Detection of Nicotine and Nicotine Metabolites in Units of Banked Blood

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ABSTRACT

Objectives: To determine the concentrations of nicotine and nicotine metabolites in RBC units as a means to estimate the point prevalence of exposure within the healthy donor pool.

Methods: Segments from 105 RBC units were tested for the presence of nicotine, cotinine, or trans-3'-hydroxycotinine by liquid chromatography–tandem mass spectrometry.

Results: Of the 20 (19%) units that contained detectable concentrations of nicotine, cotinine, or trans-3'-hydroxycotinine, 19 (18.1%) contained concentrations consistent with the use of a nicotine-containing product within 48 hours of specimen collection. One RBC unit contained nicotine concentrations consistent with passive exposure.

Conclusions: Chemicals from nicotine-containing products are detectable within the US RBC supply. Further investigation is needed to determine the risks of transfusion-associated exposure to nicotine and other tobacco-associated chemicals among vulnerable patient populations such as neonates.

Ensuring the safety of the blood supply is of paramount importance in transfusion medicine. To safeguard the quality of transfused blood products, the US Food and Drug Administration (FDA) and AABB establish requirements to determine the suitability of an individual to serve as a blood donor.

These requirements are meant to protect both the recipient and the donor by excluding from the donor pool individuals who introduce excess risk to the transfusion. For example, persons with exposure to communicable diseases or usage of certain medications (such as anticoagulants, chemotherapeutic or potentially teratogenic agents) are targeted for identification and exclusion.

Prior to blood collection, all nonremunerated volunteers complete a universal donor health questionnaire, participate in vital sign assessments, and undergo a limited physical examination. If all donor eligibility criteria are met, and the donor does not indicate that his or her blood should not be used, the donation is accepted. Once the blood is collected, testing for a standardized panel of infectious diseases is completed; certain units (specifically those from female donors) may undergo additional testing for HLA antibodies to reduce the risk of transfusion-related acute lung injury.¹ These precautionary measures help to significantly reduce recipient risk of exposure to blood-borne pathogens and further enhance patient safety.¹⁻³ However, despite enhanced precautionary measures, potentially hazardous substances (including heavy metals, prescription pharmaceuticals, and environmental toxins) may not be excluded from the blood supply.⁴⁻⁷ Although often clinically tolerated in adults, the risks posed by exposure to these substances in

vulnerable transfusion populations such as children, neonates, and fetuses are not well defined.

One potentially hazardous chemical that may be prevalent in the blood supply is nicotine. A toxic and addictive substance in tobacco, nicotine can be introduced via cigarettes, cigars, electronic cigarettes (e-cigarettes), hookahs, chewing tobacco, nicotine replacement therapy, or other environmental sources. Tobacco smoke is estimated to contain hundreds to thousands of chemical substances besides nicotine, many of which are known carcinogens.⁸ Toxic volatile organic compounds as well as heavy metals commonly found in cigarettes have also been identified in e-cigarettes.⁹

Although rates of cigarette smoking have declined over the past decade, it remains an active public health concern, with a prevalence of 15.5% in 2016. While smoking cessation initiatives have been successful in decreasing rates of cigarette smoking, other forms of nicotine consumption are on the rise; recent reports indicate the use of e-cigarettes from 2011 to 2015 increased by 900% among high school students, who represent an important blood donor demographic.^{10,11} Based on these reported frequencies, it is likely that many current and future blood donors smoke tobacco or use nicotine-containing products. At present, there is no federal requirement for direct testing of blood components for the presence of nicotine or any other tobacco-related chemical, and the universal donor health questionnaire does not ask about the use of tobacco or other nicotine-containing products.^{12,13} In light of the known risks associated with tobacco and nicotine exposure, work to detect and quantify the presence of nicotine analytes in blood products is needed.

The aim of this study was to determine the quantitative and qualitative presence of nicotine and its metabolites, cotinine and *trans*-3'-hydroxycotinine (3-OH-cotinine), in RBC units banked in a large, tertiary care US hospital (Figure 1). We hypothesized that nicotine and its metabolites would be detectable in 15% or less of RBC units tested, based on current rates of smoking in the United States and the widely held belief that blood donors have healthier recreational habits compared with non-blood donors.¹⁰

Materials and Methods

The study protocol was approved by the institutional review board (IRB number: 171998) at Vanderbilt University Medical Center (VUMC).

Samples

RBC units were obtained from qualified, volunteer, nonremunerated blood donors and stored in the hospital

Figure 1 Nicotine metabolism. Exposure to nicotine via recreational tobacco products or nicotine replacement therapy leads to the metabolism of nicotine into six primary metabolites (cotinine, 3-OH-cotinine, norcotinine, norcotine, cotinine oxide, and nicotine oxide). Adapted from McGuffey et al.¹⁴

blood bank. Using a previously described approach,⁵ 107 RBC unit segments were randomly selected from the AS-3 inventory at the VUMC blood bank (approximately 2 days' worth of inventory); all blood groups were represented in this random sample (group A = 50, B = 4, AB = 2, O = 51). All segments were collected from unique donor identification numbers, ensuring that each segment was from a unique donor. Prior to shipment to ARUP laboratories, the sealed, sterile tubing segments were kept in a shipping container at 4°C and stored at 4°C at the ARUP facility prior to analysis. Of the 107 RBC units sent for analysis, 105 had sufficient volumes required to perform quantitative measurement of nicotine, cotinine, and 3-OH-cotinine (Figure 2).

Mass Spectrometry Analysis of Residual RBC Segments

Quantitative analysis of nicotine, cotinine, and 3-OH-cotinine was performed by validated liquid chromatography–tandem mass spectrometry (LC-MS/MS), previously adapted from the published method for urine specimens.¹⁵ Briefly, 100 µL each of blank dialyzed plasma, supernatant from residual RBC segments, calibrators, and quality control samples was pipetted into a Phenomenex 96-well polypropylene transfer plate. Then, 500 µL of a mixture containing 0.1 mol/L acetic acid and 1 µg/mL deuterium-labeled internal standards (nicotine-d3, cotinine-d3, 3-OH-cotinine-d3) was added to each of the wells that contained the 100-µL aliquots. Samples were thoroughly mixed and loaded onto a SPEware PSCX 96-well preassembled solid-phase extraction plate. An SPEware automatic liquid dispenser positive-pressure manifold was used to extract the specimens through the columns at a rate of one drop every 4 seconds. The

Figure 2 Study design.

columns were then washed with 1 mL 0.1 mol/L acetic acid at one drop per second (~5-10 psi), followed by 1 mL acetonitrile/methanol/nanopure water at a ratio of 10:10:80. The wells were dried for 15 minutes under high pressure (60 psi), and the bound analytes were eluted by gravity flow with 1 mL of freshly prepared methanol/ NH_4OH (98:2) into a new 96-well plate (2-mL volume). Then, 50 μL of 0.12 mol/L HCl in methanol was added to each well and the plate was covered with a plate adaptor. The 96-well plate was loaded onto a dryer (Cerex sample concentrator; SPEware) at 40°C for 30 minutes. Each sample well was then reconstituted in 100 μL of 95:5 mobile phase A/B composition. The plate was covered with a silicone mat, mixed for 1 minute on a plate vortex, and then loaded onto the LC-MS/MS. The LC-MS/MS system consisted of a CTC PAL HTC-xt-DLW autosampler (LEAP) with Agilent 1260 Infinity series binary pump, degasser, and column oven. The LC column was a Restek Raptor biphenyl column (2.1 $\times$ 50 mm, 2.7- μm particle size) with a Phenomenex SecurityGuard UHPLC C18 (2.0 mm internal diameter) guard cartridge and holder. The mass spectrometer was an AB Sciex Triple Quad 4000. The LC runtime was 4.5 minutes with a flow rate of 0.4 mL/min. The method employed gradient elution using a mobile phase consisting of 5 mmol/L ammonium formate and 0.05% formic acid in water (mobile phase A) and 0.05% formic acid in methanol (mobile phase B). The limit of quantification was 2 ng/mL for each analyte,

and quantification was performed using MultiQuant Software (SCIEX). Cotinine has a longer half-life than nicotine and is commonly used to assess nicotine exposure. Nonsmokers tend to have serum cotinine concentrations less than 3 ng/mL. Individuals with passive tobacco exposure typically have serum cotinine concentrations less than 8 ng/mL, and the cutoff to distinguish between a heavy passive smoker from a nonsmoker can range from 10 to 20 ng/mL of cotinine.^{16,17} Active heavy smokers will have cotinine concentrations greater than 100 ng/mL. For additional information regarding method analysis parameters as well as chemicals and supplies used, see the supplemental section (all supplemental materials can be found at *American Journal of Clinical Pathology* online).

Results

Of the 105 RBC units tested, 20 (19.0%) had measurable concentrations of nicotine or its metabolites, and 19 (18.1%) contained concentrations consistent with the use of a nicotine-containing product within 48 hours of specimen collection. The single remaining RBC unit contained a nicotine concentration consistent with passive exposure.

The range of concentrations of nicotine, cotinine, and 3-OH-cotinine varied between units (n = 20; **Figure 3**). Nicotine was detected in 11 (55%) of these units

with a mean (SD) concentration of 5.8 (4.4) ng/mL (95% confidence interval [CI], 3.8-7.8 ng/mL). Cotinine and 3-OH-cotinine were quantifiable in all 20 units with a mean (SD) concentration of 114.1 (70.1) ng/mL (95% CI, 83.4-145.0 ng/mL) and 34.0 (21.1) ng/mL (95% CI, 24.8-43.3 ng/mL), respectively. One RBC unit had trace amounts of cotinine (6 ng/mL) and 3-OH-cotinine (4 ng/mL) and no measurable nicotine.

Discussion

Blood transfusion exposes recipients to both known and unknown substances, with unclear clinical risks and ramifications. Current federal regulations prioritize identification and avoidance of a narrow subset of exposures, with an appropriate emphasis on minimizing infectious risk. However, risks associated with donor environmental exposures have not been explored as extensively in the literature. As blood banks are not mandated to monitor smoking habits of donors, and no questions exist on the universal donor questionnaire assessing tobacco use, the prevalence of tobacco-related chemicals present in the blood supply is not known and infrequently recognized as a potential source of exposure to the transfused patient. Furthermore, the risks associated with exposure to tobacco-related chemicals among pediatric transfusion recipients differ from those of adults, as their unique physiology,

rapid growth, and incomplete development may increase susceptibility to injury from exogenous substances.

This investigative study demonstrates that chemicals from tobacco-containing products are detectable within the US RBC supply. A total of 19% ($n = 20$) of tested RBC units contained detectable concentrations of nicotine or its metabolites, consistent with either active ($n = 19$) or passive ($n = 1$) exposure to a nicotine-containing product. This is consistent with trends in tobacco use nationally, as the prevalence of current cigarette smoking among adults is 15.5%. Our blood bank is located in the state of Tennessee, which in 2016 had a reported cigarette exposure use prevalence of 22.1%.¹⁸ However, blood donors, as a group, are often viewed as a “healthier” population than nondonors, as they must pass a series of health screening tests to be eligible to donate.^{19,20} It is furthermore inferred that donors are of a more health-conscious mindset and less likely to engage in unhealthy social or recreational behaviors. Thus, the finding of slightly elevated rates of tobacco exposure among the small sample of randomly chosen donors (19%) compared with the general public (15.5%) was surprising.

It is important to remember that the measurements presented in this study indicate exposure to nicotine, which should not be strictly considered a surrogate marker for smoking. It is possible that the prevalence of cigarette smoking is, in fact, lower among donors than the national average and that a significant source of nicotine was from

Figure 3 Concentrations of nicotine (A), cotinine (B), and 3-OH-cotinine (C) in units of banked blood. Nicotine or its metabolites, cotinine and 3-OH-cotinine, were detected in 20 clinical products of banked blood. The cutoff for active vs passive smoking used in this study is indicated by the dotted line in the cotinine plot (B). The analysis was performed by an adapted liquid chromatography–tandem mass spectrometry from a previously published method.¹⁰ Mean concentration and 95% confidence intervals are displayed for each analyte.

smoking cessation aids such as nicotine gum, nicotine patches, or even e-cigarettes (which many misconstrue as a “healthier alternative” to cigarettes). However, even though other smoking-related chemicals may be absent in these products, nicotine is believed to impair critical aspects of fetal development, including retinoic acid signaling pathways.²¹

Furthermore, we hypothesize that the detection of nicotine analytes in banked blood might serve as a surrogate marker for other toxic chemicals being introduced via transfusions. Increased carbon monoxide (CO) or hemolyzed RBCs as a result of these chemicals would result in a less efficacious transfusion that could potentially lead to additional transfusions or donor exposures in a pediatric recipient. It has been well characterized that smokers have detectable concentrations of nicotine and nicotine metabolites; it has also been previously reported that CO accumulation and hemolysis are both associated with smoking. In a study of 410 blood donors, approximately 6% of donated blood was shown to have an increased CO percent attributed to cigarette smoking.²² Increases in CO in the bloodstream can adversely affect oxygen delivery to the tissues, resulting in a blunted or negative response to the transfusion, potentially leading to the need for additional transfusions. Furthermore, oxidative free radicals and other chemicals from cigarette smoke have been shown to cause RBC damage and hemolysis.²³ These concerns are especially relevant for critically ill neonates or small children who may be exposed to large volumes (on an mL/kg basis) of blood obtained from a single donor for a variety of reasons, including cardiac bypass for repair of congenital heart disease or extracorporeal membrane oxygenation for cardiorespiratory failure.²⁴ Many of these patients would likely receive irradiated RBCs, which would be expected to add to the hemolysis of the unit. Finally, we note that polycyclic aromatic hydrocarbons contained in cigarette smoke are difficult for the developing fetus and neonate to clear from their circulation due to immature glucuronidation activity.²⁵ These compounds are known to complicate the growth and development of fetuses and may have deleterious effects on the neonate as well.²⁶⁻²⁸

As far as we know, this is the first study in the published literature to quantify the concentration of nicotine or nicotine metabolites in units of RBCs collected from donors who met all FDA-required donation standards. However, we do acknowledge that the current study has recognizable limitations. The reported concentrations of nicotine and its metabolites in our RBC units may or may not represent a clinically significant amount, as our study was not designed to establish an association between nicotine concentrations and clinical

outcomes. In addition, we did not examine the role of nicotine and metabolite changes with the age of the product, as this was a single point prevalence study. It is possible that nicotine or its metabolites are degraded by storage. Based on the findings presented in this study, additional investigations are warranted to further refine and assess the effects of tobacco-related substances on pediatric, neonatal, and fetal transfusion recipients. We hope that this report inspires additional research into this important subject.

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