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Canadian Consensus Meeting on Cyclosporine Monitoring: Report of the Consensus Panel

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The Bureau of Drug Research, Health Protection Branch of the Government of Canada, requested of the transplant and clinical chemistry professional groups that a consensus meeting be held, to develop a "state-of-the-art" consensus paper on cyclosporine (CsA) monitoring. The often confusing scientific literature on CsA monitoring, created largely as a result of the use of different nonspecific and specific methods for measurement of CsA, the use of different sample matrices in which the drug is measured, and the varied criteria used for defining renal toxicity or rejection had led to this request. In response, the Canadian Society of Clinical Chemists in conjunction with the Canadian Transplant Society planned and organized the Canadian Consensus Meeting, which was held at Minaki, Ontario, May 11 through 13, 1990. Twenty-one scientists representing the clinical, methodological, and pharmacological aspects of CsA presented to 72 attendees the latest research data on mechanisms of the immunosuppressive and nephrotoxic activity of CsA; immunosuppressive and nephrotoxic activities of the major human metabolites of CsA; distribution of CsA between blood plasma, blood cells, and tissues; specific methods for measuring CsA; the impact on clinical outcome of adjusting CsA dosage on the basis of results of monitoring the parent drug concentration in whole blood; CsA pharmacokinetics; and methods for detecting rejection. Based on a detailed consideration of all data pertinent to the therapeutic drug monitoring of CsA, a set of recommendations was developed, as requested by the Canadian Health Protection Branch, by a consensus panel consisting of the nine individuals who co-authored this paper. At the conclusion of the meeting these recommendations and the rationale for them were presented for critical review to all who attended the meeting.

During the conference there was considerable debate regarding the following: (*a*) the appropriateness of one sample matrix over another (whole blood, plasma, serum, or plasma ultrafiltrate) for measuring CsA in transplant patients, and (*b*) the relative merits of the area-under-the-curve method compared with trough concentrations for regulation of CsA dosing. Although all of the transplant centers represented at the meeting use CsA monitoring to adjust dosage, several investigators in the transplant field still believe that adjusting CsA dosage in response to changes in clinical indices provides good long-term clinical outcomes in transplant patients. Future studies will be required to resolve this issue.

It is the hope of the consensus panel that the following recommendations and the rationale for them will provide the type of practical information that could foster an improvement in the center-to-center consistency in the practice of cyclosporine therapeutic drug monitoring. This report is therefore regarded as a complement to the Hawk's Cay consensus report (*1*), which described the areas of controversy but did not make specific recommendations on the time of sample collection, the method for measurement of CsA, or the sample matrix in which to measure the drug.

Recommendations and Guidelines

1) The recommended time for collection of the sample in which to measure CsA is 11–12 h after the last CsA dose for patients receiving two doses per day and 23–24 h after the last CsA dose for patients receiving one dose per day.

2) It is recommended that the time of food intake in relation to CsA dosing be consistent, because effects of food on CsA absorption can be considerable.

3) Information as to the amount and time of administration of the last dose should be submitted together with the request for CsA determination.

4) In most clinical situations during the immediate post-transplant period, the maximum recommended frequency of monitoring is once every 24 h.

5) Until one matrix is shown to be clinically superior to another, analytical reasons, including the pressing need for center-to-center consistency, should be the basis for selection of sample matrix. This is the basis for selection of whole blood as the recommended sample. EDTA is the recommended anticoagulant. It is important to assure artifact-free collection of the blood specimen. Therefore, for patients receiving the drug through an in-dwelling catheter, venipuncture from the contralateral arm is required.

6) In vitro, CsA has long-term stability over a wide range of temperatures. If required, samples can be shipped at ambient temperatures.

7) Use of a validated specific method for measuring the parent drug is recommended.

8) Performance characteristics for CsA measurements should meet the criteria for acceptable clinical laboratory practice.

9) No data currently support the monitoring of CsA

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metabolites as a guide to dosage adjustment in transplant patients. Should one or more metabolites subsequently be shown to be clinically important, it is recommended that they be measured with a specific method.

10) Target or therapeutic ranges have been empirically derived. Their definition is subject to all of the difficulties resulting from multiple confounding factors. Tables are provided listing representative ranges and factors such as the method used for measurement of CsA that may influence the derivation of each target range and, where available, the criteria used for defining rejection and nephrotoxicity.

11) It is recommended that laboratories provide same-day (before the next dose) turnaround assay for acute transplant patients.

12) To maintain the quality of the method for measuring CsA, participation in an external quality-improvement program is essential in addition to an effective internal quality-assurance program. It is recommended that a pure, well-characterized CsA preparation be developed to make possible the effective standardization of CsA measurements from laboratory to laboratory.

Time of Sample Collection

Most transplant centers throughout the world specify that the time for collection of the specimen in which CsA is to be measured be just before administration of the next dose (*2*). As is the case for the majority of therapeutic drugs, this selection of the trough time is based on its reproducibility; it occurs after absorption of the drug is complete, which places it in a more reproducible part of the drug concentration-vs-time curve; CsA toxicity correlates better with trough concentrations than with either peak or peak minus trough increments (*3*). Several investigators have proposed other sample times: 5 or 6 h after the last dose (*1*) or multiple timed samples collected throughout the dosage interval for determination of the area under the CsA concentration in blood-vs-time curve (AUC), and the average steady-state concentration (*4, 5*). On the basis of this information, standard pharmacokinetic formulas are used to determine what dosages are subsequently required to achieve target steady-state concentrations of CsA. Future studies are needed to test the value of this approach by directly comparing it with the standard trough-concentration approach in relation to achieving target concentrations in blood and the desired clinical outcome. The major practical limitations of AUC monitoring are the need for extended hospitalization or clinic visits, the frequency of blood sampling required each time the patient's CsA status is to be evaluated, and the cost. Despite these important practical difficulties this proposal has the potential advantage of providing a pharmacokinetically more rigorous approach to determining the patient's subsequent dosage regimen (*4–6*). In situations where it is difficult to attain the desired trough concentrations, pharmacokinetic profiling has been helpful in defining the nature of the problem (e.g., poor absorption or abnormal clearance of CsA) and in establishing an effective dosage regimen (*2*).

Sample Stability

CsA in EDTA-anticoagulated blood, without any additives or preservatives, is stable at either room temperature or at 2–8 °C for up to seven days. Samples can then be shipped at ambient temperatures to central processing locations. For prolonged storage, samples should be kept at −20 °C. In the presence of preservatives, samples are stable for up to four months at room temperature (*7*).

Sample Matrix

To date, there are neither pharmacological nor clinical studies in which CsA concentrations, measured specifically in different sample matrices (whole blood, plasma, serum, or plasma ultrafiltrate) prepared from individual patients, were compared to determine which of these correlates best with pharmacological effects and clinical events such as rejection or nephrotoxicity. It is difficult to draw meaningful conclusions about the comparative clinical value of different sample matrices from the literature because of confounding factors, e.g., the use of different methods for CsA determination, different dosing intervals, the temporal relationship between clinical events and monitoring CsA concentration, the concomitant use of other immunosuppressive agents, the lack of "gold standards" for use in determining the degree of immunosuppression and nephrotoxicity, and the presence of other risk factors that may predispose a transplant patient to nephrotoxicity (*1, 2, 8, 9*).

Until appropriate comparative studies are performed, the primary factors upon which a rational choice of sample matrix can be based are analytical factors and the need for center-to-center consistency of results. The analytical reasons for recommending whole blood as the sample matrix are the greater signal-to-noise ratio made possible by the higher (twofold on average) concentration in whole blood as compared with plasma prepared at 37 °C, and the requirement that plasma be separated from blood cells under stringent conditions of time and temperature. The use of plasma prepared under different temperature and (or) time conditions for CsA determination would require multiple target ranges for therapeutic drug monitoring. The use of whole-blood CsA concentrations makes it easier to compare results from center to center. Furthermore, for transplant patients who may need to have their CsA measured in different laboratories, measurement of concentrations in whole blood will give the most comparable results, especially when validated liquid-chromatographic or specific monoclonal-antibody immunoassays are used for the measurement (*2, 8, 10*).

Method for Measurement

The specific measurement of CsA in which only parent drug and not the metabolites are quantified can be accomplished by two procedures: "high-pressure" liquid chromatography (HPLC) and immunoassays involving a selective monoclonal antibody (*11–16*).

The former method, when well-validated, has been considered the reference method for the specific measurement of the drug (*1, 2, 8*). Several reversed-phase HPLC procedures for CsA determination have been described (*8, 17–24*). The major differences among them are the sample preparation procedure, the sample volume, the HPLC column, and the mobile phase. The absolute analytical recovery of the drug should be >80%, with the recovery relative to an internal standard being in the range of 90–110%.

Performance Characteristics

The error for the method (either HPLC or selective immunoassay) at 50 μg/L should be no greater than 10 μg/L and at a concentration of 300 μg/L should be no greater than 30 μg/L. This requires that the standard

deviation of the analytical method be less than or equal to 5 and 15 μg/L, at those respective concentrations. The rationale for this is that the concentration of CsA measured in blood has been decreasing as clinical protocols have been developed. In addition, several centers have shown that concentrations that decrease with time after the transplant are efficacious (*25*). A recent report indicates that for one center the median CsA concentration observed with a specific assay was 135 μg/L, with 33% of the samples having concentrations less than 100 μg/L (*26*). Medical decisions regarding therapy are being made at the 100 μg/L level.

Results by the analytical method (either HPLC or immunoassay) should be compared with those by a validated HPLC procedure as the reference method (*x*-axis) for at least 200 specimens of blood from persons with all transplant types (kidney, liver, pancreas, heart, heart–lung, bone marrow). Standard statistical evaluation (see reference *27*, for example) of the linear-regression data should be used, including, but not limited to, the slope and standard deviation of the slope, the *y*-intercept and standard deviation of the intercept, and the standard deviation of the estimate ($s_{y/x}$, standard error of the regression, standard error of the residuals). An appropriate statistical test (*t*-test) should be performed on the slope and intercept to determine that they are not statistically different from 1.00 or 0.0, respectively, at the 95% confidence interval. We recommend that the slope differ by no more than 10% from the line of identity (slope = 1.00; acceptable 0.90–1.10), the intercept by no more than 15 μg/L, or the $s_{y/x}$ by no more than 15 μg/L.

The analytical method should be validated for accuracy. The validation of the assay depends on the availability of pure CsA standard material. Standards are available from the Sandoz Co. and from the United States Pharmacopoeia. The latter material has been checked for purity and assayed in several reference laboratories and, thus, is preferred. Precautions should be taken when drying and desiccating the reference material before standards are prepared.

The consensus meeting panel strongly advocates development of a secondary reference material in a whole-blood matrix, assayed by reference laboratories, to assist in improving accuracy of CsA testing. A potentially promising reference procedure for confirming the CsA concentration in this reference material would be liquid chromatography/mass spectroscopy, with deuterated CsA as an internal standard.

The analytical recovery of CsA and the internal standard should be determined in the assay—absolute recovery, not recovery relative to the internal standard. It has been shown that recoveries of CsA and the common internal standards cyclosporin D and dihydrocyclosporin C are not the same when hexane washes are used in the analysis (*28, 29*).

A set of "challenge samples" containing both metabolite and parent drug, and preferably in a whole-blood matrix, should be analyzed. The concentration of CsA in the challenge sample should be determined by reference laboratories. These could be part of an external quality-assurance program, such as the Canadian Quality Assessment Program. In validating the assay, at least 10 such challenge samples should be run. Thereafter, the consensus panel recommends the analysis of at least one set of challenge samples per year.

Cyclosporine Metabolites

More than 20 metabolites of CsA have been identified (*30–39*). Several studies have been published during the past few years in which the immunosuppressive (*32–34, 37, 40–45*) and toxic activities (*46–49*) of these metabolites have been investigated. Although further investigation is still required, some conclusions can be made from the data currently available.

In vitro experiments indicate that metabolites are considerably less immunosuppressive and toxic than is CsA. The immunosuppressive and toxic activities of the metabolites appear not to be associated (*47*). In vivo studies in animals have been hampered by the lack of sufficient quantities of metabolites and of a suitable model (*43, 50*). Preliminary results, obtained with rats, suggest that the metabolites are less immunosuppressive than CsA, which corroborates the in vitro findings. Clinical studies have indicated that the routine monitoring of metabolites is not warranted for the majority of clinical situations (*51–57*). If future data indicate a clinical requirement for metabolite measurement, then a specific measurement for each metabolite of significance would be warranted.

Therapeutic Range

Historically, the therapeutic range for CsA has been difficult to establish, due in large part to the variation in matrix and selectivity of the assays as well as the clinical criteria used for rejection and toxicity (*2, 30, 58–70*). Taking into account two of the recommendations proposed here—namely, the use of selective assays with standards and samples in a whole-blood matrix for routine monitoring—should facilitate the establishment of a consensus therapeutic range. Table 1 lists therapeutic ranges for solid-organ transplantation from several Canadian and United States centers where whole blood and selective assays are used. Where available, the criteria for rejection and CsA toxicity are also listed (*71–75*). It is important that each transplant center, before adopting a reference range established elsewhere, ensure that the methods of analysis and the criteria for diagnosis of clinical events are the same as those originally used to establish this range. The institution should then confirm the adequacy of this reference range for use in their own transplant population.

Quality Assurance

Several quality-assessment programs have described their experience to date regarding center-to-center reproducibility when a given method is used for measuring CsA, the recovery of cyclosporine added to drug-free or pooled specimens of whole blood from the transplant patients, and the detection of CsA in samples of pooled drug-free whole blood (*7*). These programs have been helpful in providing feedback to participating laboratories about the consistency of their performance and about how their performance compares with that of other laboratories. In the future it will be important for these programs to provide suggestions as to the possible causes of a particular problem and how to improve performance. One further recommendation is the development of a well-characterized CsA preparation in whole blood to provide all laboratories a common source of a single material against which to compare CsA standards in routine use.

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Table 1. Comparison of Therapeutic Ranges among Various Transplant Centers Using a Whole-Blood Matrix

Center	Analytical method	Dosing interval	Sample time	Other immuno-suppressants	Therapeutic range, μg/L	Transplant	Diagnosis of rejection and (or) toxicity
Health Sciences Ctr. Winnipeg, MB	Selective RIA	BID	Trough	Aza, Pred	200–300 <1 m 100–200 >1 m	K	Biopsy, >15% ↑ Cr
Hosp. of the Univ. of Pennsylvania Philadelphia, PA	HPLC	QD	Trough	Aza, Pred	100–250 <3 m 80–125 >3 m 250–350 <3 m 100–150 >3 m	K K L L, H	Biopsy, >25% ↑ Cr
Mayo Clinic Rochester, MN	HPLC HPLC	QD BID	Trough Trough	Pred Pred	250–350 150–250 <2 m 80–200 >2 m	L K	—
Ottawa General Hosp. Ottawa, ON	Selective RIA	BID	Trough	Aza, Pred	>200 post-tx <200 >1 w	K	Biopsy, ultrasound, ↑ Cr
St. Joseph's Hosp. Hamilton, ON	Selective RIA	BID	Trough	Aza, Pred	150–250 <3 m 100–150 >3 m	K	Biopsy, ultrasound, >10% ↑ Cr
Toronto General Hosp. Toronto, ON	Selective RIA	BID	Trough	Aza, Pred	100–300 100–400	K L	Biopsy, ↑ Cr
University Hospital London, ON	Selective RIA	BID	Trough	Pred	250–450 <3 m 100 >3 m 400 <3 m 200–300 3–12 m 100–200 >1 y 300–600 <3 m 200–350 3–12 m 100–200 >1 y	K H L 	Biopsy, 10–20% ↑ Cr
Univ. of Cincinnati Cincinnati, OH	HPLC	BID	Trough	Aza, Pred	150–250 <6 m 75–150 >6 m 150–300 >6 m 75–150 >3 m	K K L H	Biopsy, >10% ↑ Cr
Univ. of Minnesota Minneapolis, MN	HPLC	BID	Trough	Aza, Pred	150–200 <1 m 100–150 1–12 m 50–100 >12 m 200–250 200–300	K K K L H	Biopsy, >25% ↑ Cr
Univ. of Pittsburgh Pittsburgh, PA	HPLC	BID	Trough	Aza, Pred	150–250	K	—
Univ. of Texas Medical Center Houston, TX	Selective RIA	QD	AUC	Pred	370 target	K	—
Vancouver General Hosp. Vancouver, BC	Selective RIA	BID	Trough	Aza, Pred	300–400 <1 w 250–400 <1 m 200–300 <3 m 100–200 <6 m 100–150 <2 y 300–600 <1 w 200–450 <1 m 200–350 <3 m 200–300 <6 m 150–250 <1 y 300–450 <1 w 300–400 <1 m 200–300 <3 m 100–200 <2 y	K L H 	Biopsy, >10% ↑ Cr >10% ↑ Cr >10% ↑ Cr
Victoria General Hosp. Halifax, NS	Selective RIA	BID	Trough	Aza, Pred	250–350 <2 w 200–250 <3 m 150 target >3 m	K	Biopsy, ↑ Cr

K: kidney transplant recipients; L: liver transplant recipients; H: heart transplant recipients; Aza: azathioprine; Pred: prednisone; Pred: prednisone; Cr: creatinine; QD: once daily; BID: twice daily; AUC; area under the curve; m: month; w: week; y: year; tx, transplantation.

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