Assessment of smoking status in patients with peripheral arterial disease

Simon D. Hobbs, MBChB, Antonius B. M. Wilmink, MD, Donald J. Adam, MD, and Andrew W. Bradbury, MD, Birmingham, United Kingdom

Objective: To assess the utility of a novel rapid urinary cotinine assay to detect and quantify the level of smoking in patients with peripheral arterial disease.

Methods: This was a cross-sectional study in a vascular surgical outpatient department of a large teaching hospital. Participants were 100 consecutive patients presenting to a hospital outpatient clinic with a diagnosis of intermittent claudication confirmed by a positive Edinburgh claudication questionnaire and an ankle-brachial pressure index of less than 0.9. Main outcome measures were patient-offered smoking history, exhaled breath carbon monoxide levels, urinary cotinine levels as measured by a novel rapid assay, and laboratory-measured creatinine-adjusted urinary cotinine levels.

Results: Fifty-five subjects declared that they were current smokers, 40 declared that they were ex-smokers, and 5 declared that they were never-smokers. Of the 40 ex-smokers, 6 subjects (15%) had urinary cotinine levels greater than 500 ng/mL (regular smokers), and a further 2 (5%) had urinary cotinine levels between 100 and 500 ng/mL (light, irregular, or passive smokers). The rapid urinary cotinine assay had a sensitivity and specificity of 100% and 98%, respectively, in its ability to detect active smoking, and the degree of smoking correlated well with laboratory creatinine-corrected urinary cotinine levels (Spearman coefficient, 0.805; P < .001). By contrast, exhaled carbon monoxide had a sensitivity and specificity of 95% and 89%, respectively, and although it correlated well with urinary cotinine (Spearman coefficient, 0.782; P < .001), it was found on linear regression analysis to be unreliable in differentiating light smokers from nonsmokers.

Conclusions: Patient-offered smoking history is unreliable because there is no correlation between the patient-reported number of cigarettes smoked per day and urinary cotinine levels. The novel rapid assay for urinary cotinine described here is superior to exhaled carbon monoxide measurement in detecting the level of smoking exposure among patients with intermittent claudication, and its results correlate well with laboratory-measured cotinine. (J Vasc Surg 2005;41: 451-6.)

Smoking is by far the single most important risk factor for peripheral arterial disease (PAD). Continued smoking is associated with disease progression, suboptimal results after vascular and endovascular intervention, and a 50% reduction in 5-year mortality after both medical and surgical treatment. It is universally recognized that complete and permanent cessation of smoking is by far the most clinically effective and cost-effective intervention in patients with PAD (nicotine replacement therapy/buproprion, £2000 per life-year gained; statins, £13,000 per life-year gained). Despite this, the great majority of patients get little or no evidence-based treatment for their nicotine addiction, and, as a result, smoking cessation rates remain predictably and unnecessarily low. Two major hurdles are establishing the severity of nicotine addiction at baseline and assessing compliance with therapy. Patient-offered smoking history is unreliable, routine measurement of plasma or urinary cotinine (widely held as the gold standard) is impracticable in the everyday clinical setting, and exhaled carbon monoxide (CO) reflects only very recent cigarette consumption. The aim of this study was to compare a novel rapid assay for urinary metabolites of nicotine (SmokeScreen; Surescreen Diagnostics Ltd, Derby, UK) with standard laboratory measurement of urinary cotinine and exhaled CO in a hospital vascular outpatient setting.

METHODS

Local ethics committee approval was obtained. One hundred consecutive patients who had a diagnosis of intermittent claudication and who provided written informed consent entered the study (Table I). Intermittent claudication was defined as a positive Edinburgh claudication questionnaire coupled with the finding of an ankle-brachial pressure index of less than 0.9 in the affected leg. No patient refused to participate in the study; however, two patients were excluded from entering the study because they were unable to satisfactorily complete assessment of exhaled breath CO because of either an inability to breath-hold for 20 seconds (n = 1) or an inability to blow into the CO analyzer with a sustained breath (n = 1).

Patients were questioned regarding current smoking status, the number of cigarettes smoked per day, and pack-years smoked. CO concentrations were measured in an exhaled breath sample by using Micro CO (Micro Medical, Rochester, UK). All patients had the breathalyzer test explained to them in detail and were given the opportunity to practice. Patients were asked to exhale fully before...
inhaling fully and holding their breath for 20 seconds. After
the breath-hold, patients were asked to exhale slowly into
the Micro CO and were encouraged to exhale fully to
sample alveolar air. All participating patients were able to
complete the test to the satisfaction of the clinician. Be-
cause the test has a good reproducibility, one CO reading
was deemed sufficient. CO values were expressed in parts
per million (ppm), where 0 to 6 ppm indicates a non-
smoker, 7 to 10 ppm indicates a light smoker, 10 to 20 ppm
indicates a regular smoker, and more than 20 ppm indicates
a heavy smoker, as recommended by the manufacturer of
the analyzer. A CO level of 7 ppm or more has been
demonstrated to have the greatest sensitivity and specificity
for differentiating between smokers and nonsmokers.

Urine was collected from patients at the time of their clinic
appointment for SmokeScreen analysis. An aliquot of the
same sample was stored at 

SmokeScreen is a disposable colorimetric assay that
measures all the major urinary metabolites of nicotine,
including cotinine. It is therefore specific to cigarette
smoke and will not be affected by ambient air pollution.
Two milliliters of urine is collected in a syringelike attach-
ment that is added to the reagents in a sealed unit. If
nicotine metabolites are present within the urine, a pink/
orange color change will occur over a 6-minute period. The
degree of color change correlates with the concentration of
nicotine metabolites present. A color chart that takes into
account the concentration of the urine is used to semiquan-
tify smoking habit into none, light, moderate, heavy, and
very heavy. The lower limit of detection is reported to be
60 ng/mL of urinary cotinine, the intra-assay variability
is 92.7%, and it has a good interobserver correlation ($r =
0.61$). The SmokeScreen assay was performed with the
operator blinded to both the patient-reported smoking
status and the CO result. Urinary cotinine was measured in
an accredited laboratory with a Cotinine Microplate EIA
(Cozart Bioscience Ltd, Abingdon, UK) with a detection
limit for cotinine of 12 ng/mL, and urinary creatinine was
measured by a standard technique on a Roche P800 ana-
lyzer (Roche Diagnostics, Lewes, UK). For the purpose of
this study, cotinine-adjusted urinary cotinine values were
used as the gold standard test for quantifying smoking
status, because the test is noninvasive and allows closer
comparison with the SmokeScreen assay. Because fluid
intake influences the concentration of urinary cotinine and
because urinary creatinine excretion is fairly constant, the
urinary cotinine level was corrected for urinary creatinine
by a previously described regression adjustment. Our
standard laboratory reference range indicates that levels less
than 100 ng/mL are consistent with a nonsmoker, and
levels greater than 500 ng/mL indicate a regular smoker.
Cotinine values of 100 to 500 ng/mL indicate a light
regular smoker, an irregular smoking habit, or significant
passive smoking.

**RESULTS**

Fifty-five subjects declared that they were current
smokers, 40 were ex-smokers, and 5 had never smoked
(Table II). All of the declared smokers had laboratory
urinary cotinine levels consistent with a regular current
smoking habit, and all of the never-smokers had urinary
cotinine levels less than 100 ng/mL (nonsmoking range).
However, of the 40 ex-smokers, 6 subjects (15%) had
urinary cotinine levels greater than 500 ng/mL (regular
smoker), and a further 2 (5%) had urinary cotinine levels
between 100 and 500 ng/mL (light regular smoker, irreg-
ular smoking habit, or significant passive smoking; Fig 1).
Thus, 8 (13%) of 63 active smokers, identified by increased
cotinine levels, denied their smoking habit on direct ques-
tioning. Among smokers, there was no correlation between
the patient-reported cigarettes per day and corrected

### Table I. Demographic details, comorbidities, and medical management of patients recruited

<table>
<thead>
<tr>
<th>Variable</th>
<th>Never smokers</th>
<th>Ex-smokers</th>
<th>Current smokers</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. subjects</td>
<td>5</td>
<td>40</td>
<td>55</td>
<td>100</td>
</tr>
<tr>
<td>Age, y, median (IQR)</td>
<td>73 (55.5-79)</td>
<td>70 (65.25-77)</td>
<td>65 (60-71)</td>
<td>68 (62.3-74.5)</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>2:3</td>
<td>34:6</td>
<td>35:20</td>
<td>71:29</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>2</td>
<td>11</td>
<td>11</td>
<td>24</td>
</tr>
<tr>
<td>Myocardial infarction/angina</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Coronary artery bypass graft</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Cerebrovascular accident</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2</td>
<td>10</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3</td>
<td>29</td>
<td>34</td>
<td>66</td>
</tr>
<tr>
<td>Medication</td>
<td>3</td>
<td>32</td>
<td>42</td>
<td>77</td>
</tr>
<tr>
<td>Antiplatelet agent</td>
<td>3</td>
<td>29</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>Statin</td>
<td>1</td>
<td>26</td>
<td>28</td>
<td>57</td>
</tr>
<tr>
<td>Angiotensive</td>
<td>3</td>
<td>10</td>
<td>11</td>
<td>22</td>
</tr>
</tbody>
</table>

IQR, Interquartile range; ACE, angiotensin-converting enzyme.
urinary cotinine (Spearman coefficient, \(r = 0.034; P = 0.81\); Fig 2).

Using laboratory creatinine-adjusted urinary cotinine values of 100 ng/mL to denote a current smoking habit, SmokeScreen produced two false-negative tests and no false-positive tests. This resulted in a sensitivity and specificity of 100% and 98%, respectively; a positive predictive value of 0.98; and a negative predictive value of 1. The false negatives had corrected urinary cotinine values of 155 and 191 ng/mL. With regard to CO in expired breath, a level of 7 ppm or more was used to denote a positive result. Micro CO produced four false positives (CO (ppm):corrected urinary cotinine level (ng/mL): 7:72, 9:52, 7:34, and 7:11) and three false negatives (CO:corrected urinary cotinine level: 3:2950, 4:1212, and 3:191). This resulted in a sensitivity and specificity of 95% and 89%, respectively, and a positive predictive value and negative predictive value of 0.94 and 0.92, respectively.

Figs 3 and 4 show the comparison of SmokeScreen and exhaled CO measurement against creatinine-adjusted urinary cotinine in terms of their ability to quantify smoking status in each subject. Both SmokeScreen (Spearman coefficient, \(r = 0.805; P < .001\)) and exhaled CO (Spearman coefficient, 0.782; \(P < .001\)) measurements correlated well with corrected urinary cotinine results. To investigate this further, linear regression analysis was performed by using creatinine-adjusted urinary cotinine as the dependent variable and by using SmokeScreen (using nonsmoker as the baseline level) and exhaled CO (0-6 ppm) as independent variables (Table III). The regression analysis indicated that exhaled CO at 7 to 10 ppm cannot reliably distinguish light smokers from nonsmokers and that SmokeScreen cannot reliably differentiate heavy smokers from very heavy smokers.

**DISCUSSION**

Complete and permanent cessation of smoking is by far the single most clinically effective and cost-effective intervention in patients with PAD, yet it receives little or no attention in most vascular surgical clinics. Exhaled CO
measurement is used extensively in smoking-cessation programs to provide an accurate assessment of baseline smoking status, the severity of nicotine addiction, and compliance with therapy. Biomarker measurement allows treatment to be tailored to individual patient requirements (such as the appropriate dose of nicotine-replacement therapy), provides motivating and re-enforcing feedback, and improves smoking-cessation rates.\textsuperscript{16,18-20} This study confirms that patient-offered smoking history is unreliable both in terms of smoking status and, in admitted smokers, the level of smoking (nicotine addiction), because there was no correlation between the patient-reported number of cigarettes smoked per day and urinary cotinine levels. In part, this lack of correlation could be due to underreporting by the subject, because many subjects deny
or play down their smoking habit. Another explanation is that because of wide variations in the number of puffs taken per cigarette and in the depth of each inhalation, the number of cigarettes smoked is a poor surrogate marker of consumption. In this study, 13% of current smokers (as detected by urinary cotinine) denied that they were currently smoking. Although confronting patients with this information requires care, biomarker feedback, properly used, can help patients to discuss their nicotine addiction more openly, thus helping them to seek and obtain appropriate treatment.

Measurement of exhaled CO is quick and easy to perform in the clinical setting and had a sensitivity and specificity of 95% and 89%, respectively, for correctly identifying current smokers. All four false positives had levels in the light-smoker range (7-10 ppm) that could be explained by passive smoking, exposure to pollution, or an underlying inflammatory lung condition (eg, asthma or chronic obstructive airway disease). Nevertheless, on the basis of CO alone, these four patients would have been falsely “accused” of being smokers, possibly leading to a loss of trust between the patient and doctor. The three false negatives are probably explained by the short (approximately 4 hours) half-life of CO, because Micro CO will detect only smoking within the previous 12 hours. This limits the utility of CO in the assessment of light or erratic smokers, especially if it is used early in the morning before a cigarette has been smoked. Thus, although, in this study, there seems to be a strong correlation between CO and adjusted urinary cotinine, the linear regression model clearly indi-

![Image](https://example.com/image.png)

**Fig 4.** Box plot to show comparison between exhaled breath carbon monoxide (CO) and corrected urinary cotinine. An expired CO of 0 to 6 ppm represents a nonsmoker, 7 to 10 a light smoker, 11 to 20 a smoker, and more than 20 a heavy smoker (Spearman correlation coefficient, .782; \( P < .001 \)).

**Table III.** Linear regression analysis of the relationship between SmokeScreen/exhaled carbon monoxide and corrected urinary cotinine

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>SE</th>
<th>t</th>
<th>P value</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>SmokeScreen* result</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.567</td>
</tr>
<tr>
<td>Light</td>
<td>2160.4</td>
<td>526.5</td>
<td>4.10</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>3144.1</td>
<td>385.4</td>
<td>8.16</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Heavy</td>
<td>3866.7</td>
<td>423.3</td>
<td>9.14</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Very heavy</td>
<td>3651.4</td>
<td>549.3</td>
<td>6.65</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Exhaled CO (ppm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.537</td>
</tr>
<tr>
<td>7-10</td>
<td>913</td>
<td>526.5</td>
<td>1.74</td>
<td>.086</td>
<td></td>
</tr>
<tr>
<td>11-20</td>
<td>3058</td>
<td>397.9</td>
<td>7.64</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>&gt;20</td>
<td>3666</td>
<td>385.1</td>
<td>9.52</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

CO, Carbon monoxide.

*For SmokeScreen, the nonsmoker category was used as the baseline, and for exhaled CO, 0 to 6 ppm was used as the baseline.
icates that exhaled CO cannot be used to distinguish light smokers from nonsmokers.

Cotinine, the primary metabolite of nicotine, can be detected in serum, saliva, and urine; has a long half-life (approximately 16 hours); fluctuates much less than CO on a day-to-day basis; and can quantify smoking habit over the preceding 3 to 4 days. Despite these obvious advantages, urinary cotinine measurement is rarely undertaken in clinical practice because the laboratory assay is time consuming and expensive, and delay occurs because testing must often be batched. The resulting delay clearly reduces the effect that testing can have on the consultation and treatment and limits is use predominantly to that of a research tool.

In this study, urinary cotinine testing with SmokeScreen was clearly superior to exhaled CO and correlated well with laboratory-measured corrected urinary cotinine. In particular, SmokeScreen could distinguish between light smokers and nonsmokers, and this is important because there is no safe level of smoking. SmokeScreen is, therefore, a useful addition to the clinician’s armamentarium. It will facilitate the optimization of smoking-cessation rates, thus improving the natural history of PAD and the results of medical, vascular, and endovascular therapies.

We thank Mr Tim Marshal, a medical statistician from the University of Birmingham, for his statistical advice. We thank Philippa Goddard and Julie Stinson from the Department of Biochemistry at Heartlands and Solihull NHS Trust, Birmingham, for their help with laboratory analysis of urinary cotinine and creatinine, and Yvonne Hall, vascular nurse specialist, for her help with patient identification and recruitment.

REFERENCES