I, Kpandja Diawe, hereby submit this original work as part of the requirements for the degree of Doctor of Philosophy in Epidemiology (Environmental Health).

It is entitled:
Effects of Environmental Exposures on: Pneumocystis jirovecii Pneumonia (PcP) Hospital Admissions; and Antibody Levels to Major Surface Glycoprotein among HIV-Infected Patients from San Francisco

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Effects of Environmental Exposures on: *Pneumocystis jirovecii* Pneumonia (PcP) Hospital Admissions; and Antibody Levels to Major Surface Glycoprotein among HIV-infected Patients from San Francisco

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Abstract

Pneumocystis is an opportunistic pathogen for subjects with a compromised immune system, including patients with HIV+ infection, post organ-transplant patients, malignancy cancer patients and those receiving immunosuppressive drugs. Previous studies showed that about 85% of HIV-infected patients developed PcP at some time during their illness. However, with the introduction of HAART and the use of PcP prophylaxis, the frequency of PcP has decreased over time. In spite of this reduction in incidence, PcP currently is the second leading cause of morbidity among HIV-infected patients in the U.S. Although PcP causes serious outcomes in immunocompromised patients, some epidemiologic features are still puzzling scientists. Researchers are still struggling to agree on the mode of transmission and the effects of environmental factors.

The Major Surface Glycoprotein (Msg) is a crucial protein complex in Pneumocystis pathogenicity and is involved in host-organism interaction. This protein is encoded by a multicopy gene family and is capable of antigenic variation to allow Pneumocystis to evade the host immune response. However, the immunologic study of this organism has been hindered because Pneumocystis is difficult to grow, making it difficult to obtain native Msg in a large amount for Seroepidemiology studies. Recently, studies have used Msg as the main antigen to develop different recombinant fragments. Although studies showed that there are geographic variations and seasonal variation in antibody responses to Msg, no study has analyzed the effects of environmental factors on antibody levels to Msg fragments.
In the first aim of the study, the case-crossover design was used to identify environmental factors associated with PcP hospital admissions in San Francisco General Hospital (SFGH). Environmental data were collected from the National Air Quality database. Data from 457 HIV+ patients with advanced stages of HIV+ disease were analyzed. A significant seasonal variation of PcP hospital admissions was found (p<0.05). Increased of temperature and SO$_2$ levels was significantly associated with PcP hospital admissions (p<0.05). However, the effects of SO$_2$ were modified by the presence of CO.

In the second aim of the study, the influence of environmental factors on antibody levels to Msg fragments was determined. One hundred and thirty nine serum specimens sampled at the time of PcP admissions were analyzed. The levels of environmental factors at admission and two weeks before admission were measured, and Tobit regression models were used to determine the association between the environmental factors and antibody levels. It was found that after controlling for other environmental parameters, temperature measured at the time of admission was significantly associated with IgG antibody responses to both MsgC and MsgA. However, temperature measured two weeks before hospital admission was only significantly associated with IgG antibody levels to MsgA. There was a significant seasonal variation in antibody levels to MsgA, but not to MsgC.

In conclusion, this study shows that among environmental factors, temperature and SO$_2$ are independent risk factors for hospital admissions for HIV+ patients with PcP infection in San Francisco. It also shows that temperature fluctuations have significant effects on antibody levels to Pneumocystis infection.
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This dissertation has two parts and each part will be published in a different journal.

Dissertation Part I:

**Title:** Effects of Environmental Exposures on *Pneumocystis jirovecii* Pneumonia (PcP) Hospital Admissions among HIV-Infected Patients from San Francisco

Dissertation Part II:

**Title:** Effects of Environmental Exposures on Antibody Levels to Major Surface Glycoprotein (Msg) among HIV-Infected Patients from San Francisco

IRB approval

The study was approved by the University of California San Francisco institutional review board and the University of Cincinnati institutional review board.
Chapter One: Introduction, Background, Hypothesis, Specific Aim, and Significance to Environmental Health

Introduction

PcP is an opportunistic pathogen for subjects with a compromised immune system, including HIV-infected patients, post organ-transplant patients, malignancy cancer patients and patients receiving immunosuppressive drugs (1-2). With the onset of the HIV+ epidemic, PcP was identified as a serious disease in this patient group, and is considered a leading AIDS-defining disease (1-3). Previous studies showed that up to early 1990s, about 85% of HIV-infected patients will develop PcP at some time during their illness (1, 3-4). Another study has shown that PcP was often the first opportunistic disease that HIV-infected patients encountered and was most likely to be a leading cause of their death (1). In the mids-1990s Highly Active Antiretroviral Therapy (HAART) and antimicrobial prophylaxis were introduced to improve the quality of life among HIV-infected patients and to reduce the incidence of opportunistic infections (1, 5-11). The introduction of HAART and the use of PcP prophylaxis have brought down the rate of PcP incidence among HIV+ patients (1, 12). A recent report by Buchacz et al.
analyzing the trends of AIDS-defining opportunistic infections in the U.S between 1994 and 2007 showed a significant decrease in opportunistic infections including a 30% decrease in PcP incidence between 1994 and 1997 (12). In spite of this reduction, PcP currently is the second leading cause of morbidity among HIV-infected patients (1, 12). Furthermore, recent studies showed a relationship between *Pneumocystis* and chronic obstructive pulmonary diseases (COPD) (14). It was reported that Pneumocystis was a major contributor to the decline of lung function in COPD patients (13).

A well-known risk factor for PcP in HIV+ patients is low CD4 cell count. Studies have shown that HIV+ patients with CD4 cell count less than 200 cells/µL are more likely to develop PcP than patients with CD4 cell count above 200 cells/µL (14-15). An early report showed that PcP incidence was about 20 cases per 100 person-years in HIV+ patients with CD4 cell count less than 200 cells/µL (16). However, a more recent study showed that 10 to 15% of HIV+ patients with CD4 cell greater than 200 cells/µL developed PcP (17). Besides CD4 cell count, other factors such as HIV viral load, use of PcP prophylaxis, sex, race, and HIV transmission category have been associated with PcP occurrence (1). Nevertheless, the risks associated with some of these factors are not well documented. From the above summary, it is clear that Pneumocystis is likely to remain a serious threat to HIV+ patients and is evolving to cause new problems in other patient groups such as COPD patients (1, 3).

Although PcP causes serious outcomes in immunocompromised patients, some epidemiologic features are still puzzling scientists. Researchers are still struggling to agree on the mode of transmission, and the effects of environmental factors. One study used data from the Centers for Disease Control and Prevention (CDC) to show that PcP
incidence was associated with gardening and camping or hiking (18). From that study, it seems like there were some environmental factors that contributed to the occurrence of PcP. The main goal of the present study is to identify those environmental factors associated with PcP hospital admissions in San Francisco.

**Hypothesis and Specific Aim**

**Hypothesis:** Short-term exposures to climatic factors and ambient air pollutants in San Francisco are important predictors of PcP hospital admissions among HIV-infected patients.

**Specific Aim:** To investigate the effects of ambient temperature, humidity and air pollution factors (SO₂, NO₂, CO, ozone, and PM10) on PcP hospital admissions.

**Significance and Relevance to Environmental and Public Health**

Recent identification of *Pneumocystis* colonization in chronic obstructive pulmonary disease (COPD) patients indicates that PcP may be more prevalent than previously believed (19-22). In addition, studies have shown that PcP is not strictly limited to immunocompromised subjects, as PcP cases have been rarely reported in healthy subjects (23). Although a large number of studies have been performed to assess PcP risk factors such as low CD4 cell counts and non-adherence to PcP prophylaxis, the influence of environmental factors on PcP occurrence has not been well studied. For example, PcP has been found to be associated with gardening and camping or hiking in HIV+ patients (18). It is possible that some environmental factors
contributed to this association. Identifying those factors may help people at risk of developing PcP to take precautions under certain environmental conditions. With increasing trends of immunocompromising diseases (24), the results of this study will be a useful tool for healthcare providers to use in recommending lifestyle changes to prevent the acquisition of PcP. In the U.S and other temperate countries, it is well-known when the influenza season begins and ends. Also, there is evidence that PcP admissions peak four months after the influenza season (25). This knowledge, plus the results of the present study, can be used as a convincing tool to encourage the use of PcP chemoprophylaxis during certain seasons.

Chapter Two: Background and Methods

This study addressed PcP seasonality in San Francisco cohort of HIV patients by measuring meteorological and air pollution parameters on the same subject at different time points. Statistical methods were employed to relate differences in exposure levels to PcP hospital admissions.

Background

Geographic variations in PcP incidence have been reported in many studies (25-29). In 1991, Hoover et al. found that, in the U.S, PcP was more common in certain cities (Chicago and Pittsburgh) compared to other cities (Baltimore and Los Angeles) (25). In addition, Lundgren et al. showed that the proportion of PcP cases was higher in certain parts of Europe, especially in the northern regions (28). Despite these geographic
variations in PcP incidence, the effects of climate and air pollution on PcP incidence are unclear.

**Climatic Factors and PcP**

Several studies have analyzed the influence of seasonality on *Pneumocystis* colonization (30-33), PcP incidence (25, 34-44) and PcP clinical outcomes (45-46). Some of these studies found non-significant effects of seasonality on PcP incidence, *Pneumocystis* colonization and PcP clinical outcomes (40-43). Among the studies that have analyzed the influence of seasonality on PcP incidence, some of them have included meteorological parameters in the analysis (34-39). However, the results of these studies are conflicting and vary from country to country (25, 34-38) and sometimes from cohort to cohort within the same country (34, 38). Studies of PcP admissions in Spain and England found a significant monthly variation in PcP incidence (34, 36-37). In these countries, it was shown that most PcP cases occurred in winter. However, in Germany, Canada, and the US, studies have shown that PcP incidence peaks in summer (25, 35, 45). In London, England, two conflicting results have been reported (34, 38). One study showed that PcP was most common in summer and positively correlates with temperature (38), but the other study showed that PcP was most common in winter (34). In 1991, Hoover et al. analyzed the patterns of upper respiratory infections (URI) and PcP in HIV-infected patients from Multicenter AIDS Cohort Study (MACS) (25). Part of their results showed that PcP hospital admissions as the first AIDS-defining illness were influenced by season and location of the patients. PcP admissions were shown to peak between May and June and most of the admissions occurred in Chicago and Pittsburgh. Without including meteorological
parameters such as ambient temperature, ambient humidity and rainfall, it is difficult to tease out the effects of seasonality on PcP hospital admissions in the U.S from this study. Also, they did not analyze seasonal variation by city. Subsequently, Bacchetti et al, in 1994, analyzed seasonal and other short-term influences on AIDS incidence in the U.S using data from the CDC (44). They showed that PcP incidence peaks in March. The above data show that the seasonal pattern of PcP hospital admission is not clear.

Ambient temperature has been shown, in a few studies, to influence the incidence of PcP (34-35, 37-39). However, the results of these studies are conflicting. Some studies found that there is a positive correlation between PcP incidence and ambient temperature (35, 38-39). Yet, other studies showed that the incidence of PcP was inversely correlated with ambient temperature (37). One study found no correlation between PcP incidence and ambient temperature (34). Recently, Sing et al. analyzed the effect of ambient temperature, precipitation, wind force and wind speed on PcP incidence in Munich, Germany. After controlling for clinical and demographic factors, they found that most of PcP cases occurred in May and August (summer), and there was a positive correlation between PcP incidence and ambient temperature (35). Their study was the first study to use robust regression modeling techniques to control for confounding factors with a large sample size to analyze the effect of ambient temperature, precipitation, wind force and wind speed on PcP incidence.

The effects of ambient humidity on PcP incidence are unclear. Only one study has analyzed the effect of ambient humidity on PcP incidence in humans (37). In 2004, Varela et al. showed non-significant effect of ambient humidity on PcP incidence. The same result was found when Demanche et al. analyzed the effect of ambient humidity
on *Pneumocystis* colonization in macaques (32). However, in rat models, Icenhour et al. showed that high relative humidity is associated with *Pneumocystis* infections (30). Other studies have also shown positive correlations between relative humidity and respiratory infections (47-48). Arundel et al. found that incidence of lower respiratory infections is lower in environment with high ambient humidity compared to environment with low ambient humidity (48). Since PcP is a lower respiratory disease, it is possible that its occurrence is associated with ambient humidity. However, this association has not been properly investigated.

The above referenced literature shows that a study is needed to elucidate the effect of meteorological parameters on PcP incidence. Such research should be more comprehensive by including both climatic and air pollution measurements.

**Air Pollution and PcP**

Air pollutants are well-known risk factors for pulmonary diseases (49-53). It has been shown in different studies that an increase in air pollution parameters such as CO, NO₂, ozone, SO₂, PM₁₀, and PM₂.₅ was associated with impaired lung function, with the potential of increased morbidity and mortality (54). Different mechanisms have been proposed to explain how air pollution causes increased morbidity and mortality. Studies showed that exposure to air pollution increased oxidative stress activities that result in depletion of cell oxidants, direct cytotoxicity (including mitochondrial dysfunction) and altered phagocytic function (55). These biological activities are more likely to cause inflammatory responses and acute exacerbations, thus increasing the likelihood of seeking medical care. In a well organized review of the effects of air pollution on pulmonary and systematic response, Hogg et al. showed that inhalation of air pollution
factors triggered both of responses, thus compromising patients’ quality of life (56). These responses may have led to seeking treatment at a hospital.

PcP is a type of pneumonia that manifests itself differently from the community acquired pneumonia (CAP). CAP is a serious pulmonary disease causing morbidity and mortality in different age groups. It is a heterogeneous disease because of its multiple causative pathogens including pyogenic bacteria (*Streptococcus pneumoniae*), atypical organisms (e.g. mycoplasma), viruses, and fungi. PcP is often considered as a separate disease because it occurs mainly in immunocompromised patients. Many studies have reported the effects of air pollution on hospital admissions or emergency room visits due to CAP (57). In most of those studies, PcP appears to be excluded from the analysis. One possible reason for this exclusion might be that PcP is a rare disease found in relatively small number of subjects, making it difficult to obtain a large number of PcP patients for an epidemiologic study. With the HIV+ pandemic, the compiled data in different cohorts have sufficient PcP information that can be used for epidemiologic studies. Studies that analyzed the influence of air pollutants on other respiratory infections are described below, together with a discussion of effects on PcP admissions.

Ozone (O₃) is an air pollutant which has been associated with hospital admissions for many diseases, including respiratory and cardiovascular diseases (57-62). Asthma and chronic obstructive pulmonary diseases (COPD) are the respiratory diseases which have been most frequently associated with ozone. In a study which analyzed the effects of air pollution on hospitalization for pneumonia and COPD in multiple U.S cities, the authors showed that during warm seasons, ozone and PM10 increased hospital admissions for pneumonia (61). Ozone alone was found to increase
pneumonia admissions by 0.41%. In Birmingham, Alabama, Schwartz et al. reported a significant relative risk of 1.14 for pneumonia admissions with a 50-unit increase in ozone level (63). Nevertheless, Zanobetti et al. reported a protective effect of ozone on pneumonia admissions when the effects of air pollution on emergency room admissions in Boston were analyzed (59). Furthermore, Karr et al. showed that ozone reduced the risk of bronchiolitis when the effects of chronic and subchronic exposure to air pollution on bronchiolitis were studied (62). In Los Angeles, Linn et al. showed that ozone was not associated with respiratory diseases when analyzing the effects of air pollution on daily hospital admissions (64). From this summary, it appears that there are conflicting findings on the effects of ozone on respiratory diseases.

The effects of CO on pulmonary diseases have been reported in many studies. Most studies showed that an increase in CO levels was associated with an increase in hospital admissions for COPD, asthma, and pneumonia (59, 64-66). In some areas, studies showed that the effects of CO on respiratory diseases were less pronounced, compared to other pollutants (64). Other studies showed an important effect of CO on respiratory diseases (65-66). Linn et al. found that in Los Angeles, the effects of CO on respiratory disease were less severe compared to the effects of NO₂ and PM₂.5. In Rome, Fusco et al. found that 5.5% of asthma hospitalizations and 4.3% of COPD hospitalizations were attributed to environmental CO levels, and these effects were more important than the effects of ozone and NO₂ (67). In addition, Sunyer et al. showed that emergency room admissions for COPD increased by 11% for each unit increase in CO levels. The above information indicates a relationship between CO and respiratory diseases. Although the directions of the effects seem to be known for some
respiratory diseases, such as COPD, asthma, and pneumonia (66-67), directions of the effects on PcP, which has a different disease progression, are unknown.

Particulate matter especially PM$_{10}$, was first identified to be associated with increased frequency of respiratory diseases. High levels of particulate matter have been associated with hospital admissions and mortality in different studies (49, 60-61, 68-71). In an analysis of the effects of air pollution on COPD, Chen et al found that an increase in PM$_{10}$ was associated with an increase in COPD hospital admissions in the elderly in Vancouver (72). Furthermore, relative risks of 1.19 for pneumonia admissions and 1.27 for COPD admissions have been linked to PM$_{10}$ levels in Birmingham, Alabama (60). In Seattle, Washington, Sheppard et al. found that the relative risk of asthma hospitalization associated with a 19-unit increase in PM$_{10}$, was 1.05 with a 95% confidence interval of [1.02-1.08] (73). Studies also showed contributing effects of PM$_{10}$ on hospital admissions for pneumonia. Medina Ramón et al. found that in most U.S cities, an increase in PM$_{10}$ levels was associated with pneumonia hospitalization especially in warm seasons (61). In Birmingham, UK, Wordley et al. showed that PM$_{10}$ levels were significantly associated with pneumonia and other pulmonary diseases (69). However, in Drammen, Norway, Ofteda et al. found a non-significant effect of PM$_{10}$ on respiratory diseases (74). These studies show that there are mixed effects of PM$_{10}$ on respiratory diseases.

NO$_2$ and SO$_2$ are other pollutants that have been shown in different studies to contribute to increases in morbidity and mortality due to pulmonary diseases. O’Connor et al. showed that NO$_2$ and SO$_2$ were associated with lower respiratory function when the acute effects of air pollution on asthma in some U.S cities were analyzed (75). With
respect to specific diseases, NO₂ and SO₂ have been found to be associated with COPD, asthma, and pneumonia hospital admissions (57, 64, 68, 71-72). Neupane et al. determined the correlation between these two pollutants and hospital admissions for CAP in Hamilton, Ontario (57). They found that NO₂ was significantly associated with CAP admissions, but the effect of SO₂ was not significant (57). Similar results were found when Walter et al. determined the effects of air pollution on pulmonary diseases admissions rate in the West Midlands (76). In Los Angeles, Linn et al. have shown that NO₂ was associated with respiratory diseases (64). Chen and his group found that in Vancouver, Canada, NO₂ was significantly associated with COPD hospital admissions (72). The same finding was reported by Anderson et al. in Denmark, when they analyzed the effects of air pollution on COPD admissions (71). Studies reported the effects of SO₂ on respiratory diseases with conflicting conclusions (75, 77). Although few studies have reported a non-significant effect of SO₂ on respiratory diseases (57, 74, 76), it is scientifically known that SO₂ has serious health effects in humans (75, 78).

In summary, these above studies showed that across the world, air pollution caused different health problems in different locations, however, the specific effects on PcP is unknown. Nevertheless, based on PcP manifestations in the lung, it is possible to assume that the effects of high level of air pollution on lung function might exacerbate the symptoms and cause the patients to seek medical care. In this study HIV-infected patients from San Francisco were used to investigate the effect of air pollutants on PcP hospital admissions.
Methods

Design

A case-crossover design was employed to assess the effects of environmental factors on the first PcP hospital admissions in HIV-infected patients from San Francisco. This design has been used in many epidemiologic studies to determine the effects of air pollution on hospital admissions due to respiratory diseases (59, 61). Case-crossover is a type of matched case-control design where a subject serves as his/her own control (79). For each environmental factor, the significance of the relative effects of exposure levels at the same subject's case and control times were tested to assess the effect of exposure on PcP hospitalization using conditional logistic regression. The advantages of this design include: 1) control group did not have to be recruited, and 2) subject-specific confounders such as clinical and demographic characteristics were controlled without model specification. Three different control times were analyzed for each patient. Since Pneumocystis is a slow growing organism with an incubation period of about two months, the controls were chosen around two weeks, one month, and two months before PcP hospitalization.

Study Location and Patient Population

SFGH is a university-affiliated hospital in San Francisco which has an AIDS chest clinic. The chief of this AIDS chest clinic, Dr. Laurence Huang, has long been involved in HIV-related lung diseases. In one of his studies, he followed HIV-infected patients who developed PcP to determine factors associated with their PcP disease
progression. The protocol of the study is such that a HIV+ patient, who goes to the hospital with PcP, is recruited and demographic and clinical information are collected. Outpatient follow-up is done at 3-4 weeks, 60 days, and then every 6 months following the admission. This research study uses patients who were admitted to SFGH with PcP from 1997 to 2008 and evaluated using a standard diagnostic protocol. As previously reported (80), PcP status was assessed by direct patient interview at the time of enrollment and by review of medical laboratory microbiology/pathology records. Diagnosis of acute PcP was made by microscopic demonstration of the organism by either Giemsa-stained induced sputum or bronchoalveolar lavage specimens.

**Exposure Characterization**

Three days average exposure levels, the day of diagnosis and the first two days prior diagnosis, were analyzed to define exposure at the time of PcP hospital admission (case identification). A 3-day time window was also used to define average control exposure levels. The exposure for the two weeks before admission was an average exposure on day 14, 15, and 16. The exposure for the one month before admission was an average exposure on day 29, 30, and 31, and the exposure for the two months before admission was an average exposure of day 59, 60, and 61. The months of admissions were divided into four seasons. Seasons were defined as winter (December to February), spring (March to May), summer (June to August), and fall (September to November).
Environmental Data

Since patients involved in this study were relatively young and more likely to be involved in daily activities prior to hospital admission, i.e. going to work and doing outdoor activities away from their residents, citywide exposure levels instead of their residential levels were obtained. Exposure data from all the Environmental Protection Agency (EPA) monitoring stations in San Francisco were considered in calculating the mean exposure levels of each pollutant (81). All the stations were within three mile of the SFGH. However, only one station had complete daily data from 1997 to 2008, and that station was used to assign patients their exposure levels. Climatic data were obtained from weather warehouse which is the online weather portal source (82). Its data come from the National Oceanic and Atmospheric Administration (NOAA), the National Climatic Data Center (NCDC), and the National Weather Service (NWS). The same method as above was used to estimate the citywide climatic factors exposure. But, here we considered stations within 20 miles of the SFGH and with completed data.

Statistical Analysis

The first stage of the analysis included the characterization of the 457 HIV + patients who were hospitalized with a diagnosis of PcP for the first time between 1997 and 2008, as determined by the SFGH HIV+ patient database. Median (interquartile range) or counts (percents of total) were obtained to describe continuous and discrete characteristics, respectively. Summary measures, including means, standard deviations, and coefficients of variation of environmental factors were also obtained. Distributional properties, such as central location, spread, symmetry, and extreme data
points were reviewed to assist in the interpretation of parameter estimates obtained from regression analyses. Dependencies among pairs of environmental factors were assessed by calculating Pearson product moment correlations, and analyses of variance were performed to test seasonal differences between mean values of each environmental factor. All environmental factors, except temperature and ozone, varied greatly across dates of measurements, so a log-transformation was applied to ensure that the estimated correlations and regression coefficients would not be unduly influenced by extreme values.

The second stage of the analysis assumed a case-crossover design, and employed conditional logistic regression to evaluate the effect of each environmental factor on PcP hospitalization. For each environmental factor, the odds of PcP hospitalization with respect to an increase in the average level of an environmental factor during the three-day interval around the date of hospitalization was obtained, versus a change in the environmental factor when PcP hospitalization did not occur (control). Three different controls were analyzed: 2 weeks, one month, and two months before hospitalization. Odds ratios were estimated for a 5-unit increase in temperature and ozone, and a one-unit increase in log–transformed values of the other environmental factors. The linearity of the logistic regression model for measuring the association between PcP hospitalization and each environmental factor was tested by assuming a generalized additive model (GAM) with a smoother to fit a restricted cubic spline function. Based on graphical and statistical assessments (p < .05), the appropriate linear spline function was employed, if necessary, to capture an observed functional form that was not linear. Forward stepwise regression was used to combine
environmental factors that were significantly related to PcP hospitalization (p< 0.15) in a multivariate regression model. The final multivariate model at each time included environmental factors which were significant (p< .05) and at most one other factor (p> .05). An adjustment for the multiple testing of the effects of environmental factors on PcP hospitalization was not made, as these analyses were, a priori, the specific aims of the study. Finally, the homogeneity of odds ratios across patients obtained in the univariate regression models of each environmental factor was tested by the Cochran-Mantel-Haenszel test. SAS for Windows, version 9.2 (SAS Institute, Cary, NC) was used to carry out all statistical analyses, and a 5% significance level was assumed, unless stated otherwise. Graphs were generated using Microsoft Excel.

Chapter Three: Results, Discussion and Conclusions

Results

From January 1997 to December 2008, 457 consecutive HIV-infected patients were admitted to SFGH with PcP. At admission, the median age of the patients was 40.5 years, and most of them were white (48%). The majority were men (89%). They had advanced HIV disease with a median (interquartile, IQR) CD4 cell count of 31 cells/µL (14-64) and a median (IQR) HIV viral load of 1.75 x 10^5 copies/mL (7.32 x 10^4-3.41 x 10^5). Three hundred and ninety-five of them (86%) had received PcP prophylaxis within the three months before admission. (Table 1).

During the twelve-year study period, the mean values of all air pollution parameters were below the national air quality standards (83). Moderate positive
correlations were found between \( \text{SO}_2 \) and \( \text{NO}_2 \) and between \( \text{SO}_2 \) and \( \text{CO} \) (\( r = 0.66 \) and 0.59, respectively). Ozone was negatively correlated with \( \text{NO}_2 \) and \( \text{CO} \) (\( r = -0.63 \) and -0.59, respectively), but \( \text{CO} \) was positively correlated with \( \text{NO}_2 \). The correlations between pairs of the other air pollutants were low (Table 2). A significant difference in \( \text{PcP} \) hospital admission rates was found across seasons (\( p = 0.05 \)). Most admissions occurred in summer (129 admissions) followed by spring (125 admissions). Winter was the season with the least number of admissions (91 admissions). When the mean temperature at the time of admissions by season was determined, the peak of \( \text{PcP} \) admissions in summer coincided with the peak in mean temperature (Fig 1).

In single pollutant models using two weeks before admission as controls, it was found that temperature and \( \text{SO}_2 \) were significantly associated with \( \text{PcP} \) hospital admissions (\( p=0.04 \) and 0.02, respectively). An increase of 5°F of temperature was associated with a significant increase in \( \text{PcP} \) hospitalization (OR [95% CI]: 1.41 [1.14-1.75]). One-unit increase of log-transformed \( \text{SO}_2 \) was associated with a significant increase in \( \text{PcP} \) hospital admissions (OR [95% CI]: 1.62 [1.08-2.44]). The effects of the other variables, such as \( \text{NO}_2 \), ozone, \( \text{PM}_{10} \), \( \text{CO} \), and humidity were not statistically significant (Table 3). Similar results were found when one month before admission was used as controls (Table 5). However, when two months before admission were used as controls, only temperature was significantly associated with \( \text{PcP} \) hospital admissions (Table 7).

In multivariate analysis using two weeks before admission as controls, the independent effects of temperature and \( \text{SO}_2 \) were significantly associated with \( \text{PcP} \) hospital admissions. However, the effects of \( \text{SO}_2 \) on \( \text{PcP} \) hospital admissions were
modified by the presence of CO. The interaction effect of SO\textsubscript{2} and CO was significant (p=0.03). The magnitude of the effects of SO\textsubscript{2} on PcP admissions varied at different levels of CO. At lower quartile of CO (5.89 PPB), the effects of SO\textsubscript{2} were higher (OR: 2.45), but at upper quartile of CO (6.59 PPB), the effects of SO\textsubscript{2} were lower (OR: 1.34) (Table 4). When one month before admission was used as controls, the effects of temperature, and SO\textsubscript{2} were still significant, but the effect of SO\textsubscript{2} interaction with CO was no longer significant (Table 6). Only temperature was found to have significant effects on PcP hospital admissions when two months before admission were used as controls (Table 8).

**Discussion and Conclusions**

In the present study, well-characterized subjects with advanced HIV infection were used to show that there was a significant seasonal variation in PcP hospital admissions, and significant effects of temperature and SO\textsubscript{2} on these admissions. It was also found that CO modified the effects of SO\textsubscript{2} on PcP admissions.

The association of PcP hospital admissions with increased in temperature found in this study is consistent with some, but not all, previous studies. Most studies have shown that PcP admissions are more common in summer (25, 35, 38-39). However, a few reports showed that PcP admissions were more common in winter (34, 37). The inconsistency in the findings may be due to geographic or seasonal variations of *Pneumocystis* genotypes. Some studies have reported significant geographic and seasonal variation of *Pneumocystis* genotypes (84-85). Beard et al. showed that genotype frequency distribution patterns varied by patients’ place of diagnosis (84). In
addition, Miller et al. found that there were seasonal variation in *Pneumocystis* genotypes distributions, and ambient temperature was significantly correlated with the identification of certain *Pneumocystis* genotypes (85). It is possible that in some locations the most virulent genotype is common in summer, but in other locations it is common in winter. Although Miller and his group have failed to demonstrate an association between specific genotypes and PcP severity (85), others have reported worse outcomes from PcP in patients with certain genotypes (86-87).

The study design and cohort differences can also result in conflicting findings. In this study a case-crossover design was used for the first time to analyze the effects of climate on PcP admissions. Cross-sectional designs were previously used to relate the incidence of PcP to climatic factors. One of the disadvantages of using cross-sectional design is that all the confounding and effect modifying factors have to be controlled for in the analysis stage. However, if some subjects have missing data on a factor that needs to be controlled for, the estimate of the effects can be biased.

Recently, Sing et al. used a robust regression analysis to determine the effects of climatic factors on PcP hospital admissions in Germany. That study and the present study used sophisticated statistical methods to show that an increase in temperature was associated with an increase in PcP admissions. However, the case-crossover design used in this study was able to provide two advantages. First, the controls were not sampled, thus reducing the time and expenses and eliminating sources of bias. Second, clinical and demographic characteristics were controlled for by making within-subject comparisons. Another difference between this study and other studies was the use of daily averaged temperature readings instead of monthly averaged temperature.
readings. Also, in this study, we used a citywide average by averaging climatic factors across stations with complete data.

In relation to other respiratory diseases, studies have shown a significant seasonal variation in the occurrence of other respiratory infections (88-89). In the analysis of the seasonality of tuberculosis in the United Kingdom (UK), Douglas et al. showed that tuberculosis peaked in summer (88). In another study, Chew et al. showed a significant seasonal variation in viral infections in Singapore (89). That study reported that respiratory syncytial virus infections peaked between March and August, but influenza A virus infections peaked in June and between December and January. However, only the occurrence of respiratory syncytial virus infections was significantly associated with high temperature and low relative humidity (89).

Although there are no previous studies to compare to this study, air pollution is known to cause respiratory diseases. In this study, it was found that an increase in SO$_2$ was significantly associated with an increase in PcP admissions. The effects of SO$_2$ on respiratory disease hospital admissions have been reported with contradictory findings. A few studies have shown that an increase in SO$_2$ level was associated with an increase in admissions for respiratory diseases such as COPD, asthma, and CAP (75-77). Walters et al found that an increase in SO$_2$ was significantly associated with asthma hospital admissions in winter. Furthermore, Martins et al showed that an increase in SO$_2$ level was significantly associated with pneumonia and influenza hospital admissions (77). However, other studies have reported a non-significant effect of SO$_2$ on respiratory disease hospital admissions (57, 76). Although it is difficult to explain how SO$_2$ might increase PcP hospital admissions, we can rely on the evidence
that exposure to SO$_2$ causes nose and throat irritation, bronchoconstriction and
dyspnea (90) to suggest that these manifestations promote *Pneumocystis* colonization
or aggravate the pre-existing PcP symptoms forcing patients to seek medical care.
However, the antagonistic relationship between SO$_2$ and CO found in this study, with
CO decreasing the effects of SO$_2$ only when two weeks before admission are used as
controls, makes one believe that the reported association may be due to chance.
Previous epidemiologic studies showed that an increase in CO levels was associated
with an increase in respiratory disease hospital admissions. Depending on the disease,
studies have reported more than a four-fold increase in the risk of admissions
associated with CO levels (64, 66-67). In this study, CO was found to be protective and
to reduce the effects of SO$_2$. Unfortunately, it is difficult to find a good explanation to the
association. These two pollutants have different sources. SO$_2$ is formed after
combustion of fuel containing sulfur, and most of SO$_2$ in air in the U.S comes from
power plants. However, CO is a traffic related pollutant, and its presence in air is mostly
from motor vehicles (78). Nevertheless, the differences in sources will not be enough to
explain the association reported in this study.

The non-significant effects of the other air pollution parameters such as ozone,
CO, NO$_2$ and PM$_{10}$ on PcP hospital admissions were surprising. Ozone is known to
cause changes in the lung function that could lead to disease complications and
hospital admissions (91-93). In fact, many studies showed that ozone levels were
associated with other hospital admissions for other respiratory diseases (65, 77). The
effects of PM$_{10}$ on respiratory diseases hospital admissions have been reported (64,
69). In addition, NO$_2$ has been also linked to hospital admissions for respiratory
diseases (64, 71, 76). The possible reasons for these lacks of association are: 1) we are dealing in this study with a slow progressing disease which is serious only in a small group of people with special conditions; 2) HIV+ patients used in this study were in advanced stage of their illness with very low CD4 cell count and very high HIV viral load. It is possible that the stage of their HIV disease made them insensitive to the exposure to some of these ambient pollutants; and 3) San Francisco is among the less polluted cities in the U.S. Perhaps the levels of these variables were below the thresholds that could cause respiratory complications.

This study has several limitations. First, the weather stations and air pollution monitoring stations were used to determine individual exposure levels. This method may lead to exposure underestimation since monitoring stations may not provide detailed information about microclimate in which each patient was living. Second, despite assigning the citywide exposure level to each subject, some of the monitoring stations did not have data for the entire study period of our study. Those stations were excluded from the calculation of the average exposure. Third, this study was done in a single city with subtle differences in seasons, so the results may not be generalizable.

In conclusion, this study shows that among climatic and ambient air pollutant constituents, temperature and SO₂ are independent risk factors for hospital admissions for HIV-infected patients with PcP to San Francisco General Hospital. Further multicenter studies are needed to identify if these factors are also predictors of PcP in other geographic locations. Animal model studies are also needed to better understand the biological mechanism behind the impact of climatic and air pollution on PcP occurrence.
### Table 1. Descriptive Characteristics of 457 HIV-infected Patients with PcP

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>Median [25th, 75th] or percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>457</td>
<td>40.05 [34.98, 45.39]</td>
</tr>
<tr>
<td>Male*</td>
<td>408</td>
<td>89 %</td>
</tr>
<tr>
<td>Race*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>125</td>
<td>27 %</td>
</tr>
<tr>
<td>White</td>
<td>217</td>
<td>48 %</td>
</tr>
<tr>
<td>Other</td>
<td>115</td>
<td>25 %</td>
</tr>
<tr>
<td>CD4 Cell Count (Cells/µL)</td>
<td>449</td>
<td>31 [14, 64]</td>
</tr>
<tr>
<td>HIV RNA (Copies/ mL)</td>
<td>430</td>
<td>$1.75 \times 10^5$ [7.32x10^4, 3.41x10^5]</td>
</tr>
<tr>
<td>Total Number of PcP Episodes</td>
<td>528</td>
<td>-</td>
</tr>
<tr>
<td>Episode*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>457</td>
<td>87 %</td>
</tr>
<tr>
<td>&gt;1</td>
<td>71</td>
<td>13 %</td>
</tr>
<tr>
<td>PcP Prophylaxis*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>395</td>
<td>86 %</td>
</tr>
<tr>
<td>No</td>
<td>62</td>
<td>13 %</td>
</tr>
</tbody>
</table>

*p <0.05 tests the difference between proportions. Note: Numbers vary slightly due to missing data*
Table 2. Correlation Coefficients between Pairs of Environmental Factors

<table>
<thead>
<tr>
<th></th>
<th>Temperature</th>
<th>Humidity</th>
<th>SO₂</th>
<th>NO₂</th>
<th>CO</th>
<th>Ozone</th>
<th>PM₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>1.00</td>
<td>-0.26</td>
<td>-0.24</td>
<td>-0.31</td>
<td>-0.26</td>
<td>0.10</td>
<td>-0.06</td>
</tr>
<tr>
<td>Humidity</td>
<td>1.00</td>
<td></td>
<td>-0.11</td>
<td>-0.19</td>
<td>-0.05</td>
<td>-0.11</td>
<td>-0.22</td>
</tr>
<tr>
<td>SO₂</td>
<td></td>
<td>1.00</td>
<td>0.66</td>
<td>0.59</td>
<td>-0.49</td>
<td></td>
<td>0.36</td>
</tr>
<tr>
<td>NO₂</td>
<td></td>
<td></td>
<td>1.00</td>
<td>0.88</td>
<td>-0.63</td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td>CO</td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
<td>-0.59</td>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td>Ozone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
<td>-0.23</td>
<td></td>
</tr>
<tr>
<td>PM₁₀</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
</tbody>
</table>
**Table 3.** Unadjusted Odds Ratios [95% CI] Measuring the Association between Each Environmental Factor and PcP Hospital Admissions Using Two Weeks before Admissions as a Control Time

<table>
<thead>
<tr>
<th>Environmental Exposure (units)</th>
<th>OR [95% CI]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°F)</td>
<td>1.41 [1.14-1.75]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SO₂ (PPB)</td>
<td>1.80 [1.15-2.83]</td>
<td>0.01</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>0.61 [0.20-1.92]</td>
<td>0.40</td>
</tr>
<tr>
<td>NO₂ (PPB)</td>
<td>0.91 [0.59-1.41]</td>
<td>0.68</td>
</tr>
<tr>
<td>PM₁₀ (µg/m³)</td>
<td>0.92 [0.70-1.22]</td>
<td>0.58</td>
</tr>
<tr>
<td>Ozone &lt;20 (PPB)</td>
<td>1.10 [0.73-1.64]</td>
<td>0.66</td>
</tr>
<tr>
<td>Ozone &gt;20 (PPB)</td>
<td>0.86 [0.44-1.69]</td>
<td>0.66</td>
</tr>
<tr>
<td>CO (PPB)</td>
<td>0.77 [0.49-1.23]</td>
<td>0.27</td>
</tr>
</tbody>
</table>

**Note:** p-value tests the significance of the effect of each independent variable on PcP hospital admissions.
Table 4. Adjusted Odds Ratios [95% CI] Measuring the Independent Effects of Multiple Environmental Factors on PcP Hospital Admissions Using Two Weeks before Admissions as a Control Time

<table>
<thead>
<tr>
<th>Environmental Exposure (Unit)</th>
<th>CO Levels</th>
<th>OR [95% CI]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°F)</td>
<td></td>
<td>1.47 [1.15-1.88]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SO₂ (PPB)</td>
<td>5.89</td>
<td>2.45 [1.10-5.45]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SO₂ (PPB)</td>
<td>6.59</td>
<td>1.34 [1.07-2.60]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SO₂ xCO (PPB)</td>
<td></td>
<td>0.41 [0.18-0.92]</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Note: p-value tests the significance of the effect of each independent variable on PcP hospital admissions
Table 5. Unadjusted Odds Ratios [95% CI] Measuring the Association between Each Environmental Factor and PcP Hospital Admissions Using One Month before Admissions as a Control Time

<table>
<thead>
<tr>
<th>Environmental Exposure (Unit)</th>
<th>OR [95% CI]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°F)</td>
<td>1.20 [1.01-1.43]</td>
<td>0.04</td>
</tr>
<tr>
<td>SO₂ (PPB)</td>
<td>1.62 [1.08-2.44]</td>
<td>0.02</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>0.72 [0.25-2.07]</td>
<td>0.55</td>
</tr>
<tr>
<td>NO₂ (PPB)</td>
<td>0.83 [0.55-1.25]</td>
<td>0.38</td>
</tr>
<tr>
<td>PM₁₀ (µg/m³)</td>
<td>1.08 [0.83-1.40]</td>
<td>0.56</td>
</tr>
<tr>
<td>Ozone &lt;20 (PPB)</td>
<td>1.10 [0.79-1.53]</td>
<td>0.58</td>
</tr>
<tr>
<td>Ozone &gt;20 (PPB)</td>
<td>0.80 [0.47-1.37]</td>
<td>0.42</td>
</tr>
<tr>
<td>CO (PPB)</td>
<td>0.64 [0.41-1.01]</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Note: p-value tests the significance of the effect of each independent variable on PcP hospital admissions.
Table 6. Adjusted Odds Ratios [95% CI] Measuring the Independent Effects of Multiple Environmental Factors on PcP Hospital Admissions Using One Month before Admissions as a Control Time

<table>
<thead>
<tr>
<th>Environmental Exposure (Unit)</th>
<th>OR [95% CI]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°F)</td>
<td>1.23 [1.02-1.48]</td>
<td>0.03</td>
</tr>
<tr>
<td>SO₂ (PPB)</td>
<td>2.48 [1.25-4.95]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SO₂xCO (PPB)</td>
<td>0.82 [0.40-1.71]</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Note: p-value tests the significance of the effect of each independent variable on PcP hospital admissions
Table 7. Unadjusted Odds Ratios [95% CI] Measuring the Association between Each Environmental Factor and PcP Hospital Admissions Using Two Months before Admissions as a Control Time

<table>
<thead>
<tr>
<th>Environmental Exposures (Unit)</th>
<th>OR [95% CI]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°F)</td>
<td>1.33 [1.15-1.53]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SO₂ (PPB)</td>
<td>1.32 [0.93-1.88]</td>
<td>0.12</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>0.39 [0.14-1.08]</td>
<td>0.07</td>
</tr>
<tr>
<td>NO₂ (PPB)</td>
<td>0.90 [0.65-1.25]</td>
<td>0.54</td>
</tr>
<tr>
<td>PM₁₀ (µg/m³)</td>
<td>0.92 [0.70-1.20]</td>
<td>0.52</td>
</tr>
<tr>
<td>Ozone &lt; 20 (PPB)</td>
<td>1.02 [0.98-1.05]</td>
<td>0.34</td>
</tr>
<tr>
<td>Ozone &gt;20 (PPB)</td>
<td>0.98 [0.93-1.03]</td>
<td>0.40</td>
</tr>
<tr>
<td>CO (PPB)</td>
<td>0.80 [0.56-1.16]</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Note: p-value tests the significance of the effect of each independent variable on PcP hospital admissions.
**Table 8.** Adjusted Odds Ratios [95% CI] Measuring the Independent Effects of Multiple Environmental Factors on PcP Hospital Admissions Using Two Months before Admissions as a Control Time

<table>
<thead>
<tr>
<th>Environmental Exposure (Unit)</th>
<th>OR [95% CI]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°F)</td>
<td>1.77 [1.33-2.36]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SO₂ (PPB)</td>
<td>1.33 [0.91-1.96]</td>
<td>0.14</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>0.69 [0.22-2.13]</td>
<td>0.52</td>
</tr>
</tbody>
</table>

*Note:* p-value tests the significance of the effect of each independent variable on PcP hospital admissions

**Figure 1.** Total Number of PcP Admissions by Season and Mean Temperature
Dissertation Part II

Title: Effects of Environmental Exposures on Antibody Responses to Major Surface Glycoprotein among HIV-Infected Patients from San Francisco

Chapter One: Introduction, Background, Hypothesis, Specific Aims, and Significance to environmental health

Introduction

The Major Surface Glycoprotein (Msg) is a crucial protein complex in *Pneumocystis* pathogenicity and is involved in host-organism interaction (94-98). This protein is encoded by a multicopy gene family capable of antigenic variation to allow *Pneumocystis* to evade the host immune response (97-98). Studies have shown that the *Pneumocystis* genome can carry up to 100 Msg genes, but the expression is limited to only one isoform of Msg at a given time on the surface of the organism (97). However, the immunologic study of this organism has been hindered because *Pneumocystis* is difficult to grow, making it difficult to obtain native Msg in large amounts for seroepidemiology studies (99). Different approaches, consisting of developing recombinant antigens of *Pneumocystis jirovecii*, have shown promise in serologic studies of *Pneumocystis* (100-101). Recently, studies have used Msg as the main antigen to develop different recombinant fragments (94, 102). For example, Daly
et al. used Msg to generate three key recombinant fragments named MsgA, MsgB, and MsgC which correspond to a single Msg isoform (Fig 1.) (94). When *Pneumocystis* infection occurs, the immune system reacts against these antigen fragments by producing specific antibodies to MsgA, MsgB, and MsgC. When they compared antibody responses to these antigens in HIV-infected patients from San Francisco, they found that the highest antibody levels were to MsgC (95). In addition, it has also been shown that MsgC was the best recombinant to use for the analysis of antibody responses in HIV+ patients infected with *Pneumocystis*. The findings by Daly et al. imply that MsgC is an important fragment to consider when analyzing serum antibody levels to Msg in HIV-infected patients.

These four clones differ from one another in amino acid sequences and trigger independent antibody responses. Recently, we assessed how effective it will be to use antibody levels to MsgC as a diagnostic tool for PcP (103). We used serum specimens from HIV-infected patients to discriminate between patients with PcP and patients with community acquired pneumonia hospitalized in San Francisco. We found an increase in sensitivity and specificity from specimens drawn at the time of admissions to specimens drawn 3-4 weeks after admissions. The results suggested that specimens drawn 3-4 weeks after admissions were more sensitive and more specific in discriminating between pneumonia caused by *Pneumocystis* and pneumonia due to other causes. This was the first study to show that serology using recombinant antigen Pneumocystis antigens offers promise in the diagnosis of PcP (103). However, to actually use antibody levels as a diagnostic tool, it is important to determine other factors affecting antibody
levels. The main goal of the present study was to determine the effects of environmental factors on antibody levels to Pneumocystis infection.

**Hypothesis and Specific Aim**

**Hypothesis**: Short-term exposures to climatic and ambient air pollution are important predictors of IgG antibody levels to Msg fragments among HIV-infected patients.

**Specific Aim**: To use serum specimens of a subset of patients admitted to the SFGH for PcP between 2000 and 2008, to investigate the effects of short-term exposures to temperature, humidity and air pollution on antibody responses to Msg fragments.

**Significance and Relevance to Environmental and Public Health**

Previously, the seroepidemiology of *Pneumocystis jirovecii* was hampered by the lack of suitable reagents to properly characterize antibody reactivity to *Pneumocystis* antigens. Therefore, clinical and demographic characteristics associated with antibody responses to *Pneumocystis* were difficult to identify. With advanced techniques in development of Msg fragments, it is now possible to determine the factors which influence antibody responses to *Pneumocystis* infection. The development of Msg fragments by Daly et al. has opened a new door to the seroepidemiology studies of *Pneumocystis*. Since the development of Msg fragments, we have learned a lot about how HIV-infected patients respond to *Pneumocystis* infection compared to healthy people (94), how the responses vary among HIV-infected patients, and how some
demographic factors including age, smoking, and location of the patients affect the antibody responses to *Pneumocystis* infection (2). However, what we haven’t learned is how environmental factors affect the antibody responses to Msg fragments. In HIV/AIDS patients or in other patients where the immune system is already compromised, it is crucial to identify external factors that could affect antibody production. Therefore, knowledge of the effects of environmental factors on antibody responses against *Pneumocystis* infection will help caregivers to encourage anti-*Pneumocystis* prophylaxis use in the periods when these factors are at high levels.

**Chapter two: Background and Methods**

**Background**

Geographic variations in antibody responses to *Pneumocystis* infection has been reported in different studies (94, 104-105). Smulian et al. found geographic differences in prevalence of antibodies to high molecular weight class of antigens in HIV-infected patients when they evaluated the geographic variations in humoral responses to *Pneumocystis* infection among five regions (U.S, Haiti, Mexico, Africa, Korea) (104). However, in that study, they used crude rather than specific antigens. In 2002, Daly et al. used specific Msg antigens to assess the geographic variations in antibody reactivity to *Pneumocystis* among the above regions (94). They found no significant differences in antibody responses to Msg fragments among these regions.

To the present, no study ever analyzed the effect of environmental factors on antibody responses to Msg fragments among HIV-infected patients. We recently
showed non-significant seasonal variation in antibody responses to MsgC between PcP cases and controls when we analyzed long term antibody responses to MsgC clones (2). The lack of significance in that study can be explained by only a small sample size being available for analysis, and also by the type of comparison we did. In that study, we compared cases and controls in terms of seasonal variation in their antibody responses to MsgC. Lately, we showed significant seasonal variation in antibody levels to MsgA, but not to MsgC when we analyzed the seasonal variation in antibody responses to MsgA and MsgC among immunocompetent Chilean children (106). Given that animal studies have shown seasonal variations in quantity of lymphocytes, neutrophils, CD4 and CD8 cells, and IL-6(107-109), we should expect a seasonal variation in antibody responses to Msg fragments. Since no study has analyzed the effects of climatic and ambient air pollution factors on antibody levels to Pneumocystis infection, it is difficult to fully understand how these environmental factors influence PcP disease progression.

**Climatic Factors and Antibody Levels**

Previous animal studies showed that temperature had a significant effect on the immune system (108, 110-111). Nelson et al. found that mice kept at low temperature have a reduced IgG level compared to those kept at high temperature (108). In another study, Lacetera et al. showed that summer with an extreme heat wave was associated with depressed cellular immunity in dairy cows(110). Furthermore, it has also been shown that the seasonal variation of daylight influences melatonin and vitamin D levels
in the body, and this influence can lead to immune dysfunction (112). In humans, the effects of ambient temperature on immune responses to specific infections such as *Pneumocystis* infection have rarely been studied.

The studies of the influence of ambient humidity on immune responses are scarce. A few animal studies done so far used temperature humidity index (THI) to estimate the effects of both temperature and humidity on immune responses(110). In 2002, Lacetera et al. analyzed the effects of seasonality on immunologic parameters of dairy cows. They found that, in colostrum, there were no significant differences in IgG, IgM, and IgA between animals exposed to high THI and those exposed to low THI during pregnancy. Like other human diseases, the influence of ambient humidity on antibody responses to *Pneumocystis* has not been analyzed.

**Air Pollution and Antibody Levels**

The effects of air pollution on immune function have been reported in few studies (113-116). Air pollution has been shown to elicit local and systemic inflammatory responses and to suppress host defenses (56). In general, exposure to air pollution stimulates inflammatory responses, and these inflammatory responses lead to a decrease or an increase of some immune biomarkers. One study showed that chronic exposure to air pollution resulted in increased levels of IgA, IgM, and complement component C3 (117). Larebeke et al. found that people living in more polluted areas had a significant higher number of CD3 and CD56 cells than those living in less polluted areas when they analyzed the effects of air pollution on immunologic and biologic biomarker (114). Hadnagy et al. also showed that IgG, IgM, and IgA levels in people
living in more polluted areas were altered (117). Picciotto et al. analyzed the effects of air pollution on the distribution of lymphocyte and immunophenotypes in cord and maternal blood at the time of delivery. They found that mothers from areas with high levels of air pollution had lower percentage of total T-cell and CD4, and lower CD4/CD8 ratio. Those mothers also had a higher percentage of natural killer cells (118).

Broad effects of ozone on immune system have been shown in different studies (119). Amato et al., when reviewing the effects of climate change on environmental factors in respiratory allergic diseases, showed that ozone exposure increased the level of inflammatory cells and mediators such as IL-6, IL-8, granulocyte-microphage colony stimulating factors, and fibronectin in Bronchoalveolar Lavage (BAL) (113). In another study, it was shown that exposure to ozone suppresses the development of cell mediated immunity with a significant increase in T cells and B cells in lungs (120). Other studies also showed similar results when they analyzed the effects of ozone on immune markers (119, 121-122). Nevertheless, Zwick et al. found no significant difference between high exposure and low exposure group in terms of IgE levels and white blood cell count when they analyzed the effects of ozone on cell mediated immune system in children (123).

Other pollutants such as CO, NO₂, SO₂, and PM₁₀ also have effects on immune function. In the analysis of the effects of NO₂ on immune system, Gilmour et al. showed that exposure to NO₂ for 3 hours was associated with significantly higher levels of antigen-specific IgE, IgA, and IgG (124). Another study showed that workers exposed to NO₂ had higher number of CD3, CD4, CD8, and an elevated level of IgG (125). In addition, Ehrlich et al. showed that mice chronically exposed to NO₂ have impaired
serum neutralization antibody responses to influenza virus vaccine, and the unvaccinated mice exposed to NO\textsubscript{2} also had altered levels of different serum immunoglobulins (126).

Exposure to high levels of SO\textsubscript{2} has been associated with increase in IL-6 and tumor necrosis factor levels (115-116, 122). Other studies showed that in mice there was a suppression of antibody formation after the exposure to SO\textsubscript{2} (127). Zarkower et al. found that exposure to SO\textsubscript{2} for about four months enhanced antibody production, but the exposure after six months suppressed the antibody production (127). This study showed that SO\textsubscript{2} can have both positive and negative effects on immunity, and these depend on the duration of the exposure.

Particulate matter (PM) also alters immune functions. Leonardi et al. analyzed the effects of PM on immune biomarkers and found that the number of B cells, CD4 cells, CD8 cells, and NK lymphocytes increased with increasing concentration of PM (128). Total IgG was shown to increase with increased PM concentrations.

The effects of CO on immune function have been less studied compared to other pollutants. However, Snella et al. showed that pigs exposed to CO for four weeks have increased number of pulmonary alveolar microphages and polymorphonuclear leukocytes (129). Since these pollutants are ingested by lung microphages, it is possible that the ingestion affects the antibody production. This interference can result into increase or decrease in antibody levels depending on the disease and the immune marker being studied.

The lack of literature on how these environmental parameters influence antibody responses against \textit{Pneumocystis} infection can be explained by the difficulties
researchers faced in the past to find a suitable reagent to properly characterize antibody reactivity to *Pneumocystis* antigens. With a new technique developed by Daly et al. (94-96, 102), it is now feasible to identify and quantify antibody levels in HIV-infected patients with PcP.

**Methods**

**Study Design and Population**
A cross-sectional design was used in this part of the study. Exposure levels to climatic and air pollution factors were measured at the time of PcP admission and two weeks before admission. The subjects involved in this part of the study are a subset of patients admitted for PcP at San Francisco General Hospital from 1997 to 2008. A sample of 139 patients admitted to the hospital between 2000 and 2008 had blood specimens available for antibody analysis.

**Clinical and Demographic Data Collection**
At the time of admission, clinical and demographic characteristics were collected. Some of the subjects’ characteristics available were age, gender, race, CD4 cell counts, HIV viral load, use of PcP prophylaxis within the last three months, and serum albumin level. Serum specimens were drawn at the time of admissions. The specimens were stored at -80c and shipped to Cincinnati Veterans Affairs Medical Center where antibody levels were measured.
Exposure Characterization

To measure the environmental exposure levels affecting antibody responses, an average exposure over a 3-day time period at the time of admission (the day of admission, one day before admission, and two days before admission) was calculated. To analyze the lag effects of these environmental factors, the exposure level at two weeks before admission was measured. The lag exposure level was an average exposure of day 14, 15, and 16 before admission.

Environmental Data

The same environmental data described above were used. However, in this part daily air pollution and climatic data from 2000 to 2008 were obtained.

Isolation and Expression of Msg Fragments

A method that was previously shown was used to generate Msg fragments (94-95, 102). Briefly, oligonucleotides were designed on the basis of the known sequence of the msg gene of P. jirovecii and were used in polymerase chain reaction (PCR) to generate 3 overlapping fragments of the msg gene. The sequence of the PCR products was confirmed, and they were cloned into the pET30 expression vector (Novagen) in the correct reading frame and were expressed in Escherichia coli. The 3 recombinant proteins were called “MsgA,” “MsgB,” and “MsgC”. Recombinant protein expressed from the pET30 vector without insert was used as a control antigen. The recombinant proteins were expressed in inclusion bodies within E. coli and were purified by standard methods.
IgG ELISA

ELISA was performed following a previously reported procedures (102). Briefly, the reactivity of each serum specimen to Msg was corrected by subtraction of the reactivity of that serum to phosphate-buffered saline (mean OD Msg – mean OD phosphate-buffered saline). The results were quantitated using a method similar to that of Bishop and Kovacs (130), using a standard curve specific for each construct. Test sera were assayed at dilutions that fit the linear portion of the standard curves, and units of reactivity were calculated. Samples whose values were below the standard curve were assigned the lowest possible value of 1 U.

Statistical Analysis

The methods used above in the first stage of the analysis were also used in this part of the study to describe the Clinical and demographic characteristics of the 139 patients with blood specimens available at the time of admission. However, the second stage of the analysis has changed because the outcome here is continuous. Since some PcP patients had antibody levels below the limits of detection and censored to ‘1’, Tobit regression was used to evaluate the effect of each environmental factor on IgG antibody levels. For each environmental factor, the estimate effects on antibody levels were obtained. The effects were estimated for a 10% increase in mean levels of each environmental factor. Forward stepwise regression was used to combine environmental, clinical, and demographic factors that were significantly related to antibody levels (p<0.15) in a multivariate regression model. The final multivariate model included
environmental factors which were significant \((p < .05)\) and at most one other factor \((p > .05)\). SAS for Windows, version 9.2 (SAS Institute, Cary, NC) was used to carry out all statistical analyses, and a 5% significance level was assumed, unless stated otherwise. Graphs were generated using Microsoft Excel

**Chapter Three: Results, Discussion and Conclusions**

**Results**

**Patients’ Demographic Characteristics.** In a previous study where we analyzed serum antibody levels to major surface glycoprotein in the diagnosis of PcP in HIV+ patients, PcP cases were 110 patients admitted to the SFGH for PcP and the 63 controls were patients with other types of pneumonia (103). In that study, the analysis focused on first episode of PcP. Since in the present study the goal was to identify environmental factors that influence antibody responses in PcP patients, we used only PcP patients. However, we have increased the number by including patients whose specimens were drawn during the second episode of PcP. Therefore, from January 2000 to December 2008, 139 PcP patients admitted in SFGH for PcP with blood specimens available at the time of admission for analysis were identified. Their median age was 42.10 years, and most of them were whites (52%). The majority of them were males (86%). They were in advanced stage of HIV+ with low median CD4 cell count (32 cells/µL) and high median HIV viral load \((1.30 \times 10^5 \text{ copies/mL})\). Most of them (76%) were on PcP prophylaxis within the last three months before admission (**Table 1**).
Effects of Environmental Factors on IgG Antibody Level to MsgC. For IgG antibody responses to MsgC, only temperature was significantly associated with IgG antibody levels to MsgC in the univariate analysis. A 10% increase in mean temperature at the time of admission was significantly associated with an increase in IgG antibody levels to MsgC (p=0.01). An increase in humidity and NO₂ was associated with high antibody levels to MsgC, but high levels of SO₂, PM₁₀, ozone, and CO decreased antibody levels even though the effects were not statistically significant. Exposure to these factors two weeks before admission had non-significant effects on antibody levels to MsgC (Table 2). In multivariate analysis controlling for CD4 cell count, age, gender, serum LDH levels, HIV viral load, and other environmental factors, temperature at admission was still significantly associated with antibody levels to MsgC. Increase in serum LDH level was significantly associated with a decrease in the antibody levels (Table 3). The effects of the other variables and their interactions with temperature in multivariable analysis were not significant (results not shown). The seasonal variation of IgG antibody responses to MsgC was not statistically significant. The levels were higher in summer, but lower in winter.

Effects of Environmental Factors on IgG Antibody Levels to MsgA. For IgG antibody levels to MsgA, an increase in temperature and ozone at the time of admission was significantly associated with an increase in IgG antibody levels to MsgA in univariate analysis (p<0.01, p=0.02 respectively). However, only exposure to temperature at two weeks before admission was significantly associated with antibody levels to MsgA (p=0.03) (Table 4). In multivariable analysis, only temperature at the time of admission and at two weeks before admission were significantly associated with
antibody levels to MsgA \((p<0.01)\) (Table 5). Increase in serum LDH levels was significantly associated with decrease in IgG antibody responses to MsgA. There was a significant seasonal variation in antibody responses to MsgA. Patients who were admitted in summer had the highest mean antibody levels to MsgA, but those admitted in winter had the lowest mean antibody levels to MsgA \((p<0.05)\). Clinical and demographic factors such as age, CD4 cell count, HIV viral load, race, and gender were also included in the multivariable models, but their effects were not significant (results not shown).

**Discussion and Conclusions**

This part of the dissertation identified environmental factors associated with IgG antibody levels to both MsgC and MsgA using patients admitted at the SFGH. The results showed that only temperature at the time of admissions was significantly associated with IgG antibody levels to MsgC; and temperature at admission and at two weeks before admission was significantly associated with IgG antibody levels to MsgA. There was also a significant seasonal variation in antibody levels to MsgA, but not to MsgC.

The effects of seasonality on antibody responses to MsgA, but not to MsgC are consistent with our previous reports (106). In one of our previous publication, long-term serologic responses to Msg fragments were analyzed using patients from the Multicenter AIDS Cohort Study (MACS) (2). In that study, it was found that there was geographic variation in antibody levels to MsgC variants, but the variation among seasons was not significant. In addition, in our recent publication involving the analysis
of antibody responses to Msg fragments in healthy children from Chile, it was found that the effects of seasonal variation on antibody responses to MsgC were not significant. In that study significant seasonal variation of antibody responses to MsgA was found. However, in contrast to Chile study in which MsgA levels were found to be higher in spring, in the present study, antibody levels to both MsgA were higher in summer.

Using animal models, studies have shown that there is seasonal influence on the levels of lymphocytes, neutrophils, CD4 and CD8 cells, and IL-6(107-109). Furthermore, it has also been shown that the seasonal variation of daylight influences melatonin and vitamin D levels in the body, and those influences can lead to immune dysfunction(112). It is possible that the seasonal changes of these immune parameters are responsible for the variations reported in this study. However, there is no good answer to why only antibody responses to MsgA are affected by seasonal variation. It is possible that the variability and the orientation of MsgA toward the external surface of the protein make MsgA more exposed to seasonal variation compared to MsgC. This exposure to external environment might trigger different antibody levels. The findings that an increase in temperature is associated with an increase in antibody levels are consistent with some, but not others, previous studies. Some studies have shown that an increase in temperature causes an increase in the immune function (108), but others found the opposite (108, 110-111). Nelson et al. showed that low temperature causes a significant reduction in IgG levels (111). However, Lacetera et al. showed that in extremely hot conditions, cellular immunity in dairy cows was more likely to be depressed (110). In the present study, the moderate temperature changes commonly seen in San Francisco was able to affect the levels of antibodies to Msg fragments among HIV+ patients.
For the effects of air pollution on antibody responses to Msg fragments, this study was the first to assess the association between air pollution parameters and antibody levels to Msg fragments. In general, studies showed the effects of air pollution on immune system (55, 113). Air pollution elicits local and systemic inflammatory responses and suppresses host defenses (56). Exposure to air pollution initiate inflammatory responses and these inflammatory responses cause the decrease or increase of some immune responses. A study reported that IgG, IgM, and IgA levels in people living in more polluted areas are altered (117). Furthermore, mothers from areas with high level of air pollution had lower percentage of total T-cell and CD4 cells, and lower CD4/CD8 ratio (118).

When considering a single pollutant effects on antibody levels, only ozone were significantly associated with antibody levels to MsgA. However, these significant effects disappeared after adjusting for other environmental factors. Antibody levels to MsgC were not associated with any environmental factors, either in a single pollutant model or in multivariate model. Ozone is a well-known pollutant that affects the immune system (119). It high level in environment was linked to increase in the level of IL-6, IL-8, granulocyte-microphage colony stimulating factors, T cells and B cells ratio in lungs, and fibronectin in BAL (113, 119-120, 122, 131). Similar effects of ozone on immune system were found in this study, but only in the unadjusted model.

The other pollutants namely CO, PM$_{10}$, SO$_2$, and NO$_2$ were not significantly associated with IgG antibody responses to Msg fragments. It has been documented in other studies that an increase in air pollution factors was associated with a significant increase or decrease in antibody levels (114-119). Some studies revealed that high
exposure to SO₂ was associated with an increase in IL-6 and tumor necrosis factors level (115, 122). Others showed that high levels of SO₂ suppressed antibody formation (127). One study reported that exposure to NO₂ was associated with significantly higher level of antigen-specific IgE, IgA, and IgG (124). Furthermore, higher exposure to NO₂ has been linked with higher number of CD3, CD4, CD8, and IgG (125). Similar effects have been found for PM₁₀ (128). The effects of CO on immune system are not well documented. These different types of associations were not seen in this present report. However, the design of this study is different from other studies. This study was performed in HIV-infected patients, whose immune system was already compromised. Perhaps a different result would have been found if immunocompetent patients were used for the analysis. Also, this study determined the effects of air pollution on antibody levels to a specific infection.

This study shows that only the variation of temperature significantly affects antibody levels to Msg fragments. Its increase is associated with an increase in antibody levels. However, the clinical significance of this increase in antibody levels still needs to be determined. We have previously shown that HIV+ patients who die from PcP had significantly higher antibody level to MsgC variants prior to death (2). Based on that study, it is attempting to postulate that increase in temperature causes increase in antibody responses to Pneumocystis, and this increase in antibody level is a prognostic marker of death. Nevertheless, for the present study, we have insufficient data to relate antibody levels to death. More studies are needed to analyze this possible cascade of events where temperature affects antibody levels which can be used to prognosticate on the outcome.
This study has several limitations. First, the study was done in a city that has subtle climatic variation with air pollution levels below the national standard levels. This can also explain the non-significant effects of air pollutants on antibody levels. Second, weather station and air pollution monitoring stations were used to determine individual exposure level. This method may lead to exposure underestimation since monitoring stations may not provide detailed information about microclimate in which each patient might be living. Finally, since the study was conducted in a single location, the results may not be generalized.

In conclusion, this study has shown that temperature has significant effects on antibody levels to Pneumocystis infection. It also showed that antibody levels to MsgA were more sensitive to environment factors compared to antibody levels to MsgC, but this was only seen in univariate analysis. More studies are needed to confirm the findings of the present study. Also, studies are needed to see if these factors affect antibody responses to other infectious diseases.
Tables and Figures of Dissertation Part II

**Table 1.** Descriptive Characteristics of the 139 HIV-infected Patients with PcP having Specimens Available at the Time of Admission

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>Median [25th, 75th] or percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>139</td>
<td>41.70 [36.60, 47.30]</td>
</tr>
<tr>
<td>Male</td>
<td>119</td>
<td>86 %</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>45</td>
<td>32 %</td>
</tr>
<tr>
<td>White</td>
<td>72</td>
<td>52 %</td>
</tr>
<tr>
<td>Other</td>
<td>22</td>
<td>16 %</td>
</tr>
<tr>
<td>CD4 Cell Count (Cells/µL)</td>
<td>139</td>
<td>32 [8, 73]</td>
</tr>
<tr>
<td>HIV RNA (Copies/ mL)</td>
<td>135</td>
<td>1.30 x10⁵ [4.84x10⁴, 4.10x10⁵]</td>
</tr>
<tr>
<td>PcP Prophylaxis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>106</td>
<td>76 %</td>
</tr>
<tr>
<td>No</td>
<td>33</td>
<td>24 %</td>
</tr>
</tbody>
</table>
Table 2. Univariate Effects of Environmental Exposures on IgG Antibody Responses to MsgC

<table>
<thead>
<tr>
<th>Environmental Exposure (Unit)</th>
<th>Exposure at the Time of Admission</th>
<th>Exposure at Two Weeks before Admission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Effect</td>
</tr>
<tr>
<td>Temperature (°F)</td>
<td>58.05</td>
<td>0.64</td>
</tr>
<tr>
<td>SO₂ (PPB)</td>
<td>1.67</td>
<td>0.02</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>74.29</td>
<td>-0.24</td>
</tr>
<tr>
<td>NO₂ (PPB)</td>
<td>13.03</td>
<td>-0.02</td>
</tr>
<tr>
<td>PM₁₀ (ug/m³)</td>
<td>18.62</td>
<td>0.25</td>
</tr>
<tr>
<td>Ozone (PPB)</td>
<td>21.90</td>
<td>0.11</td>
</tr>
<tr>
<td>CO (PPM)</td>
<td>415.72</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Note: p-value tests the significance of the effect of each independent variable on IgG antibody responses to MsgC.
Table 3. Multivariate Effects of Environmental Exposures on IgG Antibody Responses to MsgC

<table>
<thead>
<tr>
<th>Environmental Exposure (Unit)</th>
<th>Exposure at the Time of Admission</th>
<th>Exposure at Two Weeks before Admission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Effect</td>
</tr>
<tr>
<td>Temperature (°F)</td>
<td>58.05</td>
<td>0.52</td>
</tr>
<tr>
<td>PM$_{10}$ (µg/m$^3$)</td>
<td>18.62</td>
<td>1.56</td>
</tr>
</tbody>
</table>

Note: p-value tests the significance of the effect of each independent variable on IgG antibody responses to MsgA.
### Table 4. Univariate Effects of Environmental Exposures on IgG Antibody Responses to MsgA

<table>
<thead>
<tr>
<th>Environmental Exposure (Unit)</th>
<th>Exposure at the Time of Admission</th>
<th>Exposure at Two Weeks before Admission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Effect</td>
</tr>
<tr>
<td>Temperature (°F)</td>
<td>58.05</td>
<td>1.86</td>
</tr>
<tr>
<td>SO₂ (PPB)</td>
<td>1.67</td>
<td>0.02</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>74.29</td>
<td>-0.65</td>
</tr>
<tr>
<td>NO₂ (PPB)</td>
<td>13.03</td>
<td>-0.04</td>
</tr>
<tr>
<td>PM₁₀ (μg/m³)</td>
<td>18.62</td>
<td>-0.11</td>
</tr>
<tr>
<td>Ozone (PPB)</td>
<td>21.90</td>
<td>0.11</td>
</tr>
<tr>
<td>CO (PPB)</td>
<td>415.72</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**Note:** p-value tests the significance of the effect of each independent variable on IgG antibody responses to MsgA.
Table 5. Multivariate Effects of Environmental Exposures on IgG Antibody Responses to MsgA

<table>
<thead>
<tr>
<th>Environmental Exposure (Unit)</th>
<th>Exposure at the Time of Admission</th>
<th>Exposure at Two Weeks before Admission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Effect</td>
</tr>
<tr>
<td>Temperature (°F)</td>
<td>58.05</td>
<td>1.80</td>
</tr>
<tr>
<td>SO₂ (PPB)</td>
<td>1.67</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Note: p-value tests the significance of the effect of each independent variable on IgG antibody responses to MsgC

Fig 1. Msg Recombinant Fragments
References

15. D'Egidio GE, Kravcik S, Cooper CL, Cameron DW, Fergusson DA, Angel JB. Pneumocystis jiroveci pneumonia prophylaxis is not required with a CD4+ T-cell count < 200 cells/microl when viral replication is suppressed. AIDS. 2007;21(13):1711-5.


81. EPA. National Air Quality System.


Appendix A: Role in the Study

Study Design and Hypothesis Formulation

The cohort used in this study was designed by Dr. Laurence Huang at San Francisco General Hospital to determine factors associated with PcP progression in HIV-infected patients. Kpandja Djawe, in conjunction with Dr. Peter Walzer, proposed to use this cohort to determine the effects of environmental factors on PcP hospital admissions. The use of Case-Crossover design was proposed by Kpandja Djawe, in conjunction with Drs. Peter Walzer and Linda Levin. Kpandja Djawe, Along with Dr. Peter Walzer developed the hypothesis and the specific aims. Advice on how to choose the appropriate control times was given by Drs. Peter Walzer, Laurence Haung, and Robert Miller.

Data

The clinical and demographic characteristics were collected in SFGH by Dr. Laurence Huang research assistants. The environmental data was obtained by Kpandja Djawe from publicly available databases. Free air pollution data was obtained the EPA Air Quality System. The climatic data was purchased from Weather Warehouse with Dr. Walzer’s grant money. The MSG recombination and the ELISA analysis were done at Cincinnati Veterans Affairs Medical Center by Dr. Kieran Daly and Ms. Judy Koch.

Literature Review
Literature review was performed by Kpandja Djawe

**Statistical Analysis**

The use of logistic regression in part I of dissertation and the use of Tobit regression in part II of the dissertation were proposed by Kpandja Djawe, in conjunction with Dr. Linda Levin. Analysis was done in SAS by Kpandja Djawe with supervision of Dr. Linda Levin. Data interpretation was made by Kpandja Djawe with advice from Drs. Linda Levin and Peter Walzer.

**Writing**

Kpandja Djawe was the original author of the writing. Drs. Peter Walzer, and Linda Levin, Ralph Buncher, and Ranjan Deka performed editing and comment the text.

**Appendix B: Future Research**

In part I of this study, we found that increase in temperature and SO2 was significantly associated with increase in PcP hospital admissions in San Francisco. This raised questions that need to be addressed in future studies. First, how are environmental factors affect PcP admissions in other areas of the country? I plan to investigate several key problems in this area of air pollution and hospital admissions. A multicenter study needs to be developed to see if these factors are also predictors of PcP in other geographic locations in the country. There are HIV/AIDS cohorts such as Multicenter AIDS Cohort Study (MACS) and Women Interagency HIV Study (WIHS) with centers in major cities of the U.S. These cohorts can be a possible data source for this type of project. Second, what is the biological mechanism behind the effect of environmental factor on PcP admissions and on antibody responses? Animal model
study needs to be developed to address this question. Third, what is the effect of air pollution on other opportunistic respiratory disease hospital admissions?