Assessment of the Measurement Error in Cyclosporine Levels Drawn Between Peripheral and Central Sources

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ABSTRACT

Objectives: *Cyclosporine is often monitored by drug levels drawn through central venous catheters (CVCs), which may be falsely elevated due to reversible drug adsorption onto the catheter. Therefore, we assessed the correlation between cyclosporine levels drawn peripherally and through CVCs.*

Methods: *Bone marrow transplantation patients had a weekly collection of both peripheral and CVC draws from dual-lumen catheters simultaneously to assess cyclosporine levels after research ethics approval. Our primary outcome was the proportion of paired samples that were incongruent—defined as the mean of the CVC level being greater than 2 standard deviations from the peripheral level mean.*

Results: *After approaching 27 eligible patients, 20 patients (77.8%) provided samples. Of 53 paired samples, seven were incongruent (13.2%). Peripheral and CVC levels correlated (*r = *0.91) and agreed well.*

Conclusion: *Despite potential for preanalytical error due to adsorption, cyclosporine infusion and monitoring via CVCs produce results similar to monitoring via peripheral blood draws.*

Cyclosporine is a cornerstone of immunosuppressive therapy for graft-versus-host-disease (GVHD) after bone marrow (BMT) and solid organ transplantation. Inappropriate cyclosporine levels can result in acute kidney injury, hypertension, infections, graft rejection, and GVHD. Therefore, drug monitoring to maintain levels in the therapeutic range is crucial. As repeated peripheral vein access can be logistically challenging for transplant patients, central venous catheters (CVCs) are routinely placed for infusions and bloodwork. However, CVCs present unique patient safety concerns, including the discarding of excessive amounts of blood to produce an undiluted sample,[1] complications of the insertion and removal of the catheter itself, and the potential for the introduction of preanalytical errors when drug is reversibly adsorbed onto the CVC.[2]

Reversible drug adsorption on CVCs causing falsely elevated cyclosporine and tacrolimus levels has been reported in the literature in both in vitro and in vivo studies.[2,3] This may occur despite protocols to prevent cross-contamination of lines in multilumen CVCs when administering these immunosuppressive medications.[2,4] Currently, there are no published guidelines specific to the use of catheters vs peripheral blood draws for monitoring immunosuppressive therapies for GVHD in BMT patients.[5] Protocols for other conditions and drugs differ among centers and reasons for immunosuppression. In renal transplant literature, there are published guidelines for monitoring immunosuppressive therapies, but none

specifically suggest monitoring drug levels through a peripheral line vs a CVC.[6,7]

It is currently unknown whether drawing cyclosporine levels peripherally or from a CVC is optimal for the measurement of a correct cyclosporine level for monitoring. Additional patient discomfort associated with a peripheral blood draw in a patient who has an in situ CVC is also a perceived concern. To address this knowledge gap, we instituted a quality improvement initiative to assess the difference between cyclosporine levels drawn peripherally and through multilumen CVCs. We hypothesized that the difference in cyclosporine levels would be larger than would be explainable by laboratory variation in measurement.

Materials and Methods

Study Design

This study was performed prospectively on a BMT ward in a tertiary care center in Ontario, which regularly has six to 10 BMT inpatients admitted for transplantation. At our center, BMT patients admitted for transplant routinely have a dual-port CVC (polyurethane valved peripherally inserted central catheter, Bard Access Systems, Salt Lake City, UT), where the intention is to keep one port exclusively for drug administration and the other port exclusively for blood draws. Patients then have daily cyclosporine levels drawn through the central line with routine morning bloodwork until discharge. Typical practice is that if cyclosporine levels appear incongruent with the clinical context, the next level is drawn through the CVC with a paired peripheral sample, with the latter being considered the reference standard. Cyclosporine dosages are usually changed in 25% increments by the treating physician if not in the therapeutic range.

Patients were identified as eligible by the pharmacy team if they were receiving an allogeneic BMT as an inpatient. After consent, enrolled patients had cyclosporine levels drawn both peripherally and through the multilumen polyurethane valved CVC within a 30-minute time period once per week. To minimize patient discomfort, nurses with more experience with peripheral draws were assigned to study patients during patient assignment at the beginning of each shift. If a discrepancy was observed, the peripheral result was considered the correct drug level. Patients exited the study when they were discharged, switched to oral cyclosporine, transferred off the ward, or requested to withdraw.

Cyclosporine Assay

After drawing both samples and labeling with respective source sites, samples were analyzed using the Abbott Architect i1000 (Abbott, Lake Bluff, IL) within 6 hours of acquisition using the Abbott immunoassay method for cyclosporine A. The paired peripheral and central samples were each tested in duplicate to quantify the analytical variation on individual samples and facilitate optimal evaluation of the "between collection site" difference. Precision of the assay was monitored with Bio-Rad Lyphochek Whole Blood Immunosuppressant Control—Levels 1, 3, and 4 (Bio-Rad, Hercules, CA).

Data Collection

Data were abstracted using electronic health record review. The information collected included both peripheral and central cyclosporine levels from the pair drawn, age, sex, cyclosporine dose (along with the number of dose adjustments), renal function, and indication for BMT. Patients were assigned a unique study ID, and the patient log was kept in a locked cabinet in the inpatient BMT ward pharmacy office.

Primary Outcome

The primary outcome was the proportion of paired samples that were incongruent—defined as the mean of the central sample being greater than 2 standard deviations apart from the mean of the peripheral sample. The standard deviation was calculated using the formula of % coefficient of variation (CV) = standard deviation (SD)/mean of paired samples. The analytical performance of the cyclosporine assay was determined from three quality control samples, which allowed us to calculate the CV at three levels and then combine these to get an estimate of CV for the expected analytical range of the assay. The CVs chosen for analysis include the CV of the assay calculated throughout the study, the typical CV obtained from the 6 months prior to the inception of the study, and 25%. This latter threshold—chosen as a clinical change value of 25%—would be considered a significant difference and potentially lead to changes in management.

Secondary Outcomes

We assessed whether cyclosporine levels derived from a central or peripheral source were in a normal distribution. If so, we assessed correlation between central and peripheral levels using a scatterplot and by calculating the Pearson's correlation coefficient. Difference plots were used to assess agreement, as well as biases, of central and peripheral levels.

To determine if differences between central and peripheral draws led to a change in management, we also assessed if the cyclosporine dose was changed in accordance to the peripheral draw if the paired samples were incongruent.

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Sample Size Calculation

To determine a sample size for a noninferiority study, we assumed that there is a 5% total incongruence of hypothetical-paired peripheral samples and a 15% higher amount of total incongruent (20% total) samples when CVC and peripheral samples are prospectively collected. We set a type 1 error rate of 0.05, a power of 80%, and a noninferiority margin of 5%. From these calculations, our minimum sample size was 45 paired samples.

Analysis

Data was imported into Microsoft Excel (Microsoft Corporation, Redmond, WA) and statistical analyses were performed using the add-on software Analyse-it version 2.24 (Analyse-it Software, Leeds, UK).

Results

During our study, 27 patients were identified as eligible and approached for consent, of which 21 (77.8%) were enrolled. The most common reason for patients not enrolling was perceived discomfort of the additional venipuncture. Of the patients enrolled, 20 patients contributed 53 paired samples. The mean age of patients providing samples was 44 (SD, 11.3) years, approximately half were male (55%), and acute myeloid leukemia (AML) and/or myelodysplastic syndrome (MDS) was the most common indication for BMT (12 with AML/MDS, four patients with acute lymphoid leukemia, two patients with chronic myeloid leukemia, and two patients with lymphoma). One patient consented and was enrolled but became ineligible before having any samples drawn because the patient was switched to oral cyclosporine. Two patients requested withdrawal from the study due to discomfort experienced from the peripheral venipuncture.

Primary Outcome

The CV of the assay during the study was determined to be 10.7%. Using this CV, the number of incongruent samples was 7/53 (13.2%). Absolute mean cyclosporine levels of these samples are displayed in Table 1. Using the CV calculated at the time of the sample size calculation, the number of incongruent samples was 8/53 (15.1%). When the CV was set at 25%, the number of incongruent samples was 3/53 (9.4%). One sample that was incongruent using all 3 definitions had a cyclosporine infusion protocol violation where the drug was infused in the lumen used for blood draws.

Table 1
Absolute Mean Cyclosporine Levels of Incongruent Samples Based on the Study Coefficent of Variation

Sample No.	Absolute Mean Cyclosporine Level (ng/mL)		Notes Regarding Changes in Management
	Central	Peripheral	
1	574	310	Cyclosporine dose was not changed; patient with the known cyclosporine infusion protocol violation, where the drug was infused in the lumen, used for blood draws
2	222	181	Cyclosporine dose was not changed; levels were considered incongruent
3	224	177	Cyclosporine dose was not changed; levels were considered incongruent
4	246	199	Cyclosporine dose was not changed; levels were considered incongruent
5	159	126	Cyclosporine dose changed; levels were considered incongruent but change did not occur due to peripheral level
6	350	285	Cyclosporine dose changed; levels were considered incongruent but change did not occur due to peripheral level (only one of the peripheral levels reported, which was 337 ng/mL)
7	331	272	Cyclosporine dose changed due to peripheral levels

Secondary Outcomes

The means from levels drawn centrally and peripherally were found to be in a normal distribution based on q-q plots and levels from the two sources correlated well Figure 1A. The single outlier represents the levels drawn with the infusion protocol violation. After excluding the sample that was collected after the clinical protocol violation, the *r* value was 0.91 (0.85-0.95). Difference plots of absolute values observed vs target levels with the outlier removed demonstrated a small proportional bias between the peripheral and central samples Figure 1B. When compared against the peripheral as a standard, the central samples on average were not significantly higher (6.4 ng/mL, 95% confidence interval at −2.4 to 15.2).

We retrospectively assessed dose changes occurred in incongruent samples (based on the study CV) with knowledge of the correct peripheral cyclosporine levels (Table 1). Only in one instance did knowledge of the peripheral cyclosporine level change management. A patient

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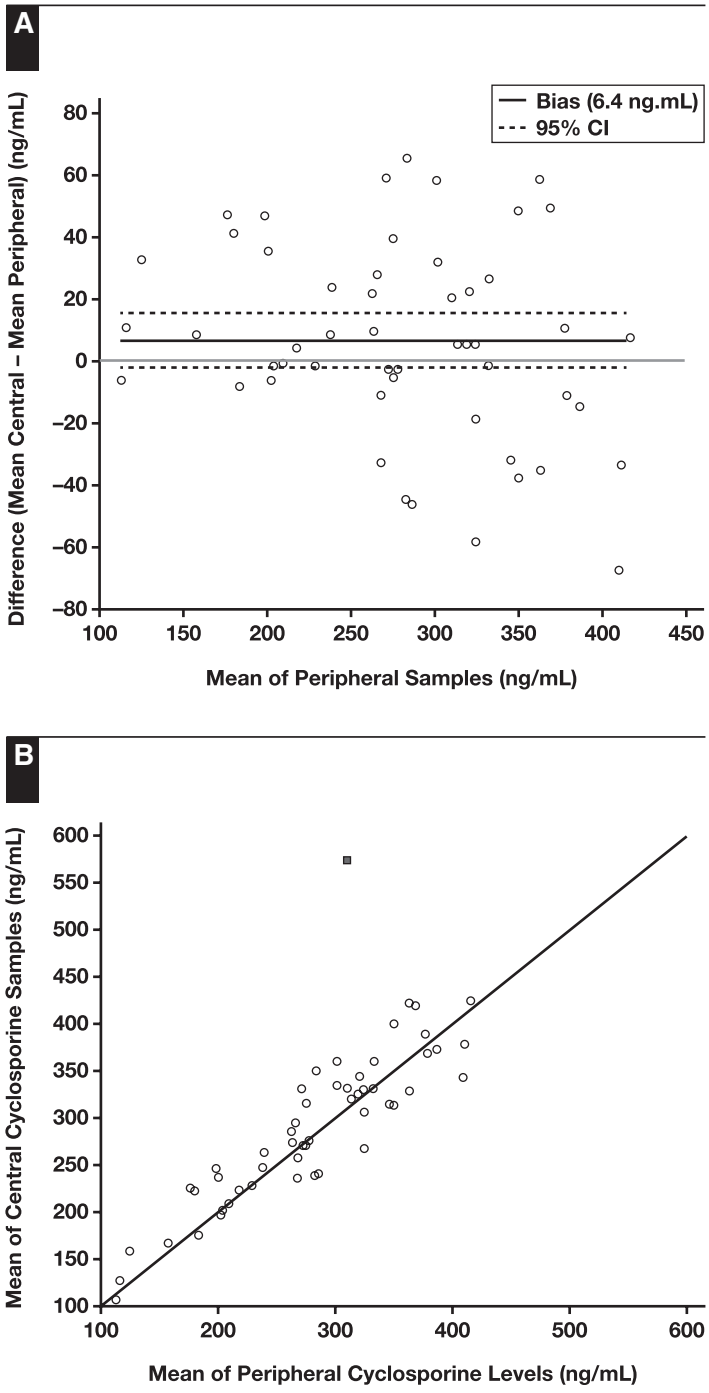

Figure 1 Correlation and difference plot between cyclosporine levels drawn centrally and peripherally. **A**, Scatterplot demonstrating correlation between the mean levels from central and peripheral cyclosporine samples. Each circle represents a paired sample taken from a patient. The colored square represents a paired sample taken from the patient with the known cyclosporine infusion protocol violation, where the drug was infused in the lumen used for blood draws. **B**, The difference plot with an outlier with the known cyclosporine infusion protocol violation removed demonstrates agreement overall between the central and peripheral sampling. No significant positive bias was seen with central sampling (6.4 ng/mL, 95% confidence interval at −2.4 to 15.2). When including the outlier, the positive bias is higher but nonsignificant (11.3 ng/mL, 95% confidence interval at −1.7 to 24.3).

on an IV cyclosporine dose of 150 mg twice a day had a paired cyclosporine draw that demonstrated a central level of 331 ng/mL and peripheral level of 272 ng/mL. The most responsible physician changed the dose to 175 mg IV twice a day to target a higher therapeutic level. The dose was changed in two other cases where levels were

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incongruent, but this was based on both the central and peripheral levels being outside the therapeutic range. In four cases, peripheral levels did not change management despite peripheral and central samples being incongruent.

Discussion

In this prospective study comparing cyclosporine levels from peripheral and CVC sources that enrolled 20 patients and analyzed 53 samples, we found that drug levels correlated well despite the risk for falsely elevated CVC results due to adsorption. While differences were found between sources used for sampling, the majority of incongruent samples were not of sufficient magnitude to change management. We found approximately one-fifth of patients declined to participate, often citing potential discomfort associated with the peripheral draw. Two patients discontinued the study because of this discomfort, despite venipuncture being performed by experienced staff.

Previous reports discuss that adsorption of cyclosporine and similar drugs like tacrolimus onto CVCs used for infusing and sampling bloodwork may be a preanalytical source of error that could adversely affect patients and the ability to dose medications accurately.[2,3] This was confirmed in our study when one subject had a line protocol breach resulting in a highly discrepant CVC result. Contamination can persist even after the catheter has been flushed or the intravenous drug has been discontinued.[2] Maintaining these medications in their therapeutic range is optimal for the care of BMT patients receiving immunosuppression for GVHD.[8,9] Guidelines and practice manuals lack information regarding the phenomenon of contaminated samples due to adsorption and do not provide specific recommendations to administer and monitor immunosuppressive medications such as cyclosporine through a CVC for patient comfort.[5-7,10] The package inserts for both cyclosporine and tacrolimus also do not warn of this phenomenon nor provide recommendations for access for drug infusion. We suggest, based on the results of our study, that administering cyclosporine and drawing levels through a CVC is a safe practice, providing that blood for testing is obtained through a lumen, which is not used for medication administration. This also needs to be balanced against known risks of CVCs, such as catheter-related infections.[11]

This study does have limitations. We did not institute a target "therapeutic level" to allow for real-world variation in practice. Our center used a polyurethane CVC; this material has been observed to have less adsorption compared to CVCs made of silicone.[2] Awareness of a protocol to draw samples both centrally and peripherally may have produced an observer (or Hawthorne) effect,[12] where attentiveness may have reduced accidental infusions of medication into the same lumen, which would have produced more of a discrepancy between CVC and peripheral sampling. In addition, the protocol requested that nurses with more experience with peripheral draws were assigned to study patients. This may have also had the effect of increasing the quality of sampling and compliance to using the correct CVC lumens for infusion and sampling. This study was not powered to look for differences in clinical outcomes that might occur with more accurate dosing through peripheral sample draws. Finally, these results cannot be extrapolated to other medications that have been observed to adsorb onto CVCs.[5,13]

In conclusion, in BMT patients needing repeat bloodwork to monitor cyclosporine levels, a polyurethane CVC can be used for infusion and monitoring if separate lumen are used for these activities. In those patients requiring repeat bloodwork who have such a CVC, routine peripheral venous sampling cannot be recommended because the added accuracy cannot be reconciled with the additional discomfort of peripheral sampling. In centers using a CVC to obtain cyclosporine levels, physicians and other care providers should be mindful of cross-contamination when using CVCs and investigate with a peripheral sample where appropriate.

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