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Research Article

The Impact and Correction of Analysis Delay and Variability in Storage Temperature on the Assessment of HbA1c from Dried Blood Spots - an IMI DIRECT Study -

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ABSTRACT

Aims: Dried Blood Spot (DBS) sampling is a frequently used method to obtain Haemoglobin A1C (HbA1c) in clinical studies of free-living populations. Under controlled conditions, DBS sampling is a valid and robust alternative to traditional Whole Blood (WB) sampling. The objective of this analysis was to investigate the impact of storage conditions on the validity of HbA1c assessed from DBS collected in free-living and to develop a method to correct for this type of error.

Methods: Overall, 14,243 DBS cards from 2,237 IMI DIRECT participants at risk of developing diabetes were analyzed using non-linear regression analysis. 4,272 HbA1c levels from WB from the same 2,237 participants were used to validate the predictive performance of the method.

Results: The delay between DBS sample collection and analysis, in combination with different storage temperatures, caused inflation of measured HbA1c levels. An E_{\max} model was used to correct inflated HbA1c levels according to individual analysis delay and storage temperatures. Corrected HbA1c showed higher agreement to WB results, compared to the uncorrected HbA1c from DBS cards (Pearson correlation coefficients of 0.61 and 0.69 for reported and corrected vs. WB, respectively, $p = 5.92 \times 10^{-36}$). The mean HbA1c derived from WB was $5.6 \pm 0.29\%$ (38 ± 3.2 mmol/mol); from DBS, $5.8 \pm 0.40\%$ (40.0 ± 4.4 mmol/mol) and $5.6 \pm 0.33\%$ (38 ± 3.6 mmol/mol) (before and after correction, respectively).

Conclusions: Analysis delay and storage temperature influence the assessment of HbA1c from DBS cards. This correction method provides an opportunity to account for the storage conditions and to improve the precision and accuracy of DBS card-derived HbA1c levels under field conditions.

Keywords: Analysis delay; Correction method; Dried blood spot cards; HbA1c; IMI DIRECT; NONMEM

ABBREVIATIONS

WB: Whole Blood; DBS: Dried Blood Spot; fsOGTT: Frequently Sampled Oral Glucose Tolerance Test; IMI: Innovative Medicines Initiative; DIRECT: Diabetes Research on Patient Stratification; HPLC: High-Performance Liquid Chromatography; SAEM: Stochastic Approximation Expectation Methods; IMP: Monte Carlo Importance Sampling; OFV: Objective Function Value; AIC: Akaike Information Criterion; AUC: Area under the Curve; FPG: Fasting Plasma Glucose

BACKGROUND AND OBJECTIVE

HbA1c is a well-established biomarker in diabetes mellitus and reflects long-term (4-6 weeks) blood glucose concentrations [1]. The use of HbA1c as a diagnostic measure is part of the "Standards of Care" by the American Diabetes Association based on the recommendations of the International Expert Committee [2]. Further, HbA1c is used as a longitudinal marker to observe disease progression and to evaluate the success of therapeutic intervention [3]. Traditionally, HbA1c is measured using Whole Blood (WB) samples taken by venipuncture. Compared to alternative blood sampling methods, the collection of blood by venipuncture is more expensive and associated with greater participant burden and logistic challenges regarding sample handling and processing [4]. One alternative blood sampling method is to use a dry matrix where a very small volume of blood obtained from finger puncturing is put on a matrix paper [5]. HbA1c can be measured from these Dried Blood Spots (DBS). Assessments using DBS cards are cheaper, safer and more acceptable to study participants than WB sampling [6]. Because of these advantages, the DBS method is highly appealing in research settings. This is especially true in longitudinal or large population-based studies with repeated HbA1c measurements, as the DBS approach can help reduce costs and minimize inconvenience to participants [7].

The comparability of both sampling methods was recently evaluated in a meta-analysis of seventeen heterogeneous studies [8] and DBS validity has been shown under normalized sample collection, transportation and storage settings. Only a few studies focused on the influence of DBS storage conditions, i.e. specific

temperatures or storage times, on the resulting HbA1c measure [9-12]. It was shown that the accuracy and precision to assess HbA1c from DBS cards stored for more than seven days at room temperature is compromised. So far, there is a lack of knowledge about the stability of DBS card-assessed HbA1c under varying conditions that include long-term storage at variable temperatures.

Within the IMI (Innovative Medicines Initiative) DIRECT (Diabetes Research on Patient Stratification) study, more than 2,000 people at risk of developing diabetes were recruited and in total about 14,000 HbA1c values were collected by DBS cards every 4.5 months during an observation period of 48 months to monitor the individual disease progression [13]. As the collection of the DBS cards varied regarding the storage time and condition, this cohort provides a good opportunity to assess the impact of storage conditions on measurement validity. The aim of this analysis was to investigate the effect of long-term storage at different conditions on the reliability of HbA1c levels obtained from DBS cards that were routinely collected in the IMI DIRECT study. Further, the impact of analysis delay was quantified using a mathematical model to correct for storage time. To investigate the predictive performance of the correction method, a comparison of the corrected and the reported HbA1c levels from DBS cards versus HbA1c levels derived from WB analysis, as well as of the HbA1c levels versus other biomarkers for glycaemic control obtained from a Frequently Sampled Oral Glucose Tolerance Test (fsOGTT), was evaluated.

RESEARCH DESIGN AND METHODS

Study Design

This analysis was performed on longitudinal, repeated HbA1c measurements in non-diabetic participants that engaged in a study within the IMI DIRECT consortium. A detailed study description has been published previously [13]. People at risk of developing diabetes were recruited at four different data collection centers (A-D) [13,14]. To study disease progression, DBS cards were used to obtain HbA1c measurements and further, beta cell function and insulin sensitivity were determined using 75 g fsOGTTs at months 0, 18 and 48. For the fsOGTTs, study participants were called into their respective study



center A-D. In addition to the fsOGTT samples, fasting blood samples from each patient were taken and immediately stored at -80°C . At the time of our analysis, data from 0 up to 18 months were available and used for model development.

Bioanalytics

At the beginning of the study, each participant and the clinic staff were instructed how to handle the DBS cards according to the internal study protocol: two blood spots were put on the provided filter paper and kept at room temperature for drying for at least 2 hours before packing them in sealed plastic bags. The participants were asked to store the bags at room temperature and send them immediately to their local study center. From there, the cards were shipped to center A within three days for analysis. All shipments from center B, C and D to center A were performed by regular mail resulting in variable timing and storage conditions. Moreover, details concerning sample collection, processing and storage of DBS cards before shipment to center A differed between the centers; many cards were shipped with longer delays than recommended in the study protocol due to numerous logistical barriers.

Only at center A were all participants invited to have their DBS samples taken by a nurse at the clinical site. The DBS cards were registered, dried, immediately stored at -20°C and analyzed within one week.

At center B, participants took their DBS at home and sent them to the study center to be collected and stored at room temperature before being shipped to center A for analysis. Shipment was done every second week but, because samples were collected continuously, storage time at room temperature at center B varied between one to fourteen days.

At center C, some participants came to the center for DBS sampling; others took their samples at home and sent them to the center. The samples were stored at room temperature until shipment to center A for analysis. Usually the DBS cards were shipped on the day of receipt from the patients. When same-day shipment was not possible, the DBS cards were stored at $4-8^{\circ}\text{C}$. Shipment from center C to center A took approximately three to seven days.

At center D, DBS samples were collected at home by the participants and hand-delivered or sent by mail to the study center with variable delay (range from one day to more than a week). At the study center, the DBS cards were stored at 4°C before being sent in batches to center A on a monthly basis.

WB samples were used to measure HbA1c for the purposes of this DBS validation study. The analysis of WB samples was undertaken at center E (no data were collected here). The WB samples were assumed to represent the true HbA1c levels for the two available time points (month 0 and 18). The analysis of all WB samples was performed using High-Performance Liquid Chromatography (HPLC) (Tosoh G8 HPLC Analyzer) [15]. The Tosoh G8 HPLC Analyzer utilizes Standard Ion-Exchange method of HbA1c measurement. The HbA1c determination derived from DBS cards was performed at center A using the immunoturbidimetric assay on Konelab 20XT Clinical Chemistry analyzer, both from Thermo Fisher Scientific. The validation of the DBS as sample material was performed at center A. The analytical total Coefficients of Variation (CV %) for DBS were 5.3-6.5%. The concordance between both assays has been investigated elsewhere [16], where WB samples were analyzed using the HPLC method and compared to DBS cards analyzed with HPLC, as well as the immunoturbidimetry method.

Data analysis

The impact of the analysis delay on HbA1c inflation was investigated with a non-linear regression analysis using the software NONMEM (V. 7.3, ICON Development Solutions, Ellicott City, MD, USA) with the graphical user interface Pirana (V. 2.9.5). Throughout the analysis, the Stochastic Approximation Expectation Methods (SAEM) algorithm with the interaction option, followed by a step of Monte Carlo Importance Sampling (IMP) algorithm was used. Model selection was based on several criteria such as the changes in the NONMEM Objective Function Value (OFV) [17], goodness-of-fit plots, and the precision of parameter estimation [18].

The decrease of the OFV by 3.84 points for the addition of 1 parameter (chi-square, $p < 0.05$ with 1 degree of freedom) was considered as statistically significant between two nested models [19]. For non-nested models, the Akaike Information Criterion (AIC) was computed to determine if one model was superior to the other. In the current analysis, AIC was defined as $\text{OFV} + 2 \times \text{number of parameters}$ [20].

The model building process was performed in a stepwise procedure. First, the baseline model was developed by testing different mathematical functions. A linear, E_{max} with and without Hill factor, and several exponential functions were tested to describe the relationship between measured HbA1c levels and analysis delay. Further, a log transformation of analysis delay was tested to account for the non-normal distribution of analysis delay. In a second step, the center was tested as a covariate.

SAS (V. 9.4) was used for dataset preparation. Graphical visualization of NONMEM results was performed with R (V. 3.2.5) and the graphical user interface RStudio (V. 1.0.44).

To validate the correction method, WB-derived HbA1c levels were used. Bland-Altman plots [21] and regression analysis were performed to compare reported and corrected HbA1c from DBS cards with HbA1c from WB samples. For further validation, glucose exposure, reflected by the Area under the Curve (AUC) of the glucose concentration-time profile obtained from the fsOGTT, as well as the Fasting Plasma Glucose (FPG) and 2h-glucose, were employed. Pearson correlation coefficients between these glucose related biomarkers and HbA1c levels were calculated; paired significance tests for correlation differences were computed and used for model evaluation [22].

RESULTS

Dataset

Overall, 2,237 participants fulfilled the inclusion criteria for the IMI DIRECT prediabetes cohort [13], 76% of whom were male. The median age was 62 years (range from 30 to 75 years) at enrollment and the median weight was 84.2 kg (range from 43.0 to 152 kg). 1,275 participants were enrolled at center A, 332 at center B, 147 at center C and 493 at center D. In total, 14,243 HbA1c values obtained from DBS cards were available and used for model development. The validation dataset consisted of 4,272 HbA1c measurements derived from WB samples, 2222 from the fsOGTT performed at month 0, and 2050 at month 18, respectively. The mean HbA1c derived from WB is $5.6 \pm 0.29\%$ (38 ± 3.2 mmol/mol); from DBS, $5.8 \pm 0.40\%$ (40.0 ± 4.4 mmol/mol). A summary of the characteristics of the key variables of the IMI DIRECT cohort is already described elsewhere [23].

Analysis delay was calculated as the time (in days) between DBS



sampling and the DBS assay date. The overall analysis delay in the dataset used for model development ranged from 0 to more than 400 days. Only 10.7% of the samples was analyzed within one day after sample collection and 50.4% within one week. Approximately 90% of the samples were analyzed within a timespan of four weeks. Less than 0.2% of the DBS cards were stored for more than 12 weeks (Figure 1). Detailed information on temperature variation during this time was unavailable. Supplementary figure S1 shows the relationship between analysis delay and reported HbA1c, restricted to a delay of 100 days.

Data analysis

An E_{max} model with Hill factor best described the relationship between analysis delay and inflated HbA1c levels (Equation 1), reflected by the lowest AIC value compared to other tested structural models. The E_{max} value was estimated for each study center separately to account for differences in DBS card storage conditions. The inclusion of the study center as a covariate was significant ($p = 7.86 \times 10^{-211}$). The maximal inflation of HbA1c reflected by the E_{max} value in center A was small compared with the other three centers (0.411 [mmol/mol] compared to 10.9 [mmol/mol], 7.40 [mmol/mol] and 6.76 [mmol/mol] for centers B, C and D, respectively). The EC50 reflects the analysis time in days that is related to a half maximum inflation. A log transformation of the analysis delay had no benefit on the correct method, so it was reject in the final model. The observed HbA1c levels and the center-specific E_{max} functions versus the analysis delay are shown in figure 2; parameter estimates are presented in table 1.

Equation 1:

$$HbA1c_{reported} = HbA1c_{at\ sampling\ date} + \frac{E_{max(centre)} * analysis\ delay_{DBS\ card}^{Hill}}{EC50^{Hill} + analysis\ delay_{DBS\ card}^{Hill}}$$

To correct reported HbA1c levels, the center-specific E_{max} function was shifted in parallel along the y axis to intersect the reported HbA1c level. The new intercept of the y axis and the shifted E_{max} function was noted as the corrected HbA1c level and can be calculated using equation 2.

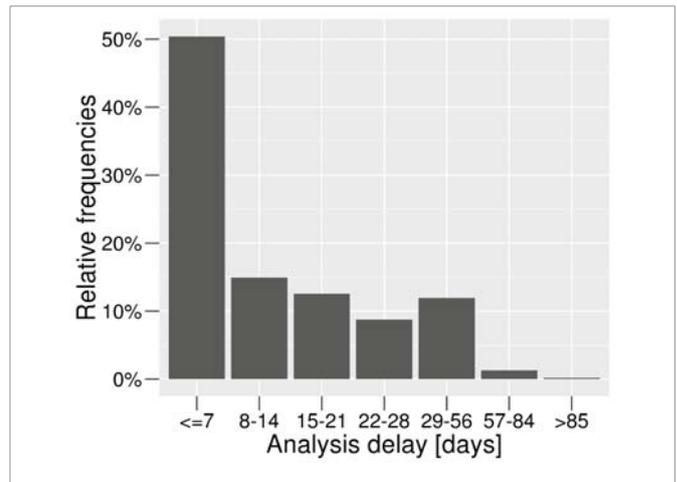


Figure 1: Histogram of the relative frequencies of analysis delay for the Dried Blood Spot (DBS) cards. More than 50% of all DBS cards were analysed in the first week after sampling, while 1.4% were stored for more than 8 weeks.

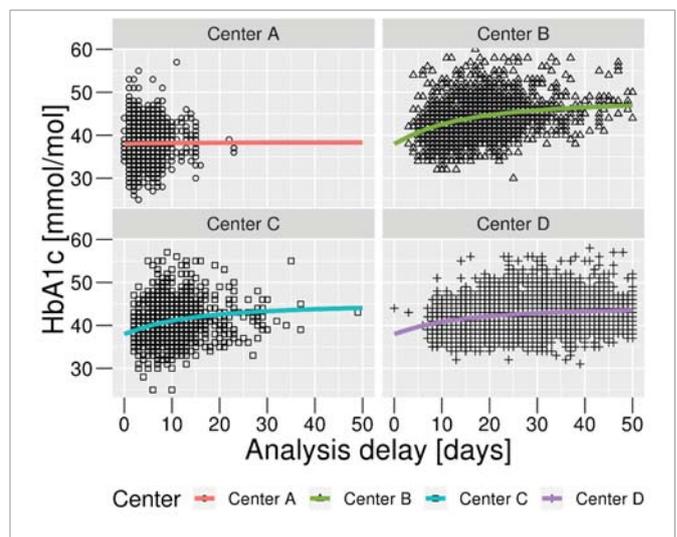


Figure 2: Center-specific E_{max} functions to describe the relationship between HbA1c obtained from Dried Blood Spots (DBS) cards and analysis delay. The points, triangles, squares and crosses indicate the reported HbA1c derived from DBS cards of center A, B, C and D, respectively. The colored lines indicate the model prediction for each center (center specific E_{max} function).

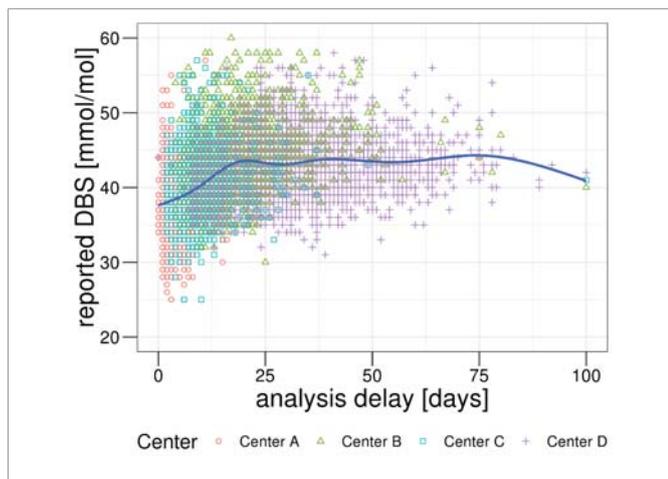


Figure S1: Reported HbA1c levels obtained from the Dried Blood Spot (DBS) cards [mmol/mol] vs. analysis delay (in days) restricted to a delay of 100 days. The blue line indicates a trend line, the shape and color represents the four study centers. The red circles indicate center A, where most of the DBS cards were analysed within 14 days. The green triangles indicate center B, the blue squares and the purple crosses center C and D, respectively.

Table 1: Parameter estimates of the correction model.		
Parameter	Value (RSE[%])*	Description
Model parameters		
Intercept [mmol/mol]	38.0 (0.2)	Mean HbA1c at sampling date
E_{max} (Center A) [mmol/mol]	0.411 (85)	Maximal HbA1c increase of Center A
E_{max} (Center B) [mmol/mol]	10.9 (6)	Maximal HbA1c increase of Center B
E_{max} (Center C) [mmol/mol]	7.40 (7)	Maximal HbA1c increase of Center C
E_{max} (Center D) [mmol/mol]	6.76 (6)	Maximal HbA1c increase of Center D
EC50 [days]	13.4 (11)	Analysis delay of half-maximum HbA1c increase
Hill	1.15 (9)	Hill factor
Variability		
PRV [CV%]	8.8 (0.6)	proportional residual variability
* residual standard error		



Equation 2:

$$HbA1c_{corrected} = HbA1c_{reported} \cdot \frac{E_{max(centre)} * analysis_delay^{Hill}}{EC50^{Hill} + analysis_delay^{Hill}}$$

To validate our correction method, the corrected HbA1c, as well as the reported HbA1c levels from DBS cards, were compared to HbA1c levels derived from WB samples. Mean HbA1c levels derived from DBS cards were $5.8 \pm 0.40\%$ (40.4 ± 4.39 [mmol/mol]) before and $5.6 \pm 0.33\%$ (38.0 ± 3.59 [mmol/mol]) after correction. The mean HbA1c derived from WB was $5.6 \pm 0.29\%$ (37.6 ± 3.17 [mmol/mol]).

Figure 3 shows the reported and corrected HbA1c values from DBS cards vs. WB, split into two groups: analysis delay \leq seven days (within the stability window according to literature), and greater than seven days (outside stability window) [9]. Samples with an analysis delay outside the stability window were corrected appropriately, the corrected HbA1c levels being spread more evenly around the line of identity. Correlation between corrected HbA1c levels from DBS and WB sampling were stronger compared to the reported ones (Pearson correlation coefficient of 0.61 and 0.69 for reported and corrected vs. WB, respectively, $p = 5.92 \times 10^{-36}$). Furthermore, Bland-Altman plots were examined to check for bias in the correction method. After correction, HbA1c levels had significantly better concordance with WB sample results compared to uncorrected HbA1c DBS values (p -value $< 2.2 \times 10^{-16}$). The mean difference between HbA1c from DBS cards and WB for all samples was -0.2% (-2.16 [mmol/mol]) before, and -0.03% (-0.32 [mmol/mol]) after correction. Figure 4 shows the Bland-Altman plots for each center before and after correction.

Using the HbA1c levels obtained from WB samples as a diagnostic marker for prediabetes ($HbA1c \leq 48$ [mmol/mol] and ≥ 40 [mmol/mol]) or diabetes ($HbA1c > 48$ [mmol/mol]), 24% of the samples would be associated with prediabetes and 0.4% with diabetes. Using the reported or the corrected DBS samples, 45% or 23% of the samples results in diagnosis of pre-diabetes and 4.6% and 0.2% in a diagnosis of diabetes, respectively. Using the reported DBS,

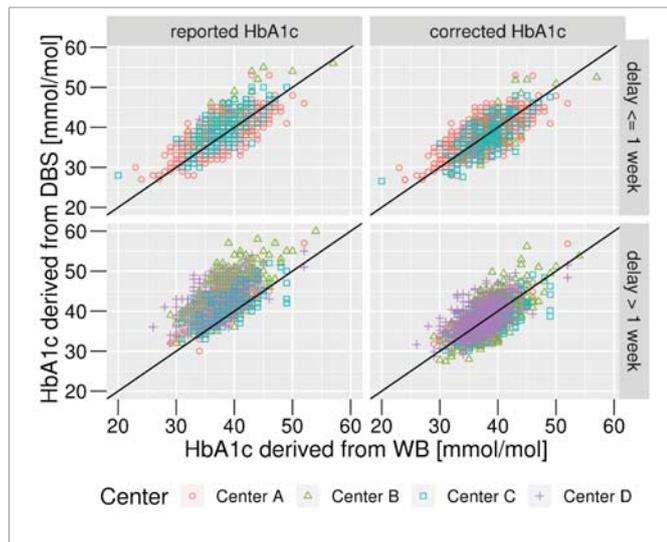


Figure 3: HbA1c levels derived from Dried Blood Spot (DBS) cards (left: reported levels, right: corrected levels) vs. Whole Blood (WB) samples regarding the analysis delay within (top) and outside (bottom) the stability window of one week reported in literature. Each point, triangle, square and cross indicates one observation from one of the four centers. Black lines are lines of identity.

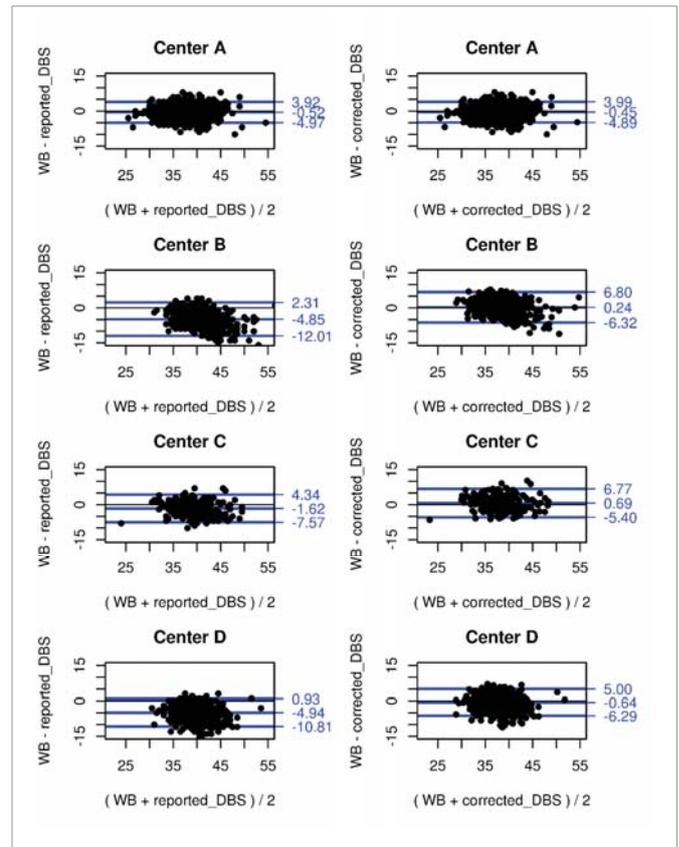


Figure 4: Bland-Altman plots: differences between Whole Blood (WB) HbA1c [mmol/mol] and Dried Blood Spot (DBS) derived HbA1c vs. the mean of the two measurements. The bias between the two methods is represented by the gap between the X axis, corresponding to zero difference in black, and the parallel blue line to the X axis. The middle blue line represents the mean difference between the two assays. The upper and lower blue lines represent the agreement limits ($1.96 \times$ standard deviation). The columns show HbA1c derived from DBS cards, as reported values (reported_DBS, left) and corrected values (corrected_DBS, right). Data are shown separately for each center (A-D) in the panels.

27% of the samples results in a false positive diagnosis of pre-diabetes and 4.3% of diabetes. After the correction the false positive rate for diagnosis prediabetes or diabetes is reduced to 0.9% (pre-diabetes) and 0.4% (diabetes).

Further, all HbA1c levels were compared to biomarkers obtained from the fsOGTT. Supplementary figure S2 illustrates a significantly stronger correlation between corrected HbA1c and the AUC of glucose compared to reported HbA1c ($r^2_{uncorrected} = 0.274$, $r^2_{corrected} = 0.351$, $p = 1.61 \times 10^{-19}$). The relationship between the AUC of glucose compared to WB for FPG, the correlation coefficient also increased after correction ($r^2_{uncorrected} = 0.248$, $r^2_{corrected} = 0.342$, $p = 1.42 \times 10^{-28}$). Corrected HbA1c and WB showed a similar relationship to 2h-glucose ($r^2_{WB} = 0.229$, $r^2_{corrected} = 0.229$), FPG ($r^2_{WB} = 0.348$, $r^2_{corrected} = 0.341$) and AUC ($r^2_{WB} = 0.358$, $r^2_{corrected} = 0.351$); statistically, there were no differences between correlation coefficients ($p > 0.30$).

DISCUSSION

In this study, we observed a significant increase in HbA1c levels with an increasing analysis delay of the DBS cards under real-life conditions, a topic that has not been investigated previously. The IMI DIRECT study was not explicitly designed to address this research question; however, the huge amount of data collected in IMI DIRECT

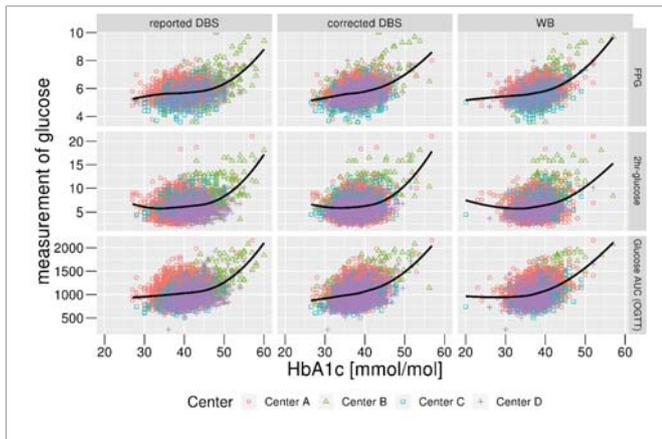


Figure S2: Correlation analysis between HbA1c measurements (reported and corrected HbA1c derived from Dried Blood Spot (DBS) cards and HbA1c derived from Whole Blood (WB)) and fsOGTT related glucose measurements (Fasting Plasma Glucose (FPG), 2h-glucose, Area Under the Curve (AUC)). Each point, triangle, square and cross indicate one observation from one of the four centers. Black lines represent the LOESS regression analysis.

provides a unique possibility to investigate and quantify the impact of up to 400 days of storage.

In accordance with the published literature, DBS cards immediately stored at -20°C showed a negligible influence of storage time on the resulting HbA1c levels [11]. In our study at center A, the DBS samples were handled in this manner and confirmed this finding. A maximum inflation of 0.04% (0.411 [mmol/mol]) during storage is usually not considered as clinically relevant. However, some of the HbA1c values obtained from DBS cards collected at study centers B-D were highly inflated, due to extended storage time at variable storage temperature.

With the exception of the study by Buxton et al., which investigated the stability of DBS cards stored in the freezer for at least three months [11], most of the published stability studies investigated a shorter analysis delay and smaller sample size compared to our analyses. Nevertheless, short-term studies indicate that inflation of HbA1c values is directly related with storage time [9,24].

Fokkema et al. investigated the stability of DBS cards over ten days at room temperature and reported an increase in HbA1c of 0.4% (from 7.2% (55.0 [mmol/mol], day 0) to 7.6% (60.0 [mmol/mol], day 10) [10]. In center B, where cards were stored at room temperature for up to 14 days, our correction method would predict a HbA1c of 7.6% (59.5 [mmol/mol]) assuming an HbA1c level of 7.2% at sampling date and a storage of ten days. Thus, the degree of inflation attributable to extended storage time and variable temperature of our correction method concurs with previous findings.

In addition to the comparison to WB-derived HbA1c levels, we compared our corrected HbA1c levels with glucose related biomarkers obtained from fsOGTTs. We detected a statistically stronger correlation between FPG and AUC of the glucose concentration-time profile during the fsOGTT and the corrected HbA1c, in contrast to the reported value. The correlation coefficients after correction are not statistically different from those of WB and the glucose related biomarkers additionally supporting the validity of the presented correction method.

The relationship between analysis delay and HbA1c inflation is described by an E_{\max} model that could possibly be explained by the

mechanism of HbA1c formation. Free haemoglobin irreversibly reacts with glucose in a non-enzymatic reaction to form HbA1c [25]. Under ex-vivo conditions, the higher the temperature, the easier the formation of HbA1c in the stored blood sample [26]. Limited by decreasing concentrations of the two reactants over time, an E_{\max} model for the description of the change in HbA1c in relation to the analysis delay appropriately captures the reaction. To account for handling and storage temperatures at the four different study centers, specific E_{\max} values reflected the different conditions. The estimated E_{\max} values were increasing with increasing storage temperatures; storage at -20°C (center A) is related to a small maximal effect of inflation (0.411 mmol/mol); storage at 4°C (center D) as well as $4-8^{\circ}\text{C}$ (center C) is related to a maximal increase of 6.76 mmol/mol and 7.40 mmol/mol, respectively; and, storage at room temperature for up to two weeks (center B) had the highest influence on HbA1c, with a maximum effect of 10.9 mmol/mol.

The storage conditions of our DBS samples varied across the four study centers. Within-center specific details of storage conditions for each DBS card were not available. Our correction method uses analysis delay as a predictor for HbA1c inflation during storage. For evaluation of this multicenter study, we have to consider that all DBS samples had to be shipped to center A, where DBS cards were analyzed immediately or frozen at -20°C . The temperature during shipment was assumed to be close to room temperature. As the specific conditions are not known for all samples, our correction method provides an approximation. Precise information about temperature fluctuations during storage, the glucose and haemoglobin concentrations in the sample, as well as humidity levels, all could in theory improve model performance further.

The IMI DIRECT study was conducted to investigate disease progression and not the effect of long-term storage on DBS. Nevertheless, even with this caveat, our correction method appropriately corrects for storage temperature and analysis delay. It remains to be seen whether corrected HbA1c levels can help identify progression subgroups and new biomarkers within the IMI DIRECT cohort.

For other researchers to apply our correction method, it might be necessary to adjust the model parameters to their specific study conditions. For example, the E_{\max} value is expected to be correlated to storage temperature; the higher the temperature, the higher the E_{\max} value. Furthermore, baseline HbA1c levels might also have an impact on the E_{\max} value, when a broader range of HbA1c levels is considered. In our case, participants at risk of developing diabetes were studied. We hypothesize a negative relation between HbA1c baseline and the possibility of glycation of the not-yet-glycated haemoglobin. If the overall HbA1c baseline is low, an increase in HbA1c due to the high amount of not yet glycated hemoglobin in the samples could be more likely to be observed. However one can also argue that with high HbA1c baseline, plasma glucose might also be higher, increasing the potential for the glycation of proteins. Such dependent relationships should be tested and adjusted for, whenever appropriate.

CONCLUSION

Our study shows that analysis delay and non-ideal storage conditions of DBS samples have a significant impact on the inflation of the resulting HbA1c value. Our developed correction method, however, seems to adequately adjust for HbA1c instability in such instances. Storage conditions of DBS cards should be carefully monitored and controlled, ideally at -20°C ; however, whenever this



is not possible, our correction method can be used to adjust for the attributable error.

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