Herman Veenhof, Remco A. Koster, Jan-Willem C. Alffenaar, Aad P. van den Berg, Marco R. de Groot, Erik A.M. Verschuuren, Stefan P. Berger, Stephan J.L. Bakker and Daan J. Touw*

Clinical application of a dried blood spot assay for sirolimus and everolimus in transplant patients

https://doi.org/10.1515/cclm-2019-0053
Received January 15, 2019; accepted March 8, 2019; previously published online April 2, 2019

Abstract

Background: Monitoring of immunosuppressive drugs such as everolimus and sirolimus is important in allograft rejection prevention in transplant patients. Dried blood spots (DBS) sampling gives patients the opportunity to sample a drop of blood from a fingerprick at home, which can be sent to the laboratory by mail.

Methods: A total of 39 sirolimus and 44 everolimus paired fingerprick DBS and whole blood (WB) samples were obtained from 60 adult transplant patients for method comparison using Passing-Bablok regression. Bias was assessed using Bland-Altman. Two validation limits were pre-defined: limits of analytical acceptance were set at >67% of all paired samples with 20% of the mean of both samples and limits of clinical relevance were set in a multidisciplinary team at >80% of all paired samples within 15% of the mean of both samples.

Results: For both sirolimus and everolimus, Passing-Bablok regression showed no differences between WB and DBS with slopes of 0.86 (95% CI slope, 0.72–1.02) and 0.96 (95% CI 0.84–1.06), respectively. Only everolimus showed a significant constant bias of 4%. For both sirolimus and everolimus, limits of analytical acceptance were met (76.9% and 81.8%, respectively), but limits or clinical relevance were not met (77.3% and 61.5%, respectively).

Conclusions: Because pre-defined limits of clinical relevance were not met, this DBS sampling method for sirolimus and everolimus cannot replace WB sampling in our center at this time. However, if the clinical setting is compatible with less strict limits for clinical relevance, this DBS method is suitable for clinical application.

Keywords: dried blood spots; immunosuppressants; microsampling; validation.

Introduction

Lifelong therapy with immunosuppressive drugs is a cornerstone in the prevention of rejection of allografts in transplant patient care [1]. Because of their narrow therapeutic range, many immunosuppressive drugs, including the mammalian target of rapamycin inhibitors everolimus and sirolimus are subject to therapeutic drug monitoring (TDM) to allow for balancing between toxic- and sub-therapeutic drug concentrations. Tacrolimus is currently the most widely used calcineurin inhibitor in kidney transplant patient care [2]. However, the recent TRANSFORM trial suggests efficacy of maintenance therapy with everolimus in combination with low dose tacrolimus is comparable to a standard regimen of tacrolimus and mycophenolate mofetil [3, 4]. An additional advantage is the reduced viral infection risk. This might lead to an increase in everolimus use in transplant patients.

Traditionally, venous blood samples are used for monitoring of immunosuppressive drug concentrations and patients have to travel to the hospital on a regular basis to have their blood drawn. To decrease the burden
for patients, dried blood spot (DBS) home sampling has been developed among various micro sampling methods for several drugs, including immunosuppressants, to enable home sampling [5–16]. For this, a drop of blood from a fingerprick is applied to a sampling card and dried. This card is sent to the laboratory by mail a few days prior to routine check-up of the patient in the hospital. At the time of the check-up, blood-drug concentrations and creatinine levels will be available for the clinician and the patient. Current challenges of DBS implementation include the influence of the hematocrit and logistical hurdles [9, 13, 17, 18]. Although DBS analytical methods can meet the required analytical standards, analysis of clinically collected samples does not always result in sufficient agreement between the standard (venous) method and the novel fingerprick DBS method [17]. Therefore, a clinical validation study showing interchangeability between DBS and venous sampling is required before clinical application [18]. This is shown for tacrolimus, cyclosporin A and creatinine [5, 7–15, 19]. For sirolimus, Dickerson et al. report agreement between fingerprick DBS and venous samples in 25 pediatric transplant patients, where mean DBS concentrations were on average 0.8 μg/L lower than venous samples [15]. This difference between the two methods increased with increasing concentrations of sirolimus. Willemse et al. reported agreement between everolimus fingerprick DBS and venous samples in 20 patients with cancer with a mean ratio of whole blood (WB) to DBS concentrations of 0.90 [20]. The current Clinical and Laboratory Standards Institute (CLSI) guideline suggests at least 40 paired samples for comparison, therefore, the number of samples collected in both studies for cross-validation was low [21]. In addition, no clinical validation study for everolimus using fingerprick DBS has been published for transplant patients. Therefore, the aim of this study was to clinically validate our method for analyzing sirolimus and everolimus in DBS to enable implementation in routine care.

Materials and methods

Patients and sample collection

Patient samples were collected from adult transplant patients during routine clinical check-ups in the hospital. Because of the nature of this study, the need to provide written informed consent by the patients was waived by the Ethics Committee of the University Medical Center Groningen (Metc 2011.394). A trained phlebotomist obtained both the venous and DBS samples within 10 min of each other using a collection method described elsewhere [9, 22, 23]. In short, after a fingerprick, two drops of blood were allowed to fall freely on a Whatman FTA DMPK-C sampling paper (GE Healthcare, Chicago, IL, USA). The WB samples were analyzed within a day as they were part of routine care. DBS are stable for at least 7 days at room temperature, therefore the DBS samples were allowed to dry for 24–74 h at room temperature and packed in zip lock plastic mini bags with a desiccant [24–26]. Upon receiving the DBS samples in the laboratory, the samples were inspected for spot quality based on predefined criteria [22, 23, 27]. DBS samples fit for analysis were stored at −20 °C until analysis. DBS samples are stable for at least 29 weeks at −20 °C so analysis occurred within this timeframe [25].

Equipment, conditions and procedures

Our reference procedure was a measurement of sirolimus and everolimus in WB obtained by venipuncture, with a previously validated analysis method performed on a Thermo Fisher Scientific (Waltham, MA, USA) triple quadrupole Quantiva LC-MS/MS system with a Vanquish HPLC system [28]. For the DBS analysis of sirolimus and everolimus, a previously validated method was used, which was the aforementioned Thermo Quantiva LC-MS/MS [26, 25]. The analytical range for both the WB and DBS assay for sirolimus and everolimus was 1.0–50.0 μg/L.

Hematocrit of the venous samples was measured using an XN10/XN20 hematology analyzer (Sysmex, Kobe, Japan).

Statistical analysis

Statistical analysis was performed using Analyse-it® Method Validation Edition for Microsoft Excel version 4.18.6 (Analyse-it, Leeds, UK) and Microsoft Excel (Microsoft Inc., Redmond, WA, USA). Method comparison was done using Passing-Bablok regression analysis and a Bland-Altman analysis was used for bias calculation [29, 30]. Because no official guideline exists for clinical validation of DBS assays, we set two limits of acceptance a priori. The first is the limit of analytical acceptance which is based on the EMA guidelines for cross-validation and the 2018 version of the FDA guideline for studies required to bridge two analytical methods [31, 32]. As acceptance criteria, both FDA and EMA guidelines state that at least two-thirds (67%) of the paired samples should be ±20% of the mean of both methods. The second is the limit of clinical relevance which was set at a range of 85%–115% around the ratio of the paired DBS and WB samples for at least 80% of the samples. This range was chosen by a multidisciplinary team consisting of clinicians, pharmacists and analysts and was chosen based on the therapeutic window given in the summary of product characteristics of 3–8 μg/L for everolimus and 4–12 μg/L for sirolimus trough concentrations for stable transplant patients >3 months after transplantation [33, 34]. A difference of 15% in the acceptable range ratio for a high everolimus trough concentration (8 μg/L) in WB would lead to a DBS concentration range of 6.8–9.2 μg/L. For a low everolimus trough concentration (3 μg/L) in WB this would lead to an acceptable DBS concentration range of 2.6–3.5 μg/L. These values are comparable to the acceptable variability of 15% for accuracy and precision that are mentioned in the FDA and EMA guidelines for bioanalytical methods [31, 32]. If 80% of all patients are within this range this was deemed feasible by the clinicians.
The predictive performance of the DBS analytical method was established using the method described by Sheiner and Beal [35]. In short, DBS concentrations were used to predict WB concentrations. For each paired WB and DBS sirolimus and everolimus sample, the slope and intercept of the Passing-Bablok regression was calculated using the whole population of sirolimus and everolimus samples, respectively, excluding the data of that specific paired sample. The error of this prediction is determined by bias and imprecision. The bias is the median difference between the predicted and true concentration and is shown by the median prediction error (MPE) and the median percentage prediction error (MPPE). The imprecision is the variance of the predicted values which is measured by the root median squared prediction error (RMSE) and the median absolute percentage prediction error (MAPE). For analyzing the predictive performance the following equations were used:

\[
\text{median prediction error (MPE)} = \text{median (predicted WB – WB)} \quad (1)
\]

\[
\text{median percentage prediction error (MPPE)} = \text{median} \left( \frac{100\% \times |\text{predicted WB – WB}|}{\text{WB}} \right) \quad (2)
\]

\[
\text{root median squared prediction error (RMSE)} = \sqrt{\text{median (predicted WB – WB)}^2} \quad (3)
\]

\[
\text{median absolute percentage prediction error (MAPE)} = \text{median} \left( \frac{100\% \times |\text{predicted WB – WB}|}{\text{WB}} \right) \quad (4)
\]

In accordance with other studies, acceptable values for MPPE and MAPE were set at <15% and at least 67% of all samples should have an absolute prediction error of <20% [5, 20].

**Results**

**Patients and samples**

A total of 90 paired DBS and WB samples were taken from 60 adult transplant patients between January 2017 and December 2017. All DBS cards had at least one spot of sufficient quality for analysis. Three samples were excluded because no paired WB sample was taken. Another three samples were excluded because the WB and DBS sample were not taken within 10 min of each other. One sample was excluded because it was not a trough concentration. A total of 39 paired sirolimus and 44 paired everolimus samples were available for method comparison from 29 and 27 unique transplant patients, respectively. The hematocrit ranged from 0.23 to 0.51 (v/v) with a mean hematocrit of 0.40. All hematocrit values were within the analytically validated range, which means that the hematocrit value had no influence on the DBS analytical results [24]. Mean concentrations of sirolimus and everolimus in WB and DBS can be found in Table 1. All evaluated concentrations were within the analytically validated range [24]. Patient demographics and transplantation type can be found in Tables 2 and 3.

**Clinical validation**

**Sirolimus**

For sirolimus, the Passing-Bablok analysis fit was \( y = 0.86x + 0.44 \) (95% CI slope, 0.72–1.02; 95% CI intercept –0.23 to 1.11) showing no significant constant or systematic difference as can be seen in Figure 1. The correlation coefficient was 0.93. The Bland-Altman plot (Figure 2) shows that the mean ratio of WB and DBS sirolimus concentrations is 1.00 (95% CI 0.93–1.07), without significant bias. The 95% limits of agreement (LoA) are 0.60 and 1.40, which is wider than the limits of analytical acceptance which were set at 0.80 and 1.20. Nine out of 39 values

**Table 1:** Mean drug concentrations, range and SD of sirolimus and everolimus in WB and DBS.

<table>
<thead>
<tr>
<th>Drug concentrations</th>
<th>n</th>
<th>Mean ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirolimus in WB, µg/L</td>
<td>39</td>
<td>5.0 ± 2.4 (1.9–10.9)</td>
</tr>
<tr>
<td>Sirolimus in DBS, µg/L</td>
<td>39</td>
<td>4.7 ± 1.9 (1.8–9.7)</td>
</tr>
<tr>
<td>Everolimus in WB, µg/L</td>
<td>44</td>
<td>5.4 ± 2.6 (1.2–14.3)</td>
</tr>
<tr>
<td>Everolimus in DBS, µg/L</td>
<td>44</td>
<td>5.0 ± 2.4 (1.9–10.9)</td>
</tr>
</tbody>
</table>

DBS, dried blood spot; SD, standard deviation; WB, whole blood.

**Table 2:** Patient demographics and transplantation type.

<table>
<thead>
<tr>
<th>Patient demographics and clinical laboratory data</th>
<th>n</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>56</td>
<td>61 (23–77)</td>
</tr>
<tr>
<td>Sex</td>
<td>56</td>
<td>38 male (67.9%), 18 female (32.1%)</td>
</tr>
<tr>
<td>Time from transplantation</td>
<td>56</td>
<td>2 years, 3 months, 5 days (10 days–22 years, 7 months)</td>
</tr>
</tbody>
</table>

**Table 3:** Patient transplantation type per sample type.

<table>
<thead>
<tr>
<th>Transplantation type</th>
<th>Sirolimus samples</th>
<th>Everolimus samples</th>
<th>Total samples</th>
<th>Unique patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>30</td>
<td>0</td>
<td>30</td>
<td>22</td>
</tr>
<tr>
<td>Lung</td>
<td>2</td>
<td>7</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Stem cell</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Kidney</td>
<td>0</td>
<td>37</td>
<td>37</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>44</td>
<td>83</td>
<td>56</td>
</tr>
</tbody>
</table>
(23.1%) fell outside the limits of analytical acceptance. For the limits of clinical relevance this was 15/39 (38.5%). For the predictive performance, bias was small with an MPE of $-0.008 \mu g/L$ and an MPPE of $-0.16\%$. The predictive performance of imprecision as measured by the RMSE was small with a value of 0.56 $\mu g/L$. The MAPE was within acceptable limits (<15%) with a value of 11.07%. The acceptance limit for MAPE (at least 67% of the samples with a value <20%) was met with 30 out of 39 values (76.9%) (Figure 3).

**Everolimus**

For everolimus, the Passing-Bablok analysis fit was $y = 0.96x + 0.37$ (95% CI slope, 0.84–1.06; 95% CI intercept −0.11 to 1.11). The dashed line is the 95% CI, also showing no significant constant or systematic difference as can be seen in Figure 4.

![Figure 1: Method comparison for sirolimus concentrations in WB and DBS (n = 39). The continuous line is the Passing-Bablok regression line $y = 0.86x + 0.44$ (95% CI slope, 0.72–1.02; 95% CI intercept −0.23 to 1.11). The dashed line is the 95% CI.](image1)

![Figure 2: Method comparison for sirolimus concentrations in WB and DBS (n = 39). The continuous line is the Bland-Altman bias estimation of 1.00 (95% CI 0.93–1.07). The dashed line is the 95% LoA and the dotted/dashed line is the limit of clinical relevance set at 15%.](image2)

![Figure 3: Percentage prediction error or predicted to measured sirolimus concentrations with acceptable prediction error set at −20% and 20%.](image3)
The correlation coefficient was 0.97. The Bland-Altman plot (Figure 5) shows that the mean ratio of WB and DBS everolimus concentrations is 1.04 (95% CI 1.00–1.08), which is a small but significant bias of 4%. The 95% LoA are 0.78 and 1.30, which is wider than the limits of analytical acceptance which were set at 0.80 and 1.20. Only eight out of 44 values (18.2%) fell outside the limits of analytical acceptance. For the limits of clinical relevance this was 10 out of 44 (22.7%). For the predictive performance, bias was small with an MPE of 0.003 μg/L and an MPPE of 0.13%. The imprecision as measured by the RMSE was small with a value of 0.39 μg/L. The MAPE was within acceptable limits (<15%) with a value of 7.9%. The acceptance limit for MAPE (at least 67% of the samples with a value <20%) was met with 39 out of 44 values (88.6%) (Figure 6). One outlier of −72.5% was observed. The outlier prediction error shown in Figure 6 can likely be explained by the low concentration of everolimus (1.2 μg/L in WB), which is just above the lower limit of quantification of the method. In this setting, the influence of the intercept (−0.49) becomes paramount, resulting in a predicted value of 0.33 μg/L, giving a prediction error of −72.5%.

**Discussion**

This study showed good agreement between DBS sirolimus and everolimus concentrations and venous WB concentrations in transplant patients over a concentration range relevant for TDM of trough concentrations. No correction factor is needed to calculate WB values from DBS concentrations.
values. For sirolimus and everolimus 76.9% and 81.8%, respectively, of all DBS concentrations fell within limits of analytical acceptance. Therefore, this method met the requirements set in the EMA guideline for cross-validation and FDA guidelines for bridging studies [31, 32]. The predictive performance of the sirolimus and everolimus DBS method complied with the predefined criteria of >67% of all samples to have a prediction error of <20%. However, the limits set for clinical relevance (>80% of the samples with <15% of the mean) were not met with a value of 77.3% and 61.5% for sirolimus and everolimus, respectively.

Because tacrolimus is the most widely used immunosuppressant in our center to prevent renal allograft rejection, the amount of patients in our institution receiving either sirolimus or everolimus is limited. Therefore, patients from all transplantation types (Table 3) were asked to provide samples. The heterogeneous patient population is a strength of this study, hematocrit values of all patients were within the analytically validated limits and mean hematocrit values were comparable between the different groups of transplant patients (data not shown).

Because a clinical validation of a DBS fingerprick method shows strong resemblance to a cross-validation, the CLSI guideline recommends to include at least 40 patient samples [21]. Although the study by Willemsen et al. showed good agreement between WB and capillary blood, the performed power calculation resulting in 20 samples necessary was done prior to this result. The power calculation was based on the assumption that venous blood and DBS are the same matrix and no effect of the hematocrit is expected [20]. It is, however, well-known that hematocrit can affect DBS assays and sometimes results in unacceptable biases [24, 25, 36]. Capillary collected blood consists of a mixture of venous blood, arterial blood and interstitial fluid which is not the same matrix as a venous WB sample. Therefore, we think making an assumption that the matrix of capillary blood is the same as venous WB is not recommended. Following the CLSI guideline for finding a sample size would, in our opinion, be more appropriate. The recommendation of 40 samples in the CLSI guideline is based on regression analysis described by Linnet, where the amount of samples necessary for a cross-validation can be calculated based on the analytical characteristics of the assay [37]. If Linnets’ calculation would be followed for the everolimus DBS assay used by Willemsen et al., the recommended number of samples is 40, and if Linnets’ calculation would be followed for the sirolimus DBS assay used by Dickerson et al. the recommended number of samples is 37 [19, 36]. Because of the exclusion of several sirolimus samples the required amount of 40 samples was not met. However, with the amount of 39 paired samples available, we do not think that the absence of one paired sample has a great influence on the clinical validation.

For everolimus, our results are in part in agreement with Willemsen et al. [20]. Our method did not show a constant or proportional bias as shown by Willemsen et al. where a small but significant proportional bias was found in the Passing-Bablok regression. In addition, they demonstrated a ratio of 0.90 in the Bland-Altman comparison, where our method shows a small but statistically significant ratio of 1.04. It should be noted that the Bland-Altman comparison by Willemsen et al. is shown as a ratio of WB/DBS which is in contrast with this study where the ratio is shown as DBS/WB. However, the spread of the relative difference in our method (Figure 5) and corresponding LoAs are wider than in the method used by Willemsen et al. This is especially true for the low trough concentrations (1–5 μg/L). Although not statistically significant, the analytical validation showed a trend towards more bias at lower concentrations (3 μg/L) compared to higher concentrations (10 μg/L) for everolimus [24]. This might be an explanation for the observed spread of relative difference. Other clinical validation studies usually have few samples and very few samples in the low concentrations range. However, in a study on tacrolimus, 22.2% (n = 63) of the lower (trough) concentrations exceeded ±20% limits of the DBS to WB concentration ratio [5]. In this study, the area under the curve (AUC) was calculated for both DBS and WB based on trough concentrations and three sampling points at t = 1, t = 2 and t = 3 h after medication intake. For the AUCs, 90.3% (n = 63) of the paired AUC values were within 20% limits of DBS to WB ratio suggesting higher tacrolimus concentrations show less spread compared to trough concentrations. It is unlikely that the hematocrit has caused these differences, because previous research shows that hematocrit effects are most prominent at high concentrations of everolimus and sirolimus (50 μg/L) and low hematocrits (<0.23 v/v) [24, 25]. Re-evaluation of the data stratified for either transplantation type or time from transplantations showed that these two factors are not of influence on the results (data not shown). In future studies, introduction of duplicate analysis of both WB and DBS samples or analysis of two individual blood spots might reduce the observed spread in the lower (1–5 μg/L) concentration range. In addition, incurred sample reanalysis (ISR) is recommended for both WB and DBS samples to assess the spread of individual patient samples. Two major differences present in the study by Willemsen et al. are the much broader concentration range of trough concentration samples (3.6–28.5 μg/L in WB) and the broader
limits of clinical relevance that were used in comparison to this study [20]. Because dosing of everolimus in patients with cancer is performed in steps of 2.5 mg and the target trough concentration range is much wider (up to 19.2 μg/L), a larger clinical limit is accepted [38, 39]. In transplant patients, dosing can be done in steps of 0.25 mg and the target trough concentration range is 3–8 μg/L, therefore, a much narrower limit of clinical relevance is adjudicated. To the best of our knowledge, no guideline is available to determine limits of clinical relevance for DBS. The available literature suggests that setting a limit of clinical relevance should be done in a multidisciplinary team taking into account the clinical application of the method, the patient characteristics and the properties of the analytical methods [5, 20]. In our study, the everolimus DBS method does not meet the limits of clinical relevance set by our team and, at this time, cannot replace conventional WB sampling in the TDM of transplant patients where low trough concentrations are targeted. For sirolimus, Dickerson et al. showed a statistically significant difference of −0.8 μg/L in the Bland-Altman analysis where our method showed no bias [15]. The range of sirolimus concentrations in Dickerson et al. is 4–18 μg/L which is higher than the range of 1.7–10.9 μg/L in our study. The observed increased bias for higher trough concentrations (>10 μg/L) shown in Dickerson et al. might also be present using our method. Although results are shown as a ratio, samples with a WB concentration of >7.5 μg/L (n = 6) also showed lower concentrations in DBS (Figure 1) in this study. Excluding these samples yields a slope of 1.04 in Passing-Bablok regression, this explains the observed slope of 0.86 in the Passing-Bablok regression analysis for all sirolimus samples. However, excluding these samples does still result in not meeting the limits of clinical relevance. Another possibility is that this is a random phenomenon because the amount of samples with sirolimus WB concentration >7.5 μg/L is limited. Additional samples in the range of 5–15 μg/L are needed to assess this. For sirolimus, the limits of clinical relevance are not met in this study and the same trend as for everolimus is present where samples with a concentration of 1–5 μg/L showed the greatest bias. This might be caused by the same factors mentioned before for everolimus. Therefore, at this time, the sirolimus DBS method cannot replace conventional WB sampling in the TDM of transplant patients with low trough concentrations.

In our study the DBS samples were obtained by trained phlebotomists at the hospital and not by the patients themselves at home. Considering DBS methods are intended for home-sampling this might be a limitation of our study. However, the instructions and sampling methods are the same for both phlebotomist and patient. Patients receive instructions before home sampling is initiated including practicing a fingertip under the supervision of a trained phlebotomist. This should be sufficient for appropriate sampling at home if a patient or caregiver is willing and able to perform home sampling, in addition, paper and video instruction are available [40].

In the area of transplantation, where narrow therapeutic windows are followed for TDM of immunosuppressants, there are strict requirements for the analytical performance of assays measuring immunosuppressants in blood. With the current data, this clinical DBS validation study showed that not all predefined requirements set were met. Although Passing-Bablok analysis showed no systematic or constant differences between WB and DBS samples, the spread of samples did not meet the predefined limits of clinical relevance. However, as these limits were set by a local multidisciplinary team these may vary between settings and centers [18]. In addition, in a limited resources setting, where no WB bioanalytical method exists for sirolimus and everolimus, the DBS assay presented here could be used to allow TDM. If future studies show optimization of DBS assays using ISR, and if logistical challenges surrounding DBS home sampling can be overcome, the DBS method could be implemented in routine transplant patient care [9, 13, 18]. This would help in reducing patient burden, quickly achieving target trough levels the first months after transplantation and flexible monitoring of graft function.

Acknowledgment: We would like to thank the phlebotomists of the ‘prikpoli’ of the UMCG for obtaining the DBS samples.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: This work was supported by the Netherlands Organization for Health Research and Development (ZonMw, The Hague, The Netherlands) Funder id: http://dx.doi.org/10.13039/501100001826, grant 836044004 and the Tekke Huizinga Fund. This study was performed using the infrastructure and data provided by the TransplantLines Biobank and Cohort Study, which is registered at ClinicalTrials.gov under Identifier NCT03272841.

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
References


