Physiological Measures and Novel Sputum Biomarkers to Distinguish Subjects with Mild to Moderate COPD from Asymptomatic Current Smokers, Former Smokers and Never-Smokers

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Karsta Luettich, Patrick Vanscheeuwijck, Nveed Chaudhary, Julia Hoeng, Manuel Peitsch

Philip Morris International R&D, Philip Morris Products S.A. (part of Philip Morris International group of companies)

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Objective

Identification of physiological measures and sputum biomarkers to enhance early detection of COPD in asymptomatic smokers
## Study Description

Non-interventional, parallel-group, case-controlled study to:

### Primary Objective
- Identify a biomarker or panel of biomarkers for the differentiation of subjects with mild to moderate COPD (GOLD stage I and II), asymptomatic current smokers, former smokers and never-smokers using gene and protein analyses in biological samples

### Secondary Objective
- Determine optimal methods of biomarker analysis in sputum, nasal and blood samples
- Compare physiological measurements and quality of life (QoL) across the study groups

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Overarching Scientific Questions

What is the biological impact of smoking?

Which of the smoking effects are reversible?

Which of the smoking effects are irreversible?

What is the COPD-specific biology in smokers?
Study Population Overview

60 Current smokers (CS)
- FEV1/FVC ≥ 70%
- FEV1 ≥ 80%

60 Former smokers (FS)
- FEV1/FVC ≥ 70%
- FEV1 ≥ 80%

60 Never-smokers (NS)
- FEV1/FVC ≥ 70%
- FEV1 ≥ 80%

60 COPD*) Smokers
- FEV1/FVC ≤ 70%
- FEV1 ≥ 50%

*) Stage I and IIa based on GOLD 2009 guidelines
## Demographics

<table>
<thead>
<tr>
<th></th>
<th>Never-smokers (NS)</th>
<th>Former smokers (FS)</th>
<th>Current smokers (CS)</th>
<th>COPD smokers (COPD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>55.2 (7.3)</td>
<td>56.6 (7.0)</td>
<td>55.2 (7.0)</td>
<td>57.4 (7.0)</td>
</tr>
<tr>
<td>Gender [Male/Female]</td>
<td>35/25</td>
<td>35/25</td>
<td>35/25</td>
<td>35/25</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>26.5 (3.7)</td>
<td>27.2 (3.5)</td>
<td>27.6 (3.6)</td>
<td>26.3 (3.7)</td>
</tr>
<tr>
<td>Smoking history [Pack-years]</td>
<td>0</td>
<td>28.2 (13.6)</td>
<td>33.5 (14.5)</td>
<td>44.8 (21.9)</td>
</tr>
<tr>
<td>Heart rate [bpm]</td>
<td>63.2 (10.4)</td>
<td>64.8 (10.2)</td>
<td>68.4 (10.1)</td>
<td>69.7 (9.6)</td>
</tr>
<tr>
<td>Respiratory rate [breaths per min]</td>
<td>15.7 (2.3)</td>
<td>15.2 (2.1)</td>
<td>15.7 (2.1)</td>
<td>14.8 (2.1)</td>
</tr>
<tr>
<td>FEV1 [% predicted]</td>
<td>111.8 (13.9)</td>
<td>108.3 (12.4)</td>
<td>100.8 (11.8)</td>
<td>75.6 (18.1)</td>
</tr>
<tr>
<td>FEV1/FVC [%]</td>
<td>73.8 (5.9)</td>
<td>72.1 (4.1)</td>
<td>70.4 (5.4)</td>
<td>54.5 (8.8)</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD) with the exception of gender (n)
BMI: Body mass index; FEV1: forced expiratory volume in 1 second
Gas Transfer Assessment

- Significant differences in TLCO % predicted between COPD and current, former and never-smokers (NS ≈ FS > CS > COPD; p<0.001 and <0.001, respectively)

- No differences in other gas transfer parameters (KCO % predicted, $V_A$ % predicted or $V_{IN}$ % predicted)

- TLCO < 80% predicted seen in some study subjects with normal spirometry including former smokers and asymptomatic current smokers

**TLCO (% predicted) by study group** (NS: never-smokers; FS: former smokers; CS: current smokers; COPD: subjects with COPD). Data are presented as box-whisker plots reflecting the first and third quartile (lower and upper boundary of box, respectively), median (line), mean (open diamond) and minimum values above the lower fence/maximum values below the upper fence (lower and upper whisker, respectively) for the corresponding study group. Outliers are represented by open circles.
Lung Sound Analysis (Stethographics)

- Significant differences in inspiratory crackle rate between COPD and current smokers and between current and never-smokers (p=0.0030 and p=0.0042, respectively)

- Presence of increased “pendelluft” in COPD smokers compared with asymptomatic smokers, particularly with deep breathing

- Rank score of combined lung sound parameters (total acoustic score) significantly higher in COPD compared with current smokers (46.75 vs 13.25; p<0.0001)
Fractional exhaled nitric oxide was slightly lower in COPD smokers than in disease-free smokers, and in current compared with former smokers ($p > 0.05$)
Chest HRCT Imaging

- Significantly higher emphysema scores in subjects with mild to moderate COPD compared with current asymptomatic smokers (p<0.001), and in the current smokers compared with never-smokers (p=0.001)

- Emphysematous lesions seen in all control groups, with nearly one third of current smokers with normal lung function exhibiting mild to moderate emphysema on CT

- No difference in small airway disease scores between COPD and asymptomatic smokers

- Pulmonary nodules more frequently identified in COPD patients and current smokers than in former and never-smokers
- Significantly higher modified BODE scores in subjects with mild to moderate COPD compared with current asymptomatic smokers (p<0.001), and in the current smokers compared with never-smokers (p=0.001)
## Biomarker Identification

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Analytical Method</th>
<th>Description</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induced Sputum</td>
<td>Proteomics</td>
<td>Best representation of the inflammatory state of the upper airways. Contains cells of interest and protein mediators</td>
<td>✓</td>
</tr>
<tr>
<td>Whole Blood (Lymphocytes)</td>
<td>Transcriptomics, Proteomics, Lipidomics</td>
<td>Identify correlations with data identified from the sputum. Blood represents the ideal and most convenient matrix for large-scale biomarker measurements</td>
<td>✓ (T, L)</td>
</tr>
<tr>
<td>Nasal fluid</td>
<td>Proteomics</td>
<td>A number of respiratory researchers have shown strong correlations between inflammation in the lung and nose. May represent a convenient matrix for large-scale studies</td>
<td>✓</td>
</tr>
<tr>
<td>Nasal scrapes</td>
<td>Transcriptomics, Proteomics</td>
<td>May represent a convenient matrix for large-scale studies if correlations are seen with the sputum</td>
<td>✓ (T)</td>
</tr>
<tr>
<td>Nasal lavage</td>
<td>Transcriptomics, Proteomics</td>
<td>May represent a convenient matrix for large-scale studies if correlations are seen with the sputum</td>
<td>✓ (P)</td>
</tr>
</tbody>
</table>
Induced Sputum Analysis

Proteomics Workflow

Sputum Supernatants
- Digestion & isobaric labeling (TMT)
- Liquid chromatography
- Tandem mass spectrometry
- Computational analysis

Transcriptomics Workflow

Sputum Pellets
- RNA extraction and QC
- RNA processing and labeling
- Chip hybridization and scan
- Computational analysis

Transcriptomics Workflow
Boxplot for the measured weights of the induced sputum samples (left) and measured protein concentrations for the sputum supernatants (after protein precipitation; right). * indicates the p-values < 0.05 compared with NS (Welch t-test)
Differentially Abundant Sputum Proteins

- log₁₀(fdr p-value) vs. -log₂(fold-change) for each comparison:
  - CS vs. NS
  - CS vs. FS
  - FS vs. NS
  - COPD vs. NS
  - COPD vs. FS
  - COPD vs. CS

Graphs illustrate the distribution of differently expressed proteins:
- UP (grey)
- DOWN (red)

Fold-change and p-value analysis for each comparison.
The Sputum Proteome Reflects Smoking

- Mucin/trefoil proteins, xenobiotic metabolism enzymes, antiproteases, and proteins involved in redox processes were increased in sputum of CS compared with that of NS.

- Blood plasma-derived proteins and immunoglobulins were decreased in sputum of CS compared with that of NS.
Comparing the sputum proteome of COPD smokers with that of asymptomatic smokers identified 13 differentially abundant proteins including antiproteases and proteins linked to the mucosal immune response.

Smoking alone significantly affects the expression of these proteins; presence of disease appears to “amplify” their dysregulation.
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Smoking alone significantly affects the expression of these proteins; presence of disease appears to “amplify” their dysregulation.
Sputum Proteins Correlate with Physiological Measures

Correlation plots with FEV1% of predicted (left panel) and TLCO predicted (right panel) for the 13 differentially abundant proteins in COPD vs. CS. The bars show the Pearson correlation coefficient and the red lines the coefficient of determination (R2) for each protein.
Differentially Expressed Genes

- log₁₀ adj. p-value

log₂ fold-change [mRNA, cells]
Sputum Transcriptome Reflects Smoking (Pellets)

- Smoking significantly affects the expression of several genes, resulting in
  - Up-regulation of xenobiotic metabolism enzyme-encoding genes and those linked to oxidative stress
  - Down-regulation of genes associated with immune responses and interferon (IFN) signaling
- Cessation largely reverses smoking-related gene expression changes
- COPD status does not further change the sputum transcriptome
Study Limitations

- Subjects enrolled based on self-reported smoking status and smoking history
- Enrollment of COPD smokers completed first
- Sample size calculated based on known distributions of white blood cell count and inflammatory mediator levels in induced sputum in similar COPD study populations
- Cross-sectional design
In this non-interventional, parallel-group, case-controlled study we have demonstrated that:

- Physiological measures such as diffusion capacity, CT chest imaging and lung sound analysis allow for differentiation of asymptomatic ('healthy') smokers from subjects with mild to moderate COPD patients (GOLD stage I and II) not identified using spirometry.

- The modified BODE QoL assessment allow differentiation of asymptomatic ('healthy') smokers from subjects with mild to moderate COPD patients (GOLD stage I and II) not identified using spirometry.

- Sputum analysis detects cigarette smoking-related alterations in the proteome and transcriptome; changes in proteome are further augmented in COPD smokers, allowing for further stratification.

- Sputum proteome analysis can distinguish COPD from asymptomatic smokers with a similar accuracy than the combination of the commonly used parameters FEV1, TLCO% and total COPD score.


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PMI High Performance Computing Team

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