2.5}), and particulate matter with aerodynamic diameter <10 µg/m³ (PM₁₀).

One of the major constraints of cross-sectional studies is the need to account for a large number of potentially confounding factors, such as differences in age, level of education, personal smoking status, number of cigarettes smoked daily, and chronic bronchitis and/or emphysema. To address this limitation, I used data on these independent variables from the Oslo Study II questionnaires and the basic clinical examination. These variables were included in the statistical analyses.

4. STUDY OBJECTIVES

4.1. Main objective

The main objective of this research project is to investigate associations between serum CC16 concentrations, among a population of elderly men in Oslo, Norway, and exposures to ambient air pollutants.

4.2. Specific objectives

1. Assess the prevalence of exposure to ambient air pollutants [nitrogen dioxide (NO₂), coarse particulate matter with an aerodynamic diameter less than 10 µm (PM₁₀), and fine particulate matter with an aerodynamic diameter less than 2.5 µm (PM_{2.5})], among a population of elderly men in Oslo, Norway.
2. Assess the prevalence of exposure to selected environmental factors; other than NO₂, PM₁₀, and PM_{2.5}, among a population of elderly men in Oslo, Norway.
3. Assess the association between serum CC16 concentrations, among a population of elderly men in Oslo, Norway, and selected ambient air pollutants and environmental exposures.
4. Assess the relationship between serum CC16 concentrations and selected ambient air pollutants, while adjusting for covariates.

5. STUDY DESIGN

Cross sectional study

A cross-sectional study design was employed for investigating associations between ambient air pollutants; specifically, NO₂, PM₁₀, PM_{2.5}, and serum CC16 concentrations among a population of elderly men living in Oslo, Norway.

6. METHODOLOGY

The following section describes the methodology that was employed for this study.

6.1. Variables

Variables	Method of verification	Scale of measurement
1. Serum Clara cell protein	The Norwegian Institute of Public Health	µg/L
2. Age	Oslo II study questionnaire	Years
3. Level of education	Oslo II study questionnaire	Years
4. Personal smoking status	Oslo II study questionnaire	Current, past, or never
5. Number of cigarettes smoked daily.	Oslo II study questionnaire	Number of cigarettes
6. Chronic bronchitis/emphysema	Oslo II study questionnaire	Yes/No
7. Modeled Exposure to Nitrogen dioxide (NO ₂) at participant's home address	AirQUIS-EPISODE model at The Norwegian Institute of Air Research	NO ₂ value (µg/m ³), one week and one year before the basic clinical examination
8. Modeled Exposure to particulate matter (PM _{2.5}) at participant's home address	AirQUIS-EPISODE model at The Norwegian Institute of Air Research	PM _{2.5} value (µg/m ³), one week and one year before the basic clinical examination
9. Modeled Exposure to particulate matter (PM ₁₀) at participant's home address	AirQUIS-EPISODE model at The Norwegian Institute of Air Research	PM ₁₀ value (µg/m ³), one week and one year before the basic clinical examination

Serum CC16 concentrations

Serum CC16 concentrations were determined by enzyme-linked immunosorbent assay, according to the manufacturer's standard protocol (BioVendor; www.biovendor.com). However, phosphate-buffered saline was used as a buffer instead of Tris-buffered saline, and the assay dilution buffer was supplemented with 0.5 percent bovine serum albumin. In addition, antibody concentrations were lowered to 1.0 and 0.2 µg/ml for the capture and detection antibodies, respectively. Absorbance was measured and quantified using a plate reader (TECAN Sunrise, Phoenix Research Products, Hayward, CA, USA) complete with software (Magellan V 1.10)

Age

The age of participants ranged from 67 to 77 years old.

Education level

Participant's education level was reported as the number of years and was recoded into three categories: <10 years, 10-12 years, and >12 years.

Personal smoking status

Each participant completed a questionnaire in which they indicated whether they were current smokers, ex-smokers, or non-smokers.

Number of cigarettes smoked daily by current smokers

Each participant completed a questionnaire in which current smokers reported the number of cigarettes smoked per day. This variable was recoded as follows: 0-4 per day, 5-9 per day, 10-14 per day, 15-19 per day, and 20 or more per day.

Chronic bronchitis and/or emphysema

Each participant completed a questionnaire in which participants self-reported experiencing (yes or no) chronic bronchitis and/or emphysema.

Ambient air pollution exposure

Ambient air pollution (NO₂, PM₁₀, PM_{2.5}) concentrations ($\mu\text{g}/\text{m}^3$) at the participant's home were estimated using the Air Quality Information System (AirQUIS-EPISODE) model (www.nilu.no/airquis) that was developed at the Norwegian Institute of Air Research (NILU). AirQUIS-EPISODE is an air dispersion model based on a geographical information system (GIS), which estimates the concentrations of NO₂, PM₁₀ and PM_{2.5}, based on hourly measurements of ambient air pollutants and meteorological data

recorded at regional monitoring stations. The main sources of NO₂, PM₁₀, PM_{2.5} during the study period were traffic exhaust, road dust, wood burning, and long-range transport (54, 55, 88).

6.2. Selection and study population

6.2.1. Study site and population

Norway is located on the western portion of the Scandinavian Peninsula, with a land area of 323,802 square kilometers and a population of approximately 4.7 million. Norway is composed of 19 counties and 431 municipalities.

6.2.2. Study area

The data collection for this thesis research project took place in Oslo, the capital of Norway. Oslo is both a county and a municipality. Oslo is the largest city in Norway with an estimated 560,000 residents. Norway's renowned population-based databases are highly advantageous for epidemiological research. The National Population Register (Skattedirektoratet, Oslo, Norway) collects information on home addresses for all Norwegians and is updated each month. The Norwegian Institute for Air Research has estimated average concentrations of ambient air pollutants (NO₂, PM₁₀, PM_{2.5}) at the home address of all participants.

6.2.3. Study population

The study population for this research project was selected from the Oslo Study II, a follow-up health screening of the Oslo Study I, which was a cohort study that investigated the epidemiological aspects of cardiovascular diseases among a population of men living in Oslo. The follow-up health screening, conducted from January to June 2000, evaluated 7,157 men between 67 and 77 years of age and living in Oslo. Two weeks prior to the health screening appointment, a letter of invitation including two confidential questionnaires, a 4-page main questionnaire and a 2-page supplementary questionnaire were mailed to these men. The questionnaires included questions about age, weight, height, smoking history, alcohol use, occupation, diet, education, marital

status, physical activity, and other characteristics (Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway). During the health screening, the questionnaires were turned in; all participants signed a letter of consent; and blood samples were obtained and stored at -20 degrees Celsius for research purposes. About two weeks after participating in the health screening, participants received feedback about their height, weight, body mass index, blood pressure, total serum cholesterol, HDL-cholesterol, triglycerides, and glucose.

6.3. Data collection

Data for this study was collected during the Oslo Study II from January to June 2000. During March and April 2006, an enzyme-linked immunosorbent assay (ELISA) was used to determine serum CC16 concentrations for 1200 blood samples that were drawn during the Oslo Study II. These 1200 blood samples had been stored in -20 degrees Celsius freezers. The 1200 blood samples were selected based on the mean NO₂ concentration at each participant's home address during the last year before the basic clinical examination. These 1200 blood samples were selected from the total blood samples collected in the following manner: 400 were randomly picked from the bottom 25 percent quartile, another 400 were selected from the 25 to 50 and 50 to 75 percent quartiles, and the last 400 were selected from top 25 percent quartile. All data were entered into a SPSS dataset, which is maintained by Norway's Institute of Public Health.

6.4. Data handling

Data cleaning was performed to limit the statistical analyses to cases with complete information on the Oslo Study II attendance variable (year, month, day, and hour) and the variables of interest (n = 1014). The distribution of serum concentrations was positively skewed so a log transformation of this variable was performed.

6.5. Data analysis

The data analyses included descriptive statistics, bivariate Spearman correlations, and linear regression models to estimate the effect of ambient air pollutants on the percent change in the concentration of serum Clara cell protein, while adjusting for other covariates. Statistical analyses were conducted using SPSS version 16 (SPSS Inc.,

Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to test for differences in means between groups, and to look for trends across categorical variables. The linear regression analyses were performed with both continuous and categorical variables.

6.5.1. Hypothesis testing

H_a: There is an association between serum CC16 concentrations and exposures to NO₂ that occurred one-week before the basic clinical examination.

H_a: There is an association between serum CC16 concentrations and exposures to PM_{2.5} that occurred one-week before the basic clinical examination.

H_a: There is an association between serum CC16 concentrations and exposures to PM₁₀ that occurred one-week before the basic clinical examination.

H_a: There is an association between serum CC16 concentrations and exposures to NO₂ that occurred one-year before the basic clinical examination.

H_a: There is an association between serum CC16 concentrations and exposures to PM_{2.5} that occurred one-year before the basic clinical examination.

H_a: There is an association between serum CC16 concentrations and exposures to PM₁₀ that occurred one-year before the basic clinical examination.

H₀: There is no association between CC16 serum concentrations and exposures to NO₂ that occurred one-week before the basic clinical examination.

H₀: There is no association between CC16 serum concentrations and exposures to PM_{2.5} that occurred one-week before the basic clinical examination.

H₀: There is no association between CC16 serum concentrations and exposures to PM₁₀ that occurred one-week before the basic clinical examination.

H₀: There is no association between CC16 serum concentrations and exposures to NO₂ that occurred one-year before the basic clinical examination.

H₀: There is no association between CC16 serum concentrations and exposures to PM_{2.5} that occurred one-year before the basic clinical examination.

H₀: There is no association between CC16 serum concentrations and exposures to PM₁₀ that occurred one-year before the basic clinical examination.

7. RESULTS

Descriptive statistics

Descriptive statistics for the study population are presented in Table 1. Unadjusted serum CC16 concentrations ranged from 1.2 to 115.2 $\mu\text{g/L}$ with a geometric mean of 9.4 $\mu\text{g/L}$. The study population included elderly men aged 67 to 77 years, with a mean age of 70.7 years. About five percent of participants reported experiencing chronic bronchitis and/or emphysema. The study sample consisted of 16.9 percent current smokers, 54 percent former smokers, and 29.1 percent non-smokers. Descriptive statistics for participant's exposures to NO_2 , PM_{10} , and $\text{PM}_{2.5}$ (one-week before the basic clinical examination) are presented in Table 2.

Bivariate Spearman correlation analyses

A summary of the bivariate Spearman correlation analyses is presented in Table 3. Age was significantly associated with $\log\text{CC16}$ ($p < 0.01$). Education was not significantly associated with $\log\text{CC16}$ ($p = 0.346$). Chronic bronchitis and/or emphysema were significantly associated with $\log\text{CC16}$ ($p < 0.01$). Smoking status was significantly associated with $\log\text{CC16}$ ($p < 0.01$). Number of cigarettes smoked per day was significantly associated with $\log\text{CC16}$ ($p < 0.01$). NO_2 , PM_{10} , and $\text{PM}_{2.5}$ exposures that occurred one-year before the basic clinical examination were not significantly associated with $\log\text{CC16}$ ($p = 0.344, 0.995, 0.793$, respectively). NO_2 exposures that occurred one-week before the basic clinical examination were not significantly associated with $\log\text{CC16}$ ($p = 0.330$). PM_{10} and $\text{PM}_{2.5}$ exposures that occurred one-week before the basic clinical examination were significantly associated with $\log\text{CC16}$ ($p = 0.005$ and 0.002 , respectively).

One-way analysis of variance (ANOVA) analyses

Results of the one-way analysis of variance (ANOVA) analyses are described below.

The average concentration of serum CC16 was lower among participants who reported having or have had bronchitis and/or emphysema (7.53 $\mu\text{g/L}$ (95 percent CI: 6.34 $\mu\text{g/L}$,

8.93 µg/L) compared to participants who did not report that they did not have chronic bronchitis and/or emphysema 9.55 µg/L (95 percent CI: 9.22µg/L, 9.88µg/L). This difference was statistically significant ($p < 0.01$). However, this difference was not statistically significant after adjusting for smoking status in a linear regression model where logCC16 was the dependent variable and bronchitis and/or emphysema and smoking status were the independent variables ($P > 0.05$).

The average concentration of serum CC16 was significantly different between current, former, and non-smokers ($p < 0.001$). The average concentration of serum CC16 was lower among current smokers, 6.97 µg/L (95 percent CI: 6.43 µg/L, 7.57 µg/L) compared to former smokers, 9.66 µg/L (95 percent CI: 9.23 µg/L, 10.11 µg/L) and non-smokers, 10.74 µg/L (95 percent CI: 10.12 µg/L, 11.39 µg/L).

Generally, the average concentration of serum CC16 declined with increased numbers of cigarettes smoked per day. For example, the average concentration of serum CC16 was 8.06 µg/L (95 percent CI: 7.38 µg/L, 8.81 µg/L) for participants who smoked 16-20 cigarettes per day, compared to 9.27 µg/L (95 percent CI: 8.66 µg/L, 9.93 µg/L) among participants who smoked 6-10 cigarettes, and 10.61 µg/L (95 percent CI: 10.05 µg/L, 11.21 µg/L) among participants who smoked 0-1 cigarette per day. This difference was statistically significant ($p < 0.001$).

Linear regression analyses

Results of the linear regression analyses are described below and in Tables 4 and 5.

The percentage change in serum CC16 concentration was 0.2 percent for each 1 µg/m³ change in PM₁₀ exposure that occurred one-week before the basic clinical examination, after adjusting for age, self-reported chronic bronchitis and/or emphysema, smoking status, and number of cigarettes smoked per day ($p < 0.05$). In addition, the percentage change in serum CC16 concentration was also 0.2 percent for each 1 µg/m³ change in PM_{2.5} exposure that occurred one-week before the basic clinical examination, after adjusting for age, self-reported chronic bronchitis and/or emphysema, smoking status, and number of cigarettes smoked per day ($p < 0.01$).

Compared to current smokers who reported smoking between 0 and 4 cigarettes per day, the percentage change in serum CC16 concentration was -10.1 percent for current smokers who reported smoking between 5 and 9 cigarettes per day, after adjusting for age, self-reported chronic bronchitis and/or emphysema ($p < 0.01$). Compared to current smokers who reported smoking between 0 and 4 cigarettes per day, the percentage change in serum CC16 concentration was -14.6 percent for current smokers who reported smoking between 10 and 14 cigarettes per day, after adjusting for age, self-reported chronic bronchitis and/or emphysema ($p < 0.01$). Compared to current smokers who reported smoking between 0 and 4 cigarettes per day, the percentage change in serum CC16 concentration was -15.8 percent for current smokers who reported smoking between 15 and 19 cigarettes per day, after adjusting for age, self-reported chronic bronchitis and/or emphysema ($p < 0.01$). Compared to current smokers who reported smoking between 0 and 4 cigarettes per day, the percentage change in serum CC16 concentration was -17.8 percent for current smokers who reported smoking 20 or more cigarettes per day, after adjusting for age, self-reported chronic bronchitis and/or emphysema ($p < 0.01$).

8. DISCUSSION

8.1. Findings

Currently, there is a paucity of studies that specifically explore associations between serum CC16 concentrations in elderly populations and exposures to ambient air pollutants. Therefore, the main objective of this thesis research project is to bridge this gap in the literature by investigating associations between serum CC16 concentrations, among a population of elderly men in Oslo, Norway, and exposures to ambient air pollutants.

In this present study, statistically significant associations were found between serum CC16 concentrations and the following: age; chronic bronchitis and/or emphysema; smoking status; number of cigarettes smoked per day; as well as PM_{10} and $PM_{2.5}$ exposures one-week before the basic clinical examination.

No associations were found between serum CC16 concentrations and the following: education level; and NO₂, PM₁₀ and PM_{2.5} exposures one-year before basic clinical examination.

The relationship between serum CC16 concentrations and PM₁₀ exposures one-week before basic clinical examination was statistically significant ($p < 0.05$). There was a 0.2 percent change in serum CC16 concentrations for each one $\mu\text{g}/\text{m}^3$ change in PM₁₀ exposure that occurred one-week before the basic clinical examination. This effect remained unchanged after adjusting for age, self-reported chronic bronchitis and/or emphysema, smoking status, and number of cigarettes smoked per day. In addition, the relationship between serum CC16 concentrations and PM_{2.5} exposures one-week before basic clinical examination was statistically significant ($p < 0.01$). There was a 0.2 percent change in serum CC16 concentrations for each one $\mu\text{g}/\text{m}^3$ change in PM_{2.5} exposure that occurred one-week before the basic clinical examination. This effect also remained unchanged after adjusting for age, self-reported chronic bronchitis and/or emphysema, smoking status, and number of cigarettes smoked per day. Thus, the effects on serum CC16 concentrations due to exposures to PM₁₀ and PM_{2.5} that occurred one-week before the basic clinical examination are of the same magnitude. Results of this study are consistent with other studies that have reported small but statistically significant increases in adverse health effects as a result of recent exposures to ambient air pollutants (17-19, 21, 35, 87).

Exposure to cigarette smoke has been reported to decrease serum CC16 concentrations (85). Among current smokers in this study, a dose-response relationship between the number of cigarettes smoked per day and serum CC16 concentrations was observed. As the number of cigarettes smoked per day increased, serum CC16 concentrations decreased (Table 5).

In human and experimental animal studies, it has been demonstrated that the serum concentration of CC16 is a sensitive biomarker to detect an increased permeability of the bronchoalveolar-blood barrier, which is one of the earliest signs of lung injury induced by ambient air pollutants (74, 76, 82, 83). Several ambient air pollutants have been observed to be associated with either increases or decreases in serum CC16 concentrations. Serum CC16 concentrations can increase by a disruption of the epithelial barrier and decrease

following a destruction of Clara cells. For example, serum CC16 concentrations have been shown to increase with acute lung injury involving disruption of the bronchoalveolar-blood barrier. Increases have been associated with smoke inhalation among firefighters (75), trichloramine among regular indoor-chlorinated swimming pool attendees (82, 84), and acute exposures to ambient air pollution, including ozone, PM_{2.5}, PM₁₀, and NO₂ (76, 85, 87). Conversely, serum CC16 concentrations have been shown to decrease with chronic exposures. Decreases have been associated with cigarette smoking (85) and occupational exposures to nitric oxides (83), and silica (86). The findings of this study indicate that the effects of PM_{2.5} and PM₁₀ exposures that occurred one-week before the basic clinical examination on serum CC16 concentrations are less than that of other known factors such as cigarette smoking.

8.2. Conclusion

The findings of this study are similar to other studies that have found evidence of associations between serum CC16 concentrations and ambient air pollutants, as well as other environmental exposures. In addition, results of this study are consistent with other studies that have reported small but statistically significant increases in adverse respiratory effects as a result of recent PM_{2.5} and PM₁₀ exposures. Lastly, our results indicate that effects of PM_{2.5} and PM₁₀ exposures that occurred one-week before the basic clinical examination on serum CC16 concentrations were less than that of other known factors such as cigarette smoking.

However, this study has its limitations. Due to the cross-sectional study design the findings of this study cannot be considered causal. Despite being a rich data source, there is a limitation to using the Oslo Study II questionnaires, that mainly being selection bias. Selection bias may have had an effect on the results of this study since elderly men with healthy profiles may be more likely to have different health-seeking behaviors than elderly men who have less healthy profiles. Also, it is possible that some unknown confounding could affect these findings. In addition, since only a single baseline blood sample was taken, it was not possible to evaluate individual variation of serum CC16 concentrations that may occur over time. Moreover, the blood samples were not taken at a uniform time of day, and therefore our data cannot evaluate diurnal variation in the individual serum CC16 concentrations. Furthermore, this study utilized self-reported

data, which could increase misclassification bias. It is important to be aware that these limitations could lead to an overestimation or underestimation of the true effects.

Strengths of this study include the use of a GIS-based air dispersion model to estimate NO₂, PM₁₀ and PM_{2.5} exposures at each participant's home address; information on smoking and other environmental exposures is included on a large homogeneous group of elderly men; and serum samples have been reported to be more stable than urine samples for determining concentrations of CC16.

In conclusion, the findings of this study support other studies that have found evidence of acute exposures to PM_{2.5} and PM₁₀ leading to increased permeability of the bronchoalveolar-blood barrier in elderly men. More research is needed to gain a better understanding of the relationship between serum CC16 concentrations and ambient air pollutants, as well as other environmental exposures. Using CC16 as a non-invasive biomarker to assess the integrity of the respiratory epithelium could help identify susceptible persons with respiratory epithelial injury and bronchial dysfunction. Individual level information about the integrity of the respiratory epithelium in elderly persons could be used develop rapid risk communication strategies, so persons most at risk of cardiopulmonary morbidity and mortality could be warned during significant ambient air pollution episodes.

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10. APPENDICES

Table 1. Descriptive statistics for the study population

Number of subjects	1014
Serum CC16 concentrations, mean (SD)	11.2 µg/L (8.9)
Serum CC16 concentrations, range	1.2 to 115.2 µg/L
Serum CC16 concentrations, geometric mean (SD)	9.4 µg/L (1.7)
Age, years, mean (SD)	70.7 (2.8)
Age, years, range	67 to 77
Education, n (percent)	
< 10 years	239 (23.6)
10-12 years	336 (33.1)
> 12 years	439 (43.3)
Chronic bronchitis and or emphysema, n (percent)	53 (5.2)
Smoking status, n (percent)	
Current	171 (16.9)
Former	548 (54.0)
Never	295 (29.1)
Number of cigarettes smoked per day by current smokers, n (percent)	
0-4	27 (15.8)
5-9	46 (26.9)
10-14	41 (24.0)
15-19	24 (14.0)
20 or more	33 (19.3)

Table 2. Descriptive statistics for participant's exposure to NO₂, PM₁₀, & PM_{2.5}

Ambient air pollutants (one-week before basic clinical examination)

Based on AirQUIS-EPISODE model

NO ₂ µg/m ³ , mean (SD)	34.6 (15.1)
PM ₁₀ µg/m ³	18.7 (10.9)
PM _{2.5} µg/m ³	15.8 (9.5)

Table 3. Summary of bivariate Spearman correlation analyses

	p-value
<i>log(CC16) with:</i>	
Age (continuous)	< 0.01
Education (categorical)	0.346
Chronic bronchitis and/or emphysema (categorical)	< 0.01
Smoking status (categorical)	< 0.01
Number of cigarettes smoked per day (categorical)	< 0.01
NO ₂ exposure one-year before basic clinical examination (continuous)	0.344
PM ₁₀ exposure one-year before basic clinical examination (continuous)	0.995
PM _{2.5} exposure one-year before basic clinical examination (continuous)	0.793
NO ₂ exposure one-week before basic clinical examination (continuous)	0.330
PM ₁₀ exposure one-week before basic clinical examination (continuous)	0.005
PM _{2.5} exposure one-week before basic clinical examination (continuous)	0.002

Table 4. Associations between serum CC16 concentration and ambient air pollutant exposures; percent change (95 percent confidence interval (CI) in serum CC16 concentration per 1 $\mu\text{g}/\text{m}^3$ change in exposure

Ambient air pollutants (AirQUIS-EPISODE model)	Crude change (95 percent CI)	Adjusted change (95 percent CI) [‡]
PM _{2.5} $\mu\text{g}/\text{m}^3$ (week before)	0.2 (0.1, 0.4) **	0.2 (0.0, 0.3) **
PM ₁₀ $\mu\text{g}/\text{m}^3$ (week before)	0.2 (0.0, 0.3) *	0.2 (0.0, 0.3) *

[‡] Adjusted for age, self-reported chronic bronchitis and/or emphysema, smoking status, number of cigarettes smoked per day.

* p < 0.05 ** p < 0.01

Table 5. Associations between serum CC16 concentrations and cigarette smoking; percent change (95 percent CI) in serum CC16 concentration per level of exposure.

		Crude change (95 percent CI)	Adjusted change (95 percent CI)
Number of cigarettes smoked per day by current smokers			
0 – 4 cigarettes	27	reference	reference
5 – 9 cigarettes	46	-11.2 (-18.3, -4.1) **	-10.1 (-17.1, -3.1) ^a **
10 – 14 cigarettes	41	-15.9 (-23.4, -8.4) ***	-14.6 (-22.0, -7.2) ^a ***
15 – 19 cigarettes	24	-16.5 (-26.2, -6.8) **	-15.8 (-25.4, -6.2) ^a **
20 or more cigarettes	33	-19.9 (-28.1, -11.6) ***	-17.8 (-26.1, -9.5) ^a ***

^a Adjusted for age, self-reported chronic bronchitis and/or emphysema.
 * p < 0.05, ** p < 0.01, *** p < 0.001