Analysis of inflammatory markers in adult Japanese smokers and non-smokers

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INTRODUCTION AND OBJECTIVE

The most important risk factor for chronic obstructive pulmonary disease (COPD) is cigarette smoking, and it has been estimated that smoking accounts for up to 95% of COPD in the developed world [Barnes 2000, Pauwels 2001]. However, the majority of smokers do not develop COPD and the precise mechanisms by which smoking causes COPD in susceptible smokers are unknown. Some serum biomarkers, including matrix metalloproteinase-9 (MMP-9), have been reported to be related to lung function decline in COPD [Higashimoto 2009]. Knowledge about biomarkers which are most affected by smoking may add some insight into the disease pathogenesis.

This observational study was performed to measure biomarkers of exposure (BoExp) to cigarette smoke constituents and biomarkers of effect (BoEff) in adult Japanese smokers and non-smokers. The main purpose of this study was to determine the levels and variability of BoEff and to understand the effect of smoking on these biomarkers. Multiplex analysis of inflammatory biomarkers was performed in plasma of study participants as a screening tool to identify additional biomarkers affected by smoking.

MATERIALS AND METHODS

This study was conducted in nine study centres in Japan from July 2007 to December 2007, and was performed according to the principles of International Conference on Harmonisation Good Clinical Practice [ICH 1996]. This study included adult smokers and non-smokers of both genders, 30 years of age and above in a ratio of 2:1 (smokers to non-smokers). Smokers were to have smoked at least 10 cigarettes per day for at least five years, whereas non-smokers were not to have smoked or used nicotine-containing products for at least one year before study entry. Smoking status was verified using a urine cotinine screening test. Key exclusion criteria included known chronic diseases and acute or recent infections.

The sample size was chosen for practical purposes. The study population was stratified by age (30-49 years and >50 years) and gender (at least 30% female) to ensure similar demographics in the study populations. Each subject had three visits, one for screening and two for biomarker assessments. Plasma prepared from 2 ml blood drawn at both assessment visits was analysed using multiplex technology (Rules-Based Medicine, Austin, USA). The Human MAP® was used to simultaneously measure 52 analytes in each sample as summarised below.

- α-1 antitrypsin (A1AT)
- α-2 macroglobulin (A2MG)
- adiponectin
- endothelin-1
- epidermal growth factor (EGF)
- epithelial neutrophil activating peptide 78 (ENA-78)
- glutathione S-transferase alpha (GST)
- granulocyte colony stimulating factor (G-CSF)
- granulocyte macrophage colony stimulating factor (GM-CSF)
- intercellular adhesion molecule 1 (ICAM-1)
- interferon γ (IFN-γ)
- immunoglobulin A (IgA)
- insulin
- interleukins (IL): IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 p40, IL-12 p70, IL-13, IL-15, IL-16
- leptin
- lymphotactin
- macrophage-derived chemokine (MDC)
- macrophage inflammatory proteins (MIP): MIP-1α, MIP-1β
- matrix metalloproteinase (MMP): MMP-2, MMP-3, MMP-9
- monocyte chemotactic protein 1 (MCP-1)
- myeloperoxidase
- regulated upon activation, normal T-cell expressed and secreted (RANTES/ CCL5)
- serum amyloid P (SAP)
- stem cell factor (SCF)
- thrombopoietin (TPO)
- thyroxin binding globulin (TBG)
- tissue factor
- tissue inhibitor of metalloproteinase 1 (TIMP-1)
- tumour necrosis factors (TNF): TNF-α, TNF-β, TNF receptor type 2 (TNF RII)
- vascular cell adhesion molecule 1 (VCAM-1)
- vascular epithelium growth factor (VEGF)
- von Willebrand factor (vWF)

All data analyses were performed according to a pre-defined statistical analysis plan. The mean value for each analyte from two consecutive visits was used for statistical analysis. The 95% confidence intervals of the means were used to indicate whether the data were different between smokers and non-smokers. For biomarkers with non-overlapping 95% confidence intervals of the means, data was further examined to identify the effect of potential confounding variables such as gender, age, and daily cigarette consumption. Due to the exploratory nature of this analysis, multiplicity adjustment was not performed.

RESULTS

1098 adult Japanese subjects (731 smokers and 367 non-smokers) were enrolled in the study. The multiplex biomarker statistical analysis was performed on samples from the per protocol population (a subset of subjects who completed the study and who had no major protocol violations). Smokers and non-smokers had similar demographic characteristics as summarised in Table 1.

Table 1: Study Population (per protocol)

Per protocol population	Statistics	Smoker	Non-smoker
Gender Male Female	 N (%) N (%)	 435 (64.9) 235 (35.1)	 226 (63.5) 130 (36.5)
Age (years)	N Mean (SD) Range	670 48.1 (11.7) 30-80	356 49.5 (12.8) 30-83
Body Mass Index (kg/m²)	N Mean (SD) Range	670 22.9 (3.5) 15.0-37.7	356 23.2 (3.3) 15.1-38.2

Differences between smokers and non-smokers were observed for the following twelve biomarkers: A1AT, A2MG, ICAM-1 (CD54), IgA, IL-7, MDC, MIP-1α, MMP-2, MMP-9, TBG, TIMP-1, and TNF RII. The results together with information regarding potential confounders are presented in Table 2.

Differences in mean values were also observed for the following biomarkers: VCAM-1 (CD106), IL-8, and IL-1ra. The 95% confidence intervals of the means were only slightly overlapping, suggesting that these biomarkers may also be affected by smoking.

There were no differences detected between smokers and non-smokers for other biomarkers including adiponectin, IL-16, leptin, RANTES (CCL5), SAP, SCF, VEGF, and vWF. For the remaining biomarkers, the variability of the values was high and many results were below the level of quantification.

Table 2: Summary of Data for Selected MAP Biomarkers

Biomarker	Smokers			Non-smokers			Potential confounders A=Age G=Gender C=cigs. per day
	N* BLOQ	Mean (SD)	LLCI95 ULCI95	N* BLOQ	Mean (SD)	LLCI95 ULCI95	
A1AT (mg/ml)	670 0	1.66 (0.27)	1.64 1.68	356 0	1.50 (0.26)	1.47 1.53	A^S
A2MG (mg/ml)	670 0	0.32 (0.06)	0.32 0.33	356 0	0.34 (0.05)	0.33 0.34	G,C
ICAM1 (ng/ml)	666 0	72.40 (25.96)	70.4 74.4	352 0	58.02 (18.40)	56.1 60.0	A^S,C
IgA (mg/ml)	669 0	1.43 (0.59)	1.38 1.47	356 0	1.71 (0.67)	1.64 1.78	A,G^{NS}
IL-7 (pg/ml)	670 46	91.5 (27.6)	89 94	356 35	86.6 (22.9)	84 89	NA
MDC (pg/ml)	670 0	260.9 (80.5)	255 267	356 0	208 (58.9)	202 214	C
MIP-1α (pg/ml)	669 4	36.96 (11.54)	36.1 37.8	356 11	34.70 (9.01)	33.8 35.6	G
MMP-2 (ng/ml)	670 0	1816.9 (688.1)	1765 1869	356 0	1957.0 (723.8)	1882 2032	A^{NS}
MMP-9 (ng/ml)	667 143	118.7 (89.7)	112 126	354 116	85.0 (61.6)	79 91	G,C
TBG (µg/ml)	670 2	58.10 (12.35)	57.2 59.0	356 0	55.26 (10.75)	54.1 56.4	A
TIMP-1 (ng/ml)	670 0	49.31 (9.74)	48.6 50.0	356 0	47.56 (7.87)	46.7 48.4	A,G,C
TNF RII (ng/ml)	670 0	2.40 (0.69)	2.35 2.45	356 0	2.26 (0.60)	2.20 2.32	A,G^S,C

*N: Total number of samples; BLOQ: Number of samples below level of quantification; LLCI95: Lower limit of 95% confidence interval; ULCI: Upper limit of 95% confidence interval; ^{S}: Indication of an effect only observed in smokers group; ^{NS}: Indication of an effect only observed in non-smokers group

DISCUSSION AND CONCLUSIONS

Multiplex analysis was used as a screening tool and has identified additional biomarkers which are potentially affected by cigarette smoking.

This exploratory analysis provides semi-quantitative information about these biomarkers, some of which may be involved in the pathogenesis of COPD. This information contributes to the selection of a biomarker panel which could be used to evaluate potential reduced-risk tobacco products.

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