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Association between smoking and presence of Mycoplasma pneumoniae in circulation leukocytes

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Summary

PRINCIPLES: The morbidity and mortality of infectious diseases due to smoking are not widely appreciated by physicians. However, cigarette smoking appears to be a major risk factor for respiratory tract and other systemic infections. Only limited data are available on the association between smoking and Mycoplasma (M) pneumoniae. We raise the question of whether smoking increases the presence of M pneumoniae in circulating leukocytes. Furthermore we have studied whether the combination of smoking and M pneumoniae infection affects circulating levels of inflammatory biomarkers.

METHODS: Prevalence of latent M pneumoniae infection was analysed in a total of 122 subjects by polymerase chain reaction. Smoking status was documented at presentation. Circulating levels of c-reactive protein, macrophage chemoattractant protein-1 and complement factor 5a were determined by commercial enzyme linked immuno-sorbent assays.

RESULTS: We found a significant association between smoking and latent M pneumoniae infection ($p = 0.009$). This association remained significant after correction for age, gender and diabetes (OR 3.4; 95% CI 1.3–9.4; $p = 0.017$). There was no correlation between circulating levels of the inflammatory biomarkers studied and smoking or M pneumoniae infection, respectively.

CONCLUSIONS: Our data indicate that smoking is associated with the presence of M pneumoniae in circulating leukocytes. This could contribute to the pro-inflammatory effects of smoking. Despite the low number of subjects included in this study, this is an interesting finding demanding further investigation.

Key words: *Mycoplasma pneumoniae; smoking; inflammation*

Introduction

Smoking is well-known major risk factor for premature mortality due to cancer, cardiovascular disease and chronic obstructive pulmonary disease [1]. It currently causes 5 million deaths per year, and if present trends continue, 10 million smokers per year are projected to die by 2025. Nonetheless, the worldwide production and consumption of cigarettes is increasing [2].

Cigarette smoking also appears to be a major risk factor for respiratory tract and other systemic infections. Both active and passive cigarette smoke exposure increase the risk of infections [1]. The morbidity and mortality of infectious diseases due to smoking are not widely appreciated by physicians.

It has been shown that community acquired pneumoniae (CAP) is associated with tobacco consumption [3]. However, only limited data are available on the association between smoking and specific infectious agents [3]. There is experimental evidence for a facilitating effect of smoking in infection with *Streptococcus pneumoniae* [4]. To the best of our knowledge, there are no data showing an association between smoking and presence of *Mycoplasma (M) pneumoniae* in circulating leukocytes.

Infections with *M pneumoniae* have been associated with COPD exacerbation [5]. A recent report described a detrimental interaction between *M pneumoniae* and cigarette smoke in a murine model, accelerating the onset of COPD [6]. After infection with M pneumoniae, which usually occurs in the respiratory tract, this infectious agent can persist in circulating leukocytes and it is thereby suspected of fuelling chronic, systemic inflammation [7–9].

We raise the question of whether smoking is associated with the presence of *M pneumoniae* in circulating leukocytes. Furthermore, we studied whether the combination of smoking and *M pneumoniae* infection affects circulating levels of inflammatory biomarkers previously associated with chronic inflammatory conditions, such as atherosclerosis or COPD [10–12].

Materials and methods

Subjects and study design

This is a cross sectional study. Subjects with stable coronary artery disease and with no history or current medication for COPD were recruited from the cardiology outpatient clinic. All subjects were of middle European ethnicity. At the time point of inclusion, all patients were asymptomatic for at least three months for their stable coronary artery disease. Patients were not included if there were clinical signs or treatment necessity for heart failure or use of nitrates was reported in the last three months. Patients were recruited between July 2004 and June 2006. Furthermore, we excluded subjects with concurrent malignant disease, severe kidney failure or who had suffered an acute coronary syndrome within the three months before enrolment.

Written informed consent was obtained from all participants. This study was approved by the Medical University of Vienna ethics committee, and complies with the declaration of Helsinki. The clinical data were entered into a computerised database. Baseline characteristics can be seen in table 1.

Definition of co-morbidities

Subjects were classified as having diabetes when under treatment for insulin-dependent or non-insulin-dependent diabetes mellitus or when they had fasting blood glucose levels ≥110 mg/dl or HbA1C levels ≥6%. Subjects were classified as smokers when they were smoking at the time of enrollment.

DNA isolation

All laboratory measurements were performed by investigators blinded to subject characteristics. Venous blood samples were drawn from the antecubital vein into EDTA tubes and DNA was extracted from whole blood using a commercially available DNA extraction kit (MagNA Pure LC DNA Isolation Kit, Roche, Basel, Switzerland) according to the manufacture's instructions.

Polymerase chain reaction

The method used for detection of *M pneumoniae* is described elsewhere [9]. Briefly, the amplification conditions consisted of an initial denaturation step at 95 °C for 5 minutes, 30 cycles of 95 °C for 1 minute, 65 °C for 1 minute and 72 °C for 1 minute, a final elongation step at 72 °C for 7 minutes followed by a cooling phase to 4 °C. The design of the sense (5'-gcc acc ctg ggg ggc agt cag-3'; 2599-2619) and antisense (5'-gag tcg gga ttc ccc gcg gag g-3'; 2807-2729) primers resulted in an amplification product with the size of 209 base pairs.

Enzyme linked immunosorbent assays

Blood samples were drawn after overnight fasting (≥10 h) at inclusion. Serum was separated within one hour and prepared for c-reactive protein (CRP) analysis, performed by use of conventional methods. In addition, serum used for monocyte chemoattractant protein-1 (MCP-1) and complement factor 5a (C5a) was prepared and kept frozen at –70 ˚C until further analyses. Commercial Enzyme Linked Immunosorbent Assays were used for the MCP-1 and C5a analyses (R&D Systems Europe, Abingdon, Oxon, UK).

Statistical analysis

Sample size was calculated with an alpha set at 5% and a beta set at 80%, assuming that 10% difference in presence of *M pneumoniae* amongst smokers compared to non-smokers would be of clinical relevance. Association between smoking and *M pneumoniae* infection was calculated using Fisher's exact test. Odds ratio (OR) for association between smoking and *M pneumoniae* infection was calculated with logistic regression. OR was adjusted for relevant covariates (age, gender and diabetes) in a multivariate regression model. For comparison of circulating levels of inflammatory markers in subjects with and without exposure to smoking and *M pneumoniae* infection, respectively, Kruskal-Wallis test or Bonferroni post hoc analysis was used for continuous data and the Pearson Chi squared test for categorical data where appropriate. A significance level of 0.05 was used throughout. The statistical analyses were performed with SPSS software, version 16.0 (SPSS Inc, Chicago).

Results

In accordance with previous findings, the prevalence of latent *M pneumoniae* infection in our study population was 66% [9, 13]. We found a significantly higher prevalence of *M pneumoniae* infections in smokers (83%) compared to non-smokers (59%). As can be seen from table 2, there

Table 1
Clinical characteristics of the exposure groups (total n = 122); Median values (interquartile range) or proportions (%) are given.

	No exposure n = 34	Smokers n = 7	M. pneumoniae positive n = 48	Both exposures n = 33	p
Age	67 (12)	67 (14)	69 (12)	56 (20)	0.01
Male gender	77%	57%	77%	85%	n.s.
Diabetes	24%	29%	25%	18%	n.s.
BMI	27 (4)	27 (6)	27 (6)	28 (6)	n.s.
Hypertension	77%	71%	83%	63%	n.s.

n.s. – not significant

was a strong association between smoking and *M pneumonia* infection (p <0.01). In multivariate regression analysis, this association remained significant after correction for age, gender and diabetes (OR 3.4; 95% CI 1.3–9.4; p = 0.017).

We divided the study population (median age 64, 78% male) into four different groups, according to their respective exposure to smoking or presence of latent *M pneumoniae* infection. All inflammatory markers measured (leukocytes, CRP, MCP-1 and C5a) might have a tendency to be higher in individuals exposed to smoking and latent *M pneumoniae* infection (table 3) compared to unexposed individuals. However, this tendency was not statistically significant, maybe due to lack of power. At an alpha level of 5%, a post hoc power analysis revealed a beta of only 23.2% for a two tailed analysis, when comparing exposed to non-exposed individuals.

Discussion

The main finding of this study is that smoking is strongly associated with presence of *M pneumoniae* in circulating leukocytes. We did not find an effect on circulating levels of inflammatory cytokines, possibly due to the lack of power for this analysis. We are, therefore, formally not allowed to draw conclusions from this finding. However, this finding can be seen as hypothesis generating. Even though there was no statistically significant systemic inflammatory response, the local inflammation and remodelling in various tissues might still be affected as described previously [6].

To the best of our knowledge, this is the first description of an association between smoking and the presence of *M pneumoniae* in circulating leukocytes. This association could be relevant in maintaining chronic systemic inflammation by fuelling low grade inflammation in circulating leukocytes through the presence of this pathogen. A recent study showed that tobacco smoke and *M pneumoniae* increase oxidative stress synergistically in the lungs [14]. Oxidative stress is a crucial component in inflammation in various organ systems. Through this interaction, the combination of two potentially pro-inflammatory stimuli, namely the presence of *M pneumonia*e and smoking, could lead to an increase in inflammation and oxidative stress in target organs of the circulating leukocytes analysed in this study.

One major limitation of this study is the low sample size in the subgroup analysis for the circulating inflammatory markers. This study was performed in a single medical centre. Thus, the external validity of its results is limited accordingly.

Funding / potential competing interests

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Table 2
Cross tabulation between smoking and presence of M. pneumoniae in circulating leukocytes.

	M. pneumoniae negative n, (%)	**M. pneumoniae positive n, (%)**	**Total n, (%)**
Non-Smokers	34 (41%)	48 (59%)	82 (100%)
Smokers	7 (18%)	33 (82%)	40 (100%)
Total	41 (34%)	81 (66%)	122 (100%)
Smoking is associated with presence of M. pneumoniae in circulating leukocytes (OR 3.4; 95% CI 1.3–9.4; p = 0.017)			

Table 3
Circulating levels of inflammatory biomarkers in groups of differently exposed individuals. Median values (25, 75 percentiles) are given. No significant differences were detected.

	No exposure n = 34	**Smokers n = 7**	**M. pneumoniae positive n = 48**	**Both exposures n = 33**
Leukocytes (103/µl)	6.6 (5.8; 7.2)	7.4 (5.7; 8.2)	6.2 (5.4; 7.5)	7.0 (6.4; 8.4)
CRP (mg/l)	0.2 (0.1; 0.5)	0.3 (0.1; 0.7)	0.2 (0.1; 0.4)	0.3 (0.1; 0.4)
C5a (ng/ml)	31 (25; 48)	27 (23; 49)	35 (22; 49)	39 (20; 50)
MCP-1 (pg/ml)	131 (97; 152)	131 (120; 139)	123 (98; 165)	132 (117; 190)

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