

Assessment of smoking status in patients with peripheral arterial disease

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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 subjects 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).¹⁻⁵ Continued smoking is associated with disease progression,^{6,7} suboptimal results after vascular and endovascular intervention,^{8,9} and a 50% reduction in 5-year mortality after both medical⁵ and surgical¹⁰ 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/bupropion, <£2000 per life-year gained; statins, £13,000 per life-year gained¹¹). 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.^{3,12} Two major hurdles are establishing the severity of nicotine addiction at baseline and assessing compliance with therapy. Patient-offered smoking history is unreliable,¹³ routine measurement of plasma or urinary cotinine (widely held as the gold standard) is impracticable in the everyday clinical setting, and exhaled carbon mon-

oxide (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 questionnaire¹⁴ 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

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Competition of interest: none

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

<i>Variable</i>	<i>Never smokers</i>	<i>Ex-smokers</i>	<i>Current smokers</i>	<i>Total</i>
No. subjects	5	40	55	100
Age, y, median (IQR)	73 (55.5-79)	70 (65.25-77)	65 (60-71)	68 (62.3-74.5)
Sex (M:F)	2:3	34:6	35:20	71:29
Ischemic heart disease				
Myocardial infarction/angina	2	11	11	24
Coronary artery bypass graft	0	3	3	6
Cerebrovascular disease				14
Cerebrovascular accident	0	3	4	7
Transient ischaemic attack	0	5	2	7
Diabetes mellitus	2	10	4	16
Hypertension	3	29	34	66
Medication				
Antiplatelet agent	3	32	42	77
Statin	1	29	35	65
Antihypertensive	3	26	28	57
ACE inhibitor	1	10	11	22

IQR, Interquartile range; *ACE*, angiotensin-converting enzyme.

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. Because the test has a good reproducibility,¹⁵ 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.¹⁵ Urine was collected from patients at the time of their clinic appointment for SmokeScreen analysis. An aliquot of the same sample was stored at -80°C for later batched laboratory analysis. All patients were screened in a Monday morning clinic between 9:30 AM and noon; thereby, the sampling times were kept as standardized as possible.

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 attachment 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 semiquantify 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 ($\kappa = 0.61$).¹⁶ 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 analyzer (Roche Diagnostics, Lewes, UK). For the purpose of this study, creatinine-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.¹⁷ 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, irregular 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 questioning. Among smokers, there was no correlation between the patient-reported cigarettes per day and corrected

Table II. Subject smoking history and results of near-patient and laboratory assessment of smoking status

Declared smoking habit	No. subjects	Age (y)	Pack-years* smoked	Exhaled CO	SmokeScreen result†	Urinary cotinine/creatinine ratio
Current smoker	55	65 (60-71)	35 (22-50)	20 (15-27)	2.45 (1-4)	2950 (1726-4981)
Ex-smoker	40	70 (65.25-77)	32.5 (20-52.25)	4 (3-7)	0.33 (0-4)	16 (12.25-51)
Never-smoker	5	73 (55.5-79)	0 (0-0)	2 (1.5-3.5)	0 (0-0)	16 (12.5-19.5)

Results represent median (IQR) unless otherwise specified.

CO, Carbon monoxide.

*Pack year = (number of cigarettes smoked per day/20) × number of years smoked.

†SmokeScreen results: 0, nonsmoker; 1, light smoker; 2, moderate smoker; 3, heavy smoker; 4, very heavy smoker. Values are expressed as mean (range).

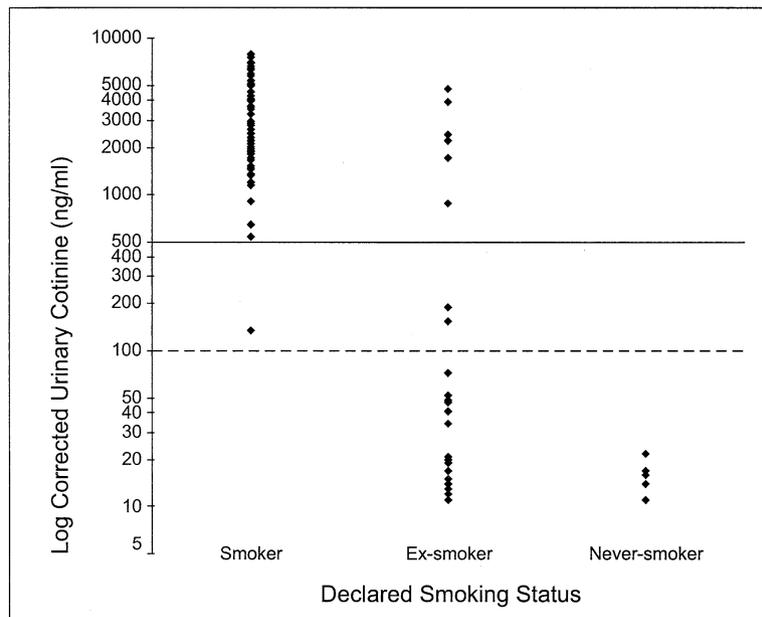


Fig 1. Declared smoking status plotted against corrected urinary cotinine. A corrected urinary cotinine more than 500 ng/mL indicates a regular smoker, values between 100 and 500 ng/mL are typical for light or erratic smokers, and nonsmokers have values less than 100 ng/mL.

urinary cotinine (Spearman coefficient, -0.034 ; $P = .81$; 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-ad-

justed urinary cotinine in terms of their ability to quantify smoking status in each subject. Both SmokeScreen (Spearman coefficient, 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.³ Exhaled CO

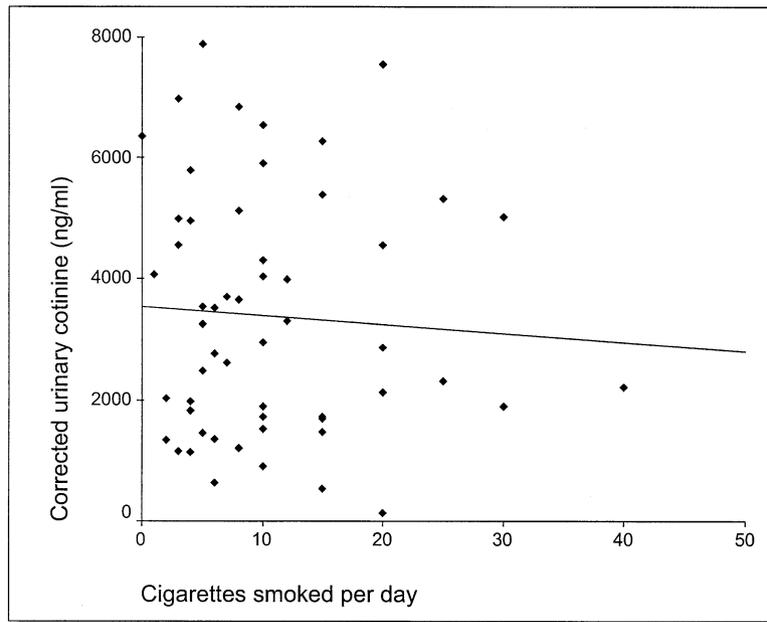


Fig 2. Correlation between declared cigarette consumption of smokers and corrected urinary cotinine. The line represents the degree of best fit ($R^2 = -0.004$).

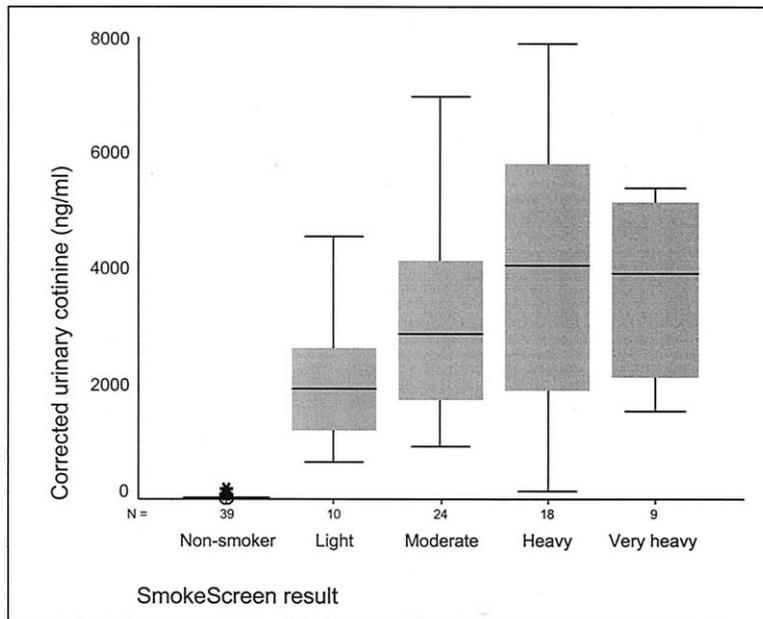


Fig 3. Box plot to show comparison between SmokeScreen and corrected urinary cotinine (Spearman correlation coefficient, 0.805; $P < .001$).

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.^{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

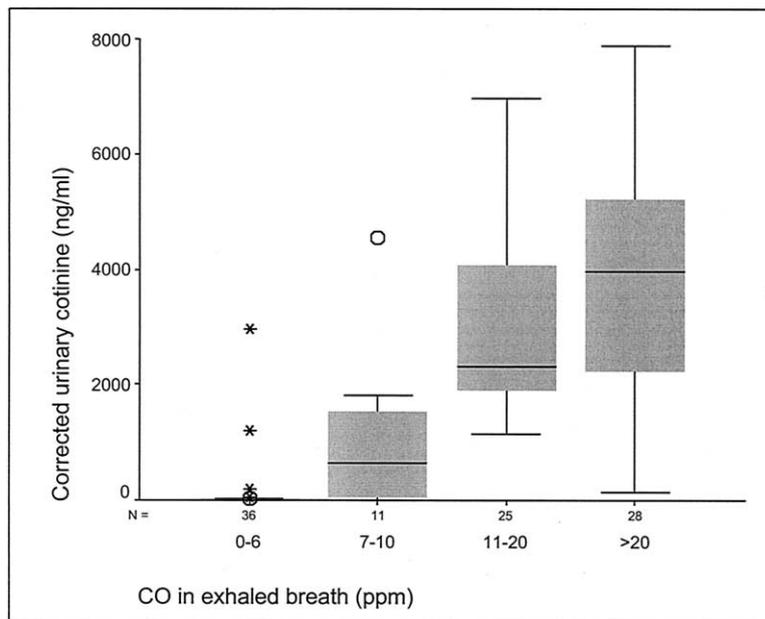


Fig 4. Box plot to show comparison between exhaled breath carbon monoxide (CO) and corrected urinary cotinine. An expired CO of 0 to 6 ppm represents a nonsmoker, 7 to 10 a light smoker, 11 to 20 a smoker, and more than 20 a heavy smoker (Spearman correlation coefficient, .782; $P < .001$).

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

Variable	Coefficient	SE	t	P value	R ²
SmokeScreen* result					0.567
Light	2160.4	526.5	4.10	<.001	
Moderate	3144.1	385.4	8.16	<.001	
Heavy	3866.7	423.3	9.14	<.001	
Very heavy	3651.4	549.3	6.65	<.001	
Exhaled CO (ppm)					0.537
7-10	913	526.5	1.74	.086	
11-20	3038	397.9	7.64	<.001	
>20	3666	385.1	9.52	<.001	

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.

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).²¹ 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-

cates 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.²² 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 its 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.

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REFERENCES

1. Kannel WB, Shurtleff D. The Framingham Study. Cigarettes and the development of intermittent claudication. *Geriatrics* 1973;28:61-8.
2. Cole CW, Hill GB, Farzad E, Bouchard A, Moher D, Rody K, et al. Cigarette smoking and peripheral arterial occlusive disease. *Surgery* 1993;114:753-7.
3. Hobbs SD, Bradbury AW. Smoking cessation strategies in patients with peripheral arterial disease: an evidence based approach. *Eur J Vasc Endovasc Surg* 2003;26:341-7.
4. Fowkes FGR, Housley E, Riemersma RA, Macintyre CCA, Cawood EHH, Prescott RJ, et al. Smoking, lipids, glucose intolerance, and blood pressure as risk factors for peripheral atherosclerosis compared with ischaemic heart disease in the Edinburgh Artery Study. *Am J Epidemiol* 1992;135:331-40.
5. Hirsch AT, Treat-Jacobson D, Lando HA, Hatsukami DK. The role of tobacco cessation, antiplatelet and lipid-lowering therapies in the treatment of peripheral arterial disease. *Vasc Med* 1997;2:243-51.
6. Jonason T, Bergstrom R. Cessation of smoking in patients with intermittent claudication. *Acta Med Scand* 1987;221:253-60.
7. Gardner AW. The effect of cigarette smoking on exercise capacity in patients with intermittent claudication. *Vasc Med* 1996;1:181-6.
8. Stonebridge PA, Bradbury AW, Murie JA. Continued smoking and the results of vascular reconstruction. *Br J Surg* 1994;81:51-2.
9. Carty CS, Huribal M, Marsan BU, Ricotta JJ, Dryjski M. Nicotine and its metabolite cotinine are mitogenic for human vascular smooth muscle cells. *J Vasc Surg* 1997;25:682-8.
10. Faulkner KW, House AK, Castleden WM. The effect of cessation of smoking on the accumulative survival rates of patients with symptomatic peripheral vascular disease. *Med J Aust* 1983;1:217-9.
11. National Institute for Clinical Excellence. Guidance on the use of nicotine replacement therapy (NRT) and bupropion for smoking cessation. Technology appraisal guidance No. 39. National Institute for Clinical Excellence. Available at: URL: <http://www.nice.org.uk>. Accessed February 16, 2002.
12. Burns PJ, Lima E, Bradbury AW. Second best medical therapy. *Eur J Vasc Endovasc Surg* 2002;24:400-4.
13. Hald J, Overgaard J, Grau C. Evaluation of objective measures of smoking status—a prospective clinical study in a group of head and neck cancer patients treated with radiotherapy. *Acta Oncol* 2003;42:154-9.
14. Leng GC, Fowkes FGR. The Edinburgh Claudication Questionnaire: an improved version of the WHO/Rose Questionnaire for use in epidemiological surveys. *J Clin Epidemiol* 1992;45:1101-9.
15. Middleton ET, Morice AH. Breath carbon monoxide as an indication of smoking habit. *Chest* 2000;117:758-63.
16. Cope G, Nayyar P, Holder R, Gibbons J, Bunce R. A simple near-patient test for nicotine and its metabolites in urine to assess smoking habit. *Clin Chim Acta* 1996;256:135-49.
17. Thompson SG, Barlow RD, Wald NJ, Van Vunakis H. How should urinary cotinine concentrations be adjusted for urinary creatinine concentration? *Clin Chim Acta* 1990;187:289-96.
18. Jamrozik K, Vessey M, Fowler G, Wald N, Parker G, Van Vunakis H. Controlled trial of three different antismoking interventions in general practice. *BMJ* 1984;288:1499-503.
19. Risser NL, Belcher DW. Adding spirometry, carbon monoxide and pulmonary symptom results to smoking cessation counselling: a randomised trial. *J Gen Intern Med* 1990;5:16-22.
20. McClure JB. Are biomarkers useful treatment aids for promoting health behaviour change? An empirical review. *Am J Prev Med* 2002;22:200-7.
21. Sato S, Nishimura K, Koyama H, Tsukino M, Oga T, Hagiuro T, et al. Optimal cutoff level of breath carbon monoxide for assessing smoking status in patients with asthma and COPD. *Chest* 2003;124:1749-54.
22. Jarvis MJ, Tunstall-Pedoe H, Feyerabend C, Vesey C, Saloojee Y. Comparison of tests used to distinguish smokers from non-smokers. *Am J Public Health* 1987;77:1435-8.

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