Evaluation of Cardiovascular-Disease-Related Biomarkers in Adult Japanese Smokers and Non-Smokers

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Introduction

Tobacco smoking is one of the main causes of cardiovascular disease (CVD). According to the World Health Organisation (WHO), smoking increases the risk of dying from coronary heart disease and stroke 2-3 fold. In contrast, cardiovascular events fall by up to 50% in people who stop smoking.(1)

It is well known that smoking has a deleterious effect on various physiological pathways (impairment of endothelial cell dysfunction, elevation of the inflammatory status, adverse influence on cholesterol metabolism, increase in oxidative stress, and alteration of platelet function).(2) Smoking results in an acceleration of the progression of atherosclerosis in the vasculature, which in turn leads to CVD. The measurement of potential CVD-related biomarkers of effect (BoEff) reflecting the changes in biological processes in response to smoking could provide valuable insight toward a better understanding of the effects of cigarette smoking in the human body.

Objective

Quantify differences in several potential well known CVD-related BoEff, i.e., HDL-cholesterol (cholesterol metabolism), high sensitivity C-reactive protein (hsCRP) (inflammation), fibrinogen (platelet function), and 11-dehydro-thromboxane B2 (11-dehydro-TBX2) (platelet function), between adult Japanese smokers and non-smokers in an observational study.

Materials and Methods

Study Design:
An observational, parallel-group multi-center study conducted in Japan with adult smokers and non-smokers (ratio of 2:1). Eligible subjects completed three study visits: Visit 1 (screening of subjects, check of eligibility criteria, and allocation to the study arm) and Visits 2 and 3 (blood and urine samples taken for assessment). The total observational period was between 6 and 22 days. The study was performed according to the principles of Good Clinical Practice and was approved by local Institutional Review Boards.

Study Population:
- Male and female subjects of Japanese origin, 30 years of age or older with stable health status
- Smokers smoked commercial cigarettes exclusively, with a regular consumption of at least 10 cigarettes per day (cpd) and refrained from using other tobacco-related or nicotine-containing products throughout the study
- Non-smokers refrained from using any tobacco-related products (including commercial cigarettes) and did not use nicotine-containing products (for 1 year prior to Visit 1 and throughout the study)

Assessments:
Potential CVD-related BoEff were measured in plasma/blood (HDL, hsCRP and fibrinogen, and white blood cells [WBC]) and urine (11-dehydro-TBX2) using validated methods.
Adverse events, physical examination, vital signs, laboratory measurements, medical history, and concomitant medication were also assessed (data not shown).

Statistical methodology:
Analysis of BoEff levels was based on the average of Visits 2 and 3. Descriptive statistics were used to summarize the results. All results were stratified by study group, gender, and age (30–49 years vs ≥ 50 years; ~50% each).

Results

Study Population

Study group	Sub-group	Subjects enrolled	Subjects completed study	Subjects valid for FAS	Subjects valid for PP
Smokers	Female	262	255	258	235
	Male	469	454	458	435
	30–49 yrs	366	355	360	334
	≥50 yrs	365	354	356	336
	<10 cpd	30	28	28	0
	10–19 cpd	352	349	351	341
	20–30 cpd	252	248	252	245
	>30 cpd	85	84	85	84
	cpd unknown	12	0	0	0
	Menthol cig.	181	176	177	160
	Non-menthol cig.	550	533	539	510
	All	731	709	716	670
Non-smokers	Female	132	130	131	130
	Male	235	230	230	226
	30–49 yrs	182	179	179	177
	≥50 yrs	185	181	182	179
	All	367	360	361	356
Overall	Female	394	385	389	365
	Male	704	684	688	661
	30–49 yrs	548	534	539	511
	≥50 yrs	550	535	538	515
	All	1098	1069	1077	1026

Note: cpd = cigarettes per day; FAS = full analysis set; PP = per protocol

Demographics

Age and Body Mass Index (BMI) per protocol Population

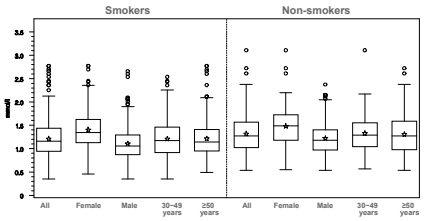
		Smokers N = 670	Non-smokers N = 356	Total N = 1026
Age (years)	Mean (SD)	48.1 (11.7)	49.5 (12.8)	48.6 (12.1)
	Range	30–80	30–83	30–83
BMI (kg/m²)	Mean (SD)	22.88 (3.53)	23.21 (3.27)	23.00 (3.44)
	Range	15.0–37.7	15.1–38.2	15.0–38.2

HDL Cholesterol

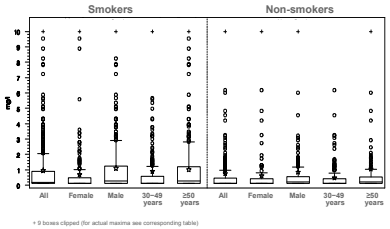
- **Lower in smokers (1.211 mmol/l) than non-smokers (1.316 mmo/l) (p<0.0001)**
- **Higher in females than in males (p<0.0001)**

	Smokers N=670	Non-smokers N=356
Mean (SD)	1.211 (0.386)	1.316 (0.395)
95% CI	1.18; 1.24	1.28; 1.36

LLOQ (lower limit of quantitation): 0.09 mmol/l



hsCRP



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- **No statistical difference between smokers (0.958 mg/l) and non-smokers (0.772 mg/l)**
- **More than 50% of values below the lower limit of quantitation (BLLOQ)**
- **High variability between subjects (>100%)**

	Smokers N=670	Non-smokers N=356
N BLLOQ	449	277
Mean (SD)	0.958 (2.400)	0.722 (2.951)
95% CI	0.78; 1.14	0.46; 1.08

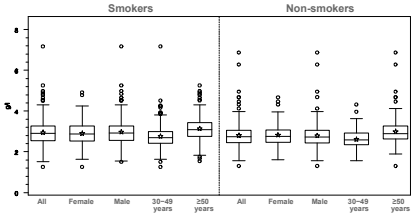
LLOQ: 0.25 mg/l; value LLOQ replaced by ½ LLOQ

Fibrinogen

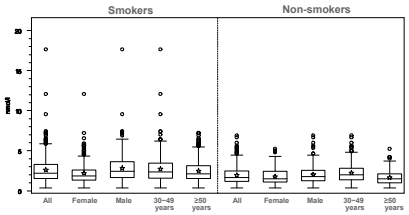
- **Higher in smokers (2.947 g/l) than non-smokers (2.803 g/l) (p<0.0001)**

	Smokers N=670	Non-smokers N=356
Mean (SD)	2.947 (0.586)	2.803 (0.584)
95% CI	2.90; 2.99	2.74; 2.86

LLOQ: 0.65 g/l



11-dehydro-TBX2



- **Higher in smokers (2.599 nmol/l) than non-smokers (1.940 nmol/l) (p<0.0001)**

	Smokers N=670	Non-smokers N=356
N BLOQ	35	277
Mean (SD)	2.599 (1.580)	1.940 (1.117)
95% CI	2.48; 2.72	1.82; 2.06

LLOQ: 0.678 nmol/l; value LLOQ replaced by ½ LLOQ

Other BoEff (data not shown)

- **Mean concentrations of leucocytes (p<0.0001) , lymphocytes (p<0.0001), monocytes (p<0.0001), haemoglobin (p=0.01), haematocrit (p=0.0044) , and 8-epi-prostaglandin F2a (p<0.0001), were statistically higher in smokers than in non-smokers**

Conclusion

The results of this study provide valuable information on the different levels of potential CVD-related BoEff in Japanese smokers versus non-smokers with different sub-analysis by gender and age. This study suggests that biological pathways involved in the pathogenesis of atherosclerosis are altered by cigarette smoking and provides a baseline for further investigation into which BoEff may be predictive for CVD risk related to cigarette smoking.

The data show statistically significant differences in BoEff concentrations between smokers and non-smokers for HDL cholesterol, fibrinogen, and 11-dehydro-TBX2 (all p<0.0001). Statistically significant differences for the concentration of leucocytes, lymphocytes, monocytes, haemoglobin, and 8-epi-prostaglandin F2a were also found. No significant differences between hsCRP concentrations were found. This is in line with a recent publication showing CRP to be of minimal additional contribution for prediction of incident cardiovascular events compared to conventional risk factors.(3)

References
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3-Melander O, Newton-Cheh C, Almgren P, Hedblad B, Berglund G, Engström G, Persson M, Smith JG, Magnusson M, Christensson A, Struck J, Morgenthaler NG, Bergmann A, Pencina MJ, and Wang TJ, 2009. Novel and conventional biomarkers for prediction of incident cardiovascular events in the community. JAMA. Jul 1;302(1):49−57

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