Correction of Urine Cotinine Concentrations for Cotinine Excretion: Is It Useful? Peter Jatlow,1,2 Sherry Mckee,2 and Stephanie S. O'Malley1 (Departments of 1 Laboratory Medicine and 2 Psychiatry, Yale University, New Haven, CT 06520; address correspondence to this author at: Yale University, Department of Laboratory Medicine, PO Box 208035, New Haven, CT 06520-208035; fax 203-688-7340, e-mail peter.jatlow@yale.edu)

The validity of plasma or serum cotinine concentrations as a marker and stable measure of chronic nicotine intake is well documented (1–6). Although considerably less exact as a measure of cigarette consumption (2, 4), cotinine concentrations are often used for that purpose as well. Applications include screening of individuals before recruitment into research studies and smoking cessation clinical trials, as an outcome marker of treatment efficacy, and for evaluation of adequacy of dosage in conjunction with nicotine replacement therapy (7). Measurement of salivary or spot urinary cotinine concentrations is frequently selected as a noninvasive alternative to blood-derived assays for reasons of logistics, cost, and subject retention (6, 8–10). As has been done with other analytes, urine cotinine-to-cotinine concentration ratios are commonly determined to correct for dilutional effects (6, 11–13), although a recent review of markers for tobacco use suggests that this step might be unnecessary (10).

Considering the disparate renal handling of cotinine and creatinine (14), we were interested in determining whether this additional laboratory procedure and calculation was justified. Because spot urinary cotinine measurements are often used as a surrogate for serum cotinine determinations, we investigated how well spot urinary cotinine measurements correlated with concurrent serum concentrations in smokers, and whether normalizing for urine creatinine concentration improved the relationship.

Samples were drawn from adults recruited into a smoking cessation treatment trial comparing three different doses of naltrexone or placebo in conjunction with a nicotine patch. In addition to meeting criteria for adequate renal and liver function and hematologic indices, participants were required to have a smoking habit of at least 20 cigarettes/day and a CO concentration 910 ppm. Mean age of the sample was 47.5 years; 50.3% were female, and 87.7% were Caucasian. Participants smoked an average of 28.3 cigarettes/day and had been smoking for a mean of 23.38 years. Urine samples were collected concurrently with the initial plasma sample, between 1700 and 1900 in the evening at the time of recruitment into the study, but before initiation of any treatment or change in smoking habits. Paired samples were successfully obtained from 340 of 349 successive eligible individuals regardless of their randomization into the various arms of the study, and both cotinine and creatinine concentrations were obtained on 330 of them. All participants voluntarily provided written informed consent for this study, which was approved by the Yale University Human Investigation Committee.

Cotinine concentrations in serum and urine were determined by reversed-phase HPLC. The procedure was modified from that of Harirahan and VanNoord (15) to enable an aqueous micro back-extraction clean-up step in place of solvent evaporation. Briefly, after addition of an internal standard, 2-phenylimidazole, cotinine was extracted from alkalinized serum or urine with a 40:60 mixture of dichloromethane–hexane. After a micro back-extraction into 0.1 mol/L H3PO4, the aqueous phase was chromatographed on a C8 reversed-phase column using a mobile phase of 100 mL/L acetonitrile buffered to pH 4.8 and containing 20 mL/L triethylamine and 0.6 g/L octanesulfonic acid. The between-day CV, in routine use, at mean serum or urine cotinine concentrations of 20 and 200 μg/L in plasma were 12% and 6.6%, and in urine, at concentrations of 200 and 2000 μg/L, they were 4.7% and 6.4%. Urine cotinine concentrations were determined on the Hitachi 747-200 with a kinetic version of the Jaffé reaction (between-assay CV for urine, 3%).

Pearson correlations were calculated to determine associations between plasma and urine cotinine concentrations and urine cotinine-to-creatinine concentration ratios, respectively. A z test was conducted to test for significant differences between the two correlation analyses.

Mean (SD) serum and urine concentrations in the 340 participants were 298 (126) μg/L and 1779 (842) μg/L, respectively, which is consistent with the smoking history of the population studied. Correlations of urine cotinine concentrations and urine cotinine-to-creatinine ratios with serum cotinine concentrations are shown in Fig. 1. Although each measure showed a significant correlation
with serum cotinine, the outcomes were significantly different ($z = 3.75; P < 0.001$). Uncorrected urine cotinine concentrations showed a much stronger correlation with serum concentrations ($r = 0.69; P < 0.0005$) than did the urine cotinine:creatinine ratios ($r = 0.41; P < 0.0005$). Reanalysis of the data after removal of outliers did not substantively change the results.

Nicotine dosage has been shown to be the major determinant of serum cotinine concentrations, but it is subject to interindividual differences in cotinine clearance and, to a lesser degree, fractional conversion of nicotine to cotinine (1–4). Urinary cotinine measurements are frequently used as a noninvasive substitute for serum or plasma cotinine and are likely as good for differentiating smokers from nonsmokers. However, urinary cotinine concentrations are also subject to any factors that might affect the renal clearance of cotinine (14, 16). Thus it was expected that the correlation between urine and serum concentrations would be less than perfect, and indeed serum concentrations explained slightly <50% of urine values. As has been done with the measurement of other constituents in spot urines, urinary cotinine concentrations are commonly corrected for creatinine concentrations to adjust for differences in urine flow (6, 11, 12). In such instances, the urine cotinine-to-creatinine ratio usually becomes the desired index.

Assuming the serum cotinine concentration to be the accepted standard for estimating chronic nicotine intake, subject to the constraints indicated above, we were interested in knowing whether correcting for creatinine improved the correlation of urinary with serum cotinine. Our data indicated that the correlation of urine cotinine-to-creatinine concentration ratios with a concurrently obtained serum cotinine is poorer than that obtained with urine cotinine concentration alone. This is not altogether surprising in that renal handling of cotinine is markedly different than for creatinine (14, 16). Renal clearance of creatinine is largely a function of glomerular filtration, which it slightly exceeds, and some tubular secretion. As has been reported and discussed by Benowitz et al. (14), the renal clearance of cotinine, ~12 mL/min, is much less than the glomerular filtration rate, which in the absence of significant plasma protein binding is consistent with renal tubular reabsorption. Because oxidation of the pyrrolidine ring leads to a reduction of nicotine’s basic characteristics, cotinine should be largely unionized within the usual physiologic urinary pH range, which is consistent with the $pK_a$ of cotinine, reported to be <5.0 (16). This, along with the concentration gradient between urine and plasma cotinine, favors renal tubular reabsorption. Thus the consequences of increased urine flow on cotinine and creatinine would differ. As verified by Beckett and Gorrod (16), increased urine flow would be expected to reduce tubular reabsorption of cotinine and enhance elimination, mitigating any dilutional effects. Urine cotinine concentrations, on the other hand, would fall proportionately under these conditions.

Data documenting the assumption that correcting cotinine concentrations for creatinine enhances the informational value of the data are limited. As a variation, normalization of urine cotinine values to reflect the mean creatinine concentration in a defined study population has been used when a comparison between individuals within that population was desired (11). This strategy was reported to yield a modest improvement in the correlation between urine and blood concentrations, whereas the cotinine:creatinine ratio did not (11). Normalization based on the relationship of urine cotinine to creatinine or urine specific gravity in a population not exposed to cigarette smoke was reported to enhance the correlation to clinical endpoints in passively exposed infants (13). Both of these studies used RIA to measure cotinine, which may have had significant cross-reactivity with the major cotinine metabolite, $trans$-3'-hydroxycotinine (17, 18). Possibly the renal excretion of this more
polar compound more closely parallels that of creatinine. On the other hand, indirectly supporting our findings, a study using liquid chromatography coupled with tandem mass spectrometry to measure urine cotinine in passively exposed children found that creatinine-corrected urine cotinine concentrations correlated less well with parental smoking history than did the uncorrected values (19).

We purposely did not incorporate strategies designed to optimize the correlation between serum and urine concentrations, which might have included initial bladder emptying and blood sampling midway through a timed collection. In fact, urinary cotinine excretion per 24 h may actually be an as good or better correlate than plasma of chronic nicotine exposure (2). Rather, our goal was to emulate the urine sampling protocol that is most feasible and almost invariably used in clinical practice as well as in clinical research and clinical trials for smoking cessation to monitor tobacco/nicotine exposure. Although one might speculate that correcting cotinine for creatinine excretion might potentially be useful for monitoring the same individual over time, the constraints resulting from the differences in renal handling of the two compounds should apply in this situation as well.

Several variables, especially smoking behavior, influence the nicotine intake that results from smoking a defined number of cigarettes, thus reducing the exactness of serum cotinine as a measure of cigarette consumption (4, 20). These limitations would apply to urine as well. However, this study did not address that issue. Our data suggest that the common strategy of correcting spot urine cotinine concentrations for creatinine content, through calculation of a cotinine-to-creatinine ratio, is not a useful exercise, at least in smokers, and may even be counterproductive. Possibly these findings do not apply at the very much lower urine cotinine concentrations seen in studies of passively exposed individuals.

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References


Fetal Nucleated Erythrocytes in Maternal Circulation

Do Not Display a Classic Membrane-associated Apoptotic Characteristic (Phosphatidylserine Exposure) Despite Being Positive by Terminal dUTP Nuclear End Labeling, Sasha Hristoskova, Wolfgang Holzgreve, and Sinhue Hahn

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The presence of fetal cells, especially fetal nucleated red blood cells (NRBCs), in the blood of pregnant women is now a widely demonstrated phenomenon and is considered as the basis for a novel noninvasive means for prenatal diagnosis. However, at present little is known about the fate of fetal cells once they enter the maternal circulation.

Fetal cells could be removed from the maternal circulation by the maternal immune system or by the induction of apoptosis by other means (1–3). These proposals are supported by reports indicating the presence of terminal dUTP nuclear end labeling (TUNEL)-positive fetal NRBCs in the maternal circulation, a feature that may be attributable to the increased oxygen concentration in the maternal circulation (4), as well as by the description of apoptotic fetal cells or their remnants in the maternal plasma.

Elimination of fetal cells by apoptosis from the maternal periphery may not be as widespread as suggested, or it may not affect all fetal cell types equally. Fetal leukocytes