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ORIGINAL ARTICLE

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Measurement of blood serum cyclosporine levels using capillary "fingerstick" sampling: a validation study

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Abstract Capillary blood sampling as a means of monitoring blood cyclosporine levels has replaced venipuncture in some medical centers. As the validity of capillary venipuncture for analysis of cyclosporine has not been documented, we sought to validate the capillary blood collection technique by comparing it with serum samples collected simultaneously by venous phlebotomy. Forty paired capillary- and venous samples were collected from 36 cardiac transplantation patients and analyzed, using a polyclonal immunoassay. The values obtained were compared using regression correlation. The correlation coefficient for all 40 samples was 0.859. However, we discovered that the first 7 capillary specimens were processed incorrectly. The correlation coefficient for the other 33 samples was 0.995 (99% confidence interval 0.987–0.998). The excellent correlation between serum samples obtained from capillary sampling and from venous sampling, together with the ease of obtaining capillary blood specimens, make "fingerstick" sampling the method of choice for monitoring cyclosporine levels in infants and children.

Key words Cyclosporine measurement · Capillary „fingerstick" sampling

Introduction

Cyclosporine is a potent immunosuppressive agent used widely in the prevention of graft rejection following solid organ transplantation in children and adults. It has a relatively narrow therapeutic range. Too low dosage results in under-immunosuppression and may lead to episodes of acute rejection. Conversely, dosage too high may result in toxicity, including nephrotoxicity, infection, hypertension, hyperkalemia, and tremors.[4]

Obtaining venous samples may be difficult, particularly in young children and infants. This is especially true for the transplantation population, where patients require frequent phlebotomy for the monitoring of drug levels. The frequency for measuring cyclosporine levels varies from daily, early after transplantation, to every 2–3 months, late after transplantation. In the immediate post-transplantation period, indwelling venous catheters may be needed to ensure vascular access. This is accompanied by the risk of catheter-related complications in an already immunosuppressed population.

Capillary blood sampling is used for several laboratory tests. This technique has several advantages. It avoids the difficulty associated with obtaining repeated venous samples, and it is less traumatic and less stressful for patients and their families. It also needs a smaller quantity of blood, an important consideration when caring for infants. Thus, capillary sampling would be a desirable method for cyclosporine monitoring. Although many hospitals currently use capillary sampling to measure blood levels of cyclosporine, no data exist validating its use in harvesting serum for cyclosporine assay using the polyclonal immunoassay technique.

The purpose of this study is to determine the correlation between cyclosporine levels of blood serum obtained by venipuncture and by capillary sampling ("fingerstick") techniques.

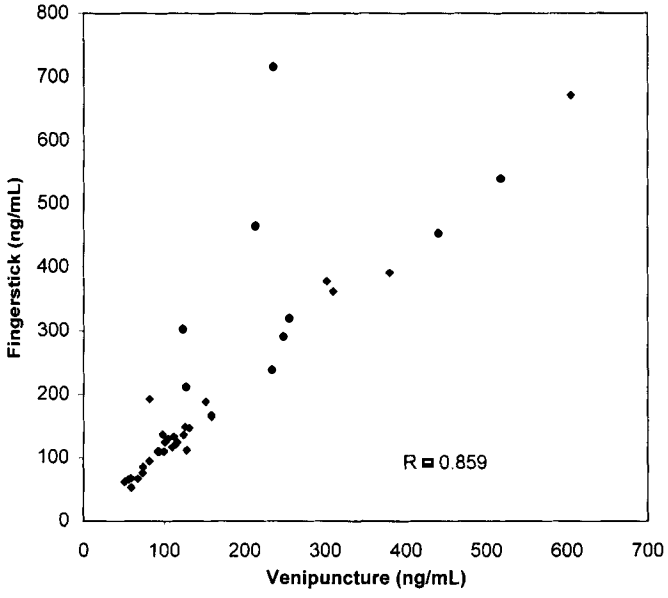

Fig. 1 Relationship between cyclosporine levels determined from venous and capillary blood samples. All data points are shown, including the initial 7 specimens that were processed incorrectly. Correlation coefficient = 0.859

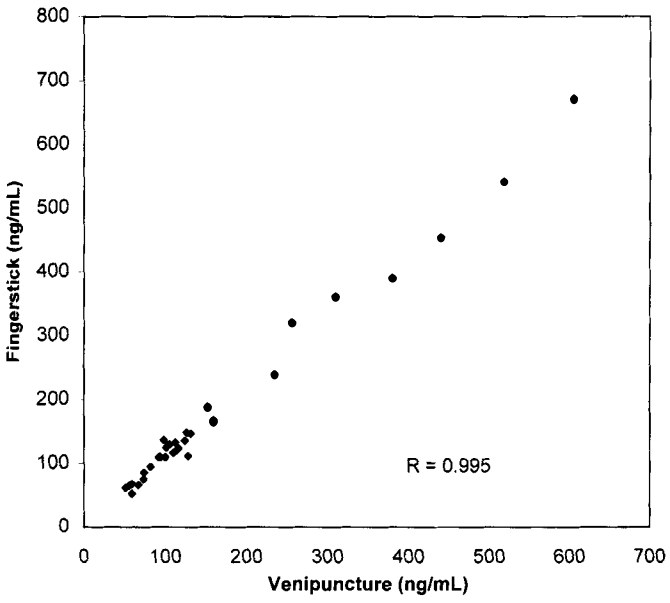

Fig. 2 Relationship between cyclosporine levels determined from venous and capillary blood samples (excluding initial 7 samples, see text). Correlation coefficient = 0.995

Materials and methods

The study population consists of 36 patients who were receiving cyclosporine following cardiac transplantation. Over a 3-month period, 40 paired blood specimens (one obtained by venipuncture and the other by "fingerstick" were obtained within 1 or 2 minutes of each other. Informed consent was obtained from the patient or the patients' parents, and the protocol was approved by the Mayo Clinic Institutional Review Board. The volume of blood collected was 1–2 ml for the venipuncture specimens, and 500 microliters for the capillary specimens. In both cases, blood was collected without the use of anticoagulant. Capillary samples were collected while trying to minimize squeezing the digit to obtain the sample. The paired blood specimens were allowed to stand at room temperature for 2 h, after which they were centrifuged at 2,000 rpm in a temperature-controlled centrifuge designed to maintain an internal temperature of 20 °C. Serum was harvested, and analysis was performed using the Abbott polyclonal immunoassay (Abbott Laboratories, Abbott Park, IL), an immunoassay designed to measure cyclosporine and its metabolites in blood serum.[2] Results were analyzed by regression correlation, using a software package (Microsoft Excel version 5.0, Microsoft Corporation, Redmond, WA). Confidence intervals were calculated using Fisher's Z-transformation.

Results

The values of the 40 paired samples are listed in the table. Early in the evaluation, we discovered that the phlebotomist collecting the capillary sample did not allow the sample to reach ambient temperature before centrifugation. This processing-error occurred with the capillary sample in the first 7 paired specimens. Each subsequent paired sample was processed in the manner described above. The relationship between the venipuncture and "fingerstick" results for the entire group of samples, including the 7 samples that were processed improperly, is shown in Fig. 1. The correlation coefficient for this group was 0.862. Figure 2 shows the same relationship after excluding the first 7 samples. The correlation coefficient for this group was 0.995 (99% confidence interval 0.987–0.998). The limits of agreement defined as ± 2 SD from the mean difference between venipuncture and capillary sample concentrations (excluding the first 7 samples) are shown in Fig. 3.

Discussion

Our results demonstrate a close correlation between the measurements of cyclosporine levels in venous and in capillary blood serum samples with use of a polyclonal immunoassay. The desired concentration of cyclosporine varies according to the individual patient's clinical circumstances. Early after transplantation we aim for a level of 250–300 ng/ml, while several months after transplantation we aim for a level of 100–150 ng/ml. Our results show close correlation within these ranges, as well as for values that are sub-therapeutic or supra-therapeutic. A correlation between cyclosporine levels of capillary and of venous samples has been described previously in a smaller population of liver transplantation pa-

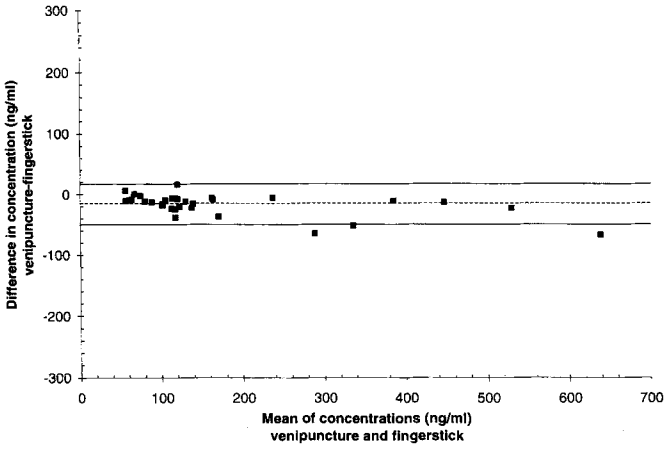

Fig. 3 Mean of paired venipuncture and capillary samples plotted on the x axis and difference between paired venipuncture samples on the y axis. Mean difference (dashed line) and ± 2 SDs from the difference (solid lines) are shown (Bland-Altman plot)

tients.[6] However, that study used a whole blood monoclonal assay technique. Our study is the first to validate the use of serum harvested from capillary specimens for cyclosporine analysis.

There are three common methods for monitoring cyclosporine concentration.[3, 7, 5] Analysis for the parent drug (cyclosporine A) may be carried out in whole blood by either monoclonal immunoassay or high-performance liquid chromatography. Monoclonal immunoassay offers the advantage of a small sample size; however, high-performance liquid chromatography is considered more specific, and is thus the reference method for whole blood analysis. Alternatively, with use of a polyclonal immunoassay, cyclosporine and its many metabolites may be measured in serum. This method provides transplantation physicians with an assessment of total cyclosporine load. However, considerable attention to detail in harvesting the serum specimen is required, because cyclosporine distributes between blood serum and erythrocytes in a temperature-dependent fashion.[9, 1] When serum is harvested for polyclonal analysis, the blood sample must be maintained at constant temperature to ensure equilibration of cyclosporine between serum and erythrocytes. The convention used by most medical centers, which is followed in the present study, is to allow the blood to stand at room temperature for at least 2 h before separating the cellular fraction and harvesting the serum.

Although relatively few medical centers use the polyclonal immunoassay in the analysis of cyclosporine blood levels (19/391 in the most recent UK International Proficiency Testing Scheme), our experience with this assay has been quite good. Indeed, using this assay to adjust cyclosporine dosage, we have achieved good survival rates following cardiac transplantation. The 1, 3, and 5-year survivals of 94.3 %, 88.9 %, and 84.0 % respectively[8] in our program, compare well with results around the world, and we remain satisfied with this particular cyclosporine assay.

Our results demonstrate the importance of proper processing of specimens, harvesting of serum, and performance of the assay procedure. In the first 7 samples, the capillary specimens were not allowed to equilibrate, contrary to standard practice for processing venous samples in our laboratory. In these samples, the correlation was quite poor. Once this equilibration was allowed to occur, the samples showed excellent correlation, with a correlation coefficient of 0.995.

Venous phlebotomy is often difficult in infants and children. Patients in the cardiac transplantation population have already been exposed to multiple medical procedures. Over time, venous access becomes challenging, and multiple attempts may be necessary to collect the required volume of blood. This can be stressful for the patient and the family. Capillary sampling has obvious advantages in terms of ease of collection and decreased stress on the patient. Based on the results of the current study, our practice at the Mayo Clinic is to use capillary sampling whenever possible, to measure cyclosporine levels in cardiac transplantation patient.

We conclude that with use of a polyclonal immunoassay procedure, cyclosporine levels may be measured accurately in capillary blood serum samples. This method requires less blood volume, avoids the difficulty of frequent venipuncture, eliminates the need for indwelling venous catheters solely for the purpose of drug monitoring, and is less traumatic than venipuncture. This makes capillary sampling the method of choice for monitoring cyclosporine levels, particularly in infants and children.

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