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Dried blood spot collection, sample quality, and fieldwork conditions: Structural validations for conversion into standard values

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Abstract

Objectives: SHARE, a pan-European panel study in 27 European countries and Israel, has collected dried blood spot (DBS) samples from approximately 27 000 respondents in 13 countries. We aim to obtain factors to convert analyte values between DBS and venous blood samples (VBS) taking account of adverse fieldwork conditions such as small spot size, high temperature and humidity, short drying time and long shipment times.

Methods: We obtained VBS and DBS from a set of 20 donors in a laboratory setting, and treated the DBS in a systematic and controlled fashion simulating SHARE fieldwork conditions. We used the 3420 outcomes to estimate from DBS analyte values the values that we would have obtained had it been feasible to collect and analyze the donors' venous blood samples.

Results: The influence of field conditions and sample quality on DBS analyte values is significant and differs among assays. Varying spot size is the main challenge and affects all markers except HbA1c. Smaller spots lead to overly high measured levels. A missing desiccant is detrimental for all markers except CRP and tHb. The temperature to which the samples are exposed plays a significant role for HDL and CysC, while too brief a drying time affects CRP and CysC. Lab-based adjustment formulae only accounting for the differences between re-liquefied DBS and venous blood do not address these fieldwork conditions.

Conclusions: By simulating adverse fieldwork conditions in the lab, we were able to validate DBS collected under such conditions and established conversion formulae with high prediction accuracy.

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1 | INTRODUCTION

The health of the general population, and especially of the older aged population, is very different between countries. Comparisons between the US Health and Retirement Study (HRS), the English Longitudinal Study of Ageing (ELSA) and the Survey of Health, Aging and Retirement in Europe (SHARE) have stimulated extensive research into the potential mechanisms responsible for the differences, and were instrumental in the establishment of two National Academy of Sciences panels (Crimmins, Kim, & Vasunilashorn, 2010; Woolf & Aron, 2013). These studies have shown that older people in Europe have health advantages when compared with their American counterparts, for example, the percentages of individuals aged 50 to 74 with at least one limitation in the activities of daily living (ADL) is 12% in the US, 10% in the UK and 7% in the EU (Avendano, Glymore, Banks, & Mackenbach, 2009).

Understanding the reasons for cross-national health differences requires that studies use comparable measurements of health. The findings by Avendano et al. (2009) were based on comparable measures but these were self-reports and may have suffered from reporting biases that may differ between countries. Cross-national differences in the quality and definition of diagnoses, prescription drug use (Crimmins et al., 2010) and reporting styles (Kapteyn, Smith, & van Soest, 2007) also likely affect the comparability of results from population studies.

It is therefore highly desirable to base cross-national comparisons of health on blood-based biomarkers. Few studies, however, use objective biomarkers. Banks, Marmot, Oldfield, and Smith (2006) found differences between the US and UK when a set of four biomarkers measured in venous blood samples were evaluated: glycated hemoglobin (HbA1c), a marker for diabetes; C-reactive protein (CRP), a marker for inflammation; and fibrinogen and HDL-cholesterol, risk factors for cardiovascular diseases (CVD). It was found, for example, that the percentage of individuals aged 50 and older with HbA1c-values greater than 6.5% (an indicator of diabetes) was 7.1% in the US and 4.1% in the UK. Martinson, Teitler, and Reichman (2011) using CRP, HDL and stress data confirmed these findings and further found that the cross-national differences, while stronger for women than men and more pronounced in lower education groups, were pervasive.

Collecting blood samples in international surveys is challenging. Although biomarkers are conventionally measured in sera obtained from venous blood samples (VBS), the costs of VBS collection are prohibitive for a large international population-representative survey. The alternative of collecting dried blood spot (DBS) samples has the advantage that DBS samples can be collected by lay interviewers at much lower costs than VBS, which require collection by phlebotomists or nurses (Brindle, O'Connor, & Garret, 2014; Williams & McDade, 2009).

This cost advantage has to be traded off against challenges in the field. While standard procedures for DBS collection have been developed for newborn screening, this normally occurs in a clinical setting. SHARE, however, a pan-European panel study of approximately 85 000 individuals aged 50+ (Börsch-Supan et al., 2013), collected DBS samples during its Wave 6 from approximately 27 000 respondents at their place of residence. Data were collected in 13 countries ranging from Israel in the South to Sweden in the North, by far the largest sampling of DBS from a representative adult population. Challenges encountered included the effects of the environmental conditions present during sample collection and shipment, the circumstances and the extent of the collaboration of the respondent, and the interviewer's ability to collect a sample correctly as trained and to record data accurately. We found these fieldwork conditions to be more adverse than anticipated in spite of extensive training of the lay interviewers for the pan-European survey (SHARE Wave 6; see Börsch-Supan, Weiss, Andersen-Ranberg, & Börsch-Supan, 2020). One of the largest challenges was to convince the lay interviewers to collect sufficiently large blood spots and to dry them properly.

An additional disadvantage of DBS is that results from DBS assays cannot be directly compared to the results one would obtain from assays of conventional VBS using standard laboratory methods (Crimmins et al., 2014; Karvanen, 2015; McDade, Williams, & Snoddgrass, 2007). Although there is variation in values obtained from analyses of VBS, the DBS values of, for example, total cholesterol, which is known to be particularly difficult to measure in DBS samples, have both a higher mean and a larger variance, influenced by many laboratory and fieldwork-related factors (Thomas et al., 2018; Crimmins et al., 2020; Bowen & Evans, 2014. In: Li & Lee, 2014). After applying parametric standardization (Karvanen, 2015) or nonparametric normalization formulae (eg, Crimmins et al., 2014), the DBS values fit the distribution of values obtained from VBS quite well. This approach has been used with HRS data to produce adjusted values for a small set of analytes (Crimmins et al., 2014).

Recent work has shown that lab-based formulae used to transform DBS values into VBS-equivalent values do not address the fieldwork conditions that may affect the quality of DBS samples collected for an international survey like SHARE (Weiss & Börsch-Supan, 2019). This finding has been replicated in two field studies, which

collected both VBS and DBS (Crimmins et al., 2020; Weiss et al., 2019).

While it is important to prevent issues from occurring in the field rather than imposing post hoc adjustments, these efforts are costly and may not be perfect. Hence, ex post conversion is necessary. In this article, we have applied a systematic validation of DBS results, using data obtained from a simulation of SHARE fieldwork conditions. We call it "structural validations" because our methodology is based on a structural model of the differences between VBS collected under laboratory conditions and DBS collected under fieldwork conditions.[1] We use the validations to establish field-based conversion formulae applicable to the SHARE DBS samples to estimate the value that we would have obtained had it been feasible to instead collect a donor's venous blood in a clinic and then analyze it using standard analytical methods (reference value).

2 | METHODS

2.1 | Collection of DBS samples in the field

SHARE collected DBS samples during its Wave 6 in 2015 in 12 European countries and Israel. We used harmonized collection protocols and DBS collection kits, thoroughly trained our interviewers and implemented interviewer monitoring throughout the fieldwork. All DBS samples arriving at the central biobank in Denmark for storage were visually inspected for number of blood spots, spot quality (smeared or overlapping, as these change the distribution of analytes in the filter paper) and spot size (small spots indicate that the interviewer did not wait until a sufficiently large blood drop had formed before collecting the blood on the filter paper). Further shortcomings noted included missing desiccant (influencing humidity protection) and spot discoloration (indicating that a wet DBS was packaged). Other uncontrollable impacts on the samples were malfunctioning national postal systems, with consequences on shipment time, and unusually high temperatures encountered during shipment. The implementation and monitoring of the DBS collection in SHARE Wave 6 has been described (Börsch-Supan et al., 2020).

2.2 | Validation structure

The aim of the validations is to simulate the environmental conditions and fieldwork effects experienced by DBS and to then measure the resulting change in biomarker values in a structured approach. The environmental data collected during fieldwork monitoring and by sample inspection enabled us to design the validation scheme.

Figure 1 shows a conceptual model of the structural differences between VBS collected in a laboratory, DBS collected in the field and its mirror image, our VBS-based DBS validation scheme structured by the same processes.

Standard VBS analyses are depicted in the center of Figure 1. For a given marker, the laboratory analysis yields a value Y that corresponds to the reference value based on serum or whole blood, depending on analyte.

The DBS field process is depicted in the left side of Figure 1, marked in blue. A donor provides capillary blood from a finger-prick, which is dropped onto a filter paper card and creates spots of varying size (dependent on the volume of the blood drop; DBS_C). This blood is dried and then experiences the various conditions found to arise during fieldwork and shipment such as differences in temperature, humidity, and drying and/or shipment time (DBS_F). In the lab, the dried blood is liquefied and assayed, yielding a value of V for the marker.

Our validations mimicked the field process but used venous whole blood to create DBS in order to obtain sufficient material for all 171 treatments from a single donor, which would not be feasible using finger pricking to obtain capillary blood. This process is depicted on the right side of Figure 1, marked in red. The donor's venous blood was collected in an EDTA-coated tube, pipetted onto a filter paper card and dried (DBS_V), and then treated under replicable laboratory conditions to simulate the typical fieldwork and shipping treatments described above (ie, heat, humidity, etc.; DBS_T). The dried blood was then liquefied and analyzed to yield the value X. For the validation experiment we also collected capillary DBS from each donor (DBS_C), yielding the value W, as well as analyzed untreated DBS_V, resulting in the value Z.

The outcomes X, Y, Z, and W are different due to three groups of effects generated by chemical and physical processes. These effects are shown in green in Figure 1.

First, the blood source is either capillary blood from a finger-prick or venous blood collected by cannula. Capillary blood is a heterogeneous mixture of plasma, interstitial fluid and blood cells while venous blood does not contain interstitial fluid. Centrifugation of venous blood to obtain serum removes all cellular material. In addition, venous blood analyzed directly or used to obtain plasma for analyses, is treated to prevent coagulation, for example, with EDTA. There are also small differences between plasma and serum in the concentrations of some analytes. We call the implications of these differences between capillary and venous blood on the assays the

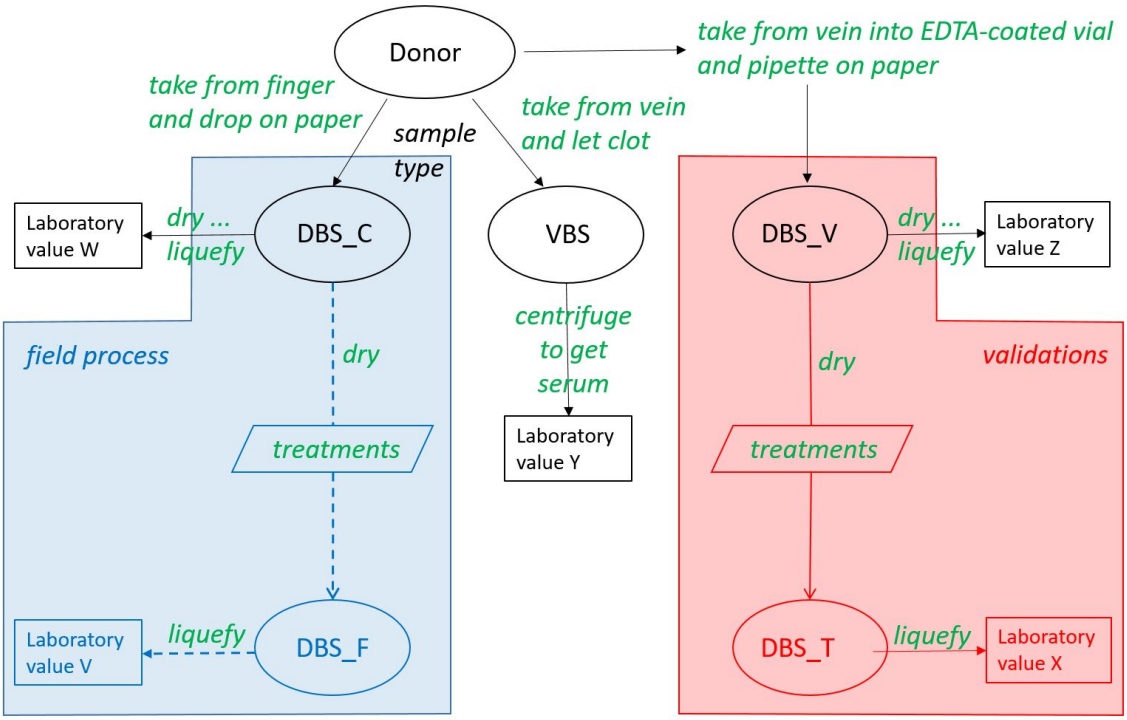

FIGURE 1 General structure

"sample-type effects." These effects have not been extensively studied for the analytes of interest here. Spooner, Ramakrishnan, Barfield, Dewit, and Miller (2010) reported different marker values for blood collected from finger prick, venous cannula and whole blood cannula when measuring paracetamol exposure. They speculated that the observed lack of interchangeability between the sampling sites may not be limited to drugs.

Second, the capillary blood is dried on filter paper. During the drying process, cell lysis occurs and releases cellular content. The dried blood is re-liquefied in the lab for analysis. These processes may influence the chemical composition of the blood and the marker molecules. We call this the "dry-liquefy effects".

The combination of sample-type and dry-liquefy effects creates differences between the outcome values W, Y, and Z. The difference between Y and Z has been extensively studied by Crimmins et al. (2014) and is 3-fold for most analytes: the difference between serum and whole venous blood, the treatment of the latter with EDTA, and it subsequent drying and reliquefaction. Isolation of the dry-liquefy effect would involve comparisons between dried/eluted plasma spots and whole blood that had undergone sonication, or freeze/thaw, to release cellular contents; such comparisons are beyond the aims of this study. The difference between Y and W is a combination of the blood source (capillary whole blood vs venous serum) and the dry-liquefy effect. The difference between W and Z has two causes: the difference between capillary blood and venous blood, and the EDTA-treatment of the latter.

The third group of effects are the original and simulated "fieldwork effects" (heat, humidity, etc.), depicted as blue and red rhombi in Figure 1. These effects have not been systematically investigated previously and are the focus of this study.

Our aim is to establish a conversion formula following this structure that is applicable to the SHARE population and estimates the value that we would have obtained had it been feasible to analyze donors' venous blood by standard analytical methods for serum, plasma or whole blood.

More formally, the aim of this study is to establish an equation, $Y = f(X, T, H)$, which computes the estimated standard value for Y from the treated DBS value X, the applicable treatment conditions T (eg, temperature, humidity, drying time, shipment time, spot size) and donor characteristics H (eg, health, age, sex) reflecting potential interaction effects (Section 3.3). Ideally, this conversion formula would have accuracy comparable to the measurement variation of the reference value Y obtained from serum or venous whole blood (Woodworth et al., 2014).

2.3 | Data

The validation study and all marker assays were performed at the University of Washington Department of

Laboratory Medicine and Pathology Biomarker Laboratory (UW).

Over a period of 10 days (July 20 to 30, 2018) venous blood was collected under laboratory conditions by a phlebotomist from 20 donors recruited by BloodWorks NorthWest, Seattle. WA. Venous blood was collected into K2EDTA vacutainers and into tubes without added preservatives or stabilizers. Immediately after collecting the venous blood, the DBS were created. Whole blood was stored at ambient temperature and was couriered to the UW laboratory within 4 hours of collection. Once received, EDTA plasma and serum by clot was separated by centrifugation at 3000 rpm for 10 minutes. Liquid whole blood (for HbA1c and tHb), serum and plasma samples (for all other markers) were created and assayed the same day. Analyses of the serum created the venous reference marker values, called *Y* in Figure 1. Venous EDTA blood samples were further processed as follows:

(i) We created DBS samples from EDTA whole blood (DBS_V). They were immediately frozen at −70°C after drying and kept frozen until analysis, resulting in the value *Z*;
(ii) Of the blood from the 20 donors, we created DBS samples that were subsequently exposed to 171 different controlled conditions (DBS_T in Figure 1) mimicking the fieldwork conditions that we encountered during the SHARE Wave 6 DBS collection, resulting in the value *X*.

At the time of the venous blood collection, the phlebotomist also collected a capillary blood sample from a fingerprick for DBS using the same technique applied during SHARE fieldwork (DBS_C). After drying and transfer to UW the DBS cards were frozen at −70°C. Filter-paper cards used to create the DBS were the same as those used during SHARE fieldwork: Ahlstrom 226 filter paper. Ahlstrom 226 is fully comparable to the Whatman 903 protein saver cards predominantly used in the US and by HRS. The CDC found no difference between the performance properties of 903 vs 226 filter papers, which also produced comparable results across analytes and testing methods (Mei et al., 2010). Desiccant used during SHARE and in the simulations of shipment times and storage was 2 g molecular sieve in a Tyvek pouch (Absorpower Service GmbH, Germany). Analyses of the DBS_C resulted in the value *W*.

The 171 different controlled conditions used in (ii) are described in the following:

- Spot size: We created dried blood spots in three sizes, using 10 μL, 30 μL or 60 μL of blood, respectively, as a large percentage of the field-collected spots had diameters < 1 cm (60 μL).
- Drying time: The samples were dried at room temperature for 5, 20, 35, or 240 minutes and then immediately packed as for shipment. The short drying times reflect frequent survey circumstances which did not permit overnight drying; the long drying time refers to full dry (Mei, 2014).
- Humidity protection:
  a. Closed polyethylene (PE) bag: The samples were put into PE bags, which then were zip-closed or left open.
  b. Desiccant: A desiccant was either placed inside the PE bag or not.
- Outside temperature: The samples (inside their PE bags and envelopes) were exposed to 5°, 20°, or 35°C for 2 hours. This mimics the exposure to (high) outside temperature under the assumptions that the fieldwork samples were exposed to the actual outside temperature for only a fraction of the shipment time.
- Shipment time: The samples were left at room temperature for additional 3, 7, or 14 days.

Our data set contains 3420 observations clustered by 20 donors, each including a certain treatment combination, the respective values for the analyzed biomarkers (see below) and three values from different kinds of "untreated" blood samples: venous blood (VBS), DBS samples from venous EDTA-whole blood (DBS_V), and DBS samples from capillary blood (DBS_C). "Untreated" in this sense means that they were of optimal size (60 μL) and were immediately frozen after the optimal drying time (240 minutes), hence, they were created under laboratory conditions as opposed to the DBS that were subjected to the simulated varying fieldwork conditions described above. The capillary DBS samples will be part of the conversion equation used for recalculating the fieldwork samples, which are actually capillary blood DBS, not venous blood DBS as in this validation study. In addition to the blood values, we had information on age, gender and body mass index (BMI) score for each donor.

All samples were analyzed for seven biomarkers according to published techniques: glycosylated hemoglobin (HbA1c, Egier, Keys, Hall, & McQueen, 2011), total hemoglobin (tHb, Frenchik, McFaul, & Tsonev, 2004), total cholesterol (TC, Lakshmy, Gupta, Prabhakaran, Snehi, & Reddy, 2010 and Lakshmy et al., 2012), high density lipoprotein-cholesterol (HDL, Huang, Kao, & Tsai, 1997; Arranz-Peña, Tasende-Mata, & Martin-Gil, 1998), triglycerides (TG, Quraishi, Lakshmy, Prabhakaran, Mukhopadhyay, & Jailkhani, 2006; Lakshmy et al., 2010), C-reactive protein (CRP, high-sensitive immunoassay, McDade, Burhop, & Dohnal, 2004; Brindle, Fujita, Shofer, & O'Connor, 2010), and Cystatin C (CysC, Vogl, 2014).

The liquid-blood assays for the VBS were performed on a Beckman-Coulter Olympus AU680 Chemistry

Analyzer for HDL, TC, TG, and CRP; CysC was analyzed with a microtiter plate assay. THb was assayed using a Sysmex XN hematology analyzer. All HbA1c tests, both on whole blood and DBS samples, were run on a Bio-Rad Variant II High Pressure Liquid Chromatography (HPLC) System.

For the analyses of the DBS samples, UW has developed standard (in-house) assays based on the above-mentioned references. For those assays, validations have shown roughly a coefficient of variation of 5% to 10% in inter-assay, inter- and intra-spot reproducibility. To keep the inherent variation of our validation samples low, for each assay all samples from a single donor were assayed together.

2.4 | Statistical analysis

We use an ordinary least squares regression to estimate the equation, $Y = f(X, T, H)$, which relates the VBS-based standard value Y to the treated DBS value X, the applicable treatment conditions T, and donor characteristics H. Our basic specification includes all treatments, major treatments squared and interacted, and donor characteristics as follows:

$$\begin{aligned} Y = {} & \beta_1 + \beta_2 \cdot X + \beta_3 \cdot dry + \beta_4 \cdot ship + \beta_5 \cdot bag \\ & + \beta_6 \cdot desiccant + \beta_7 \cdot size + \beta_8 \cdot temp \\ & + \beta_9 \cdot dry^2 + \beta_{10} \cdot ship^2 + \beta_{11} \cdot size^2 + \beta_{12} \cdot temp^2 \\ & + \beta_{13} \cdot dry \cdot size + \beta_{14} \cdot bag \cdot desiccant + \beta_{15} \cdot bag \cdot dry \\ & + \beta_{18} \cdot age + \beta_{19} \cdot age^2 + \beta_{20} \cdot bmi + \beta_{21} \cdot bmi^2 \\ & + \beta_{22} \cdot female \end{aligned}$$

We also estimated a simplified model as well as a larger model which includes all treatments and all possible interactions (up to 6-fold). The six estimated models are summarized in Table 1.

In order to select the model with the best out-of-sample prediction and to minimize in-sample overfitting, we used a cross-validation approach. We estimated all models on all but one donor and used the data from the remaining donor for out-of-sample predictions. For each model s and each donor i, the prediction error (root mean square error) was calculated as

$$RMSE_{si} = \sqrt{\frac{\sum_{k=1}^{n_i} \left(Y_i - \hat{Y}_{ik}\right)^2}{n_i}}$$

where n_i denotes the number of observations associated with donor i.

This procedure was iterated over all donors 1 to 20 to yield the prediction error for model s:

$$RMSE_s = \frac{\sum_{i=1}^{20} RMSE_{si}}{20}.$$

3 | RESULTS

3.1 | Differences among untreated samples of different sample types

We first show bivariate scatter plots of the three untreated blood-source types, beginning with values from venous EDTA-treated DBS (Z in Figure 1) vs values from capillary DBS (W in Figure 1). The differences between the red dots in Figure 2 and the equity line (dashed black) represent measurement errors in the laboratory assays plus the blood-source and the EDTA effect. The systematic part is represented by the difference between the red regression line and the equity line. This sample-type effect is statistically significant at the 1%-level for all markers. For CRP, the effect is relatively small. The coefficient of the capillary values is 0.9

TABLE 1 Estimated models

Model name	basic	simple	allint	basicH	simpleH	allintH
Treatment variables	x	x	x	x	x	x
Single interactions	x			x		
Squared treatment variables	x			x		
All possible interactions			x			x
Gender (dummy)				x	x	x
BMI score				x	x	x
BMI score squared				x	x	x
Age (years)				x	x	x
Age squared				x	x	x

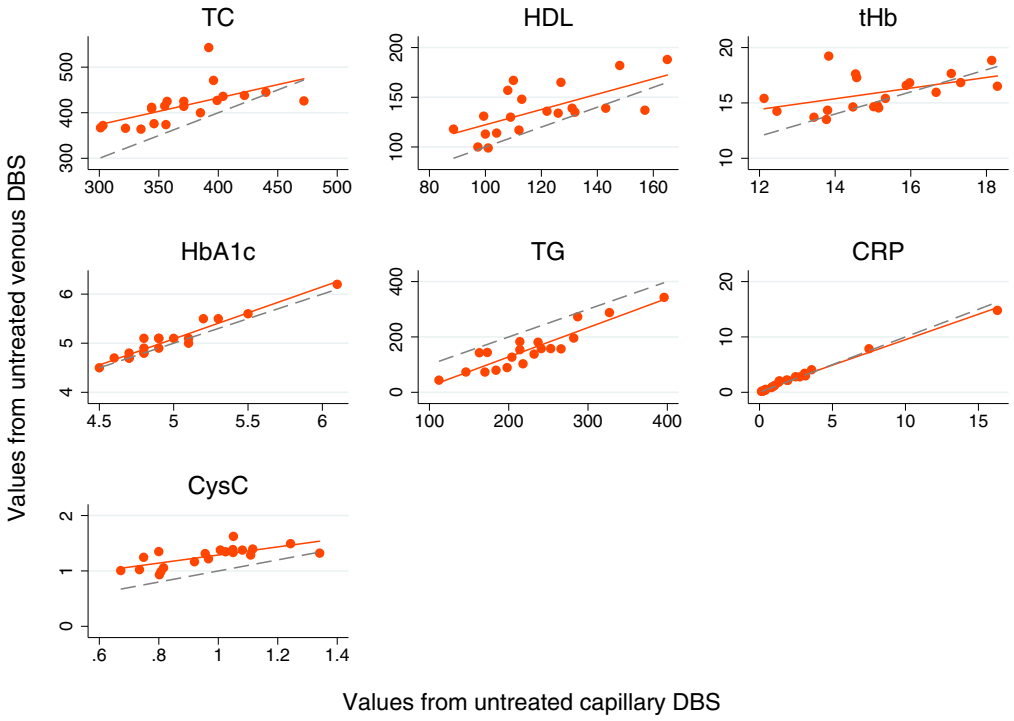

FIGURE 2 Difference between DBS from capillary and venous blood (Z vs W)

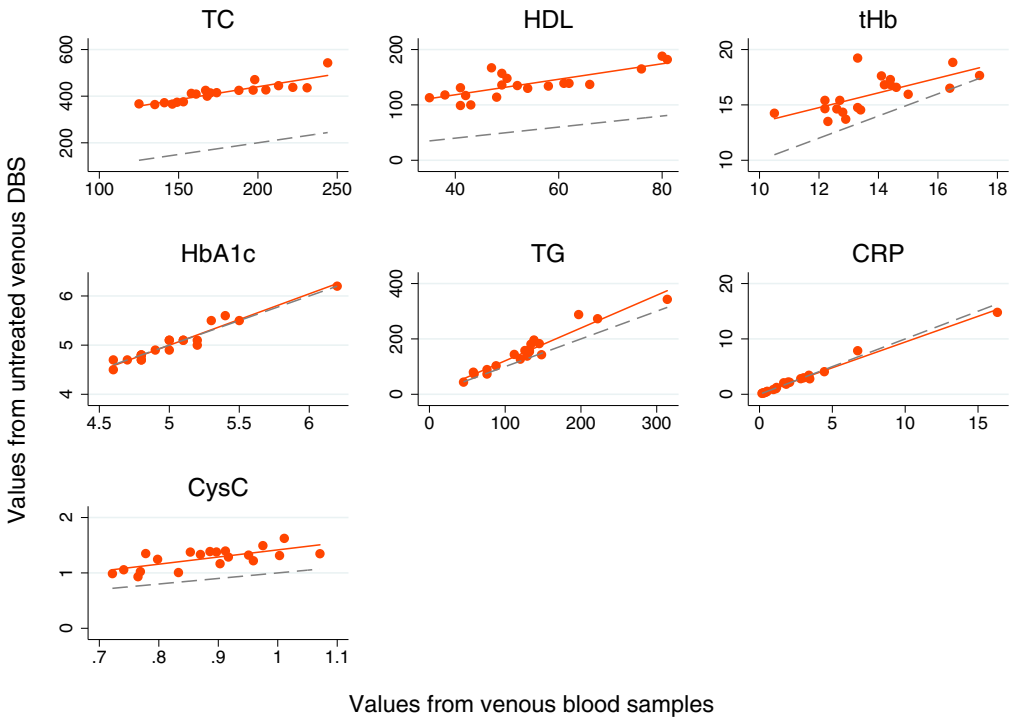

FIGURE 3 Difference between standard values and DBS from venous blood (Y vs Z)

and the intercept is little more than 0.3. However, the effect is statistically significant because the fit is very high ($R^2 = .99$).

The DBS samples in our validation study were not made from capillary blood, but instead were created from venous (EDTA-treated) blood. Values obtained from those DBS_V (Z in Figure 1) show differences from the standard values (Y in Figure 1) obtained from serum (Figure 3). Since both the standard and DBS values are based on the same blood source, namely venous blood, this identifies the dry-liquefy effect in combination with the difference between whole blood and serum and the EDTA-effect.

We find larger systematic differences between the standard values obtained from serum and the VBS-based DBS samples. They are statistically significant at the 1%-level for all markers except HbA1c and CRP. The lipids

TC and HDL show particularly large deviations; their DBS values are shifted upwards as compared to the standard values. These shifts are to be expected since the amount of blood taken from the spot (eg, a 3.2-mm punch) was arbitrary as well as was the buffer volume used to liquefy the dried blood; both define the concentration of a marker measured in a DBS.

Finally, there are systematic differences between the standard values obtained from serum (Y in Figure 1) and the values measured in DBS samples taken from a finger prick (W in Figure 1) even under laboratory conditions (Figure 4). These differences represent the combination of the sample-type and dry-liquefy effects. This combination effect is important if one wants to compare DBS and VBS results from the same donor; it is statistically significant at the 1%-level for all seven markers except CRP and CysC. THb was co-eluted with HbA1c and HDL; the protocol was not optimized for tHb to avoid affecting HbA1c or HDL.

3.2 | Treatment effects

Correcting for treatment effects requires the selection of a regression model among those presented in Table 1. Figure 5 shows the result of the cross-validation in terms of the prediction errors. The red dot indicates the lowest *RMSE* for each marker; the dot for "raw" represents the deviation of the raw values X from the standard value Y. Coefficients within each model vary only slightly with the exclusion of different donors.

Correcting for treatment effects is essential for all markers. Model "basic" (all treatments plus major treatments squared and interacted) yields low prediction errors for all markers. Adding donor characteristics improves prediction only for tHb and CysC. For TG, the difference between our "basic" model and the larger model "allint" (the latter includes all treatments and all possible interactions between them) is insignificant and numerically very small. We therefore selected the "basic" model for TC, HDL, CRP, HbA1c, and TG, and the extended "basicH" model for CysC and tHb, for the conversion equation $Y = f(X, T, H)$.

The regression coefficients of the chosen models indicate which treatments require corrections. This is depicted in Figure 6.

Spot size (effect shown for steps in 10 μL) plays an important role for all markers except HbA1c. For small spot sizes, which occurred often during the collection in SHARE, the measured values are too low. Hence, the conversion formula needs to increase them. A missing desiccant is detrimental for most markers except CRP and tHb. The measured levels of both types of cholesterol decrease when a desiccant is missing during shipment and, hence, need to be increased by the conversion formula; in turn, the measured value of TG needs to be decreased. The temperature to which the samples are exposed plays a significant role for HDL and CysC, while

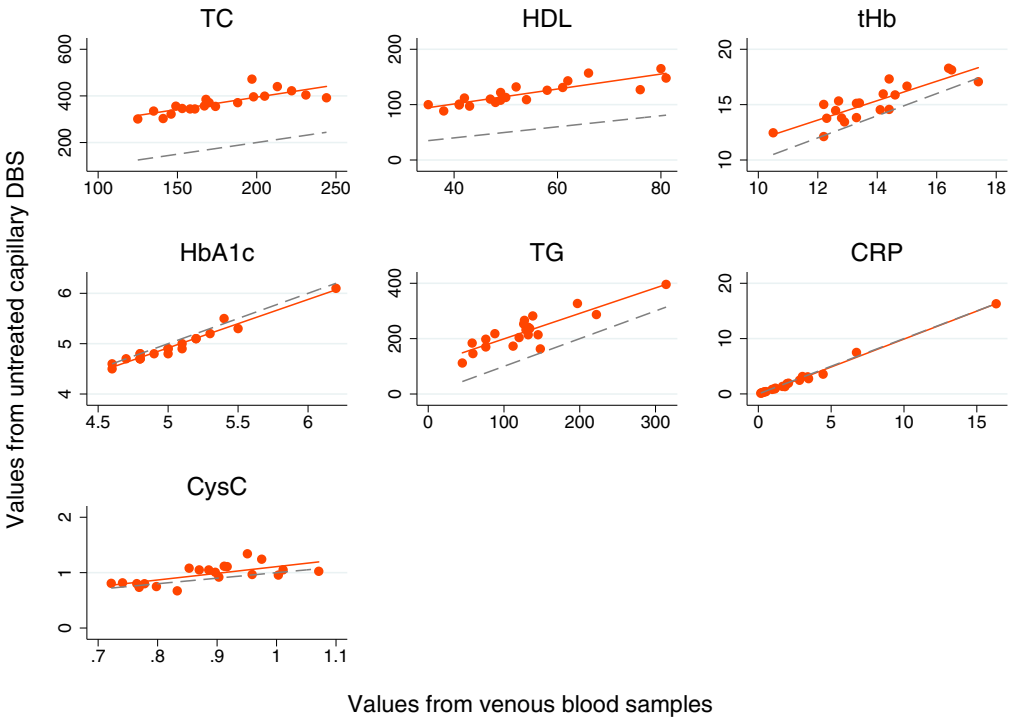

FIGURE 4 Difference between standard values and DBS from capillary blood (Y vs W)

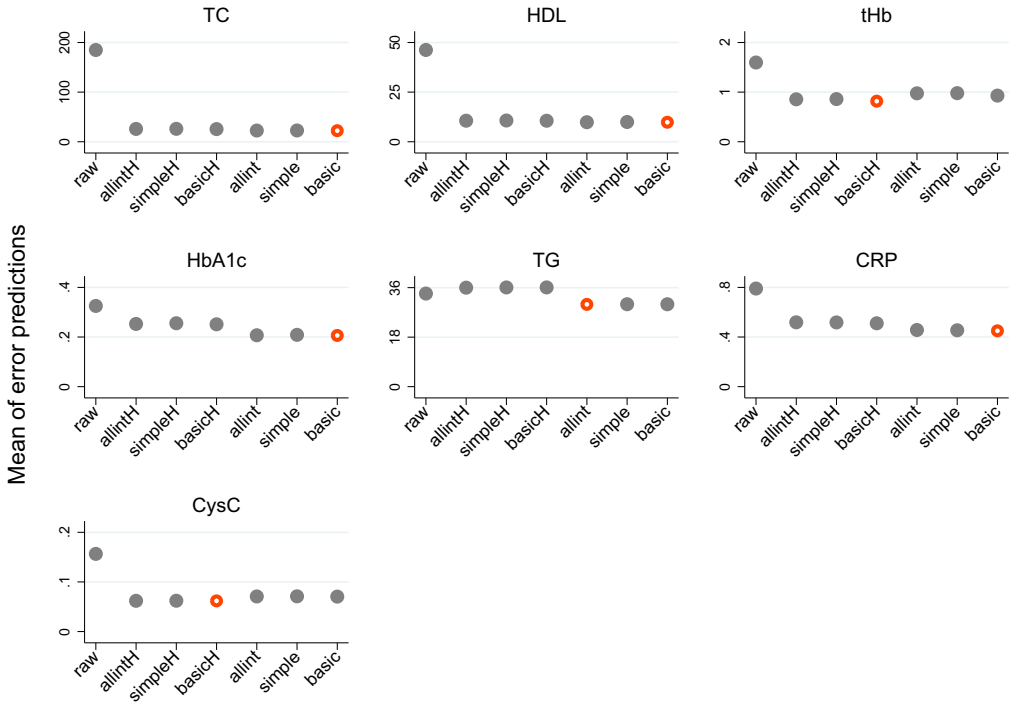

FIGURE 5 Prediction errors

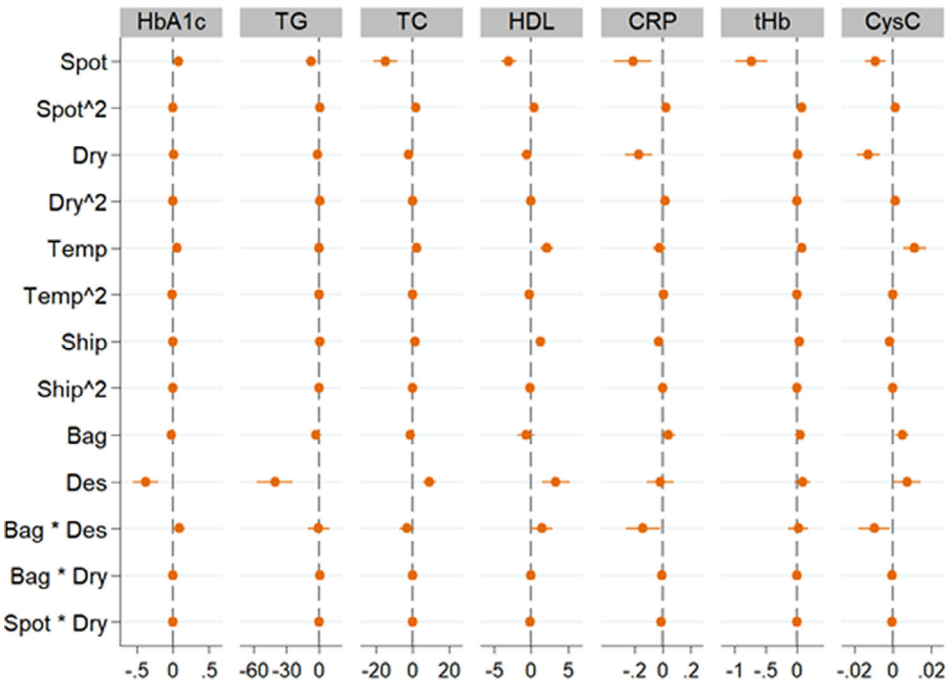

FIGURE 6 Treatment effects. *Note:* Treatment coefficients of the models chosen for conversion of all markers. Horizontal bars show confidence intervals. Standard errors are clustered in donors. Treatments are denoted by: Spot = spot size (unit = 10 μL), Dry = drying time (unit = 5 minutes), Temp = temperature exposure (2 hours.; unit = 5°C), Ship = shipment time (days), Bag = dummy for open PE bag, Des = dummy for missing desiccant; ^2 indicates squared variables, * indicates interactions

too brief a drying time affects CRP and CysC. Most other effects are very small and statistically insignificant.

3.3 | Adjusting for treatments

Figure 7 plots the raw values from treated DBS (yellow, laboratory value X) and the converted values (orange, value $\hat{Y}$) against the standard value Y, which are marked as light blue circles on the equity line. In addition, the dark blue crosses mark the values from untreated capillary blood from the donors (laboratory value W).

The yellow bars show the large variation of the raw laboratory values from DBS_T and their distance from the dashed equality line. The orange values are based on estimations accounting for the simulated fieldwork conditions using the respective model chosen in Section 3.2. The conversion has two effects: it decreases the overall

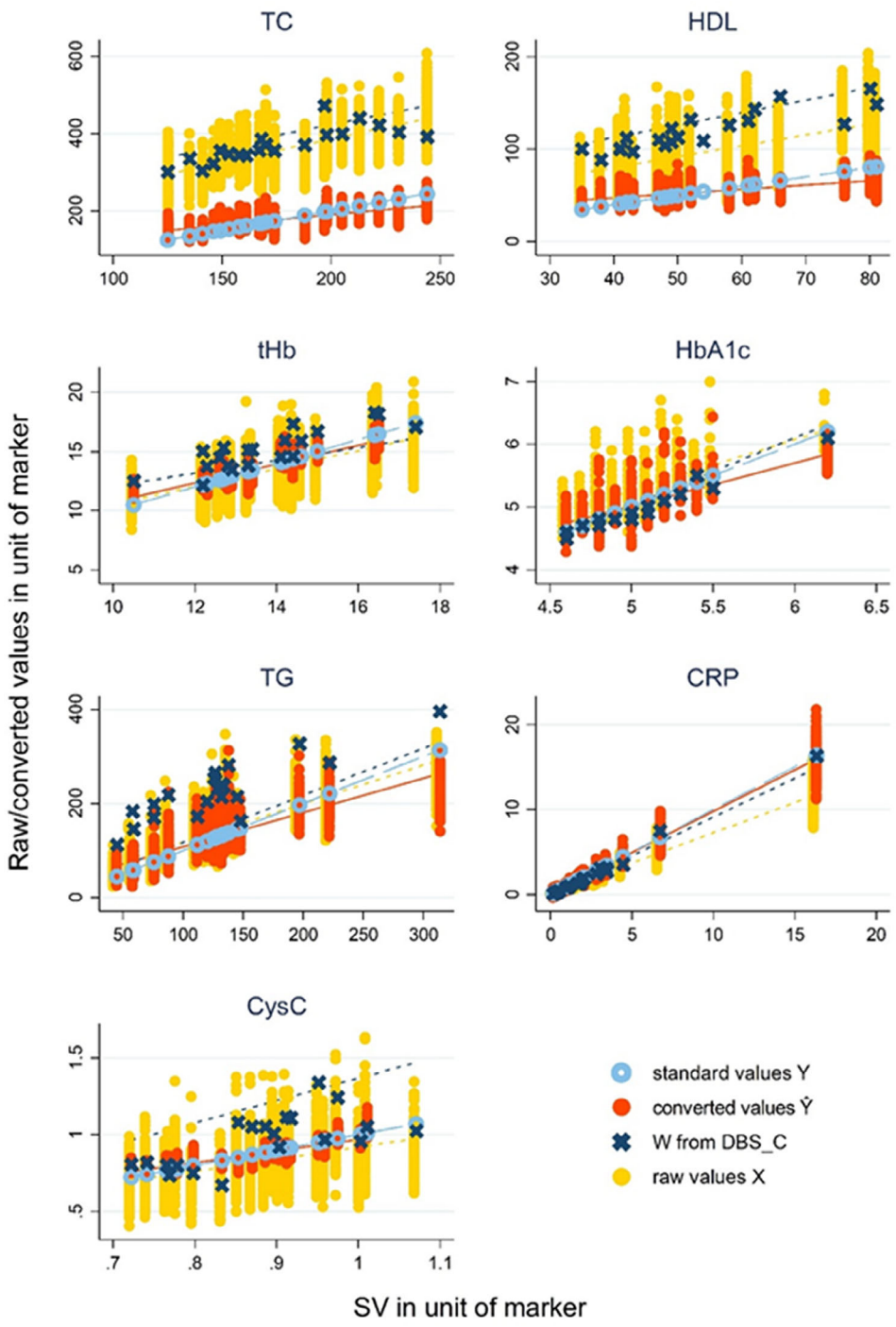

FIGURE 7 Results of conversion equation

variation and it shifts the mass of the converted values (orange) closer to the equation line, that is, the $\hat{Y}$ values are much closer to the standard values Y, which we would have expected from venous blood samples, than the treated DBS values X. The effects of decreasing the overall variation are particularly large for TC, HDL, tHb, and CysC, while the shifting effects are particularly large for TC and HDL. For HbA1c and TG the orange regression line averaging the converted values appears to be less precise than the yellow regression line averaging the raw values. However, this is only due to the least-squares effect of a linear regression, which gives the single outlier an inappropriately high weight.

While donor characteristics affect both standard values Y and treated values X directly—this is the reason to collect DBS in the first place—they should not affect Y conditional on X unless there are indirect interaction effects between donor characteristics and fieldwork effects. This is not the case for most markers in this study. Exceptions are tHb and CysC where we find a

significantly better fit of the prediction equation when we include donor characteristics, especially age. This finding holds up after cross-validation.

A possible explanation is that age influences spot size in a nonlinear fashion which is not captured by the quadratic in our prediction equation, or that age influences several treatment effects simultaneously which is not captured in the interaction effects in that equation. Pinning down these interactions will require larger sample sizes and will be the subject of further research.

4 | SUMMARY AND DISCUSSION

Collecting DBS to obtain epidemiologically important biomarkers in blood is an attractive and cost-efficient technique feasible for large population-based surveys like SHARE, especially when they cover a large number of countries. However, the disadvantages of this strategy are the many adverse environmental conditions and fieldwork effects that are difficult and costly to control perfectly ex ante. Besides minimizing the effects ex ante, it is thus essential to also correct ex post for treatment effects in the course of fieldwork and shipping, such as small spot size, high temperature and humidity, short drying time and long shipment times, since they vary greatly among respondents and create large artifacts. It was the aim of this study to develop correction formulae for each analyte since treatment effects vary across assays.

All marker values need to be corrected for varying spot sizes, with smaller spots leading to a need for an upwards correction. A missing desiccant is detrimental for all markers except CRP and tHb. The measured levels of both types of cholesterol need to be increased when a desiccant is missing during shipment, while the level has to be decreased for TG. The temperature to which the samples are exposed plays a significant role for HDL and CysC, while too short a drying time affects CRP and CysC.

By simulating adverse fieldwork and shipping conditions in the lab and relating the resulting analyte values to values obtained from venous blood under standard lab conditions, we were able to derive correction formulae with high prediction accuracy. This conversion has two effects. First, by accounting for the large differences in treatments, it decreases the overall variation. Second, by removing the systematic biases created by the different treatments, it shifts the DBS treated values much closer to the standard values that we would have expected to obtain had we collected venous blood samples. The effects of decreasing the overall variation are particularly large for TC, HDL, tHb, and CysC, while the shifting effects are particularly large for TC and HDL.

Our study was based on 171 different treatments applied to blood collected from 20 donors. The small number of donors is a limitation of this study. Further validations with a larger number of donors would likely increase the applicability of our conversion formulae and shed light on potential interaction effects between donor characteristics and fieldwork conditions.

There are several practical lessons from this analysis. First, ex ante prevention is better than ex post cures. Large multinational studies such as SHARE benefit from investigators having a thorough understanding of inter-country differences in policies and field conditions that can effect DBS collection and shipment. Most importantly, extensive interviewer training, protocol development and fieldwork monitoring is required to avoid adverse environmental conditions (eg, during excessively long shipment times) and insufficient fieldwork quality. Regarding the latter, interviewers need to be instructed and monitored to properly collect sufficiently large blood spots that fill the entire preprinted circles and do not shortcut the necessary drying times. While these efforts are costly and may not be perfect, so ex post conversion would still be needed, it will create a better starting point for the proposed corrections.

Second, since spot size turns out to be the most significant treatment effect, an accurate measurement and documentation of spot size is instrumental to apply a correction formula like the one proposed here. A separate study on ex post spot-size measurement for SHARE Wave 6 DBS is part of our current research.

Finally, the application of newly developed collection devices, which collect a fixed volume of blood, may eliminate the consideration of spot size and punching.

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AUTHOR CONTRIBUTIONS

Luzia M. Weiss: Formal analysis; methodology; writing-original draft; writing-review and editing. **Martina Börsch-Supan:** Formal analysis; methodology; writing-original draft; writing-review and editing. **Alan J. Potter:** Formal analysis; validation; writing-original draft; writing-review and editing. **Jake Cofferen:** Formal analysis; validation; writing-review and editing. **Elizabeth Kerschner:** Formal analysis; validation; writing-review and editing.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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ENDNOTE

[1] The structure underlying Figure 1 can be described by a structural equation model.

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