Plasma Cotinine Cutoff for Distinguishing Smokers From Nonsmokers Among Persons Living With HIV

To the Editors:

Cotinine is an alkaloid in tobacco leaves and the main metabolite of nicotine metabolism.[1] The cotinine half-life (20 hours) is longer than that of nicotine (2–3 hours). Hence, cotinine may be used as a biomarker of nicotine exposure and smoking, when no information about smoking status is available.[2,3] The most common plasma cotinine (P-cotinine) cutoff for distinguishing smokers from nonsmokers in the general population is 14 ng/mL (80 nmol/L) [range from 3 to 20 ng/mL (17–114 nmol/L)].[1,2,4]

In a recent study in *J Acquir Immune Defic Syndr*, the authors assessed the nicotine metabolite ratio (NMR) as a biomarker of nicotine metabolism and smoking behavior among persons living with HIV (PLWH).[5] NMR was found to be higher in PLWH (0.47) compared with what has been previously reported for the general population (0.34–0.39).[5] Moreover, higher NMR (i.e., faster nicotine metabolism) was associated with a higher rate of smoking in PLWH.[5] Thus, owing to altered nicotine metabolism, cutoff for distinguishing smokers from nonsmokers may be different among PLWH.[5,6] In this letter, we aimed to evaluate the validity of current P-cotinine cutoff (14 ng/mL) for distinguishing smokers from nonsmokers in a population of PLWH and to determine the best P-cotinine cutoff among PLWH.

We used data from the Copenhagen comorbidity in HIV infection (COCOMO) study conducted at Rigshospitalet and Amager-Hvidovre Hospital, University of Copenhagen, Denmark (NCT02382822).[7,8] The study was approved by the regional ethics committee of Copenhagen (H-15017350) and the Danish Data Protection Agency (RH-2016-20: 04369). Written informed consent was obtained from all participants. All COCOMO participants with available P-cotinine and smoking data were included in this study. Smoking data were collected using a detailed and structured questionnaire, as previously described.[7] We collected data on self-reported smoking status (current, former, never) and, in current smokers, average daily consumption of cigarettes (with or without filter), cheroots, cigars, and pipe (packages of 40–50 g per week). Moreover, we collected data on use of electronic cigarette (E-cigarette) with nicotine, nicotine substitution (nicotine containing gum or patch), and hours of passive smoking.[7] Smoking is defined as inhalation of any type of tobacco smoke. In addition, we multiplied the number of daily smoked cigarettes, cheroots, cigars, and pipe to the average amount of tobacco in each and summed up the results to calculate the use of tobacco as grams/day.[9] P-cotinine was measured at Bevital AS (www.bevital.no) using liquid chromatography/tandem mass spectrometry (LC-MS/MS).[10] We used the Mann–Whitney *U* test to compare medians. Moreover, we used area under the receiver operating characteristic curve (AUROC) and Youden Index (J) (J = sensitivity + specificity − 1) to define the best P-cotinine cutoff for distinguishing smokers from nonsmokers among PLWH. We used the McNemar test to show the statistical difference between this new cutoff and recommended P-cotinine cutoff (14 ng/mL) in the general population.

A total of 988 PLWH were included. The median [interquartile range (IQR)] age was 50 (43–58) years, 855 (87%) were men, 861 (87%) were Caucasian, and the median (IQR) BMI was 25 (22–27) kg/m^2. Nine hundred seventy-one (98%) patients received combination antiretroviral therapy (cART), 926 (94%) had plasma HIV RNA below 50 copies/mL, and the median (IQR) current CD4$^+$ T-cells was 690 (520–890) cells/μL.

The proportions of current, former, and never smokers were 300 (30%), 350 (35%), and 338 (34%), respectively. The median (IQR) of tobacco use was 15 (10–20) g/d among current smokers. Fifty-four persons (5.5%) used nicotine substitution, and 33 persons (3.3%) used electronic cigarettes with nicotine.

The median P-cotinine level according to smoking status stratified by nicotine-substitution and E-cigarette use is shown in Figure 1A. The lowest median (IQR) P-cotinine value found in never smokers is 1.3 (0–2.6) ng/mL (n = 300) and in former smokers is 1.2 (0–2.6) ng/mL (n = 285) without a history of nicotine substitution or E-cigarette use. We assumed this amount of P-cotinine to be the effect of environmental tobacco smoke (ETS) exposure. The highest median (IQR) P-cotinine value was 1375 (718–1825) ng/mL and was among current smokers with nicotine substitution but without E-cigarette use (n = 20). The median (IQR) P-cotinine value among current smokers without nicotine substitution and E-cigarette use was 959 (489–1322) ng/mL (n = 238).

The sensitivity, specificity, positive predictive value (PPV), and negative predictive values (NPV) for cotinine cutoff 14 ng/mL to classify smokers from nonsmokers in the total population were 96%, 87%, 77%, and 98%, respectively.

Median P-cotinine among former smokers with other sources of nicotine (nicotine substitution and/or E-cigarette use) was close to current smokers. Hence, in an exploratory analysis, we excluded PLWH who had other sources of nicotine and PLWH without recorded data for other sources of nicotine [n = 165/988 (17%)] and re-evaluated the validity of P-cotinine cutoff 14 ng/mL.

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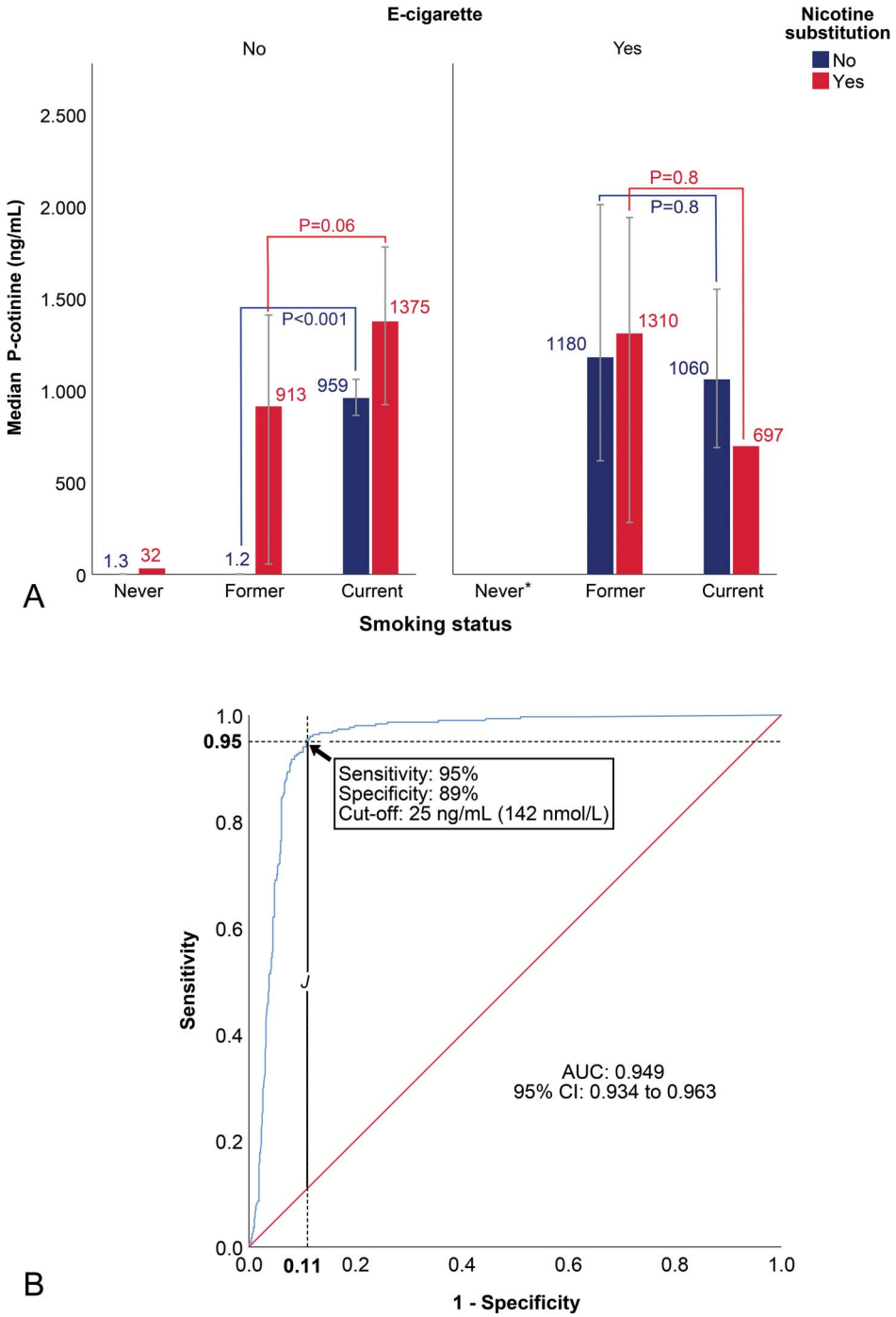

FIGURE 1. A, Median P-cotinine of PLWH according to smoking status stratified by nicotine substitution (nicotine containing gum or patch) and E-cigarette use (E-cigarette with nicotine) (Mann–Whitney *U* test applied, $P \leq 0.05$ is statistically significant). *There was no never-smoker who fits into this category; (B) Receiver operating characteristic curve (ROC) for the best P-cotinine value for distinguishing smokers from nonsmokers among total population of PLWH (n = 988).

In this subpopulation, the sensitivity, specificity, PPV, and NPV for cotinine cutoff (14 ng/mL) were 96%, 93%, 84%, and 98%, respectively.

Using AUROC among the total population of PLWH, the highest value for J (0.846) was found using P-cotinine cutoff 25 ng/mL (142 nmol/L) (area under the curve = 0.949, 95% confidence interval = 0.934 to 0.963, $P < 0.001$) (Fig. 1B). Sensitivity, specificity, PPV, and NPV for this cotinine cutoff (25 ng/mL) among the total population were 95%, 89%, 79%, and 98%, respectively. P-cotinine cutoff 25 ng/mL was statistically different from 14 ng/mL ($P < 0.001$) in distinguishing smokers from nonsmokers among PLWH.

In a sensitivity analysis excluding PLWH with other sources of nicotine and PLWH without recorded data for other sources of nicotine [n = 165/988 (17%)], we repeated AUROC analyses. The highest value for J (0.896) was at P-cotinine cut-off 19 ng/mL (108 nmol/L) (AUC = 0.978, 95% confidence interval: = 0.968 to 0.989, $P < 0.001$). Sensitivity,

specificity, PPV, and NPV for this cotinine cutoff (19 ng/mL) among PLWH without other source of nicotine were 95%, 94%, 87%, and 98%, respectively.

Previous studies have reported different P-cotinine cutoffs for distinguishing smokers from nonsmokers in the general population, with 14 ng/mL being the commonly used cutoff.[4] However, there is no established cutoff for PLWH despite evidence for a different nicotine metabolism. We determined a P-cotinine cutoff for distinguishing smokers from nonsmokers among PLWH which was found to be 25 ng/mL (142 nmol/L). In comparison with this cutoff, the commonly used cotinine cutoff (14 ng/mL) for general population had almost comparable sensitivity and specificity, although the 25 ng/mL cutoff yielded statistically different results.

Cotinine cannot differentiate between current smoking and use of other sources of nicotine, which is a limitation when there is no access to information about nicotine substitution and/or E-cigarette use. The effect of passive smoking or ETS on cotinine cutoff is another point of debate.[2] However, the median P-cotinine value related to ETS was very low among PLWH in our study.

Our study had limitations; our cohort consisted of mainly Caucasian men, and nicotine metabolism may vary according to different ethnicities and sex.[1] Hence, the validity of our cutoff should be tested in other populations of PLWH. Strengths of the study include the large population of PLWH and the detailed and accurate data on smoking. We also used the LC-MS/MS method with lower limit of detection of 0.18 ng/mL (1 nmol/L) for detection of P-cotinine.[10] This method is a sensitive and specific method for detection of low amounts of cotinine in body fluids.[11]

In conclusion, P-cotinine cannot differentiate between current smokers and nonsmokers with other sources of nicotine. Moreover, best cotinine cutoff may be different according to target population. In this study, a P-Cotinine cutoff of 25 ng/mL (142 nmol/L) had the best performance in terms of concomitant sensitivity and specificity for distinguishing smokers from nonsmokers. However, the commonly used cotinine cutoff [14 ng/mL (80 nmol/L)] established for the general population had almost comparable validity. Future studies should validate the cutoff among other populations of PLWH with different distributions of sex and ethnicity.

Omid Rezahosseini, MD[a]
Andreas Dehlbæk Knudsen, MD[a]
Marco Gelpi, MD[a]
Andreas Ronit, MD, PhD[a]
Per Magne Ueland, MD[b]
Øivind Midttun, PhD[b]
Ditte Marie Kirkegaard-Klitbo, MD[a,c]
Henrik Ullum, MD, PhD[d]
Susanne Dam Nielsen, MD, DMSc[a]

[a]Viro-immunology Research Unit, Department of Infectious Diseases 8632, Rigshospitalet, University of Copenhagen, Copenhagen
[b]Bevital AS, Bergen, Norway
[c]Department of Infectious Diseases, Amager-Hvidovre Hospital, University of Copenhagen, Hvidovre, Denmark
[d]Department of Clinical Immunology, Rigshospitalet, Copenhagen, Denmark

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IgG From HIV-1–Exposed Seronegative and HIV-1–Infected Subjects Differently Modulates IFN-γ Production by Thymic T and B Cells

To the Editors:

Interferon-gamma (IFN-γ), a pleiotropic cytokine, which is mainly produced by activated lymphocytes, has long been considered to play a pivotal role in mediating host–pathogen interactions. Understanding how cells produce IFN-γ is thus important key to eventually elucidate the mechanisms involved in the pathogenesis and therapy.[1] Specifically, in HIV-1 infection, increased plasma levels of IFN-γ have been linked to lower $CD4^+$ cell count recovery during antiretroviral therapy,[2] a parameter of great clinical importance for such patients.

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