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# Investigating the spill-over hypothesis: Analysis of the association between local inflammatory markers in sputum and systemic inflammatory mediators in plasma

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ABSTRACT

Exposure to air pollutants represents a risk factor not only for respiratory diseases and lung cancer, but also for cardiometabolic diseases. It has been hypothesised that local inflammation in the lung and systemic subclinical inflammation are linked by impaired lung function and the spill-over of proinflammatory factors from the lung into the circulation which could act as intermediaries between environmental exposures and disease risk. We wanted to investigate whether local and systemic inflammatory markers are associated, which would support the spill-over hypothesis.

Sputum and plasma samples were obtained from 257 women of the German SALIA cohort. We performed immunoassays to measure multiple biomarkers of airway inflammation in sputum as well as cytokines, chemokines and soluble adhesion molecules in plasma. Correlations were calculated and adjusted for potentially confounding variables.

Even though several significant associations were detected between inflammatory mediators in sputum and plasma, correlation coefficients were rather low ranging from $r \geq -0.20$ to $r \leq 0.20$. Comparatively stronger associations were observed between nitrite, eosinophil cationic protein, leukotrienes C/D/E4 and interleukin-8 in sputum. Notably, correlations were positive with all proinflammatory biomarkers and interleukin-1 receptor antagonist in plasma, whereas negative correlations were observed with the anti-inflammatory adipokine adiponectin.

*Abbreviations:* BMI, body mass index; COPD, chronic obstructive pulmonary lung disease; CRP, C-reactive protein; ECP, eosinophil cationic protein; HMW, high-molecular-weight; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; IL-1ra, IL-1 receptor antagonist; IP-10, interferon gamma-induced protein 10; LC, leucocyte count; LT, leukotriene; MCP-1, macrophage chemoattractant protein-1; MMP, matrix metallopeptidase; N/A, not applicable; SALIA, Study on the influence of air pollution on lung function, inflammation and aging; sE-selectin, soluble E-selectin; sICAM-1, soluble intracellular adhesion molecule-1; sTNFα-R55/75, soluble tumour necrosis factor-α receptor 55/75; TGF-β1, transforming growth factor β1; TNFα, tumour necrosis factor-α; %NC, sputum neutrophils (%).

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In conclusion, local inflammation in the lung and systemic subclinical inflammation appear mainly independently regulated in elderly women from the general population. Although we found multiple significant correlations between inflammatory biomarkers in sputum and plasma, our results do not provide clear support for the spill-over hypothesis.



## 1. Introduction

The exposure to air pollution does not only contribute to the development of respiratory diseases and lung cancer, but also to cardiovascular disease and overall mortality (Brook et al., 2010). In addition, there is also some evidence for associations with atherosclerosis, stroke (Brook et al., 2010), hypertension (Sun et al., 2008), type 2 diabetes (Krämer et al., 2010; Rajagopalan and Brook, 2012) and cancers at other sites than the lung (Raaschou-Nielsen et al., 2011a, 2011b).

All aforementioned diseases have in common that an underlying subclinical inflammation has been described as one important mechanism (albeit not necessarily as primary cause from an aetiological point of view) in their development and progression (MacNee, 2013; Sinden and Stockley, 2010; Steinvil et al., 2008; Tkacova, 2010). It has been hypothesised that the incidence of respiratory diseases as consequence of exposure to air pollution, which triggers local inflammation in the lung tissue (Ghio et al., 2000; Gilmour et al., 2004; Hetland et al., 2004; Michael et al., 2013; Seagrave, 2008), could also induce systemic inflammation. Several studies support this hypothesis by reporting an association between exposure to air pollution and systemic inflammation (Hoffmann et al., 2009; Ruckerl et al., 2006; Schwartz, 2001; Seaton et al., 1999). However, the mechanisms how local inflammation spreads throughout the body and leads to systemic subclinical inflammation remain largely unknown (Sinden and Stockley, 2010). There are two hypotheses that aim to explain this phenomenon. The first hypothesis states that exogenous particles enter the lung, pass through the lung-blood barrier and are then distributed in the circulation through the body where they may cause local inflammatory reactions in different target tissues. The second hypothesis assumes that air pollutants enter the lung, activate local immune cells, which then respond by the release of inflammatory proteins that *spill over* into the circulation. This mechanism could lead to a systemic inflammatory reaction based on locally released and then systemically distributed immune mediators. The latter "spill-over hypothesis" is commonly used to describe the deleterious health effects of air pollutants (Sinden and Stockley, 2010).

It is important to note that air pollution is not the only factor contributing to inflammatory processes in the respiratory tract. Another widespread risk factor is smoking, which is also related to systemic inflammation and increased cardiometabolic risk (Glantz and Gonzalez, 2012). In addition, several medical conditions are characterised by inflammation in the lung. These include chronic obstructive pulmonary lung disease (COPD), asthma and pulmonary infections. COPD patients show chronic airway inflammation mainly caused by inhaled substances from cigarette smoking, but also to a lesser degree from air pollution (GOLD Scientific Committee, 2013). Currently available data provide evidence for the presence of systemic subclinical inflammation in patients with COPD, because serum or plasma levels of several inflammatory mediators such as the proinflammatory cytokines interleukin-6 (IL-6) and tumour necrosis factor-α (TNFα), the acute-phase protein C-reactive protein (CRP) and the chemokine IL-8/CXCL8 are higher in COPD patients than in healthy controls (Agustí et al., 2012; Eagan et al., 2010; Garcia-Rio et al., 2010; Mohamed et al., 2013; Walter et al., 2008). Notably, COPD patients also suffer more frequently from cardiometabolic diseases and type 2 diabetes which both have an inflammatory component (Decramer et al., 2012).

The hypothesised overspill of proinflammatory mediators from the lung into the systemic circulation has been investigated in mostly small study samples based on patients with COPD, but beyond this selected group of patients the evidence for the putative association between airway inflammation and systemic inflammation in humans remains rather poor and data from population-based studies are not available.

Therefore, our study aim was to examine the association between local inflammation in the respiratory tract and systemic subclinical inflammation. For this objective we performed a cross-sectional analysis within the Study on the influence of air pollution on lung function, inflammation and aging (SALIA) cohort in Germany. Previous findings in this cohort included associations between the exposure to air pollution and a variety of health outcomes like COPD (Schikowski et al., 2005), mild cognitive impairment (Ranft et al., 2009), airway inflammation (Vossoughi et al., 2014), impaired glucose metabolism (Teichert et al., 2013) and incidence of type 2 diabetes (Krämer et al., 2010). The SALIA study was chosen for our objective because of the availablility of in-depth phenotyping in a subset of the cohort and a history of high exposure to air pollution. We used sputum concentrations of a range of inflammatory mediators and cells as indicators for airway inflammation and analysed their correlations with plasma levels of 14 pro- and anti-inflammatory mediators which could represent putative intermediaries between the exposure to air pollution and the incidence of cardiometabolic and other diseases.

## 2. Methods

### 2.1. Study design and population

We performed a cross-sectional study within the SALIA cohort (baseline 1985–1994). The data are based on results generated during the follow-up examination of $N=834$ surviving women between 2008 and 2009 (Teichert et al., 2013). Fasting blood samples were collected in a subgroup of $N=363$ women, and from this population $N=257$ women also complied to give an induced sputum sample. A drop-out analysis comparing the 257 women with both sputum and plasma samples and the remaining 106 women with plasma samples only is provided in Supplemental Table A1. Written informed consent from all study participants was collected. The study was approved by the ethics committee of the Ruhr University in Bochum (Germany) and carried out in accordance with the Declaration of Helsinki.

### 2.2. Physical examination, interview and questionnaires

Anthropometric measurements (height, weight) were conducted according to standardised protocols. Additional data were obtained using an extensive interview, which included questions on symptoms, previous diagnoses of respiratory diseases (e.g. asthma, bronchitis, COPD) as well as other chronic diseases (e.g. type 2 diabetes and previous myocardial infarction) and medication. Socioeconomic status was stratified into two categories by the maximum period of education achieved by the women or their spouses ($<10$ years vs. $\geq 10$ years). Women were grouped according to their smoking habits as never smokers, passive smokers (home, workplace), past smokers or current smokers. Data on exposure to indoor mould as covariable were also collected by questionnaire.