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Short Communication

Novel proposed cutoff values for anatabine and anabasine in differentiating smokers from non-smokers

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ABSTRACT

Objectives: Anatabine and anabasine are two tobacco alkaloids used to differentiate between tobacco users and abstainers, including users of nicotine replacement therapy. Cutoff values (>2 ng/mL for both alkaloids) have not been revised since their implementation in 2002. These values may be too high, leading to increased likelihood of misclassification between smokers and abstainers. This results in major consequences, especially adverse outcomes of transplantation when smokers were incorrectly identified as being abstinent. This study proposes that a lower threshold for anatabine and anabasine will better distinguish tobacco users from non-users and thereby improve patients' care.

Design and methods: A new and more sensitive analytical method by liquid chromatography-mass detection was developed to allow the quantification of low concentrations. Anatabine and anabasine were measured in urine samples of 116 self-reported daily smokers and 47 long-term non-smokers (confirmed by the analysis of nicotine and its metabolites). The best compromise between sensitivity and specificity allowed us to determine new cutoff values.

Results: The thresholds >0.097 ng/mL for anatabine and >0.236 ng/mL for anabasine were associated with a sensitivity of 97% (anatabine) and 89% (anabasine) and a specificity of 98% for both alkaloids. These cutoff values greatly increased the sensitivity given that it dropped to 75% (anatabine) and 47% (anabasine) when using the reference value (>2 ng/mL).

Conclusions: The cutoff values >0.097 ng/mL for anatabine and >0.236 ng/mL for anabasine appear to better differentiate tobacco users from abstainers than the current reference threshold (>2 ng/mL for both alkaloids). It may considerably impact patients' care, especially in transplantation settings in which smoking abstinence is essential to avoid adverse outcomes of transplantation.

1. Introduction

Anatabine and anabasine, minor tobacco alkaloids, were proposed as biomarkers to differentiate smokers from non-smokers. Given that they are not nicotine-derived and present only in negligible amounts in pharmaceutical grade nicotine, they are especially useful to distinguish between tobacco and nicotine replacement therapy (NRT) users [1,2].

Jacob et al. defined in 2002 the cutpoint of 2 ng/mL for both alkaloids in urine as the criterion to differentiate tobacco users and non-users [2]. The method of quantification (gas chromatography coupled with mass spectrometry) used in their study and developed in 1993 showed a limit of quantification (LOQ) of 1 ng/mL [3]. Moyer et al. (2002) also suggested the use of the threshold >2 ng/mL for anabasine (anatabine not studied), based on a liquid chromatography-tandem mass spectrometry (LC-MS/MS) procedure with LOQ = 1 ng/mL [4]. In 2014, Suh-Lailam et al. used a LC-MS/MS method with LOQ = 2 ng/mL for anabasine (anatabine not studied) to propose the cutoff of 3 ng/mL [5]. It appears that the LOQ of these methods are relatively high compared to the cutoff values, leading generally to unquantifiable concentrations of alkaloids for supposed non-users.

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Table 1
Median concentrations of tobacco biomarkers in smokers versus non-smokers.

	Smokers (n = 116) Median (Interquartile range)	Non-smokers (n = 47) Median (Interquartile range)	P
CO (ppm)	18 (13–23)	–	–
Nicotine (μg/mL)	0.58 (0.23–1.11)	0.06 (0.06–0.06)	<0.0001
Cotinine (μg/mL)	1.21 (0.85–1.71)	0.01 (0.01–0.01)	<0.0001
3HC (μg/mL)	3.72 (1.58–6.07)	0.02 (0.02–0.02)	<0.0001
Anatabine (ng/mL)	5.595 (2.013–11.286)	0.029 (0.029–0.029)	<0.0001
Anabasine (ng/mL)	1.785 (0.559–7.440)	0.029 (0.029–0.137)	<0.0001

CO, carbon monoxide; 3HC, trans-3′-hydroxycotinine.

Since then several assays by LC-MS/MS have been developed allowing quantification of lower concentrations: LOQ = 0.15 ng/mL [6], 0.4 ng/mL [7] for anatabine and 0.2 ng/mL [6], 0.5 ng/mL [7] for anabasine. Despite the emergence of these new quantification methods, the cutoff >2 ng/mL established in 2002 by Jacob et al. remains the reference to differentiate between tobacco users and abstainers [1,6,8,9]. However, several studies have drawn attention to a lack of clinical sensitivity when using these biomarkers with such cutoff values: 14% [8], 16% [6] or 60% [10] of self-reported active smokers would have been wrongly considered abstinent due to concentrations of these alkaloids below the cutoff and/or undetectable concentrations by the analytical procedure used. This high likelihood of misclassification may have major consequences, including inappropriate insurance premiums or eligibility for organ transplantation (thereby increasing adverse outcomes of transplantation if smokers are included) [10].

The aim of our study was to propose more adequate cutoff values of anatabine and anabasine to improve the differentiation between tobacco users and non-users by using a new and more sensitive analytical method.

2. Materials and methods

One hundred and sixteen self-reported daily smokers (55 men and 61 women aged 26–81 years) and forty-seven long-term non-smokers (25 men and 22 women aged 21–82 years) were recruited in the Centre Hospitalier Universitaire of Catholic University of Louvain (Namur, Belgium). Smokers used only cigarettes and had a median consumption of 20 cigarettes per day (interquartile range: 10–25). Their expired carbon monoxide (CO) level was higher than 5 ppm. Informed consent was provided from all participants. The project was approved by the hospital ethics committee and was led according to the Declaration of Helsinki and the Good Clinical Practice guidelines [11].

Urine samples from smokers and non-smokers were collected to measure free anatabine and anabasine. Other tobacco biomarkers – free nicotine, cotinine, and trans-3′-hydroxycotinine (3HC) – were measured to objectively confirm the participants' smoking status. The five biomarkers were quantified simultaneously by an in-house-developed method (liquid chromatography-mass detection) that was previously published [12]. This method allowed quantification of low concentrations with an LOQ = 0.02 μg/mL (nicotine and 3HC), 0.002 μg/mL (cotinine), and 0.029 ng/mL (anatabine and anabasine). The precision of the assay was evaluated at the LOQ and was reflected by the relative standard deviation (RSD) between three runs for each analyte. The maximal RSD was 10.3%.

The tobacco biomarkers comparison in smokers versus non-smokers was carried out by Wilcoxon rank sum test. Differences were considered significant when $P < 0.05$. Cutoff values to distinguish the two groups were defined according to the receiver operating characteristic (ROC) curves. Sensitivity (Se), specificity (Sp), positive (PPV) and negative predictive values (NPV) were assessed. PPV and NPV were calculated based on a prevalence of smoking of 20% [13]. All statistical analysis were performed using MedCalc® (version 20.109, MedCalc Software Ltd, Ostend, Belgium).

Table 2
Sensitivity, specificity, and predictive values for differentiation of smokers and non-smokers depending on the cutoff values used for anatabine and anabasine.

	Anatabine		Anabasine	
Cutoff (ng/mL)	>0.097	>2	>0.236	>2
Se (%)	97	75	89	47
Sp (%)	98	98	98	100
PPV (%)	92	90	91	100
NPV (%)	99	94	97	88

Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.

3. Results

Table 1 shows expired CO level, urinary measurement of nicotine and its metabolites, and urinary measurement of anatabine and anabasine in the two groups.

Based on the ROC curves, the cutoff values were >0.097 ng/mL for anatabine and >0.236 ng/mL for anabasine (Fig. 1). These new cutoffs

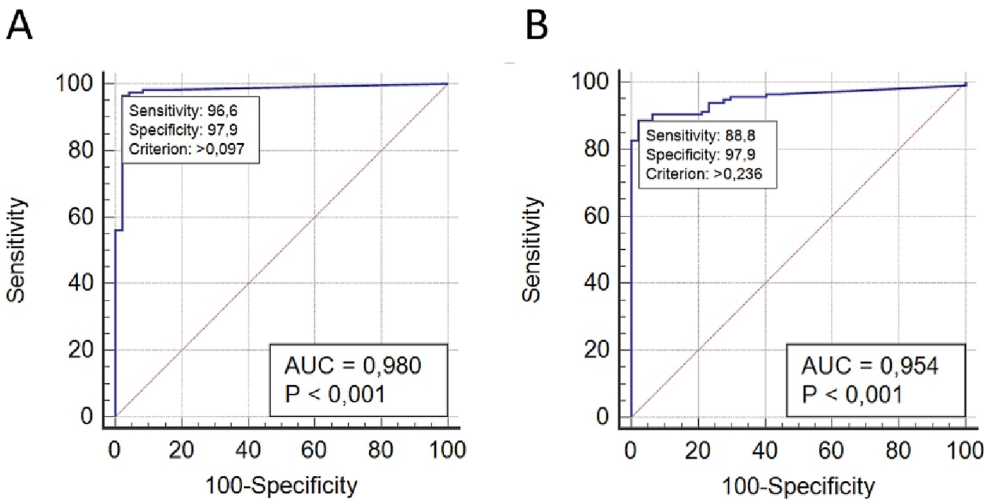

Fig. 1. Receiver operating characteristic (ROC) curves with the area under the curve (AUC) for anatabine (A) and anabasine (B).

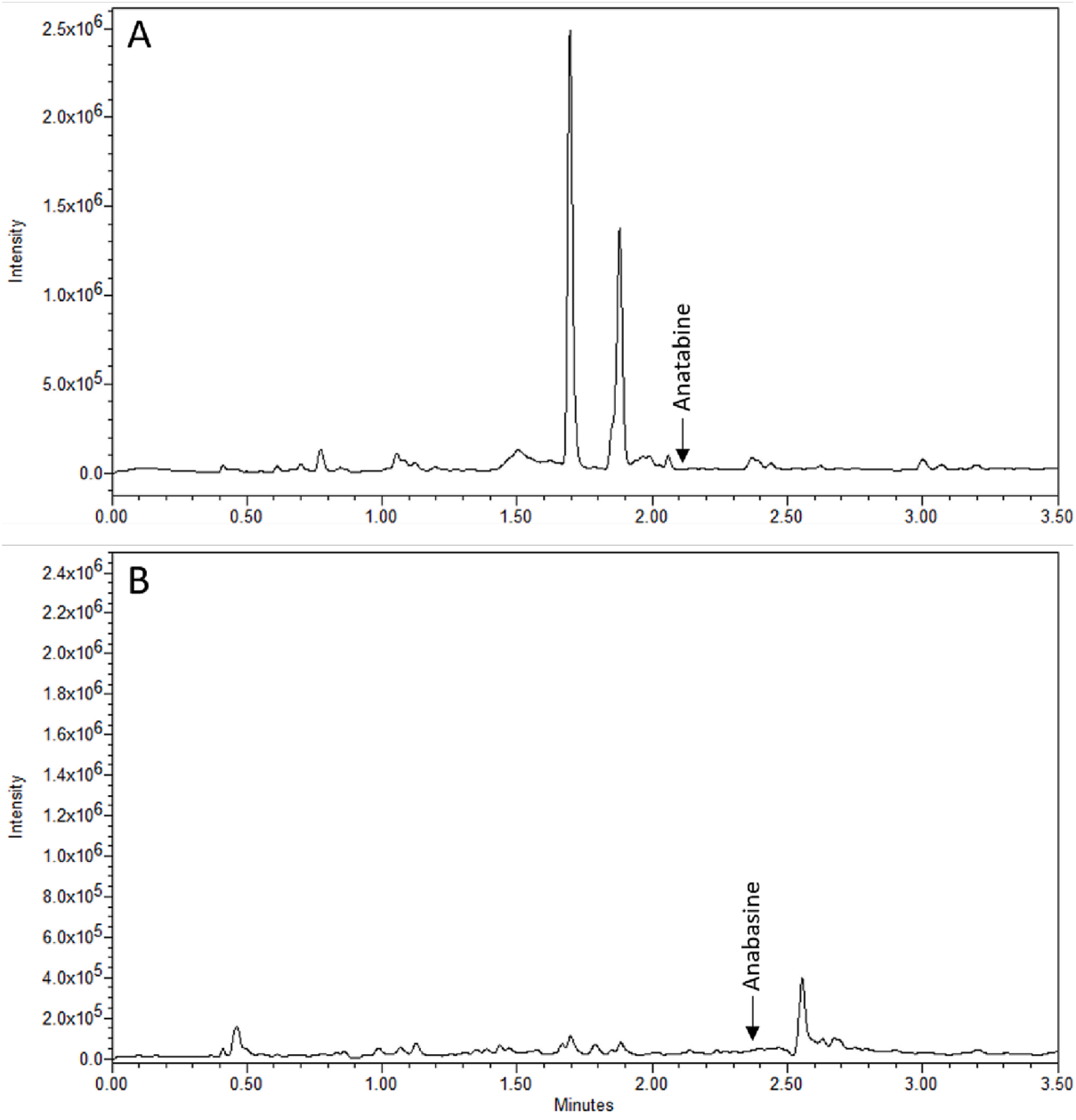

Fig. 2. Chromatograms of <0.029 ng/mL anatabine (A) and <0.029 ng/mL anabasine (B) obtained from a non-smoker's urine sample.

were the best compromise between sensitivity and specificity. Table 2 shows the diagnostic features when using these cutoffs in comparison with the cutoffs of reference (>2 ng/mL). Fig. 2 shows typical chromatograms of anatabine and anabasine in a non-smoker's urine sample.

4. Discussion

Anatabine and anabasine are two tobacco alkaloids proposed to differentiate smokers from non-smokers. They are absent in nicotine-containing medications given that they are not nicotine-derived. For this reason, these two alkaloids are especially of interest to differentiate between tobacco and NRT users [1,2]. This differentiation is crucial for insurance premiums but also for patients' care, including follow-up of patients in cessation or eligibility for organ transplantation [10]. The reference cutoff values (>2 ng/mL for both anatabine and anabasine) were determined in 2002 by Jacob et al. [2] and have not been revised since. However, several studies have pointed out the poor clinical sensitivity and put in doubt the utility of these biomarkers without updated cutoff values [6,8,10].

In order to propose more adequate cutoffs for anatabine and anabasine, we developed a new and more sensitive analytical method resulting in an LOQ of 0.029 ng/mL for both anatabine and anabasine [12]. This method allowed the quantification of lower concentrations than the one used in the study of Jacob et al. (2002) (LOQ of 1 ng/mL) which led usually to unquantifiable concentrations for supposed non-users [2,3]. We measured anatabine and anabasine, but also CO, nicotine and its metabolites, in 116 self-reported smokers and 47 long-term non-smokers. Several reference ranges for tobacco biomarkers have been proposed in the literature to differentiate smokers from non-smokers, but they are variable depending on the authors [1,4,14]. Nevertheless, median concentrations of CO, nicotine, cotinine, and 3HC well confirmed the participants' smoking status according to the cutoff values commonly suggested: >5 ppm for CO, >0.1 μg/mL for nicotine, >0.073 μg/mL for cotinine, and >0.4 μg/mL for 3HC [1,4].

According to our results, we propose here the cutoff values of >0.097 ng/mL for anatabine and >0.236 ng/mL for anabasine. These

new cutoffs were associated with a sensitivity of 97% (anatabine) and 89% (anabasine) and a specificity of 98% for both alkaloids. While using the reference cutoff (>2 ng/mL), the sensitivity dropped to 75% (anatabine) and 47% (anabasine) with specificity of 98% (anatabine) and 100% (anabasine). This result confirms the lack of clinical sensitivity associated with the reference cutoffs, as reported in other studies [6,8,10]. Interestingly, the proposed cutoff for anabasine was higher than the one for anatabine while the median anabasine concentration in smokers was found to be lower than that of anatabine. This observation could be explained by the higher baseline level observed for anabasine in non-smokers compared with anatabine.

A limitation of our study is that all samples were collected in a single location. The suggested cutoff values should be validated by the measurement of anatabine and anabasine in a larger population coming from different locations. A second limitation concerns the possible second-hand smoke exposure in studied non-smokers. We deliberately did not exclude non-smokers with passive exposure in order to take it into account for cutoff determination. This inclusion is reflected by the level of cotinine observed in the non-smokers' group (median 0.01 μg/mL) that corresponds to typical passive exposure concentrations (reference range: 0.005–0.02 μg/mL [4]). However, it could also be of interest to select a population of non-smokers without passive exposure to propose cutoff values allowing the distinction between non-smokers with or without passive exposure.

5. Conclusions

We propose using the cutoff values of >0.097 ng/mL for anatabine and >0.236 ng/mL for anabasine that will better distinguish tobacco users from non-users than the current reference cutoffs (>2 ng/mL for both anatabine and anabasine). This will impact considerably patients' care, especially in transplantation settings in which smoking abstinence is essential to avoid adverse outcomes of transplantation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] N.L. Benowitz, J.T. Bernert, J. Foulds, S.S. Hecht, P. Jacob, M.J. Jarvis, et al., Biochemical verification of tobacco use and abstinence: 2019 update, Nicotine Tob. Res. 22 (7) (2020) 1086–1097.
[2] P. Jacob, D. Hatsukami, H. Severson, S. Hall, L. Yu, N.L. Benowitz, Anabasine and anatabine as biomarkers for tobacco use during nicotine replacement therapy, Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res Cosponsored Am. Soc. Prev. Oncol. 11 (12) (2002 Dec) 1668–1673.
[3] P. Jacob, L. Yu, G. Liang, A.T. Shulgin, N.L. Benowitz, Gas chromatographic-mass spectrometric method for determination of anabasine, anatabine and other tobacco alkaloids in urine of smokers and smokeless tobacco users, J. Chromatogr. 619 (1) (1993) 49–61.
[4] Moyer TP, Charlson JR, Enger RJ, Dale LC, Ebbert JO, Schroeder DR, et al. Simultaneous analysis of nicotine, nicotine metabolites, and tobacco alkaloids in serum or urine by tandem mass spectrometry, with clinically relevant metabolic profiles. Clin. Chem. 2002 Sep;48(9):1460–71.
[5] B.B. Suh-Lailam, C.J. Haglock-Adler, H.J. Carlisle, T. Ohman, G.A. McMillin, Reference interval determination for anabasine: a biomarker of active tobacco use, J. Anal. Toxicol. 38 (7) (2014) 416–420.
[6] L.B. von Weymarn, N.M. Thomson, E.C. Donny, D.K. Hatsukami, S.E. Murphy, Quantitation of the minor tobacco alkaloids nornicotine, anatabine, and anabasine in smokers' urine by high throughput liquid chromatography-mass spectrometry, Chem. Res. Toxicol. 29 (3) (2016) 390–397.
[7] B. Wei, J. Feng, I.J. Rehmani, S. Miller, J.E. McGuffey, B.C. Blount, L. Wang, A high-throughput robotic sample preparation system and HPLC-MS/MS for measuring urinary anatabine, anabasine, nicotine and major nicotine metabolites, Clin. Chim. Acta. Int. J. Clin. Chem. 436 (2014) 290–297.
[8] P.B. Bendik, S.M. Rutt, B.N. Pine, C.S. Sosnoff, B.C. Blount, W. Zhu, J. Feng, L. Wang, Anabasine and anatabine exposure attributable to cigarette smoking: national health and nutrition examination survey (NHANES) 2013–2014, Int. J. Environ. Res. Public Health. 19 (15) (2022) 9744.
[9] McGuffey JE, Wei B, Bernert JT, Morrow JC, Xia B, Wang L, et al. Validation of a LC-MS/MS method for quantifying urinary nicotine, six nicotine metabolites and the minor tobacco alkaloids–anatabine and anabasine–in smokers' urine. PloS One. 2014;9(7):e101816.
[10] M. Feldhammer, J.C. Ritchie, Anabasine is a poor marker for determining smoking status of transplant patients, Clin. Chem. 63 (2) (2017 Feb 1) 604–606.
[11] European Medicines Agency (EMA). Good clinical practice [Internet]. 2018 [cited 2022 May 13]. Available from: https://www.ema.europa.eu/en/human-regulatory/research-development/compliance/good-clinical-practice.
[12] M.L. Colsoul, N. Goderniaux, D. Vanpee, L. Galanti, Development of an Ultra-High Performance Liquid Chromatography method for the simultaneous mass detection of tobacco biomarkers in urine, J. Chromatogr. B 1 (1210) (2022 Nov), 123476.
[13] Prevalence [Internet]. Tobacco Atlas. 2022 [cited 2022 Oct 3]. Available from: https://tobaccoatlas.org/challenges/prevalence/.
[14] A. Florescu, R. Ferrence, T. Einarson, P. Selby, O. Soldin, G. Koren, Methods for quantification of exposure to cigarette smoking and environmental tobacco smoke: focus on developmental toxicology, Ther. Drug Monit. 31 (1) (2009) 14–30.