Inflammatory Cytokines in Tobacco Consumers as Potential Biomarkers of Tobacco Effect

G. L. Prasad, Bobbette A. Jones, John W. Caraway and Michael F. Borgerding
R & D Department, R.J. Reynolds Tobacco Co., Winston-Salem NC

Abstract

Chronic cigarette smoking causes inflammation and some reports indicate that generally healthy smokers (SMK) exhibit changes in the inflammatory cytokines versus non-smokers. However, it remains to be established whether consumers of non-combustible tobacco, such as moist snuff consumers (MSC) and dual users of cigarettes and moist snuff (DU-SKMS), also experience altered inflammation. Recently in a biomarker discovery study (Study 1), we observed that in contrast to SMK, the MSC exhibited cytokine profiles similar to non-tobacco consumers (NTC). Here, we present the levels of selected inflammatory cytokines from several cohorts of natural adopters of various tobacco products participating in a different study (Study 2).
Cytokine profiles of Human InflammationMAP®v1.0 panel (Myriad RBM) were generated from plasma and saliva samples collected in the Study 2 from cohorts of generally healthy, adult SMK, MSC, DU-SKMS and NTC who fasted overnight from food and tobacco. While several analytes from both plasma and saliva were found to be significantly different among the cohorts ($p<0.05$), 11 analytes from plasma were found to be highly significantly different ($p<0.02$). The SMK cohort had the highest mean values of all 11 analytes compared to the other cohorts, followed by the DU-SKMS which had cytokine profiles similar to SMK, reflecting the level of smoking in DU-SKMS. MSC and NTC cohorts had lower mean values. Cytokine profiles were similar between MSC and NTC, consistent with the previous findings from the biomarker discovery study. Although the demographics of the two studies were notably different, six analytes, fibrinogen, ICAM-1, VEGF, MMP-9, ferritin and complement component 3, emerged as potential biomarkers that distinguish tobacco consumers. These data suggest that smoking is the likely agent driving inflammation. Overall, the inflammatory cytokine levels suggest that inflammation is increased among combustible tobacco consumers relative to MSC and NTC, with few differences detected between MSC and NTC.

Background

Study Objectives

- Explore global metabolomic changes in long-term Cigarette Smokers (SMK) and Moist Snuff Consumers (MSC)
- Discover metabolomic pathways and specific metabolites that could be used as potential biomarkers of tobacco effect

Methods

- Technology: Myriad-RBM Human InflammationMAP® v1.0: to discern inflammatory biomarker patterns in a multiplexed immunoassay for several cytokines, chemokines and acute-phase reactants.
- Samples analyzed:
  - Plasma (8 – 10 hour overnight abstention from food and tobacco); designated as “P”
  - Saliva (unstimulated); designated as “S”
- Data analysis: Multi-variate 2-group ANOVA with un-pooled variance on normalized data
- Criteria for analyte selection:
  - Significance level of $p<0.05$ or better, plus
    - Visually non-overlapping box plots (median value)

Inflammatory cytokine profiles from two Clinical Studies were created:

1. **Biomarker Discovery Research (Study 1)**
   a. Three healthy male cohorts:
      Exclusive SMK (n = 40) and MSC (n = 40); NTC (n = 40)
   b. Age: 35-60
   c. Single site, cross-sectional study conducted in NC

2. **Natural Adopters of Tobacco Products (Study 2)**
   a. Four healthy male/female cohorts:
      Exclusive SMK (n = 60) and MSC (n = 50); NTC (n = 60), and dual users of cigarettes and moist snuff (DU-SKMS) (n=50)
   b. Age 19-73
   c. Multi-site, cross-sectional study conducted in four states

Results

A number of statistically significant ($p<0.05$) differences in the abundance of various analytes were detected in the saliva and plasma samples among the cohorts from the two studies.

Study 1:

- Relative to MSC and NTC cohorts, SMK exhibited more differences in the inflammatory cytokine levels, suggesting increased inflammation.
- Fewer differences were detected between MSC and NTC cohorts.

Study 2:

Table 1. Analytes significant between Smokers and Dual Smokers/Moist Snuff

Analyte	Prob.	Group	Mean ± Std Dev
P:Matrix Metalloproteinase-2 (MMP-2):ng/mL	0.003	SMK	1116.64 ± 328.01
		DUSKMS	951.24 ± 218.13
P:C-Reactive Protein (CRP):ug/mL	0.013	SMK	3.88 ± 5.09
		DUSKMS	1.95 ± 1.88
S:Monocyte Chemotactic Protein 1 (MCP-1):pg/mL	0.032	SMK	402.000 ± 678.170
		DUSKMS	747.400 ± 910.595

Absolute values

Table 2. Analytes significant between Smokers and Moist Snuff Users

Analyte	Prob.	Group	Mean ± Std Dev
P:Intercellular Adhesion Molecule 1 (ICAM-1):ng/mL	0.003	SMK	143.93 ± 62.29
		MSC	111.55 ± 42.79
P:Fibrinogen:mg/mL	0.004	SMK	4.13 ± 1.10
		MSC	3.58 ± 0.79
P:Vascular Endothelial Growth Factor (VEGF):pg/mL	0.01	SMK	343.05 ± 158.80
		MSC	281.24 ± 51.80
P:Alpha-1-Antitrypsin (AAT):mg/mL	0.019	SMK	1.42 ± 0.36
		MSC	1.28 ± 0.25
P:Vascular Cell Adhesion Molecule-1 (VCAM-1):ng/mL	0.02	SMK	470.22 ± 113.31
		MSC	527.10 ± 136.72
P:T-Cell-Specific Protein RANTES (RANTES):ng/mL	0.021	SMK	15.89 ± 16.10
		MSC	9.59 ± 10.59
P:Haptoglobin:mg/mL	0.029	SMK	0.99 ± 0.65
		MSC	0.74 ± 0.53
P:Brain-Derived Neurotrophic Factor (BDNF):ng/mL	0.035	SMK	5.80 ± 5.85
		MSC	3.56 ± 4.90
S:Fibrinogen:ug/mL	0.014	SMK	0.1 ± 0.2
		MSC	0.3 ± 0.4
S:Alpha-1-Antitrypsin (AAT):ug/mL	0.016	SMK	1.8 ± 3.1
		MSC	5.9 ± 11.7
S:Vitamin D-Binding Protein (VDBP):ug/mL	0.031	SMK	0.100 ± 0.214
		MSC	0.220 ± 0.317

Absolute values

Table 3. Significant analytes ($p<0.02$, from plasma): Smokers higher mean across most cohorts

Analyte	Prob.	Group	Mean ± Std Dev
P:Matrix Metalloproteinase-2 (MMP-2):ng/mL	0.003	SMK	1116.64 ± 328.01
		DUSKMS	951.24 ± 218.13
P:Intercellular Adhesion Molecule 1 (ICAM-1):ng/mL	0.003	SMK	143.93 ± 62.29
		MSC	111.55 ± 42.79
P:Alpha-1-Antitrypsin (AAT):mg/mL	0.019	SMK	1.42 ± 0.36
		MSC	1.28 ± 0.25
P:Brain-Derived Neurotrophic Factor (BDNF):ng/mL	0.035	SMK	5.80 ± 5.85
		MSC	3.56 ± 4.90
P:Intercellular Adhesion Molecule 1 (ICAM-1):ng/mL	<0.001	SMK	143.93 ± 62.29
		NTC	107.09 ± 40.26
P:Vascular Endothelial Growth Factor (VEGF):pg/mL	0.001	SMK	343.05 ± 158.80
		NTC	265.53 ± 50.36
P:Tissue Inhibitor of Metalloproteinases 1 (TIMP-1):ng/mL	0.001	SMK	73.49 ± 20.97
		NTC	62.61 ± 12.13
P:Matrix Metalloproteinase-9 (MMP-9):ng/mL	0.001	SMK	219.66 ± 126.75
		NTC	156.00 ± 75.37
P:T-Cell-Specific Protein RANTES (RANTES):ng/mL	0.003	SMK	15.89 ± 16.10
		NTC	8.84 ± 8.39
P:Vascular Cell Adhesion Molecule-1 (VCAM-1):ng/mL	0.016	DUSKMS	468.84 ± 97.56
		MSC	527.10 ± 136.72
P:Matrix Metalloproteinase-9 (MMP-9):ng/mL	0.027	DUSKMS	256.20 ± 184.90
		MSC	183.02 ± 133.81
P:Ferritin (FRTN):ng/mL	<0.001	DUSKMS	136.76 ± 100.48
		NTC	72.64 ± 57.61
P:Matrix Metalloproteinase-9 (MMP-9):ng/mL	<0.001	DUSKMS	256.20 ± 184.90
		NTC	156.00 ± 75.37
P:Vascular Endothelial Growth Factor (VEGF):pg/mL	0.003	DUSKMS	322.28 ± 131.69
		NTC	265.53 ± 50.36
P:Intercellular Adhesion Molecule 1 (ICAM-1):ng/mL	0.005	DUSKMS	130.16 ± 42.75
		NTC	107.09 ± 40.26
P:Ferritin (FRTN):ng/mL*	<0.001	MSC	156.76 ± 133.23
		NTC	72.64 ± 57.61
P:Interleukin-18 (IL-18):pg/mL	0.001	MSC	194.22 ± 107.37
		NTC	140.32 ± 59.97

Absolute values, all samples from Plasma, with acceptable non-overlapping Box Plot comparisons
* Ferritin has potential confounding effect between genders at the $p<0.001$ level of significance

Results (continued)

Illustrative plots of Smokers vs Non-Tobacco Consumers

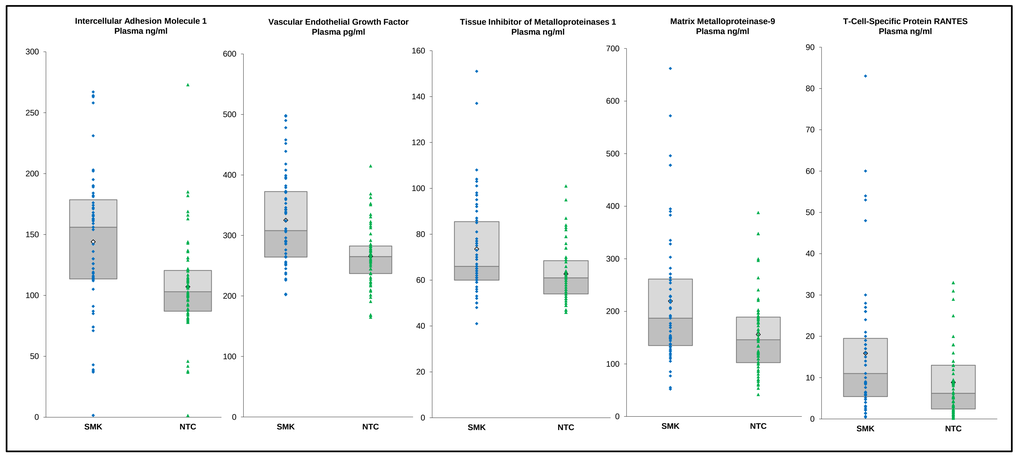

Table 4. Potential cytokine biomarkers of tobacco effect: Analytes significant at $p<0.02$ in both Study 1 and Study 2

SMK vs. MSC	Prob.	Mean ± StDev		Plots
P:Fibrinogen:mg/ml		**SMK**	**MSC**	
Study 2 (**SMK** vs. MSC)	0.004	4.1 ± 1.1	3.6 ± 0.8	
Study 1 (**SMK** vs. MSC)	0.013	7.5 ± 1.6	6.6 ± 1.8	
P:Intercellular Adhesion Molecule 1 (ICAM-1):ng/ml		**SMK**	**MSC**	
Study 2 (**SMK** vs. MSC)	0.003	143.9 ± 62.3	111.6 ± 42.8	✓
Study 1 (**SMK** vs. MSC)	0.0001	128.5 ± 42.2	95.5 ± 30.6	✓
P:Vascular Endothelial Growth Factor (VEGF):pg/ml		**SMK**	**MSC**	
Study 2 (**SMK** vs. MSC)	0.01	343.1 ± 158.8	281.2 ± 51.8	
Study 1 (**SMK** vs. MSC)	0.002	317.5 ± 98.3	260.5 ± 49.5	✓

SMK vs. NTC	Prob.	Mean ± StDev		Plots
P:Intercellular Adhesion Molecule 1 (ICAM-1):ng/ml		**SMK**	**NTC**	
Study 2 (**SMK** vs. NTC)	0.0002	143.9 ± 62.3	107.1 ± 40.3	✓
Study 1 (**SMK** vs. NTC)	<0.0001	128.5 ± 42.2	87.9 ± 32.2	✓
P:Matrix Metalloproteinase-9 (MMP-9):ng/ml		**SMK**	**NTC**	
Study 2 (**SMK** vs. NTC)	0.001	219.7 ± 126.8	156.0 ± 75.4	✓
Study 1 (**SMK** vs. NTC)	<0.0001	172.4 ± 85.4	105.7 ± 35.3	✓
P:Vascular Endothelial Growth Factor (VEGF):pg/ml		**SMK**	**NTC**	
Study 2 (**SMK** vs. NTC)	0.001	343.1 ± 158.8	265.5 ± 50.4	✓
Study 1 (**SMK** vs. NTC)	0.008	317.5 ± 98.3	264.8 ± 72.4	

MSC vs. NTC	Prob.	Mean ± StDev		Plots
S:Complement C3 (C3):ug/ml		**MSC**	**NTC**	
Study 2 (**MSC** vs. NTC)	0.017	3.6 ± 6.3	1.5 ± 1.4	
Study 1 (MSC vs. **NTC**)	0.015	1.6 ± 1.9	3.2 ± 3.6	
P:Ferritin (FRTN):ng/ml		**MSC**	**NTC**	
Study 2 (**MSC** vs. NTC) *	<0.0001	156.8 ± 133.2	72.6 ± 57.6	✓
Study 1 (**MSC** vs. NTC)	0.002	224.0 ± 189.4	118.3 ± 73.9	✓

Summary and Conclusions

- Smoking appears to drive levels of inflammation markers.
- The cytokine profiles suggest that inflammation appears to be higher in those who consume combustible tobacco, relative to MSC and NTC, with fewer differences between the latter two.
- Comparison of cytokine profiles in the two studies yielded six potential biomarkers that could distinguish tobacco consumers.

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