

Highlights

- Venous blood is often used to quantify AFB1-lysine in aflatoxin exposure studies
- We compared AFB1-lysine concentrations in paired capillary and venous blood samples
- Capillary blood may potentially substitute for venous blood to quantify AFB1-lysine

Assessing an Aflatoxin Exposure Biomarker: Exploring the Interchangeability and Correlation between Venous and Capillary Blood Samples

Balaji Srinivasan^{a,b}, Shibani Ghosh^c, Patrick Webb^c, Stacy P. Griswold^c, Kathy S. Xue^d, Jia-Sheng Wang^d, and Saurabh Mehta^{a,b}

Affiliations:

^aDivision of Nutritional Sciences, Cornell University, Ithaca, NY 14853, USA

^bInstitute for Nutritional Sciences, Global Health, and Technology (INSiGHT)

^cFriedman School of Nutrition Science and Policy, Tufts University, Boston, MA, USA

^dDepartment of Environmental Health Science, University of Georgia, Athens, GA, USA

Corresponding Author: Saurabh Mehta, Division of Nutritional Sciences, Cornell University, Ithaca, New York, 14853, smehta@cornell.edu, 607-255-2640.

Keywords: aflatoxin; mycotoxins; venous; capillary; interchangeability

Clinicaltrials.gov registration: NCT04209569

ABSTRACT

Exposure to dietary aflatoxins has been recognized as a potential threat to child nutrition and growth, in addition to being a known carcinogen. The ability to accurately assess concentration of aflatoxin in the blood of at-risk individuals is therefore very important to inform public health policies and on-the-ground programs around the world. Venous blood is frequently used to quantify biomarkers of exposure such as AFB1-lysine adducts. However, venous blood collection methods are invasive, requiring highly trained staff, which makes this method challenging to implement, especially in resource-limited settings. In contrast, capillary blood collection by fingerprick is less invasive and has the potential for application in point-of-need monitoring. The aim of this exploratory study was to investigate the correlation and interchangeability of capillary and venous human blood samples in the quantification of AFB1-lysine adduct concentration. A total of 72 venous and capillary blood samples were collected from 36 women of reproductive age (16-49 years) in northern Uganda. All sample specimens were analyzed using high-performance liquid chromatography with fluorescence detection. Regression analysis and Bland–Altman analysis were performed to compare AFB1-lysine concentrations between venous and capillary sample pairs. Bland-Altman analysis of albumin-normalized AFB1-lysine data- bias was -0.023 pg/mg-albumin and the 95% limits of agreement were 0.51 to -0.56 pg/mg-albumin for log-transformed data. There was a positive correlation between albumin-normalized venous and capillary AFB1-lysine concentrations with r of 0.71 ($p < .0001$). A lack of any accepted clinical cutoff for aflatoxin exposure makes definition of an ‘acceptable’ limit for statistical analysis and comparison of methods challenging. Our data suggests a positive correlation between albumin-normalized AFB1-lysine concentrations in venous and capillary sample pairs, but relatively weak agreement and interchangeability based on Bland-Altman analysis. Further exploration of this and other methods is needed.

1. INTRODUCTION

Aflatoxins are secondary metabolites produced by fungi of the *Aspergillus* species (mainly *A. flavus* and *A. parasiticus*) that have been established as significant environmental contaminants¹. Aflatoxins often contaminate crops such as peanuts and corn, particularly in low- and middle-income countries (LMIC)^{2,3} due to lack of proper food storage conditions that lead to fungal growth and contamination. Human exposure to aflatoxins can occur due to ingestion of contaminated foods, or indirectly from consumption of foods from animals previously exposed to aflatoxins in animal feeds⁴. It has been estimated⁵ that around 4.5 billion of the world's population is exposed to aflatoxins, which has been associated with an increased incidence of primary hepatocellular carcinoma, as well as carrying the risk of toxicity, poor birth outcomes and impaired child growth and immune functions⁵⁻⁹. Among the four types of aflatoxins commonly present in food samples, namely aflatoxins B1, B2, G1, and G2, aflatoxin B1 (AFB1) has been reported to be the most potent hepatocarcinogen of the four in experimental studies and is present in the highest concentrations in human foods². AFB1 is the most toxic of the aflatoxins, and, since 1993, has been classified as a known human carcinogen by the International Agency for Research on Cancer (IARC). A few studies have also reported maternal aflatoxin exposure during pregnancy to be associated with low weight and length at birth, as well as continued poor growth during infancy and early childhood¹⁰⁻¹⁵.

Biomarkers of aflatoxin exposure

Exposure assessment is a key component of epidemiological studies for assessing the effect of aflatoxin on human health and determining the extent of local public health risk. Biomarkers of

exposure may include the excreted toxin or its metabolites and the products of interaction between the toxin and various macromolecules such as protein- and DNA-adducts detectable in human blood, urine or tissue samples¹⁶. Currently used biomarkers of aflatoxin exposure include metabolites of aflatoxins such as aflatoxin M1 (AFM1) and AFB1-N⁷-guanine in urine^{17,18}, and AFB1-lysine adducts in blood¹⁹⁻²¹. AFB1-lysine adduct in blood is considered to reflect integrated exposures over longer time periods (2-3 months) based on longer in vivo half-life of albumin in humans when compared to urinary metabolites^{19,22} which reflects recent exposure with excretion occurring over 24–48 hours. In epidemiological studies²³⁻²⁷, quantification of AFB1-lysine adduct concentration has been shown to be useful for screening large populations for aflatoxin exposure. Figure 1 lays out in graphic form some of the main sources of aflatoxin, various modes of exposure, metabolites in human samples, and adverse effects²⁸. The major analytical techniques currently applied for measuring AFB1-lysine adduct concentration in human blood include enzyme-linked immunosorbent assay (ELISA)²⁹⁻³¹, radioimmunoassay (RIA)^{21,32,33}, and immunoaffinity chromatography followed by high-performance liquid chromatography (HPLC) with fluorescence detection^{21,30,34} and Isotope-LC-MS/MS³⁵

Venous vs. capillary blood samples

Venipuncture is a common procedure performed in hospital settings for blood collection; most laboratory reference ranges for blood analytes are based on venous blood. Studies on aflatoxin exposure have typically used venous blood for the quantification of AFB1-lysine adducts. Conventional venous blood collection methods are invasive, could potentially cause pain and needle stick injuries with a risk of contamination if not performed by highly trained and certified personnel, which makes it challenging to apply these methods, particularly in resource-limited

settings. Moreover, it has been reported that two-thirds of errors that affect laboratory test results occur in the pre-analytical phase (period before assay performance)^{36,37} and phlebotomy-related errors are regarded to account for greater than 60% of errors in this phase^{38,39}. Capillary blood sampling⁴⁰ is less invasive, does not require trained phlebotomists, and may also lead to higher acceptance of blood testing in settings where cultural traditions might interfere with patient involvement in collection of blood for analysis. Studies on greater patient involvement⁴¹ indicate

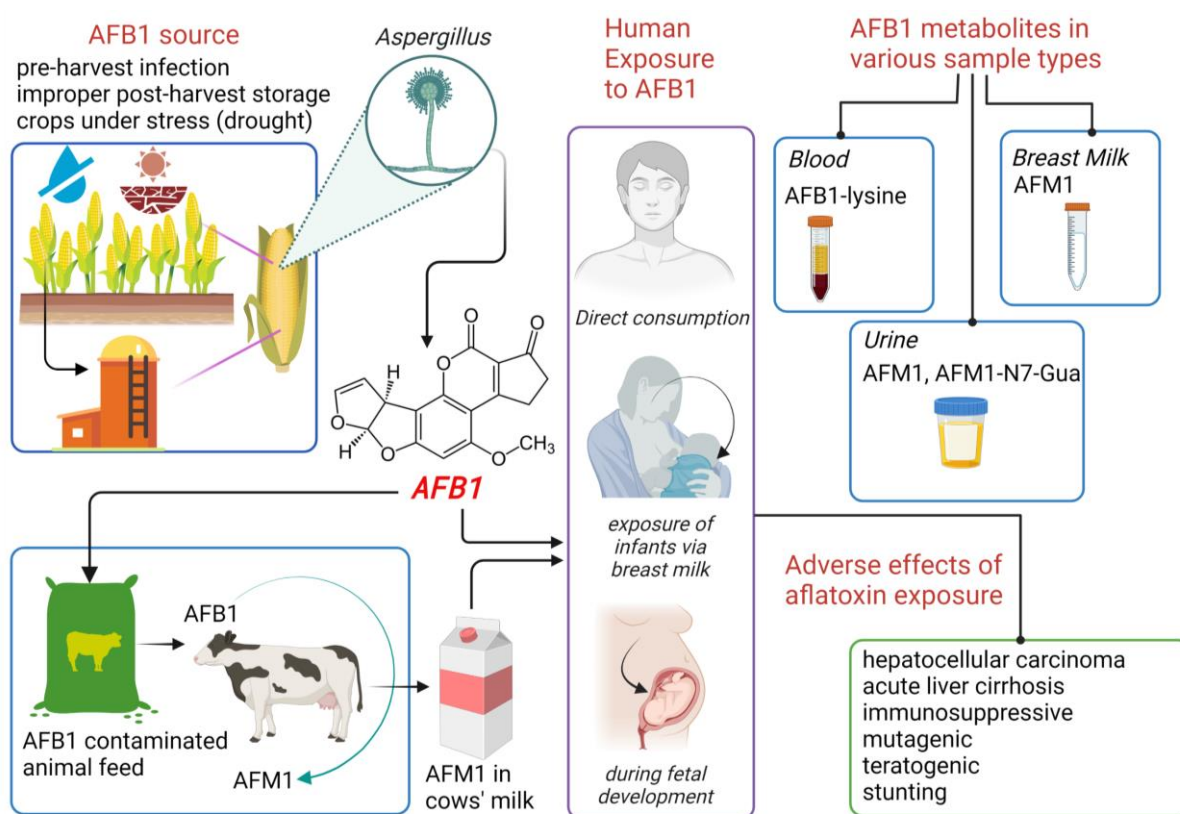


Figure 1: Schematic summarizing the source of aflatoxins, exposure modes, various metabolites of AFB1, and a list of major adverse effects of aflatoxin exposure.

improved health outcomes and treatment adherence while reducing long-term healthcare cost. Studies have also shown that patients prefer capillary blood sampling over venous sampling when frequent monitoring is required because it was less painful⁴². Moreover, capillary blood collection

is also suitable for newborns and infants as the lower blood volume with this method reduces the risk of anemia with frequent sampling⁴³.

Recent advances in point-of-care testing (POCT) technologies including the development of microfluidic chips and paper-based diagnostic technology have enabled simple and accurate capillary blood-based diagnostics at the point of care and need. According to current regulations, outside of a clinical laboratory and absent trained medical staff, the capillary is the only approved blood source for point-of-care diagnosis in field settings. However, the small sample volume (~10–250 μ L) used in capillary blood-based testing may affect the detection accuracy of a biomarker compared to laboratory-based testing with arterial and venous blood testing with larger sample volume (approximately 175 μ L - 5 mL)⁴⁴.

In adult populations, few studies comparing concentration of biomarkers in venous and capillary samples have been conducted, and fewer in acutely unwell adult populations where frequent blood tests are required for close monitoring. A cross-sectional comparison of ferritin concentration between capillary and venous samples⁴⁵ in a convenience sample of adults ($n = 20$) showed slightly elevated ferritin concentrations (mean bias of 9.9 ng/mL) in capillary samples. In healthy, non-fasted people in a non-clinical setting, a statistically significant, but clinically insignificant difference was found with moderate correlation between venous- and capillary-derived blood glucose when measured using a point-of-care, capillary-based glucometer⁴⁶. A study of capillary blood tests using the epocTM Point of Care Blood Analysis System (Alere) for analytes such as Na⁺, K⁺, glucose, lactate, creatinine, hematocrit, hemoglobin, pH and pCO₂, determined⁴⁷ that results of these assays were comparable to the reference method. The study⁴⁷ on the epocTM system

was, however, limited to paired capillary and venous blood correlation data from healthy people with the results not covering the entire analytical measurement range and therefore may not be generalizable to patients in critical conditions.

In many aflatoxin studies that quantify biomarkers of aflatoxin exposure in serum, collection of venous blood with cold chain transportation to a remote lab for testing is a commonly adopted approach. A recent study⁴⁸ has suggested dried blood spots (DBS) from capillary blood as a low-cost, viable alternative to venous blood draw for assessing AFB1 exposure with a good correlation and agreement to AFB1-lysine adduct concentration in serum samples as quantified by HPLC method with fluorescence detection. However, to our knowledge, there are no studies comparing biomarkers of AFB1 exposure in paired capillary and venous liquid blood samples.

The primary aim of this exploratory study was to investigate the correlation of AFB1-lysine adduct concentrations in paired capillary and venous blood samples from a study population consisting of 36 women of reproductive age in Agago District of northern Uganda. Both venous and capillary blood samples were collected simultaneously from each participant and serum AFB1-lysine adduct concentrations were quantified using an HPLC method described elsewhere^{48,49}.

2. METHODS

Study population

The study population consisted of women aged 16-49 years, residing in a sub-county in Agago District of northern Uganda and were not pregnant. Women were selected on the day of data collection in a convenience sample from a designated health center. The study was approved by

the Tufts Health Sciences Institutional Review Board in Boston, Massachusetts, and the Uganda National Council for Science and Technology in Kampala, Uganda. Written consent was obtained from all participants as was basic anthropometric, demographic, and health information prior to sample collection.

Sample collection

Study participants were sampled once during the study. Both venous blood and capillary blood were collected by the phlebotomist from the same arm of each participant while following standard laboratory procedures. Figure 2 shows a schematic outlining the sample collection approach. Matching venipuncture blood sample and fingerstick sample from each participant were labeled with a laboratory identification number format to enable easy identification of sample pairs. All blood specimens were subjected to the same handling and storage conditions. Blood samples were centrifuged at 4000 rpm for 5 min to separate serum and stored at -80°C. The total sample size was 36 women providing 36 venous and 36 capillary paired samples in total.

Quantification of AFB1-lysine adduct concentration

Serum samples were transported on dry ice to the Wang Laboratory at the University of Georgia, Athens, USA. The quantification of AFB1-lysine adduct concentration was performed using a high-performance liquid chromatography (HPLC)-fluorescence method (Agilent 1200, Santa

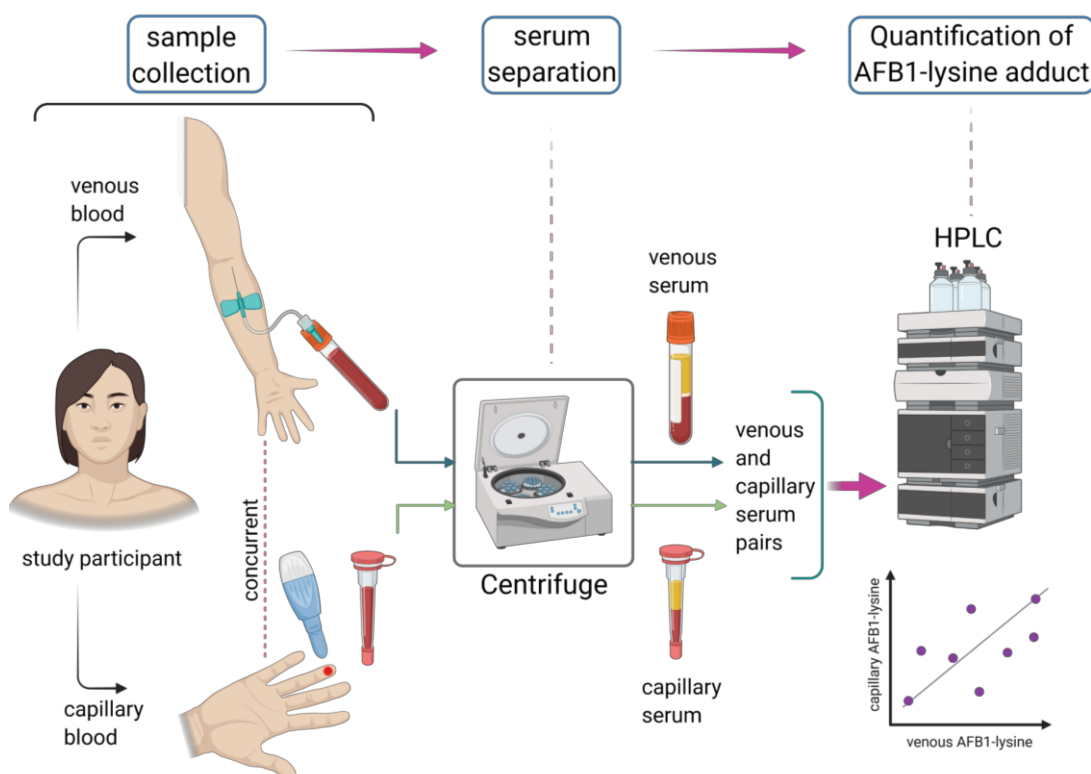


Figure 2: Sample collection for comparison of AFB1-lysine adduct in paired venous and capillary blood samples from each participant.

Clara, CA, USA). For each serum sample, the albumin concentration was quantified followed by pronase digestion to convert the AFB1-albumin adduct to a mono-AFB1-lysine adduct, concentration and purification of the AFB1-lysine adduct, and finally separation and quantification by HPLC⁵⁰ with fluorescence detection^{48,51}. The protocol for serum processing and quantification of AFB-lysine adducts has been reported previously⁵⁰. Albumin concentrations for each serum sample was experimentally quantified as previously described²¹. Briefly, concentration of human

serum albumin was determined in each sample by a bromocresol purple dye binding method⁵², which measures a stable blue-purple color complex formed between the dye and albumin with an absorption maximum at 600 nm. Standard curves for this assay were determined using human albumin. In addition, the amount of total protein was determined by Bradford-dye binding method⁵³ using a protein assay kit (Bio-Rad, Hercules, CA) calibrated with serum protein standard. This is essential for calculating the optimal volume of enzyme to be used for protein digestion. Pronase digestion steps included 150 μ L aliquots of each serum sample digested by pronase (pronase: total protein, 1:4, w: w) at 37 °C for 3 h. Under enzyme digestion, AFB1-lysine is released from the adducted albumin⁵⁴ form. The digests were then purified using Oasis MAX SPE cartridges (Waters Corporation, Milford, MA). After priming with methanol and equilibration with water, the loaded cartridge was sequentially washed by water, 70% methanol, and 1% ammonium hydroxide in methanol at a flow rate of 1 mL/min. The eluate in 2% formic acid in methanol was vacuum-dried with a CentriVapTM vacuum concentrator (Labconco Corporation, Kansas City, MO) and reconstituted with an average recovery rate of 90%.

HPLC conditions for AFB1-lysine analysis

Reproducibility of HPLC method applied in this study has previously been validated and reported^{13,14,48,55-60} in other published studies. Brief description of the HPLC conditions for AFB1-lysine is provided here- Quantification of AFB-lysine was performed on an Agilent 1200 HPLC-fluorescence system (Santa Clara, CA). The mobile phases consisted of buffer A (20 mM ammonium dihydrogen phosphate, pH 7.2) and buffer B (LC/MS grade Methanol). A ZORBAX Eclipse XDB-C18 (Agilent Technologies, Santa Clara, CA) reverse phase column (5 μ m, 4.6 $\times$ 250 mm) was used and 100 μ L was injected at a flow rate of 1 mL/min. A gradient was generated

to separate the AFB-lysine adduct in less than 25 min and the retention time of AFB-lysine was equal to approximately 12.3 min. AFB-lysine adduct was detected by fluorescence at the excitation and emission wavelengths of 405 nm and 470 nm, respectively. Quality assurance and quality control procedures were implemented during analyses, which included simultaneous analysis of one reference standard and a quality control sample daily. The limit of detection with this approach was 0.4 pg/mg albumin. The averaged coefficient of variation (CV) for serum samples analyses from over 10 different studies conducted in past 15 years ranged from 2.39 - 7.78%. Typical HPLC chromatograms of AFB1-lysine standard, as well as capillary (CC) and venous (VV) samples with near non-detectable, low

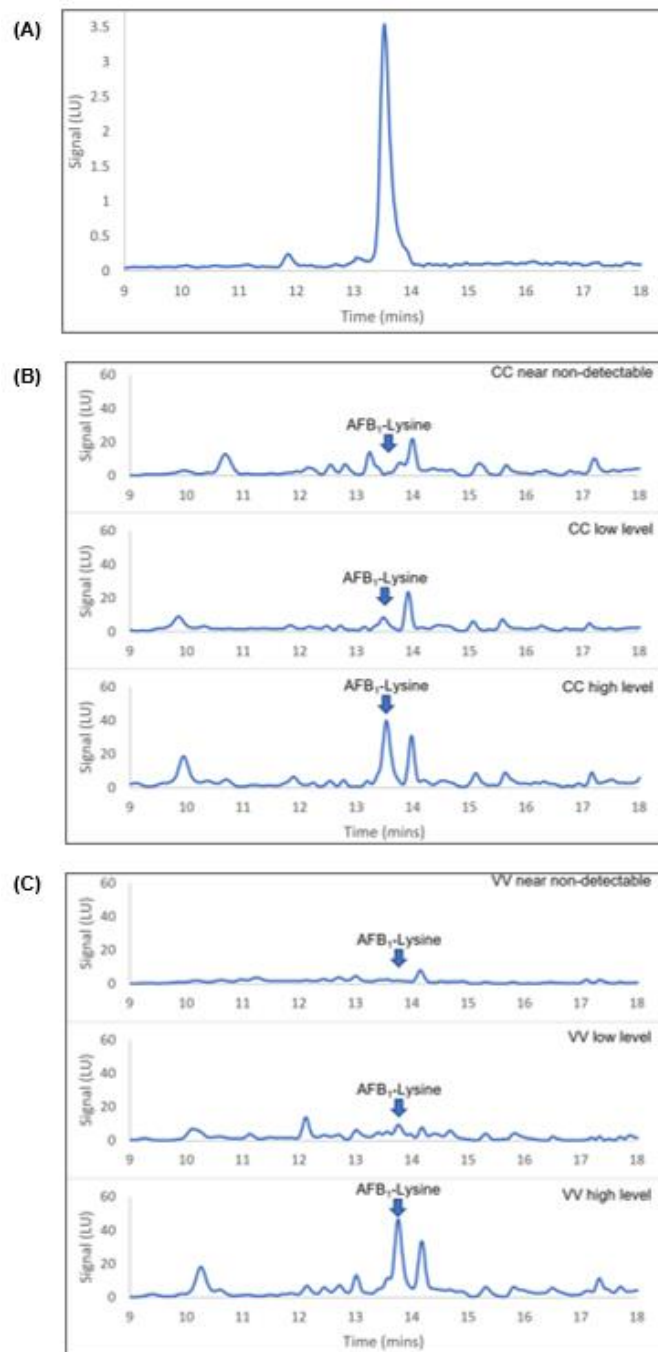


Figure 3. (A) Representative chromatograms of HPLC-fluorescence detection for AFB1-lysine in various samples. (A) AFB1-lysine standard; (B) near non-detectable, low, and high level AFB1-lysine detected in a capillary (CC) sample; (C) near non-detectable, low, and high level detected in venous (VV) sample

and high adduct levels are shown in Fig. 3. The quality control procedures included: generation of a new calibration curves were linear for AFB1-Lysine concentrations ranging from 0.01-5.0 ng/ml; pretest sample analysis procedure with AFB1-treated rat serum and normal human serum spiked with synthesized authentic AFB1-lysine with low and high concentrations; measurement of imprecisions based on 6 measurements on 3 separate days (the rates were 2.87-5.93%); measurement of inaccuracies and reproducibility for 6 repeated measurements (rates were 4.44-4.79%), measurement of recoveries based on low, middle, and high spiked AFB1-lysine adduct concentrations (rates ranged 82-95%), every 5 samples run included a separated authentic standard-spiked normal human serum samples which was under the same process. The technical person running analyses was blinded for the sample source and identifications.

Statistical analysis

All statistical analyses were performed using OriginPro 2022 (64-bit) SR1 (OriginLab Corporation, Northampton, Massachusetts). Only paired venous and capillary samples were only included in statistical analyses. Linear regression analysis with ordinary least squared estimates was performed, and Pearson's correlation coefficient (r) was used to assess correlation between capillary and venous AFB1-lysine adduct concentrations. Bland-Altman analyses⁶¹⁻⁶³ was used to analyze the bias between venous and capillary AFB1-lysine concentrations. The original data for capillary and venous AFB1-lysine concentrations was log-transformed and differences of the transformed data were confirmed to be normally distributed by Shapiro-Wilk normality test. Using Bland-Altman plots, the difference between the capillary and venous AFB1-lysine concentrations (y-axis) were plotted against the mean of the capillary and venous AFB1-lysine concentrations (x-axis). Horizontal lines were drawn at the mean difference and at the limits of agreement which

were defined as the mean difference ± 1.96 times the standard deviation (SD) of the differences. For all analyses, $p < 0.05$ was considered statistically significant.

3. RESULTS

Currently, AFB1-lysine adduct concentrations are reported normalized to total serum albumin (pg adduct/mg albumin), which requires separate assays for quantification of serum albumin in test samples. In this study, we compared AFB1-lysine adduct concentrations normalized to serum albumin as well as to total serum volume in both capillary and venous samples (paired samples only) to determine agreement.

Albumin concentration

Albumin concentration was quantified in each paired sample by bromocresol purple dye binding method. Figure 3(A) shows the distribution of albumin concentrations in

capillary and venous samples. Figure 3(B) shows a scatterplot and the linear regression fit for the set of 36 paired capillary and venous samples, with venous albumin concentration on y-axis and capillary albumin concentration on the x-axis. Results from regression analysis indicated that there

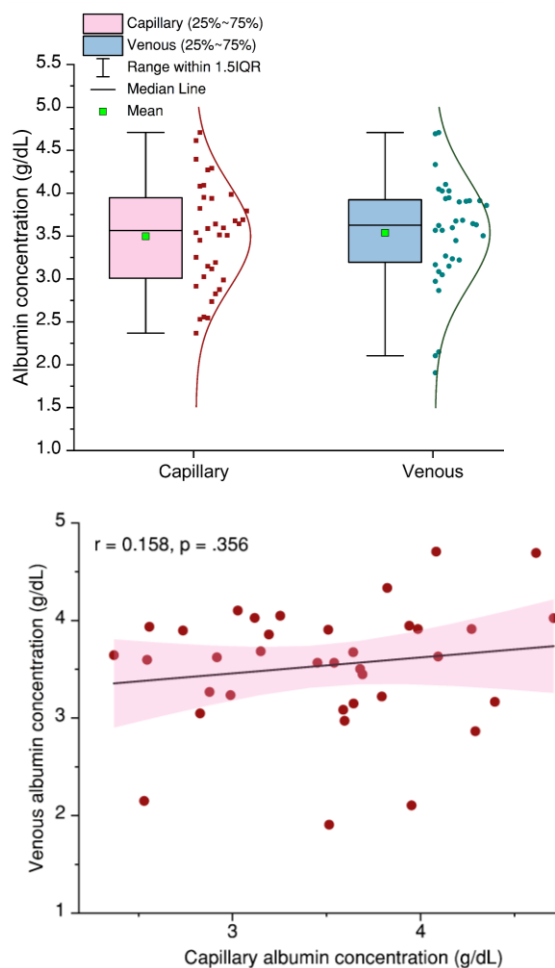


Figure 43. (A) Box plot showing a summary of capillary and venous albumin concentration data including the mean, 50'th percentile median line. (B) Linear regression analysis for correlation between capillary and venous AFB1-lysine adducts per mg albumin concentrations

was no positive correlation between venous and capillary albumin concentrations ($r = .158$, $p = .356$). The mean albumin concentration for capillary samples is 3.50 g/dL with the range of 2.37-4.71 g/dL; the mean albumin concentration for venous samples is 3.54 g/dL with the range of 2.15-4.71 g/dL. There were no significant differences in total digest.

Albumin-normalized AFB1-lysine concentration

The distribution of albumin-normalized AFB1-lysine concentrations among the capillary and venous samples was observed to be uniformly distributed as shown in Figure 35. Mean AFB1-lysine was 39.08 pg/mg-albumin (SD = 41.17, 95% CI = 26.61, 27.72). Mean albumin concentration was 3.52 g/dL (SD = 0.62, 95% CI = 3.18, 3.74). Figure 64(A) shows a scatterplot and the linear regression fit for the set of 36 paired capillary and venous samples, with albumin-normalized venous AFB1-lysine adduct

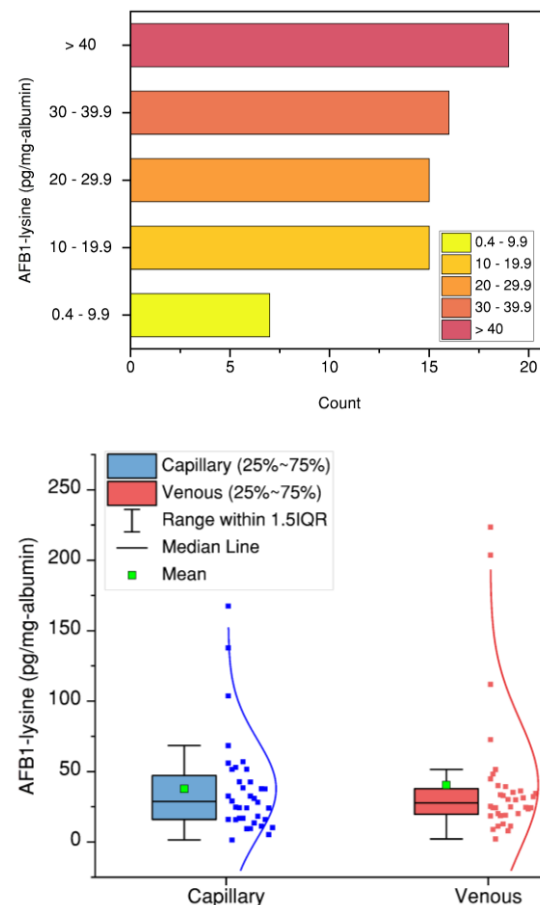


Figure 53. (A) Frequency distribution of albumin normalized AFB1-lysine adduct concentrations in the overall capillary and venous samples (B) Box plot showing a summary of capillary and venous data including the mean, 50'th percentile median line.

concentration on y-axis and albumin-normalized capillary AFB1-lysine adduct concentration on the x-axis. The fitted regression model was $[v\text{AFB1-lys}]_{\text{ALB}} = 0.9442 * [\text{cAFB1-lys}]_{\text{ALB}} + 4.8043$, where $[v\text{AFB1-lys}]_{\text{ALB}}$ and $[\text{cAFB1-lys}]_{\text{ALB}}$ represent albumin-normalized venous and capillary AFB1-lysine concentrations, respectively. There was a positive association between albumin-normalized venous and capillary AFB1-lysine concentrations with $r = .71$, $p < .0001$.

To determine interchangeability, a Bland–Altman analysis was performed, where the mean of log transformed albumin-normalized AFB1-lysine concentrations of each venous and capillary pair from participants ($n=36$) is plotted against its difference. A Bland-Altman plot for log

transformed data is shown in Figure 64(B), with the dashed blue line showing bias (mean difference) between venous and capillary, solid blue lines showing the 95% limits of agreement (LOA) and shaded areas representing 95% confidence interval limits for mean and LOA. The bias

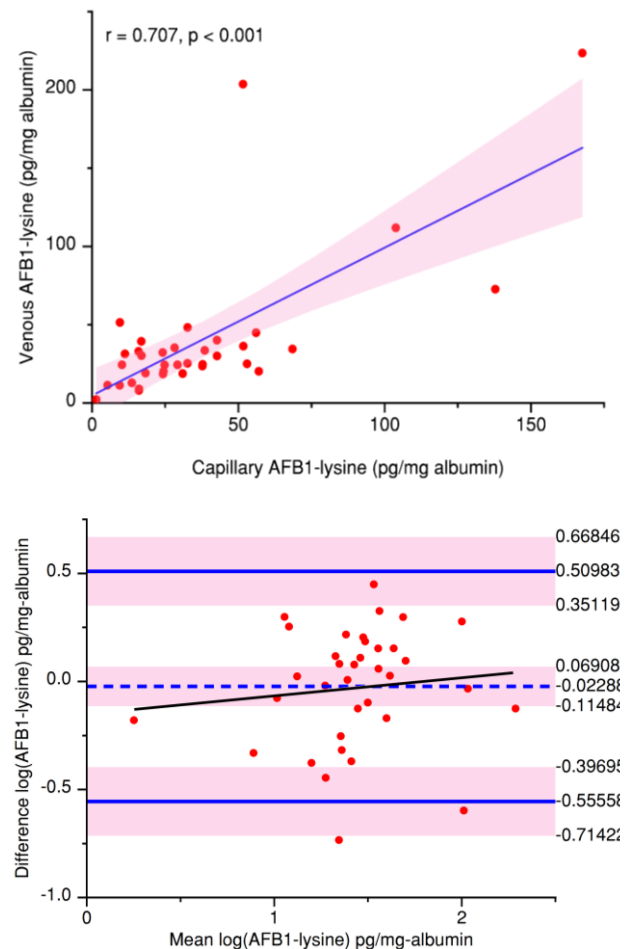


Figure 64(A) Linear regression analysis for correlation between capillary and venous AFB1-lysine adducts per mg albumin concentrations (B) Bland-Altman plot comparing log transformed capillary and venous AFB1-lysine concentration per mg-albumin concentrations. Dashed blue line shows bias (mean difference) in between venous and capillary, solid blue lines show the 95% limits of agreement (LOA) and shaded areas represent 95% confidence interval limits for mean and LOA. Solid black line shows linear regression fit for the data used in Bland-Altman plot.

was -0.023 pg/mg-albumin and the 95% LOA were 0.51 to -0.56 pg/mg-albumin for log-transformed data. Using the difference in AFB1-lysine adduct concentrations as the dependent outcome variable and the mean AFB1-lysine adduct concentration between venous and capillary sample types as an independent predictor in a linear regression, a regression fit (solid black line) with a slope of 0.086 ($r = .11$, $p = .514$) was obtained for the log-transformed data in the Bland-Altman plot in Figure 6 (B). At the 0.05 level, the slope of the regression line in Figure 6(B) is not statistically different from zero and therefore the bias between albumin-normalized AFB1-lysine in capillary and venous samples does not change with the magnitude of the measurements itself⁶⁴.

AFB1-lysine concentration normalized by total serum volume

AFB1-lysine adduct concentrations normalized by total serum volume in paired capillary and venous samples were used in an analysis similar to that to assess the albumin-normalized AFB1-lysine data. Figure 75(A) shows a scatterplot and the linear regression fit for the 36 paired capillary and venous samples, with volume-normalized venous AFB1-lysine adduct concentration (pg AFB1-lysine/ μ L) on the y-axis and volume-normalized capillary AFB1-lysine adduct concentration (pg AFB1-lysine/ μ L) on the x-axis. The fitted regression model was $[vAFB1-lys]_{VOL} = 0.8256 * [cAFB1-lys]_{VOL} + 0.2294$, where $[vAFB1-lys]_{VOL}$ and $[cAFB1-lys]_{VOL}$ represent venous and capillary AFB1-lysine concentrations normalized to serum volume, respectively. There was a positive association between volume-normalized venous and capillary AFB1-lysine concentrations with $r = .80$, $p < .0001$. A Bland-Altman plot for log transformed data is shown in Figure 75(B), with dashed red line shows bias (mean difference) between venous

and capillary, solid red lines show the 95% limits of agreement (LOA) and shaded areas represent 95% confidence interval limits for mean and LOA. For the log-transformed data, the bias was -0.027 pg/ μ L and the 95% LOA were 0.48 to -0.53 pg/ μ L. Using the difference in AFB1-lysine adduct concentrations as the dependent outcome variable and the mean AFB1-lysine adduct concentration between venous and capillary sample types as an independent predictor in a linear regression, a regression line (solid black line) with a slope of 0.175 ($r = .23$, $p = .184$) was obtained for the log-transformed data in the Bland-Altman plot in Figure 7 (B). At the 0.05 level, the slope of the regression line in Figure 7(B) is not statistically different from zero and therefore the bias between albumin-normalized AFB1-lysine in capillary and venous samples does not change with the magnitude of the measurements itself⁶⁴

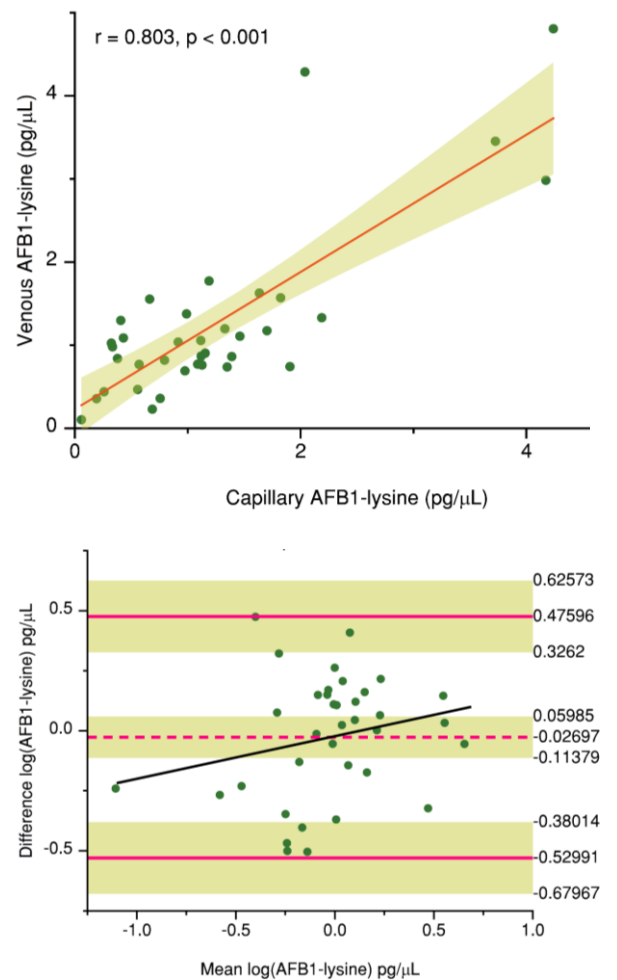


Figure 7 (A) Linear regression analysis for correlation between capillary and venous AFB1-lysine adducts per μ L (B) Bland-Altman plot comparing log-transformed capillary and venous AFB1-lysine adducts per μ L. Dashed red line shows bias (mean difference) between venous and capillary, solid red lines show the 95% limits of agreement (LOA) and shaded areas represent 95% confidence interval limits for mean and LOA. Solid black line shows linear regression fit for the data used in Bland-Altman plot.

4. DISCUSSION

The goal of this exploratory study was to evaluate whether capillary blood can be applied as a substitute to venous blood for quantification of AFB1-lysine concentration, a widely accepted biomarker in population-based studies on dietary exposure to aflatoxin. We analyzed the data for both albumin-normalized and total serum volume-normalized AFB1-lysine adduct concentrations for paired capillary and venous blood samples. With respect to the albumin-normalized AFB1-lysine concentrations - a positive association ($r = 0.71$) was observed, and Bland-Altman analysis indicated the bias was -0.023 pg/mg-albumin and the 95% LOA ranged from 0.51 to -0.56 pg/mg-albumin for the log-transformed adduct data. With serum volume-normalized AFB1-lysine concentrations – a positive association ($r = 0.81$) was observed, and Bland-Altman analysis indicated the bias was -0.027 pg/ μ L and the 95% LOA ranged from 0.48 to -0.53 pg/ μ L.

Studies comparing capillary and venous blood samples have been reported for various clinical biomarkers such as brain biomarker S100B⁶⁵, mean differential leukocyte counts⁶⁶, ferritin⁴⁵, hemoglobin⁶⁷, and glucose⁴⁶. For AFB1-lysine, a study on AFB1-lysine adducts in dried blood spots⁴⁸ from capillary blood has been compared to paired venous blood samples. To the best of our knowledge, concordance of AFB1-lysine adduct concentrations in human capillary and venous serum samples has not been reported. A high degree of agreement between capillary and venous AFB1-lysine concentrations would support the development of point-of-care testing technologies for aflatoxin exposure based on fingerprick capillary blood in population-based studies, especially useful in resource-limited settings.

Lack of clinical cut-off for serum Aflatoxin B1-lysine

Numerous studies have studied dose–response relationships between dietary intake of AFB1-contaminated food and AFB1 metabolites in blood, urine, and breast milk by applying various analytical techniques. For most clinical biomarkers with known cutoffs, it is possible to define *a priori* the limits of maximum acceptable differences in biomarker concentrations between two approaches or sample types based on biological considerations and clinical implications of the difference. However, at present there is no established clinical cutoff for aflatoxin exposure level in humans at which adverse effects of aflatoxin are most likely to occur. This is further complicated by a very wide range of goals of the population-based studies in different types of study populations. For examples, acceptable limits of agreement for studying the effect of aflatoxin exposure on stunting in newborns may be different from a study on carcinogenic effects of aflatoxin exposure in an adult population. This lack of data makes definition of an acceptable limit for statistical analysis and comparison of methods challenging. In this study, based on Bland-Altman analysis with percentage differences of the capillary and venous pairs to compare albumin-normalized AFB1-lysine adduct concentrations, it was observed that ~ 53% of the sample pairs (N = 36) had greater than 10% difference in concentrations and ~33% of the sample pairs (N = 36) had greater than 20% difference in concentrations. Whether any such differences between AFB1-lysine concentrations in capillary and venous samples could have clinically significant impact on subsequent decision making is not clear.

Effect of serum albumin concentration on albumin-normalized AFB1-lysine results

Often, the data reported by various studies of aflatoxin exposure is in the form of serum AFB1-lysine normalized to albumin concentration (pg/mg-albumin). Serum albumin has been quantified by analytical techniques such as bromocresol purple dye method^{49,68} and ELISA⁶⁹. Data on

comparison of serum albumin in paired capillary and venous sample is not available. Total protein concentration in capillary samples have been reported to be significantly lower (3.3% difference, $p < .0005$) than venous samples. A study on comparison of albumin concentration in paired capillary and venous serum⁷⁰ samples (N = 59) concluded there was no interchangeability based on correlation ($r = 0.75$) and Bland-Altman results with mean % bias of $-0.22 \pm 6.2\%$ and 95% LOA in percentage from -12.27% to 11.84% . In our study, linear regression analysis of paired capillary and venous samples (N = 36) indicated no positive correlation between capillary and venous albumin concentrations. However, this result is limited by a small sample size and a more detailed study with large sample size would be required to confirm interchangeability of capillary and venous samples for albumin concentrations in clinical settings. This potential difference in albumin concentration between capillary and venous serums samples can in turn affect the AFB1-lysine concentrations when albumin results are applied to normalize to report the results in albumin-normalized AFB1-lysine format.

Study Limitations

Although the results obtained in this work gave valuable insight into the possibility of capillary blood sample as a substitute for venous blood for quantification of AFB1-lysine in aflatoxin exposure studies, several limitations must be taken into consideration. The primary limitations of our study include its relatively small sample size of 36 participants.⁵ However, given the high prevalence of aflatoxin exposure in this population, the wider range of AFB1-lysine concentrations allowed us to obtain some preliminary results. Future work to increase the sample size and to include different populations with different dietary aflatoxin exposure levels to validate the capillary procedure are necessary. Only one capillary sample was obtained from each participant,

and the limited volume of capillary sample was not sufficient to perform a repeatability analysis of the AFB1-lysine concentration quantified from the capillary samples.

5. CONCLUSIONS

The public health and nutrition communities of low- and middle-income countries across the Tropics have increasingly recognized that aflatoxins and other related mycotoxins pose a genuine threat to human health whether via chronic diseases such as cancer or through maternal wellbeing leading to adverse birth outcomes and impaired child growth. As a result, there is growing demand for more detailed assessments of dietary exposure risks even where aflatoxin concentrations in food may be low. Hence the importance of identifying less costly, non-invasive, and logistically feasible and more accessible approaches to carrying out such assessments in field settings. The analysis presented here suggests that capillary blood samples derived from finger-pricks may potentially substitute for venous blood draws when seeking to establish levels of exposure. The correlation and interchangeability are reasonably strong in the small sample of individuals included for this study. Results should, however, be interpreted with caution and further analysis is required to establish criteria for interchangeability between capillary and venous samples and understand its clinical implications in aflatoxin exposure studies. Further research is needed to both replicate and expand on this work to conclusively establish the potential for using fingerprick capillary blood more widely in assessments of human exposure to aflatoxins via contaminated foods.

FUNDING

This publication was made possible through support provided by the Office of Acquisition and Assistance, Bureau for Management, U.S. Agency for International Development, under the terms of a Cooperative Agreement No. 7200AA18CA00044. The opinions expressed herein are those of the authors and do not necessarily reflect the views of the U.S. Agency for International Development. The funding sources did not have any role in study design, the interpretation of the study results, writing of the manuscript, or decision to submit for publication.

ACKNOWLEDGEMENTS

Edgar Agaba for his leadership and supervision of the sample collections. David Fagerlee Medical Centre for managing logistics of the sample collection and participant recruitment.

REFERENCES

1. Wild CP, Gong YY. Mycotoxins and human disease: a largely ignored global health issue. *Carcinogenesis*. 2010;31(1):71-82.
2. IARC. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monogr Eval Carcinog Risks Hum*. 2002;82:1-556.
3. Hell K, Cardwell KF, Setamou M, Poehling HM. The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, west Africa. *Journal of Stored Products Research*. 2000;36(4):365-382.
4. Leong YH, Latiff AA, Ahmad NI, Rosma A. Exposure measurement of aflatoxins and aflatoxin metabolites in human body fluids. A short review. *Mycotoxin Res*. 2012;28(2):79-87.
5. Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *Am J Clin Nutr*. 2004;80(5):1106-1122.
6. Wild CP. Aflatoxin exposure in developing countries: the critical interface of agriculture and health. *Food Nutr Bull*. 2007;28(2 Suppl):S372-380.
7. Strosnider H, Azziz-Baumgartner E, Banziger M, et al. Workgroup report: public health strategies for reducing aflatoxin exposure in developing countries. *Environ Health Perspect*. 2006;114(12):1898-1903.
8. Wild CP, Turner PC. The toxicology of aflatoxins as a basis for public health decisions. *Mutagenesis*. 2002;17(6):471-481.

9. Gong YY, Watson S, Routledge MN. Aflatoxin Exposure and Associated Human Health Effects, a Review of Epidemiological Studies. *Food Saf (Tokyo)*. 2016;4(1):14-27.
10. Groopman JD, Egner PA, Schulze KJ, et al. Aflatoxin exposure during the first 1000 days of life in rural South Asia assessed by aflatoxin B₁ -lysine albumin biomarkers. *Food Chem Toxicol*. 2014;74:184-189.
11. Turner PC, Collinson AC, Cheung YB, et al. Aflatoxin exposure in utero causes growth faltering in Gambian infants. *Int J Epidemiol*. 2007;36(5):1119-1125.
12. Lombard MJ. Mycotoxin exposure and infant and young child growth in Africa: what do we know? *Ann Nutr Metab*. 2014;64 Suppl 2:42-52.
13. Nabwire Wangia-Dixon R, Xue KS, Alcala J, et al. Nutrition and growth outcomes are affected by aflatoxin exposures in Kenyan children. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 2020;37(12):2123-2134.
14. Andrews-Trevino JY, Webb P, Shively G, et al. Aflatoxin exposure and child nutrition: measuring anthropometric and long-bone growth over time in Nepal. *Am J Clin Nutr*. 2021;113(4):874-883.
15. Khlangwiset P, Shephard GS, Wu F. Aflatoxins and growth impairment: a review. *Crit Rev Toxicol*. 2011;41(9):740-755.
16. Wild CP. Environmental exposure measurement in cancer epidemiology. *Mutagenesis*. 2009;24(2):117-125.
17. Groopman JD, Wild CP, Hasler J, Junshi C, Wogan GN, Kensler TW. Molecular epidemiology of aflatoxin exposures: validation of aflatoxin-N7-guanine levels in urine as a biomarker in experimental rat models and humans. *Environ Health Perspect*. 1993;99:107-113.
18. Ross RK, Yuan JM, Yu MC, et al. Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *Lancet*. 1992;339(8799):943-946.
19. Groopman JD, Wogan GN, Roebuck BD, Kensler TW. Molecular Biomarkers for Aflatoxins and Their Application to Human Cancer Prevention. *Cancer Research*. 1994;54(7 Supplement):1907s.
20. Qian GS, Ross RK, Yu MC, et al. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. *Cancer Epidemiol Biomarkers Prev*. 1994;3(1):3-10.
21. Wang JS, Qian GS, Zarba A, et al. Temporal patterns of aflatoxin-albumin adducts in hepatitis B surface antigen-positive and antigen-negative residents of Daxin, Qidong County, People's Republic of China. *Cancer Epidemiol Biomarkers Prev*. 1996;5(4):253-261.
22. Skipper PL, Tannenbaum SR. Protein adducts in the molecular dosimetry of chemical carcinogens. *Carcinogenesis*. 1990;11(4):507-518.
23. Xu Y, Gong YY, Routledge MN. Aflatoxin exposure assessed by aflatoxin albumin adduct biomarker in populations from six African countries: REVIEW ARTICLE. *World Mycotoxin J*. 2018;11(3):411-419.
24. Rasheed H, Xu Y, Kimanya ME, et al. Estimating the health burden of aflatoxin attributable stunting among children in low income countries of Africa. *Sci Rep*. 2021;11(1):1619.
25. Gong YY, Egal S, Hounsa A, et al. Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: the critical role of weaning. *Int J Epidemiol*. 2003;32(4):556-562.

26. Egal S, Hounsa A, Gong YY, et al. Dietary exposure to aflatoxin from maize and groundnut in young children from Benin and Togo, West Africa. *Int J Food Microbiol.* 2005;104(2):215-224.
27. Wangia-Dixon RN, Quach THT, Song X, et al. Determinants of aflatoxin exposures in Kenyan School-aged children. *Int J Environ Health Res.* 2020:1-9.
28. Torres AM, Barros GG, Palacios SA, Chulze SN, Battilani P. Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. *Food Research International.* 2014;62:11-19.
29. Chapot B, Wild C. Techniques in diagnostic pathology. 1991.
30. Wild CP, Jiang Y-Z, Sabbioni G, Chapot B, Montesano R. Evaluation of methods for quantitation of aflatoxin-albumin adducts and their application to human exposure assessment. *Cancer Research.* 1990;50(2):245-251.
31. Yu M, Chen C, Wang L, Santella R. Aflatoxin B1 albumin adduct level and risk of hepatocellular carcinoma. Paper presented at: Proc. Am. Assoc. Cancer Res.1995.
32. Gan LS, Skipper PL, Peng XC, et al. Serum albumin adducts in the molecular epidemiology of aflatoxin carcinogenesis: correlation with aflatoxin B1 intake and urinary excretion of aflatoxin M1. *Carcinogenesis.* 1988;9(7):1323-1325.
33. Sheabar FZ, Groopman JD, Qian GS, Wogan GN. Quantitative analysis of aflatoxin-albumin adducts. *Carcinogenesis.* 1993;14(6):1203-1208.
34. Sabbioni G, Ambs S, Wogan GN, Groopman JD. The aflatoxin-lysine adduct quantified by high-performance liquid chromatography from human serum albumin samples. *Carcinogenesis.* 1990;11(11):2063-2066.
35. Scholl PF, Turner PC, Sutcliffe AE, et al. Quantitative Comparison of Aflatoxin B1 and Serum Albumin Adducts in Humans by Isotope Dilution Mass Spectrometry and ELISA. *Cancer Epidemiology Biomarkers & Prevention.* 2006;15(4):823.
36. Plebani M, Carraro P. Mistakes in a stat laboratory: types and frequency. *Clin Chem.* 1997;43(8 Pt 1):1348-1351.
37. Carraro P, Plebani M. Errors in a stat laboratory: types and frequencies 10 years later. *Clin Chem.* 2007;53(7):1338-1342.
38. Lima-Oliveira G, Guidi GC, Salvagno GL, et al. Is Phlebotomy Part of the Dark Side in the Clinical Laboratory Struggle for Quality? *Laboratory Medicine.* 2012;43(5):172-176.
39. Kimani D, Kamau R, Gadde R, et al. Findings of Phlebotomy Practices in Kenya in 2010: Need for Action. *J Infect Dis.* 2016;213 Suppl 2(Suppl 2):S53-58.
40. Rooney K. Capillary Blood Sampling from the Finger. In: Guest PC, ed. *Investigations of Early Nutrition Effects on Long-Term Health: Methods and Applications.* New York, NY: Springer New York; 2018:267-272.
41. Birkeland S, Bismark M, Barry MJ, Möller S. Is greater patient involvement associated with higher satisfaction? Experimental evidence from a vignette survey. *BMJ Quality & Safety.* 2022;31(2):86.
42. Woods K, Douketis JD, Schnurr T, Kinnon K, Powers P, Crowther MA. Patient preferences for capillary vs. venous INR determination in an anticoagulation clinic: a randomized controlled trial. *Thrombosis Research.* 2004;114(3):161-165.
43. CLSI. Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Sixth Edition. In: Clinical and Laboratory Standards Institute; 2008.

44. Tang R, Yang H, Choi JR, et al. Capillary blood for point-of-care testing. *Crit Rev Clin Lab Sci.* 2017;54(5):294-308.
45. Koehler K, Marks-Nelson E, Braga CP, Beckford S, Adamec J. Validity of plasma collection cards for ferritin assessment-A proof-of-concept study. *Eur J Haematol.* 2020;104(6):554-561.
46. Topping J, Reardon M, Coleman J, et al. A Comparison of Venous versus Capillary Blood Samples when Measuring Blood Glucose Using a Point-of-Care, Capillary-Based Glucometer. *Prehosp Disaster Med.* 2019;34(5):506-509.
47. Cao J, Edwards R, Chairez J, Devaraj S. Validation of capillary blood analysis and capillary testing mode on the epoc Point of Care system. *Pract Lab Med.* 2017;9:24-27.
48. Xue KS, Cai W, Tang L, Wang J-S. Aflatoxin B1-lysine adduct in dried blood spot samples of animals and humans. *Food and Chemical Toxicology.* 2016;98:210-219.
49. Wang JS, Abubaker S, He X, Sun G, Strickland PT, Groopman JD. Development of aflatoxin B(1)-lysine adduct monoclonal antibody for human exposure studies. *Appl Environ Microbiol.* 2001;67(6):2712-2717.
50. Qian G, Tang L, Liu W, Wang J. Development of a non-antibody method for rapid detection of serum aflatoxin B1-lysine adduct. *Toxicologist.* 2010;114:248.
51. Qian G, Tang L, Wang F, et al. Physiologically based toxicokinetics of serum aflatoxin B1-lysine adduct in F344 rats. *Toxicology.* 2013;303:147-151.
52. Hill PG, Wells TNC. Bromocresol Purple and the Measurement of Albumin:Falsely High Plasma Albumin Concentrations Eliminated by Increased Reagent Ionic Strength. *Annals of Clinical Biochemistry.* 1983;20(5):264-270.
53. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976;72:248-254.
54. Sabbioni G, Skipper PL, Büchi G, Tannenbaum SR. Isolation and characterization of the major serum albumin adduct formed by aflatoxin B1 in vivo in rats. *Carcinogenesis.* 1987;8(6):819-824.
55. Lauer JM, Duggan CP, Ausman LM, et al. Maternal aflatoxin exposure during pregnancy and adverse birth outcomes in Uganda. *Matern Child Nutr.* 2019;15(2):e12701.
56. Wang JS, Qian GS, Zarba A, et al. Temporal patterns of aflatoxin-albumin adducts in hepatitis B surface antigen-positive and antigen-negative residents of Daxin, Qidong County, People's Republic of China. *Cancer Epidemiology Biomarkers & Prevention.* 1996;5(4):253.
57. Xue KS, Tang L, Shen CL, et al. Increase in aflatoxin exposure in two populations residing in East and West Texas, United States. *Int J Hyg Environ Health.* 2021;231:113662.
58. Kang MS, Nkurunziza P, Muwanika R, et al. Longitudinal evaluation of aflatoxin exposure in two cohorts in south-western Uganda. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2015;32(8):1322-1330.
59. Shuaib FM, Jolly PE, Ehiri JE, et al. Association between birth outcomes and aflatoxin B1 biomarker blood levels in pregnant women in Kumasi, Ghana. *Trop Med Int Health.* 2010;15(2):160-167.
60. Andrews-Trevino JY, Webb P, Shively G, et al. Relatively Low Maternal Aflatoxin Exposure Is Associated with Small-for-Gestational-Age but Not with Other Birth Outcomes in a Prospective Birth Cohort Study of Nepalese Infants. *J Nutr.* 2019;149(10):1818-1825.

61. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Statistical Methods in Medical Research*. 1999;8(2):135-160.
62. Giavarina D. Understanding Bland Altman analysis. *Biochem Med (Zagreb)*. 2015;25(2):141-151.
63. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;1(8476):307-310.
64. Ho KM. Using linear regression to assess dose-dependent bias on a Bland-Altman plot. *Journal of Emergency and Critical Care Medicine*. 2018;2(8).
65. Vedin T, Karlsson M, Edelhamre M, Bergenheim M, Larsson P-A. Prospective comparison of capillary and venous brain biomarker S100B: capillary samples have large inter-sample variation and poor correlation with venous samples. *International Journal of Emergency Medicine*. 2019;12(1):26.
66. Hollis VS, Holloway JA, Harris S, Spencer D, van Berkel C, Morgan H. Comparison of venous and capillary differential leukocyte counts using a standard hematology analyzer and a novel microfluidic impedance cytometer. *PLoS One*. 2012;7(9):e43702.
67. Nicholas C, George R, Sardesai S, Durand M, Ramanathan R, Cayabyab R. Validation of noninvasive hemoglobin measurement by pulse co-oximeter in newborn infants. *J Perinatol*. 2015;35(8):617-620.
68. Hill PG, Wells TN. Bromocresol purple and the measurement of albumin. Falsely high plasma albumin concentrations eliminated by increased reagent ionic strength. *Ann Clin Biochem*. 1983;20 (Pt 5):264-270.
69. Smith JW, Kroker-Lobos MF, Lazo M, et al. Aflatoxin and viral hepatitis exposures in Guatemala: Molecular biomarkers reveal a unique profile of risk factors in a region of high liver cancer incidence. *PLoS One*. 2017;12(12):e0189255.
70. Nwankwo L, McLaren K, Donovan J, et al. Utilisation of remote capillary blood testing in an outpatient clinic setting to improve shared decision making and patient and clinician experience: a validation and pilot study. *BMJ Open Qual*. 2021;10(3).

Assessing an Aflatoxin Exposure Biomarker: Exploring the Interchangeability and Correlation between Venous and Capillary Blood Samples

Balaji Srinivasan^{a,b}, Shibani Ghosh^c, Patrick Webb^c, Stacy P. Griswold^c, Kathy S. Xue^d, Jia-Sheng Wang^d, and Saurabh Mehta^{a,b}

Affiliations:

^aDivision of Nutritional Sciences, Cornell University, Ithaca, NY 14853, USA

^bInstitute for Nutritional Sciences, Global Health, and Technology (INSiGHT)

^cFriedman School of Nutrition Science and Policy, Tufts University, Boston, MA, USA

^dDepartment of Environmental Health Science, University of Georgia, Athens, GA, USA

Corresponding Author: Saurabh Mehta, Division of Nutritional Sciences, Cornell University, Ithaca, New York, 14853, smehta@cornell.edu, 607-255-2640.

Keywords: aflatoxin; mycotoxins; venous; capillary; interchangeability

Clinicaltrials.gov registration: NCT04209569

ABSTRACT

Exposure to dietary aflatoxins has been recognized as a potential threat to child nutrition and growth, in addition to being a known carcinogen. The ability to accurately assess concentration of aflatoxin in the blood of at-risk individuals is therefore very important to inform public health policies and on-the-ground programs around the world. Venous blood is frequently used to quantify biomarkers of exposure such as AFB1-lysine adducts. However, venous blood collection methods are invasive, requiring highly trained staff, which makes this method challenging to implement, especially in resource-limited settings. In contrast, capillary blood collection by fingerprick is less invasive and has the potential for application in point-of-need monitoring. The aim of this exploratory study was to investigate the correlation and interchangeability of capillary and venous human blood samples in the quantification of AFB1-lysine adduct concentration. A total of 72 venous and capillary blood samples were collected from 36 women of reproductive age (16-49 years) in northern Uganda. All sample specimens were analyzed using high-performance liquid chromatography with fluorescence detection. Regression analysis and Bland–Altman analysis were performed to compare AFB1-lysine concentrations between venous and capillary sample pairs. Bland-Altman analysis of albumin-normalized AFB1-lysine data- bias was -0.023 pg/mg-albumin and the 95% limits of agreement were 0.51 to -0.56 pg/mg-albumin for log-transformed data. There was a positive correlation between albumin-normalized venous and capillary AFB1-lysine concentrations with r of 0.71 ($p < .0001$). A lack of any accepted clinical cutoff for aflatoxin exposure makes definition of an ‘acceptable’ limit for statistical analysis and comparison of methods challenging. Our data suggests a positive correlation between albumin-normalized AFB1-lysine concentrations in venous and capillary sample pairs, but relatively weak agreement and interchangeability based on Bland-Altman analysis. Further exploration of this and other methods is needed.

1. INTRODUCTION

Aflatoxins are secondary metabolites produced by fungi of the *Aspergillus* species (mainly *A. flavus* and *A. parasiticus*) that have been established as significant environmental contaminants¹. Aflatoxins often contaminate crops such as peanuts and corn, particularly in low- and middle-income countries (LMIC)^{2,3} due to lack of proper food storage conditions that lead to fungal growth and contamination. Human exposure to aflatoxins can occur due to ingestion of contaminated foods, or indirectly from consumption of foods from animals previously exposed to aflatoxins in animal feeds⁴. It has been estimated⁵ that around 4.5 billion of the world's population is exposed to aflatoxins, which has been associated with an increased incidence of primary hepatocellular carcinoma, as well as carrying the risk of toxicity, poor birth outcomes and impaired child growth and immune functions⁵⁻⁹. Among the four types of aflatoxins commonly present in food samples, namely aflatoxins B1, B2, G1, and G2, aflatoxin B1 (AFB1) has been reported to be the most potent hepatocarcinogen of the four in experimental studies and is present in the highest concentrations in human foods². AFB1 is the most toxic of the aflatoxins, and, since 1993, has been classified as a known human carcinogen by the International Agency for Research on Cancer (IARC). A few studies have also reported maternal aflatoxin exposure during pregnancy to be associated with low weight and length at birth, as well as continued poor growth during infancy and early childhood¹⁰⁻¹⁵.

Biomarkers of aflatoxin exposure

Exposure assessment is a key component of epidemiological studies for assessing the effect of aflatoxin on human health and determining the extent of local public health risk. Biomarkers of

exposure may include the excreted toxin or its metabolites and the products of interaction between the toxin and various macromolecules such as protein- and DNA-adducts detectable in human blood, urine or tissue samples¹⁶. Currently used biomarkers of aflatoxin exposure include metabolites of aflatoxins such as aflatoxin M1 (AFM1) and AFB1-N⁷-guanine in urine^{17,18}, and AFB1-lysine adducts in blood¹⁹⁻²¹. AFB1-lysine adduct in blood is considered to reflect integrated exposures over longer time periods (2-3 months) based on longer in vivo half-life of albumin in humans when compared to urinary metabolites^{19,22} which reflects recent exposure with excretion occurring over 24–48 hours. In epidemiological studies²³⁻²⁷, quantification of AFB1-lysine adduct concentration has been shown to be useful for screening large populations for aflatoxin exposure. Figure 1 lays out in graphic form some of the main sources of aflatoxin, various modes of exposure, metabolites in human samples, and adverse effects²⁸. The major analytical techniques currently applied for measuring AFB1-lysine adduct concentration in human blood include enzyme-linked immunosorbent assay (ELISA)²⁹⁻³¹, radioimmunoassay (RIA)^{21,32,33}, and immunoaffinity chromatography followed by high-performance liquid chromatography (HPLC) with fluorescence detection^{21,30,34} and Isotope-LC-MS/MS³⁵

Venous vs. capillary blood samples

Venipuncture is a common procedure performed in hospital settings for blood collection; most laboratory reference ranges for blood analytes are based on venous blood. Studies on aflatoxin exposure have typically used venous blood for the quantification of AFB1-lysine adducts. Conventional venous blood collection methods are invasive, could potentially cause pain and needle stick injuries with a risk of contamination if not performed by highly trained and certified personnel, which makes it challenging to apply these methods, particularly in resource-limited

settings. Moreover, it has been reported that two-thirds of errors that affect laboratory test results occur in the pre-analytical phase (period before assay performance)^{36,37} and phlebotomy-related errors are regarded to account for greater than 60% of errors in this phase^{38,39}. Capillary blood sampling⁴⁰ is less invasive, does not require trained phlebotomists, and may also lead to higher acceptance of blood testing in settings where cultural traditions might interfere with patient involvement in collection of blood for analysis. Studies on greater patient involvement⁴¹ indicate

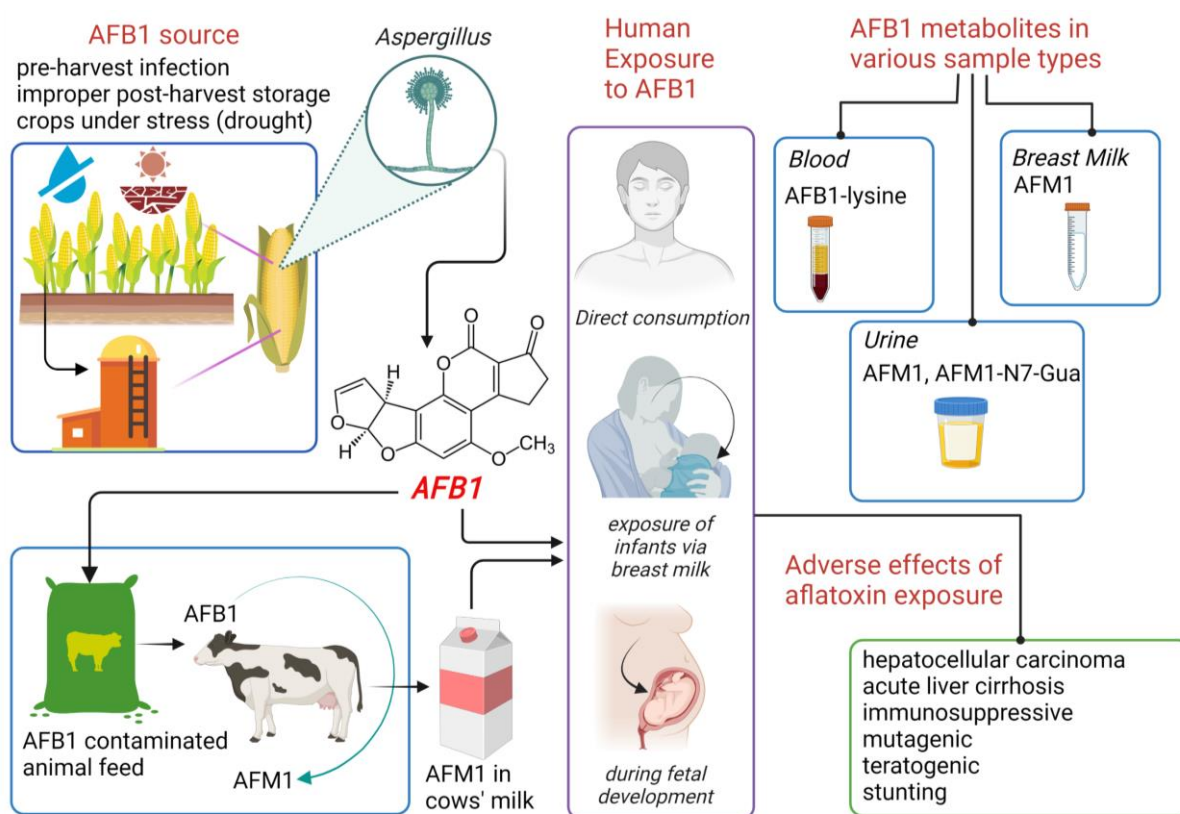


Figure 1: Schematic summarizing the source of aflatoxins, exposure modes, various metabolites of AFB1, and a list of major adverse effects of aflatoxin exposure.

improved health outcomes and treatment adherence while reducing long-term healthcare cost. Studies have also shown that patients prefer capillary blood sampling over venous sampling when frequent monitoring is required because it was less painful⁴². Moreover, capillary blood collection

is also suitable for newborns and infants as the lower blood volume with this method reduces the risk of anemia with frequent sampling⁴³.

Recent advances in point-of-care testing (POCT) technologies including the development of microfluidic chips and paper-based diagnostic technology have enabled simple and accurate capillary blood-based diagnostics at the point of care and need. According to current regulations, outside of a clinical laboratory and absent trained medical staff, the capillary is the only approved blood source for point-of-care diagnosis in field settings. However, the small sample volume (~10–250 μ L) used in capillary blood-based testing may affect the detection accuracy of a biomarker compared to laboratory-based testing with arterial and venous blood testing with larger sample volume (approximately 175 μ L - 5 mL)⁴⁴.

In adult populations, few studies comparing concentration of biomarkers in venous and capillary samples have been conducted, and fewer in acutely unwell adult populations where frequent blood tests are required for close monitoring. A cross-sectional comparison of ferritin concentration between capillary and venous samples⁴⁵ in a convenience sample of adults ($n = 20$) showed slightly elevated ferritin concentrations (mean bias of 9.9 ng/mL) in capillary samples. In healthy, non-fasted people in a non-clinical setting, a statistically significant, but clinically insignificant difference was found with moderate correlation between venous- and capillary-derived blood glucose when measured using a point-of-care, capillary-based glucometer⁴⁶. A study of capillary blood tests using the epocTM Point of Care Blood Analysis System (Alere) for analytes such as Na⁺, K⁺, glucose, lactate, creatinine, hematocrit, hemoglobin, pH and pCO₂, determined⁴⁷ that results of these assays were comparable to the reference method. The study⁴⁷ on the epocTM system

was, however, limited to paired capillary and venous blood correlation data from healthy people with the results not covering the entire analytical measurement range and therefore may not be generalizable to patients in critical conditions.

In many aflatoxin studies that quantify biomarkers of aflatoxin exposure in serum, collection of venous blood with cold chain transportation to a remote lab for testing is a commonly adopted approach. A recent study⁴⁸ has suggested dried blood spots (DBS) from capillary blood as a low-cost, viable alternative to venous blood draw for assessing AFB1 exposure with a good correlation and agreement to AFB1-lysine adduct concentration in serum samples as quantified by HPLC method with fluorescence detection. However, to our knowledge, there are no studies comparing biomarkers of AFB1 exposure in paired capillary and venous liquid blood samples.

The primary aim of this exploratory study was to investigate the correlation of AFB1-lysine adduct concentrations in paired capillary and venous blood samples from a study population consisting of 36 women of reproductive age in Agago District of northern Uganda. Both venous and capillary blood samples were collected simultaneously from each participant and serum AFB1-lysine adduct concentrations were quantified using an HPLC method described elsewhere^{48,49}.

2. METHODS

Study population

The study population consisted of women aged 16-49 years, residing in a sub-county in Agago District of northern Uganda and were not pregnant. Women were selected on the day of data collection in a convenience sample from a designated health center. The study was approved by the Tufts Health Sciences Institutional Review Board in Boston, Massachusetts, and the Uganda

National Council for Science and Technology in Kampala, Uganda. Written consent was obtained from all participants as was basic anthropometric, demographic, and health information prior to sample collection.

Sample collection

Study participants were sampled once during the study. Both venous blood and capillary blood were collected by the phlebotomist from the same arm of each participant while following standard laboratory procedures. Figure 2 shows a schematic outlining the sample collection approach. Matching venipuncture blood sample and fingerstick sample from each participant were labeled with a laboratory identification number format to enable easy identification of sample pairs. All blood specimens were subjected to the same handling and storage conditions. Blood samples were centrifuged at 4000 rpm for 5 min to separate serum and stored at -80°C. The total sample size was 36 women providing 36 venous and 36 capillary paired samples in total.

Quantification of AFB1-lysine adduct concentration

Serum samples were transported on dry ice to the Wang Laboratory at the University of Georgia, Athens, USA. The quantification of AFB1-lysine adduct concentration was performed using a high-performance liquid chromatography (HPLC)-fluorescence method (Agilent 1200, Santa

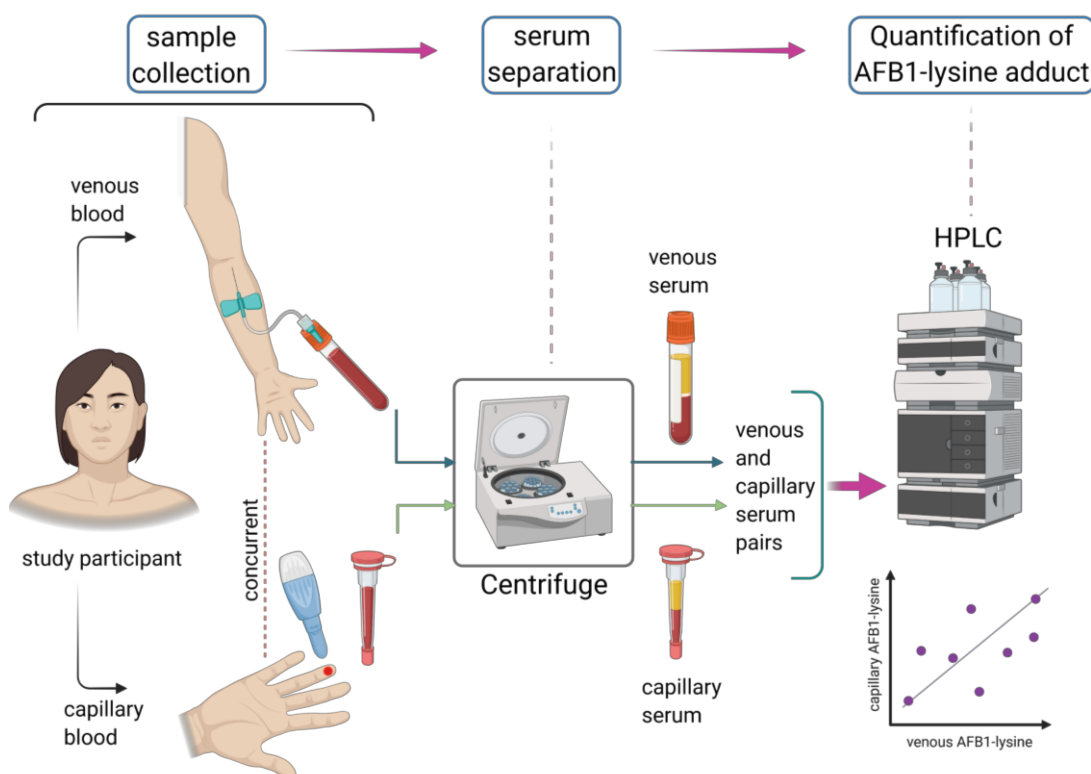


Figure 2: Sample collection for comparison of AFB1-lysine adduct in paired venous and capillary blood samples from each participant.

Clara, CA, USA). For each serum sample, the albumin concentration was quantified followed by pronase digestion to convert the AFB1-albumin adduct to a mono-AFB1-lysine adduct, concentration and purification of the AFB1-lysine adduct, and finally separation and quantification by HPLC⁵⁰ with fluorescence detection^{48,51}. The protocol for serum processing and quantification of AFB-lysine adducts has been reported previously⁵⁰. Albumin concentrations for each serum sample was experimentally quantified as previously described²¹. Briefly, concentration of human

serum albumin was determined in each sample by a bromocresol purple dye binding method⁵², which measures a stable blue-purple color complex formed between the dye and albumin with an absorption maximum at 600 nm. Standard curves for this assay were determined using human albumin. In addition, the amount of total protein was determined by Bradford-dye binding method⁵³ using a protein assay kit (Bio-Rad, Hercules, CA) calibrated with serum protein standard. This is essential for calculating the optimal volume of enzyme to be used for protein digestion. Pronase digestion steps included 150 μ L aliquots of each serum sample digested by pronase (pronase: total protein, 1:4, w: w) at 37 °C for 3 h. Under enzyme digestion, AFB1-lysine is released from the adducted albumin⁵⁴ form. The digests were then purified using Oasis MAX SPE cartridges (Waters Corporation, Milford, MA). After priming with methanol and equilibration with water, the loaded cartridge was sequentially washed by water, 70% methanol, and 1% ammonium hydroxide in methanol at a flow rate of 1 mL/min. The eluate in 2% formic acid in methanol was vacuum-dried with a CentriVapTM vacuum concentrator (Labconco Corporation, Kansas City, MO) and reconstituted with an average recovery rate of 90%.

HPLC conditions for AFB1-lysine analysis

Reproducibility of HPLC method applied in this study has previously been validated and reported^{13,14,48,55-60} in other published studies. Brief description of the HPLC conditions for AFB1-lysine is provided here- Quantification of AFB-lysine was performed on an Agilent 1200 HPLC-fluorescence system (Santa Clara, CA). The mobile phases consisted of buffer A (20 mM ammonium dihydrogen phosphate, pH 7.2) and buffer B (LC/MS grade Methanol). A ZORBAX Eclipse XDB-C18 (Agilent Technologies, Santa Clara, CA) reverse phase column (5 μ m, 4.6 $\times$ 250 mm) was used and 100 μ L was injected at a flow rate of 1 mL/min. A gradient was generated

to separate the AFB-lysine adduct in less than 25 min and the retention time of AFB-lysine was equal to approximately 12.3 min. AFB-lysine adduct was detected by fluorescence at the excitation and emission wavelengths of 405 nm and 470 nm, respectively. Quality assurance and quality control procedures were implemented during analyses, which included simultaneous analysis of one reference standard and a quality control sample daily. The limit of detection with this approach was 0.4 pg/mg albumin. The averaged coefficient of variation (CV) for serum samples analyses from over 10 different studies conducted in past 15 years ranged from 2.39 - 7.78%. Typical HPLC chromatograms of AFB1-lysine standard, as well as capillary (CC) and venous (VV) samples with near non-detectable, low

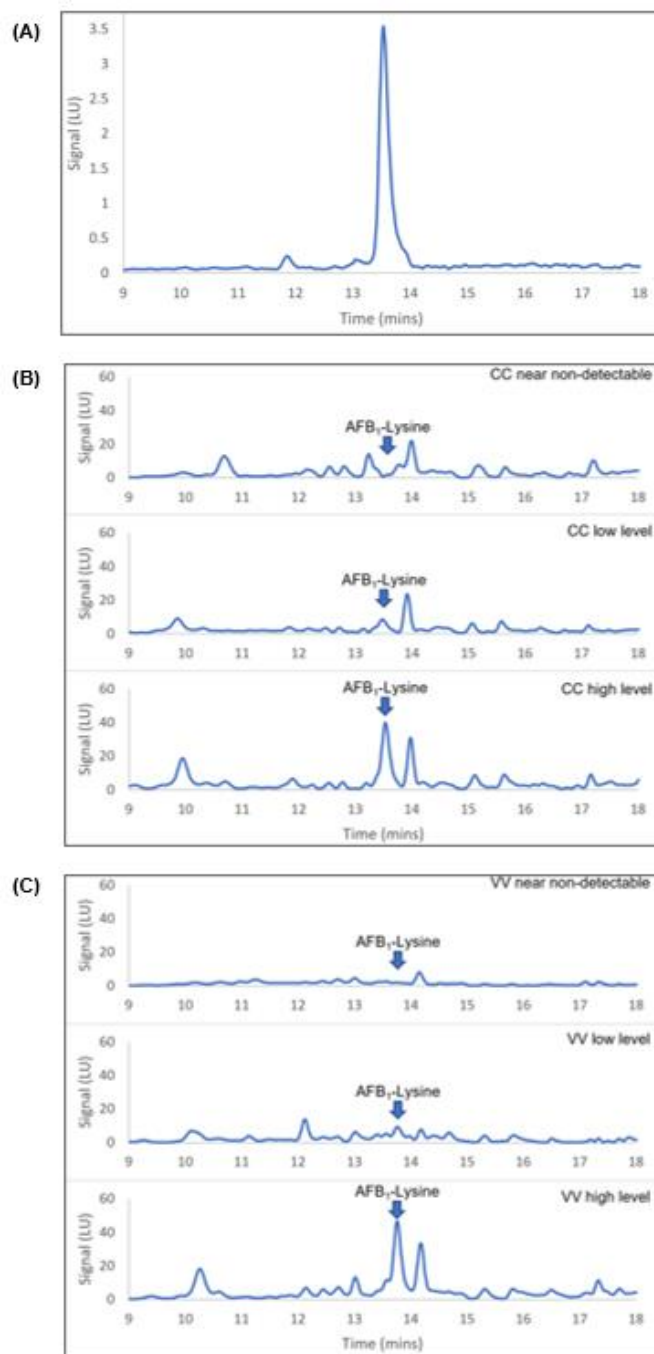


Figure 3. (A) Representative chromatograms of HPLC-fluorescence detection for AFB1-lysine in various samples. (A) AFB1-lysine standard; (B) near non-detectable, low, and high level AFB1-lysine detected in a capillary (CC) sample; (C) near non-detectable, low, and high level detected in venous (VV) sample

and high adduct levels are shown in Fig. 3. The quality control procedures included: generation of a new calibration curves were linear for AFB1-Lysine concentrations ranging from 0.01-5.0 ng/ml; pretest sample analysis procedure with AFB1-treated rat serum and normal human serum spiked with synthesized authentic AFB1-lysine with low and high concentrations; measurement of imprecisions based on 6 measurements on 3 separate days (the rates were 2.87-5.93%); measurement of inaccuracies and reproducibility for 6 repeated measurements (rates were 4.44-4.79%), measurement of recoveries based on low, middle, and high spiked AFB1-lysine adduct concentrations (rates ranged 82-95%), every 5 samples run included a separated authentic standard-spiked normal human serum samples which was under the same process. The technical person running analyses was blinded for the sample source and identifications.

Statistical analysis

All statistical analyses were performed using OriginPro 2022 (64-bit) SR1 (OriginLab Corporation, Northampton, Massachusetts). Only paired venous and capillary samples were only included in statistical analyses. Linear regression analysis with ordinary least squared estimates was performed, and Pearson's correlation coefficient (r) was used to assess correlation between capillary and venous AFB1-lysine adduct concentrations. Bland-Altman analyses⁶¹⁻⁶³ was used to analyze the bias between venous and capillary AFB1-lysine concentrations. The original data for capillary and venous AFB1-lysine concentrations was log-transformed and differences of the transformed data were confirmed to be normally distributed by Shapiro-Wilk normality test. Using Bland-Altman plots, the difference between the capillary and venous AFB1-lysine concentrations (y-axis) were plotted against the mean of the capillary and venous AFB1-lysine concentrations (x-axis). Horizontal lines were drawn at the mean difference and at the limits of agreement which

were defined as the mean difference ± 1.96 times the standard deviation (SD) of the differences. For all analyses, $p < 0.05$ was considered statistically significant.

3. RESULTS

Currently, AFB1-lysine adduct concentrations are reported normalized to total serum albumin (pg adduct/mg albumin), which requires separate assays for quantification of serum albumin in test samples. In this study, we compared AFB1-lysine adduct concentrations normalized to serum albumin as well as to total serum volume in both capillary and venous samples (paired samples only) to determine agreement.

Albumin concentration

Albumin concentration was quantified in each paired sample by bromocresol purple dye binding method. Figure 3(A) shows the distribution of albumin concentrations in capillary and venous samples. Figure 3(B) shows a scatterplot and the linear regression fit for the set of 36 paired capillary and venous samples, with venous albumin concentration on y-axis and capillary albumin concentration on the x-axis. Results from regression analysis indicated that there

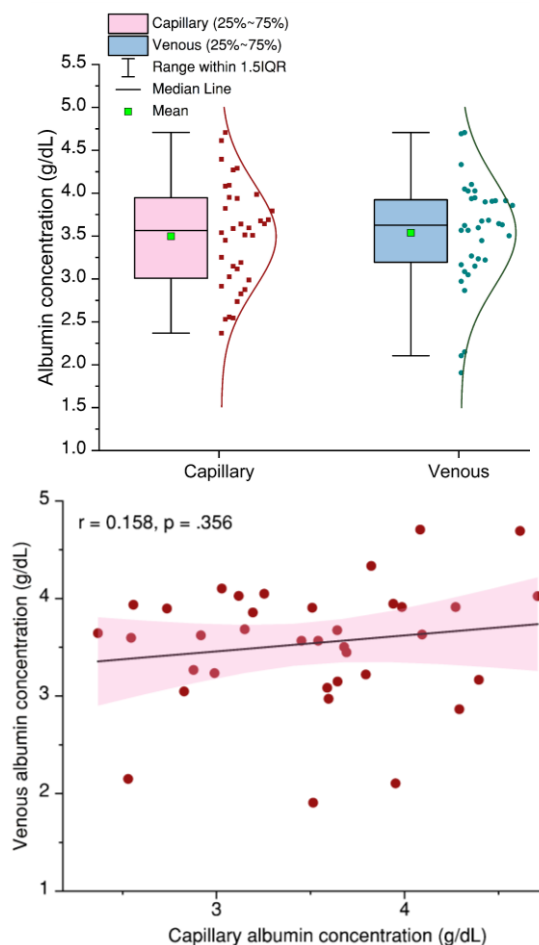


Figure 3. (A) Box plot showing a summary of capillary and venous albumin concentration data including the mean, 50th percentile median line. (B) Linear regression analysis for correlation between capillary and venous AFB1-lysine adducts per mg albumin concentrations

was no positive correlation between venous and capillary albumin concentrations ($r = .158$, $p = .356$). The mean albumin concentration for capillary samples is 3.50 g/dL with the range of 2.37-4.71 g/dL; the mean albumin concentration for venous samples is 3.54 g/dL with the range of 2.15-4.71 g/dL. There were no significant differences in total digest.

Albumin-normalized AFB1-lysine concentration

The distribution of albumin-normalized AFB1-lysine concentrations among the capillary and venous samples was observed to be uniformly distributed as shown in Figure 5. Mean AFB1-lysine was 39.08 pg/mg-albumin (SD = 41.17, 95% CI = 26.61, 27.72). Mean albumin concentration was 3.52 g/dL (SD = 0.62, 95% CI = 3.18, 3.74). Figure 6(A) shows a scatterplot and the linear regression fit for the set of 36 paired capillary and venous samples, with albumin-normalized venous AFB1-lysine adduct

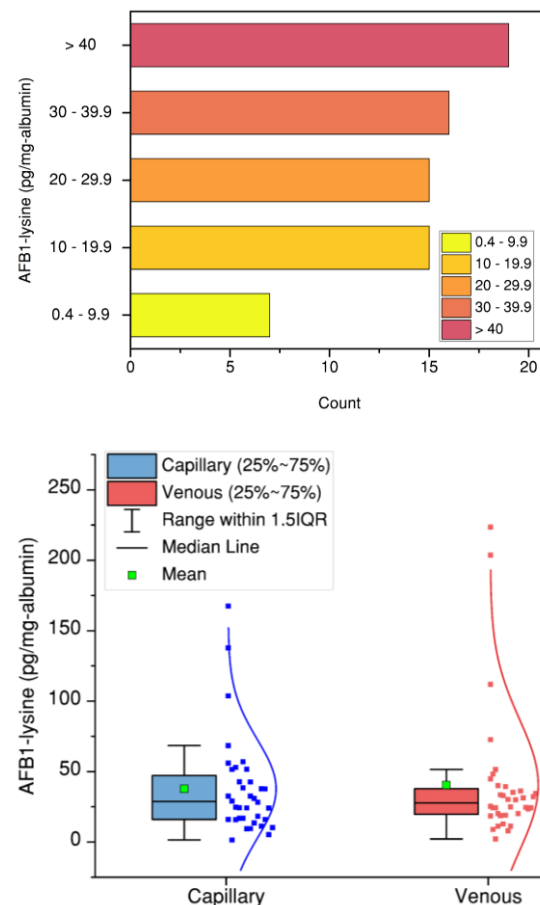


Figure 5. (A) Frequency distribution of albumin normalized AFB1-lysine adduct concentrations in the overall capillary and venous samples (B) Box plot showing a summary of capillary and venous data including the mean, 50th percentile median line.

concentration on y-axis and albumin-normalized capillary AFB1-lysine adduct concentration on the x-axis. The fitted regression model was $[vAFB1-lys]_{ALB} = 0.9442 * [cAFB1-lys]_{ALB} + 4.8043$, where $[vAFB1-lys]_{ALB}$ and $[cAFB1-lys]_{ALB}$ represent albumin-normalized venous and capillary AFB1-lysine concentrations, respectively. There was a positive association between albumin-normalized venous and capillary AFB1-lysine concentrations with $r = .71$, $p < .0001$.

To determine interchangeability, a Bland-Altman analysis was performed, where the mean of log transformed albumin-normalized AFB1-lysine concentrations of each venous and capillary pair from participants ($n=36$) is plotted against its difference. A Bland-Altman plot for log

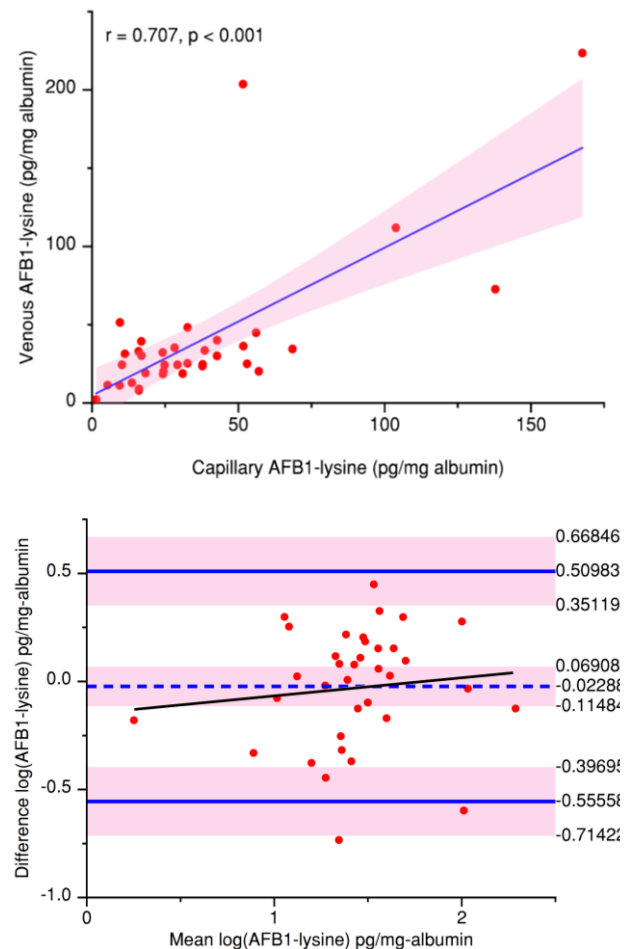


Figure 6(A) Linear regression analysis for correlation between capillary and venous AFB1-lysine adducts per mg albumin concentrations (B) Bland-Altman plot comparing log transformed capillary and venous AFB1-lysine concentration per mg-albumin concentrations. Dashed blue line shows bias (mean difference) in between venous and capillary, solid blue lines show the 95% limits of agreement (LOA) and shaded areas represent 95% confidence interval limits for mean and LOA. Solid black line shows linear regression fit for the data used in Bland-Altman plot.

transformed data is shown in Figure 6(B), with the dashed blue line showing bias (mean difference) between venous and capillary, solid blue lines showing the 95% limits of agreement (LOA) and shaded areas representing 95% confidence interval limits for mean and LOA. The bias was -0.023

pg/mg-albumin and the 95% LOA were 0.51 to -0.56 pg/mg-albumin for log-transformed data. Using the difference in AFB1-lysine adduct concentrations as the dependent outcome variable and the mean AFB1-lysine adduct concentration between venous and capillary sample types as an independent predictor in a linear regression, a regression fit (solid black line) with a slope of 0.086 ($r = .11$, $p = .514$) was obtained for the log-transformed data in the Bland-Altman plot in Figure 6 (B). At the 0.05 level, the slope of the regression line in Figure 6(B) is not statistically different from zero and therefore the bias between albumin-normalized AFB1-lysine in capillary and venous samples does not change with the magnitude of the measurements itself⁶⁴.

AFB1-lysine concentration normalized by total serum volume

AFB1-lysine adduct concentrations normalized by total serum volume in paired capillary and venous samples were used in an analysis similar to that to assess the albumin-normalized AFB1-lysine data. Figure 7(A) shows a scatterplot and the linear regression fit for the 36 paired capillary and venous samples, with volume-normalized venous AFB1-lysine adduct concentration (pg AFB1-lysine/ μ L) on the y-axis and volume-normalized capillary AFB1-lysine adduct concentration (pg AFB1-lysine/ μ L) on the x-axis. The fitted regression model was $[vAFB1-lys]_{VOL} = 0.8256 * [cAFB1-lys]_{VOL} + 0.2294$, where $[vAFB1-lys]_{VOL}$ and $[cAFB1-lys]_{VOL}$ represent venous and capillary AFB1-lysine concentrations normalized to serum volume, respectively. There was a positive association between volume-normalized venous and capillary AFB1-lysine concentrations with $r = .80$, $p < .0001$. A Bland-Altman plot for log transformed data is shown in Figure 7(B), with dashed red line shows bias (mean difference) between venous and capillary, solid red lines show the 95% limits of agreement (LOA) and shaded areas represent

95% confidence interval limits for mean and LOA. For the log-transformed data, the bias was -0.027 pg/ μ L and the 95% LOA were 0.48 to -0.53 pg/ μ L. Using the difference in AFB1-lysine adduct concentrations as the dependent outcome variable and the mean AFB1-lysine adduct concentration between venous and capillary sample types as an independent predictor in a linear regression, a regression line (solid black line) with a slope of 0.175 ($r = .23$, $p = .184$) was obtained for the log-transformed data in the Bland-Altman plot in Figure 7 (B). At the 0.05 level, the slope of the regression line in Figure 7(B) is not statistically different from zero and therefore the bias between albumin-normalized AFB1-lysine in capillary and venous samples does not change with the magnitude of the measurements itself⁶⁴

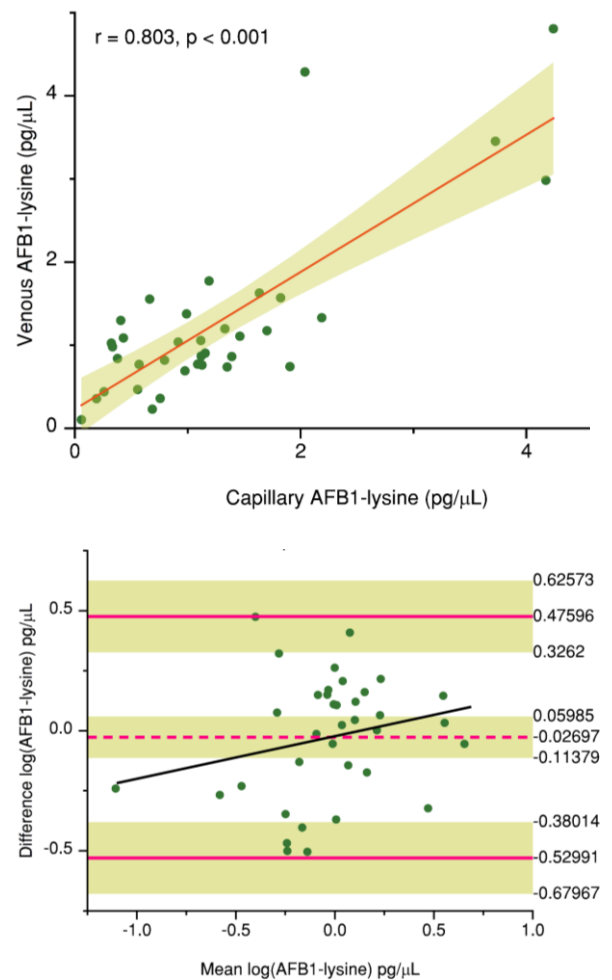


Figure 7(A) Linear regression analysis for correlation between capillary and venous AFB1-lysine adducts per μ L (B) Bland-Altman plot comparing log-transformed capillary and venous AFB1-lysine adducts per μ L. Dashed red line shows bias (mean difference) between venous and capillary, solid red lines show the 95% limits of agreement (LOA) and shaded areas represent 95% confidence interval limits for mean and LOA. Solid black line shows linear regression fit for the data used in Bland-Altman plot.

4. DISCUSSION

The goal of this exploratory study was to evaluate whether capillary blood can be applied as a substitute to venous blood for quantification of AFB1-lysine concentration, a widely accepted

biomarker in population-based studies on dietary exposure to aflatoxin. We analyzed the data for both albumin-normalized and total serum volume-normalized AFB1-lysine adduct concentrations for paired capillary and venous blood samples. With respect to the albumin-normalized AFB1-lysine concentrations - a positive association ($r = 0.71$) was observed, and Bland-Altman analysis indicated the bias was -0.023 pg/mg-albumin and the 95% LOA ranged from 0.51 to -0.56 pg/mg-albumin for the log-transformed adduct data. With serum volume-normalized AFB1-lysine concentrations – a positive association ($r = 0.81$) was observed, and Bland-Altman analysis indicated the bias was -0.027 pg/ μ L and the 95% LOA ranged from 0.48 to -0.53 pg/ μ L.

Studies comparing capillary and venous blood samples have been reported for various clinical biomarkers such as brain biomarker S100B⁶⁵, mean differential leukocyte counts⁶⁶, ferritin⁴⁵, hemoglobin⁶⁷, and glucose⁴⁶. For AFB1-lysine, a study on AFB1-lysine adducts in dried blood spots⁴⁸ from capillary blood has been compared to paired venous blood samples. To the best of our knowledge, concordance of AFB1-lysine adduct concentrations in human capillary and venous serum samples has not been reported. A high degree of agreement between capillary and venous AFB1-lysine concentrations would support the development of point-of-care testing technologies for aflatoxin exposure based on fingerprick capillary blood in population-based studies, especially useful in resource-limited settings.

Lack of clinical cut-off for serum Aflatoxin B1-lysine

Numerous studies have studied dose–response relationships between dietary intake of AFB1-contaminated food and AFB1 metabolites in blood, urine, and breast milk by applying various analytical techniques. For most clinical biomarkers with known cutoffs, it is possible to define a

priori the limits of maximum acceptable differences in biomarker concentrations between two approaches or sample types based on biological considerations and clinical implications of the difference. However, at present there is no established clinical cutoff for aflatoxin exposure level in humans at which adverse effects of aflatoxin are most likely to occur. This is further complicated by a very wide range of goals of the population-based studies in different types of study populations. For examples, acceptable limits of agreement for studying the effect of aflatoxin exposure on stunting in newborns may be different from a study on carcinogenic effects of aflatoxin exposure in an adult population. This lack of data makes definition of an acceptable limit for statistical analysis and comparison of methods challenging. In this study, based on Bland-Altman analysis with percentage differences of the capillary and venous pairs to compare albumin-normalized AFB1-lysine adduct concentrations, it was observed that ~ 53% of the sample pairs (N = 36) had greater than 10% difference in concentrations and ~33% of the sample pairs (N = 36) had greater than 20% difference in concentrations. Whether any such differences between AFB1-lysine concentrations in capillary and venous samples could have clinically significant impact on subsequent decision making is not clear.

Effect of serum albumin concentration on albumin-normalized AFB1-lysine results

Often, the data reported by various studies of aflatoxin exposure is in the form of serum AFB1-lysine normalized to albumin concentration (pg/mg-albumin). Serum albumin has been quantified by analytical techniques such as bromocresol purple dye method^{49,68} and ELISA⁶⁹. Data on comparison of serum albumin in paired capillary and venous sample is not available. Total protein concentration in capillary samples have been reported to be significantly lower (3.3% difference, $p < .0005$) than venous samples. A study on comparison of albumin concentration in paired

capillary and venous serum⁷⁰ samples (N = 59) concluded there was no interchangeability based on correlation ($r = 0.75$) and Bland-Altman results with mean % bias of $-0.22 \pm 6.2\%$ and 95% LOA in percentage from -12.27% to 11.84% . In our study, linear regression analysis of paired capillary and venous samples (N = 36) indicated no positive correlation between capillary and venous albumin concentrations. However, this result is limited by a small sample size and a more detailed study with large sample size would be required to confirm interchangeability of capillary and venous samples for albumin concentrations in clinical settings. This potential difference in albumin concentration between capillary and venous serums samples can in turn affect the AFB1-lysine concentrations when albumin results are applied to normalize to report the results in albumin-normalized AFB1-lysine format.

Study Limitations

Although the results obtained in this work gave valuable insight into the possibility of capillary blood sample as a substitute for venous blood for quantification of AFB1-lysine in aflatoxin exposure studies, several limitations must be taken into consideration. The primary limitations of our study include its relatively small sample size of 36 participants. However, given the high prevalence of aflatoxin exposure in this population, the wider range of AFB1-lysine concentrations allowed us to obtain some preliminary results. Future work to increase the sample size and to include different populations with different dietary aflatoxin exposure levels to validate the capillary procedure are necessary. Only one capillary sample was obtained from each participant, and the limited volume of capillary sample was not sufficient to perform a repeatability analysis of the AFB1-lysine concentration quantified from the capillary samples.

5. CONCLUSIONS

The public health and nutrition communities of low- and middle-income countries across the Tropics have increasingly recognized that aflatoxins and other related mycotoxins pose a genuine threat to human health whether via chronic diseases such as cancer or through maternal wellbeing leading to adverse birth outcomes and impaired child growth. As a result, there is growing demand for more detailed assessments of dietary exposure risks even where aflatoxin concentrations in food may be low. Hence the importance of identifying less costly, non-invasive, and logistically feasible and more accessible approaches to carrying out such assessments in field settings. The analysis presented here suggests that capillary blood samples derived from finger-pricks may potentially substitute for venous blood draws when seeking to establish levels of exposure. The correlation and interchangeability are reasonably strong in the small sample of individuals included for this study. Results should, however, be interpreted with caution and further analysis is required to establish criteria for interchangeability between capillary and venous samples and understand its clinical implications in aflatoxin exposure studies. Further research is needed to both replicate and expand on this work to conclusively establish the potential for using fingerprick capillary blood more widely in assessments of human exposure to aflatoxins via contaminated foods.

FUNDING

This publication was made possible through support provided by the Office of Acquisition and Assistance, Bureau for Management, U.S. Agency for International Development, under the terms of a Cooperative Agreement No. 7200AA18CA00044. The opinions expressed herein are those of the authors and do not necessarily reflect the views of the U.S. Agency for International

Development. The funding sources did not have any role in study design, the interpretation of the study results, writing of the manuscript, or decision to submit for publication.

ACKNOWLEDGEMENTS

Edgar Agaba for his leadership and supervision of the sample collections. David Fagerlee Medical Centre for managing logistics of the sample collection and participant recruitment.

REFERENCES

1. Wild CP, Gong YY. Mycotoxins and human disease: a largely ignored global health issue. *Carcinogenesis*. 2010;31(1):71-82.
2. IARC. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monogr Eval Carcinog Risks Hum*. 2002;82:1-556.
3. Hell K, Cardwell KF, Setamou M, Poehling HM. The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, west Africa. *Journal of Stored Products Research*. 2000;36(4):365-382.
4. Leong YH, Latiff AA, Ahmad NI, Rosma A. Exposure measurement of aflatoxins and aflatoxin metabolites in human body fluids. A short review. *Mycotoxin Res*. 2012;28(2):79-87.
5. Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *Am J Clin Nutr*. 2004;80(5):1106-1122.
6. Wild CP. Aflatoxin exposure in developing countries: the critical interface of agriculture and health. *Food Nutr Bull*. 2007;28(2 Suppl):S372-380.
7. Strosnider H, Azziz-Baumgartner E, Banziger M, et al. Workgroup report: public health strategies for reducing aflatoxin exposure in developing countries. *Environ Health Perspect*. 2006;114(12):1898-1903.
8. Wild CP, Turner PC. The toxicology of aflatoxins as a basis for public health decisions. *Mutagenesis*. 2002;17(6):471-481.
9. Gong YY, Watson S, Routledge MN. Aflatoxin Exposure and Associated Human Health Effects, a Review of Epidemiological Studies. *Food Saf (Tokyo)*. 2016;4(1):14-27.
10. Groopman JD, Egner PA, Schulze KJ, et al. Aflatoxin exposure during the first 1000 days of life in rural South Asia assessed by aflatoxin B₁ -lysine albumin biomarkers. *Food Chem Toxicol*. 2014;74:184-189.
11. Turner PC, Collinson AC, Cheung YB, et al. Aflatoxin exposure in utero causes growth faltering in Gambian infants. *Int J Epidemiol*. 2007;36(5):1119-1125.
12. Lombard MJ. Mycotoxin exposure and infant and young child growth in Africa: what do we know? *Ann Nutr Metab*. 2014;64 Suppl 2:42-52.

13. Nabwire Wangia-Dixon R, Xue KS, Alcala J, et al. Nutrition and growth outcomes are affected by aflatoxin exposures in Kenyan children. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 2020;37(12):2123-2134.
14. Andrews-Trevino JY, Webb P, Shively G, et al. Aflatoxin exposure and child nutrition: measuring anthropometric and long-bone growth over time in Nepal. *Am J Clin Nutr*. 2021;113(4):874-883.
15. Khlangwiset P, Shephard GS, Wu F. Aflatoxins and growth impairment: a review. *Crit Rev Toxicol*. 2011;41(9):740-755.
16. Wild CP. Environmental exposure measurement in cancer epidemiology. *Mutagenesis*. 2009;24(2):117-125.
17. Groopman JD, Wild CP, Hasler J, Junshi C, Wogan GN, Kensler TW. Molecular epidemiology of aflatoxin exposures: validation of aflatoxin-N7-guanine levels in urine as a biomarker in experimental rat models and humans. *Environ Health Perspect*. 1993;99:107-113.
18. Ross RK, Yuan JM, Yu MC, et al. Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *Lancet*. 1992;339(8799):943-946.
19. Groopman JD, Wogan GN, Roebuck BD, Kensler TW. Molecular Biomarkers for Aflatoxins and Their Application to Human Cancer Prevention. *Cancer Research*. 1994;54(7 Supplement):1907s.
20. Qian GS, Ross RK, Yu MC, et al. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. *Cancer Epidemiol Biomarkers Prev*. 1994;3(1):3-10.
21. Wang JS, Qian GS, Zarba A, et al. Temporal patterns of aflatoxin-albumin adducts in hepatitis B surface antigen-positive and antigen-negative residents of Daxin, Qidong County, People's Republic of China. *Cancer Epidemiol Biomarkers Prev*. 1996;5(4):253-261.
22. Skipper PL, Tannenbaum SR. Protein adducts in the molecular dosimetry of chemical carcinogens. *Carcinogenesis*. 1990;11(4):507-518.
23. Xu Y, Gong YY, Routledge MN. Aflatoxin exposure assessed by aflatoxin albumin adduct biomarker in populations from six African countries: REVIEW ARTICLE. *World Mycotoxin J*. 2018;11(3):411-419.
24. Rasheed H, Xu Y, Kimanya ME, et al. Estimating the health burden of aflatoxin attributable stunting among children in low income countries of Africa. *Sci Rep*. 2021;11(1):1619.
25. Gong YY, Egal S, Hounsa A, et al. Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: the critical role of weaning. *Int J Epidemiol*. 2003;32(4):556-562.
26. Egal S, Hounsa A, Gong YY, et al. Dietary exposure to aflatoxin from maize and groundnut in young children from Benin and Togo, West Africa. *Int J Food Microbiol*. 2005;104(2):215-224.
27. Wangia-Dixon RN, Quach THT, Song X, et al. Determinants of aflatoxin exposures in Kenyan School-aged children. *Int J Environ Health Res*. 2020:1-9.
28. Torres AM, Barros GG, Palacios SA, Chulze SN, Battilani P. Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. *Food Research International*. 2014;62:11-19.
29. Chapot B, Wild C. Techniques in diagnostic pathology. 1991.

30. Wild CP, Jiang Y-Z, Sabbioni G, Chapot B, Montesano R. Evaluation of methods for quantitation of aflatoxin-albumin adducts and their application to human exposure assessment. *Cancer Research*. 1990;50(2):245-251.
31. Yu M, Chen C, Wang L, Santella R. Aflatoxin B1 albumin adduct level and risk of hepatocellular carcinoma. Paper presented at: Proc. Am. Assoc. Cancer Res. 1995.
32. Gan LS, Skipper PL, Peng XC, et al. Serum albumin adducts in the molecular epidemiology of aflatoxin carcinogenesis: correlation with aflatoxin B1 intake and urinary excretion of aflatoxin M1. *Carcinogenesis*. 1988;9(7):1323-1325.
33. Sheabar FZ, Groopman JD, Qian GS, Wogan GN. Quantitative analysis of aflatoxin-albumin adducts. *Carcinogenesis*. 1993;14(6):1203-1208.
34. Sabbioni G, Ambs S, Wogan GN, Groopman JD. The aflatoxin-lysine adduct quantified by high-performance liquid chromatography from human serum albumin samples. *Carcinogenesis*. 1990;11(11):2063-2066.
35. Scholl PF, Turner PC, Sutcliffe AE, et al. Quantitative Comparison of Aflatoxin B₁ Serum Albumin Adducts in Humans by Isotope Dilution Mass Spectrometry and ELISA. *Cancer Epidemiology Biomarkers & Prevention*. 2006;15(4):823.
36. Plebani M, Carraro P. Mistakes in a stat laboratory: types and frequency. *Clin Chem*. 1997;43(8 Pt 1):1348-1351.
37. Carraro P, Plebani M. Errors in a stat laboratory: types and frequencies 10 years later. *Clin Chem*. 2007;53(7):1338-1342.
38. Lima-Oliveira G, Guidi GC, Salvagno GL, et al. Is Phlebotomy Part of the Dark Side in the Clinical Laboratory Struggle for Quality? *Laboratory Medicine*. 2012;43(5):172-176.
39. Kimani D, Kamau R, Gadde R, et al. Findings of Phlebotomy Practices in Kenya in 2010: Need for Action. *J Infect Dis*. 2016;213 Suppl 2(Suppl 2):S53-58.
40. Rooney K. Capillary Blood Sampling from the Finger. In: Guest PC, ed. *Investigations of Early Nutrition Effects on Long-Term Health: Methods and Applications*. New York, NY: Springer New York; 2018:267-272.
41. Birkeland S, Bismark M, Barry MJ, Möller S. Is greater patient involvement associated with higher satisfaction? Experimental evidence from a vignette survey. *BMJ Quality & Safety*. 2022;31(2):86.
42. Woods K, Douketis JD, Schnurr T, Kinnon K, Powers P, Crowther MA. Patient preferences for capillary vs. venous INR determination in an anticoagulation clinic: a randomized controlled trial. *Thrombosis Research*. 2004;114(3):161-165.
43. CLSI. Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Sixth Edition. In: Clinical and Laboratory Standards Institute; 2008.
44. Tang R, Yang H, Choi JR, et al. Capillary blood for point-of-care testing. *Crit Rev Clin Lab Sci*. 2017;54(5):294-308.
45. Koehler K, Marks-Nelson E, Braga CP, Beckford S, Adamec J. Validity of plasma collection cards for ferritin assessment-A proof-of-concept study. *Eur J Haematol*. 2020;104(6):554-561.
46. Topping J, Reardon M, Coleman J, et al. A Comparison of Venous versus Capillary Blood Samples when Measuring Blood Glucose Using a Point-of-Care, Capillary-Based Glucometer. *Prehosp Disaster Med*. 2019;34(5):506-509.

47. Cao J, Edwards R, Chairez J, Devaraj S. Validation of capillary blood analysis and capillary testing mode on the epoc Point of Care system. *Pract Lab Med*. 2017;9:24-27.
48. Xue KS, Cai W, Tang L, Wang J-S. Aflatoxin B1-lysine adduct in dried blood spot samples of animals and humans. *Food and Chemical Toxicology*. 2016;98:210-219.
49. Wang JS, Abubaker S, He X, Sun G, Strickland PT, Groopman JD. Development of aflatoxin B(1)-lysine adduct monoclonal antibody for human exposure studies. *Appl Environ Microbiol*. 2001;67(6):2712-2717.
50. Qian G, Tang L, Liu W, Wang J. Development of a non-antibody method for rapid detection of serum aflatoxin B1-lysine adduct. *Toxicologist*. 2010;114:248.
51. Qian G, Tang L, Wang F, et al. Physiologically based toxicokinetics of serum aflatoxin B1-lysine adduct in F344 rats. *Toxicology*. 2013;303:147-151.
52. Hill PG, Wells TNC. Bromocresol Purple and the Measurement of Albumin: Falsely High Plasma Albumin Concentrations Eliminated by Increased Reagent Ionic Strength. *Annals of Clinical Biochemistry*. 1983;20(5):264-270.
53. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72:248-254.
54. Sabbioni G, Skipper PL, Büchi G, Tannenbaum SR. Isolation and characterization of the major serum albumin adduct formed by aflatoxin B1 in vivo in rats. *Carcinogenesis*. 1987;8(6):819-824.
55. Lauer JM, Duggan CP, Ausman LM, et al. Maternal aflatoxin exposure during pregnancy and adverse birth outcomes in Uganda. *Matern Child Nutr*. 2019;15(2):e12701.
56. Wang JS, Qian GS, Zarba A, et al. Temporal patterns of aflatoxin-albumin adducts in hepatitis B surface antigen-positive and antigen-negative residents of Daxin, Qidong County, People's Republic of China. *Cancer Epidemiology Biomarkers & Prevention*. 1996;5(4):253.
57. Xue KS, Tang L, Shen CL, et al. Increase in aflatoxin exposure in two populations residing in East and West Texas, United States. *Int J Hyg Environ Health*. 2021;231:113662.
58. Kang MS, Nkurunziza P, Muwanika R, et al. Longitudinal evaluation of aflatoxin exposure in two cohorts in south-western Uganda. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 2015;32(8):1322-1330.
59. Shuaib FM, Jolly PE, Ehiri JE, et al. Association between birth outcomes and aflatoxin B1 biomarker blood levels in pregnant women in Kumasi, Ghana. *Trop Med Int Health*. 2010;15(2):160-167.
60. Andrews-Trevino JY, Webb P, Shively G, et al. Relatively Low Maternal Aflatoxin Exposure Is Associated with Small-for-Gestational-Age but Not with Other Birth Outcomes in a Prospective Birth Cohort Study of Nepalese Infants. *J Nutr*. 2019;149(10):1818-1825.
61. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Statistical Methods in Medical Research*. 1999;8(2):135-160.
62. Giavarina D. Understanding Bland Altman analysis. *Biochem Med (Zagreb)*. 2015;25(2):141-151.
63. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;1(8476):307-310.
64. Ho KM. Using linear regression to assess dose-dependent bias on a Bland-Altman plot. *Journal of Emergency and Critical Care Medicine*. 2018;2(8).

65. Vedin T, Karlsson M, Edelhamre M, Bergenheim M, Larsson P-A. Prospective comparison of capillary and venous brain biomarker S100B: capillary samples have large inter-sample variation and poor correlation with venous samples. *International Journal of Emergency Medicine*. 2019;12(1):26.
66. Hollis VS, Holloway JA, Harris S, Spencer D, van Berkel C, Morgan H. Comparison of venous and capillary differential leukocyte counts using a standard hematology analyzer and a novel microfluidic impedance cytometer. *PLoS One*. 2012;7(9):e43702.
67. Nicholas C, George R, Sardesai S, Durand M, Ramanathan R, Cayabyab R. Validation of noninvasive hemoglobin measurement by pulse co-oximeter in newborn infants. *J Perinatol*. 2015;35(8):617-620.
68. Hill PG, Wells TN. Bromocresol purple and the measurement of albumin. Falsely high plasma albumin concentrations eliminated by increased reagent ionic strength. *Ann Clin Biochem*. 1983;20 (Pt 5):264-270.
69. Smith JW, Kroker-Lobos MF, Lazo M, et al. Aflatoxin and viral hepatitis exposures in Guatemala: Molecular biomarkers reveal a unique profile of risk factors in a region of high liver cancer incidence. *PLoS One*. 2017;12(12):e0189255.
70. Nwankwo L, McLaren K, Donovan J, et al. Utilisation of remote capillary blood testing in an outpatient clinic setting to improve shared decision making and patient and clinician experience: a validation and pilot study. *BMJ Open Qual*. 2021;10(3).

Balaji Srinivasan: conceptualization, methodology, formal analysis, resources, data curation, writing – original draft, writing- review & editing, visualization, project administration. **Shibani Ghosh:** conceptualization, methodology, resources, writing- review & editing, supervision, project administration, funding acquisition. **Patrick Webb:** conceptualization, methodology, resources, writing- review & editing, supervision, project administration, funding acquisition. **Stacy P. Griswold:** conceptualization, methodology, resources, data curation, writing- review & editing, supervision, project administration. **Kathy S. Xue:** methodology, validation, data curation, writing- review & editing. **Jia-Sheng Wang:** writing- review & editing, validation, methodology, resources, supervision. **Saurabh Mehta:** conceptualization, methodology, resources, writing- review & editing, supervision, project administration, funding acquisition. All authors have read and agreed to the publisher version of the manuscript.