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Monoclonal Antibodies for Radioimmunoassay of Cyclosporine: a Multicenter Comparison of Their Performance with the Sandoz Polyclonal Radioimmunoassay Kit

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The performance of a radioimmunoassay kit containing monoclonal specific and nonspecific antibodies to cyclosporine (Sandimmun®-Kit; Sandoz Ltd., Basle, Switzerland) was compared with that of the original Sandoz polyclonal radioimmunoassay kit (Ciclosporin RIA-Kit). A total of 1320 blood and plasma samples from patients receiving cyclosporine after kidney, heart, liver, and bone-marrow transplantation were analyzed at six centers. For blood samples the median result on using the specific assay was about 50% of the polyclonal assay result after kidney and bone-marrow transplantation, about 33% after heart and liver transplantation; comparable figures for plasma samples were 70 and 40%. The monoclonal nonspecific-antibody assay produced results 10% to 140% higher than polyclonal-assay results, depending on sample matrix and transplant indication; the largest difference was seen in samples from heart- and liver-transplant recipients. Evidently the specific-antibody assay provides a convenient alternative to high-performance liquid chromatography for specific measurement of the drug, but the role of the new nonspecific antibody, possessing an even wider spectrum of cross-reactivity with cyclosporine metabolites than the original polyclonal antiserum, has yet to be defined.

Until recently, only two techniques were available for measurement of cyclosporine (Sandimmune®; Sandoz Ltd., Basle, Switzerland): high-performance liquid chromatography (HPLC) and radioimmunoassay (RIA). Most published studies on measurement of the drug as a guide to therapy are based on RIA results produced by a kit developed by Sandoz Ltd. ("Ciclosporin RIA-Kit"). In this kit a polyclonal antiserum raised in sheep is used (*1*). With this antiserum it was not possible to measure the parent compound specifically, because it cross-reacts substantially with metabolites of the drug (*2*). Almost always, results were higher than those produced by HPLC and, in some clinical settings, the disparity between HPLC and RIA results was particularly large (*3, 4*).

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Several authors have shown a clinical utility for the polyclonal RIA measurement of cyclosporine (*5–7*), but there has been a growing call for specific measurement of the compound (*8*). Providing a routine service for the measurement by use of HPLC is technically demanding, requiring instrumentation and technical skills not available in all laboratories (*9*). A specific RIA would, therefore, be of clinical value.

With these considerations in mind, a development team at Sandoz raised a series of monoclonal anti-cyclosporine antibodies from mice spleen cells (*10*). From over 180 monoclonal antibodies, two were selected for inclusion in a new RIA kit ("Sandimmun®-Kit"). One of these antibodies did not cross-react appreciably with the metabolites of cyclosporine, giving results that agreed well with those produced by a reference HPLC assay (*11, 12*). The other antibody cross-reacted substantially with the metabolites formed in man, giving even higher results than those produced by the Ciclosporin RIA-Kit (*9*). As in the original kit, a tritium-labeled cyclosporine tracer and charcoal separation are used in the new kit.

A multicenter trial was conducted to compare the results obtained by use of both of the monoclonal antibodies in the Sandimmun®-Kit with those produced by use of the polyclonal antibody supplied in the Ciclosporin RIA-Kit.

Methods

Three centers in Europe[1–3] and three in North America[5–7] participated. At these centers cyclosporine is routinely measured in samples collected from patients who are receiving the drug for a variety of clinical indications. The centers assayed 175 to 300 clinical samples by each of the three radioimmunoassays, using the same sample matrix ordinarily used in their laboratories.

Before they entered the trial, the accuracy and precision of the participating laboratories was assessed. Each laboratory was sent 22 blood or plasma samples supplemented with known concentrations of cyclosporine. These contained, in random order, a 10-point calibration curve and four replicates of three pools representing low, medium, and high concentrations of cyclosporine. The centers were asked to assay the samples by each of the three radioimmunoassays, using a separate calibration line for each assay. The samples representing the calibration line were prepared from the methanolic standard supplied with the original RIA kit. The high, medium, and low pools were made from a separate stock solution prepared from cyclosporine supplied by Sandoz. Participants were asked to make no modifications to the kit protocols.

To eliminate the possible influence of different calculation procedures, we used the raw data on disintegrations/minute

supplied by each laboratory to recalculate all the results by a single method. The calibration lines were fitted to a four-parameter logistic curve (*13*), with use of nonlinear least squares (*14*).

For analysis of samples from patients who were receiving cyclosporine, participants followed the kit protocols exactly, included the quality-control samples ordinarily included in their assay, and repeated the measurement if duplicate determinations differed by >10%. The following details were recorded for each sample: patient's identification number, transplant type, and duration of cyclosporine therapy.

All concentrations are reported as micrograms per liter. Grouped data were tested for normality and, because the distributions were found to be skewed, median results are presented. Throughout the text, the RIAs based on the monoclonal antibodies are referred to as either the "specific" or "nonspecific" assays; the Ciclosporin RIA-Kit, containing the polyclonal antiserum, is called the "original" assay.

Table 1. Distribution of Samples

Center	Matrix	Clinical indications for cyclosporine and number of samples	Total no. samples	No. patients
1	B	187–R, 65–L, 18–BM, 16–AU	286	168
2	B	213–R, 1–H, 45–BM	259	179
3	P	40–R, 60–H, 60–L, 40–BM	200	93
4	P	16–R, 32–H, 150–L	198	50
5	B	198–R	198	21
6	B	86–R, 60–H, 49–L	195	79

R, renal Tx; H, heart Tx; L, liver Tx; BM, bone-marrow Tx; AU, autoimmune diseases. Tx = transplant, B = blood, P = plasma.

Table 2. Comparison of Monoclonal Specific vs Original Polyclonal RIA

Indication	Matrix	Ratio		Correlation coeff.	n
		Median	Range		
Renal	B	0.44	0.13–1.93	0.91	684
Bone marrow	B	0.57	0.24–0.85	0.95	63
Heart	B	0.31	0.09–0.59	0.85	61
Liver	B	0.32	0.08–0.95	0.61	114
Combined data	B	0.43	0.08–1.93	0.78	922
Renal	P	0.69	0.21–1.02	0.89	56
Bone marrow	P	0.76	0.17–1.04	0.95	40
Heart	P	0.40	0.16–1.02	0.83	92
Liver	P	0.45	0.06–1.11	0.79	210
Combined data	P	0.48	0.06–1.11	0.78	398

B = blood, P = plasma.

Table 3. Comparison of Monoclonal Nonspecific vs Original Polyclonal RIA

Indication	Matrix	Ratio		Correlation coeff.	n
		Median	Range		
Renal	B	1.5	0.50–4.57	0.92	684
Bone marrow	B	1.4	1.05–2.34	0.93	63
Heart	B	2.4	0.95–5.96	0.89	61
Liver	B	2.2	0.70–6.28	0.64	114
Combined data	B	1.5	0.50–6.28	0.84	922
Renal	P	1.5	0.95–2.69	0.93	56
Bone marrow	P	1.1	0.83–1.68	0.97	40
Heart	P	1.8	1.13–3.10	0.94	92
Liver	P	1.9	0.70–6.28	0.85	210
Combined data	P	1.8	0.70–6.28	0.89	398

B = blood, P = plasma.

Results

As assessed by descriptive statistics and graphical presentation, all six participating centers conducted the assessment of performance satisfactorily. The median within-center coefficients of variation (CV) for the four replicate samples of three pools were: within-assay 5% (upper quartile 8%) and between-assay 6.5% (upper quartile 9%).

Table 1 summarizes details of the number of samples analyzed, the matrix used, and the reasons for treatment with cyclosporine. The median duration of therapy was four weeks (range one day to 205 weeks).

Tables 2 and 3 summarize the median ratios and coefficients of correlation between the two monoclonal-antibody RIAs and the original RIA for the data as a whole and with respect to the four main transplant indications from which the samples were drawn. The 18 samples from patients receiving the drug for an autoimmune disease (primary biliary cirrhosis) were not included in the final analysis, because they formed too small a group for separate analysis.

The correlation between the specific and original assays was poorest for samples collected after heart or liver transplantation and best for samples collected after transplantation of kidney or bone marrow (Table 2). The relative difference between the specific and original assay was greater for blood samples than for plasma samples for each of the transplant groups. The differences between the transplant groups for the measurement of cyclosporine in blood and plasma samples are illustrated in Figure 1*a* and Figure 1*b*, respectively. Both figures show a tendency for the results for samples from heart- and liver-transplant patients to be displaced to the right, reflecting the markedly higher results produced by the original assay for many of these samples.

The correlation between the nonspecific and the original assays was better than that between the specific and the original assays. For both matrices the correlation between the two assays was poorest for samples collected after liver transplantation. The combined data for the four transplant indications are illustrated in Figures 2*a* and 2*b*, in which results for samples from liver-transplant patients tend to be displaced to the left, because the nonspecific assay produced particularly high results for many of these samples as compared with the original assay.

For samples of both blood and plasma, the relative difference between the specific and the two nonspecific assays was greatest for samples from heart- and liver-transplant patients, the monoclonal nonspecific assay giving particularly high results in such cases. This difference was smallest for the samples from patients receiving bone-marrow transplants. The median results for blood and plasma samples, obtained by the three assays in each of the four transplant indications, are illustrated in Figures 3*a* and 3*b*, respectively.

The poor specificity of both RIAs in which antibodies that cross-react with cyclosporine metabolites were used was well demonstrated by data from samples collected from liver-transplant patients. Figure 4 depicts the distribution of the ratios between the two nonspecific assays and the specific assay for the 114 blood samples. The range of results was very much larger for the monoclonal nonspecific antibody than for the polyclonal nonspecific antibody. The median ratio for the nonspecific/specific RIA was 3.4 (range 1.1–51.5); the corresponding figure for the ratio original/specific RIA was 3.1 (range 1.1–13.2). There were similar findings for plasma samples collected from liver-transplant

recipients: median ratio nonspecific/specific RIA 4.6 (range 1.6–64.7), median ratio original/specific RIA 2.2 (range 0.9–17.6). It is clear from Figure 3 that the disparity between the specific and nonspecific RIAs was even larger for blood samples from heart-transplant patients, the median ratio nonspecific/specific being 7.7 (range 2.7–23.0).

The ratio between a nonspecific and specific measurement the drug varied not only interindividually but also intraindividually. The within-patient variation was very pronounced in a series of plasma samples collected at Center 4 during 12 months from a patient who had received a liver transplant (Figure 5). During the first month after the transplant the nonspecific/specific ratio was as high as 15.5, but it declined rapidly, reaching values near 2.5 after three months.

Discussion

Clinical samples representative of four major transplant indications—kidney, heart, liver, and bone marrow— have been analyzed for cyclosporine by three radioimmunoassays with differing specificities for the parent compound. The samples were drawn from a broad variety of patients receiving the drug, including those in the early postoperative phase and stable transplant patients.

Recent studies based on the use of the specific-antibody RIA for the measurement of cyclosporine in clinical samples have established that it produces results practically indistinguishable from those produced by a reference HPLC assay (*11, 12*). Thus, this RIA is the first immunoassay to give results that are specific for the parent compound, with

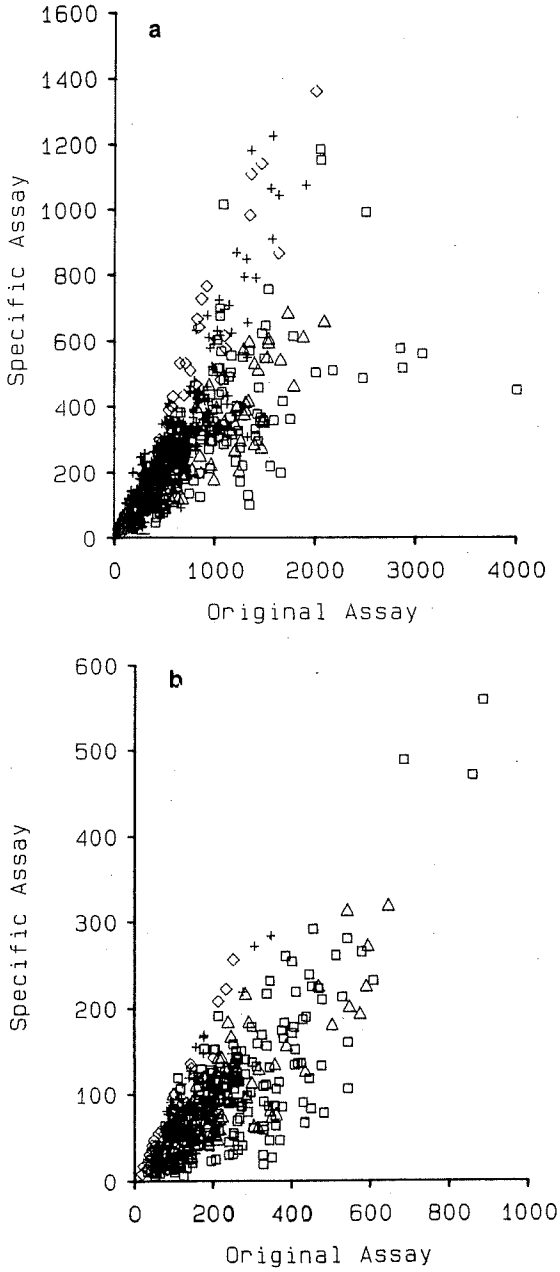

Fig. 1. Concentrations of cyclosporine measured by the original polyclonal and monoclonal specific RIA's in (*a*) 922 blood samples and (*b*) 398 plasma samples from patients receiving the drug
Transplant indications: + renal, ◇ bone marrow, △ heart, □ liver

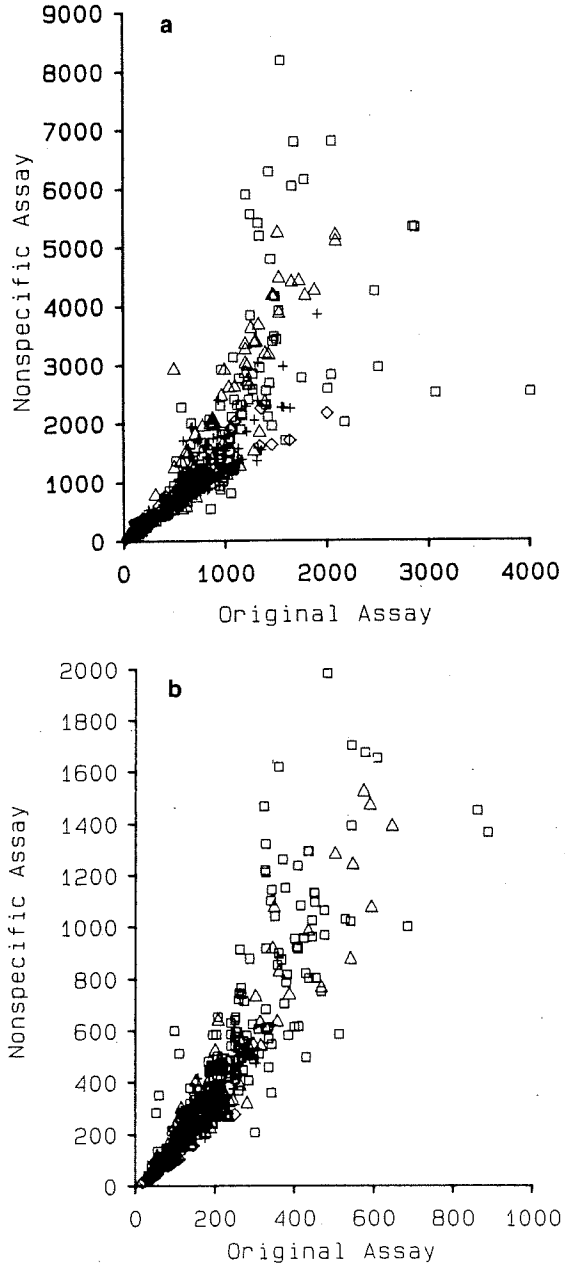

Fig. 2. Concentrations of cyclosporine measured by the original polyclonal and monoclonal nonspecific RIA's in (*a*) 922 blood samples and (*b*) 398 plasma samples from patients receiving the drug
Transplant indications: + renal, ◇ bone marrow, △ heart, □ liver

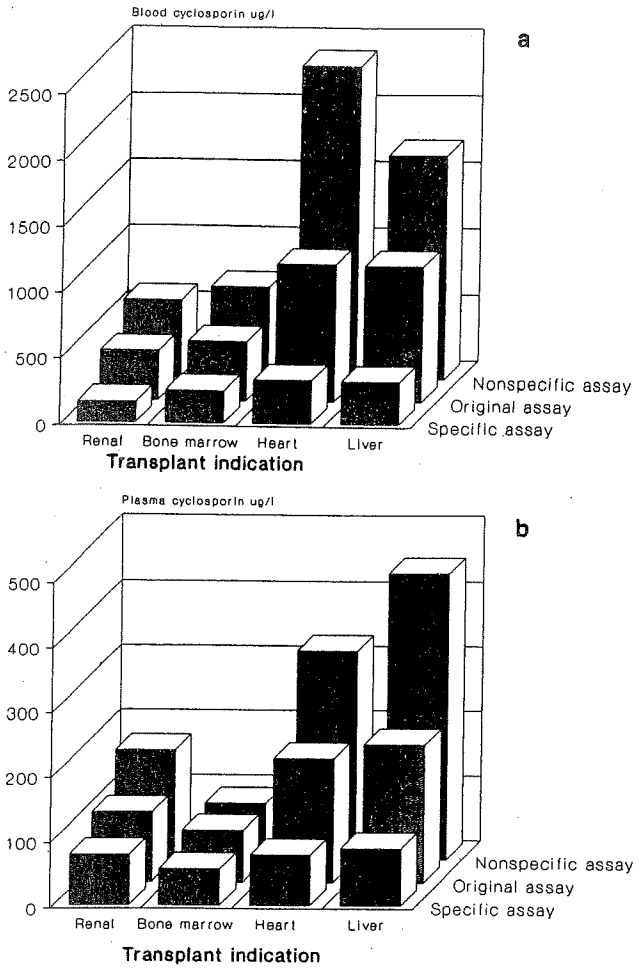

Fig. 3. Median cyclosporine concentrations measured by each of the three RIA's in four transplant indications: (*a*) in blood samples and (*b*) in plasma samples

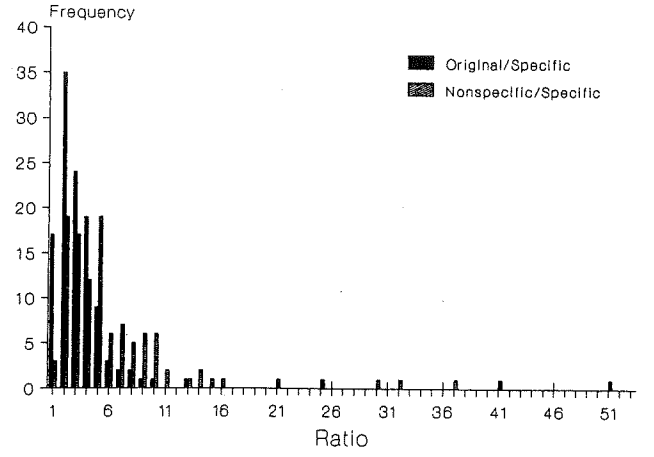

Fig. 4. Distribution of ratios comparing the measurement of cyclosporine by the original and nonspecific RIAs with the specific RIA measurement in 114 blood samples from liver-transplant patients

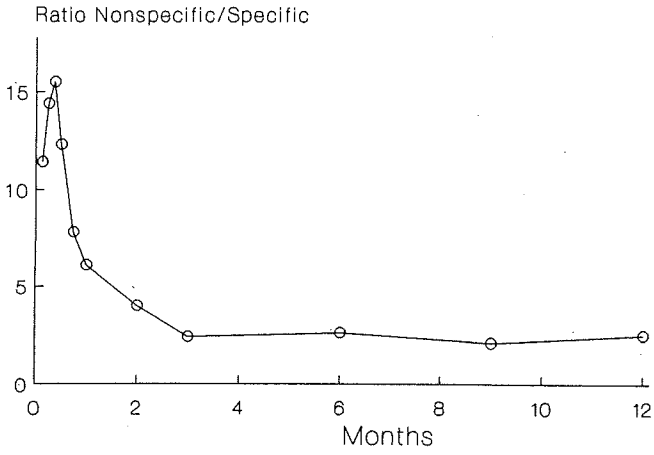

Fig. 5. Variation in the ratio nonspecific/specific RIA measurement of cyclosporine in a series of plasma samples collected from a single patient for up to 12 months after liver transplantation

no appreciable interference from the metabolites of the drug.

Correlations between the results obtained with the Ciclosporin RIA-Kit and with both antibodies supplied in the Sandimmun®-Kit were very similar with either blood or plasma as the sample matrix. The correlations were best for samples from patients receiving renal or bone-marrow transplants and were poorest for samples from heart- and liver-transplant patients. These differences reflect the relative concentrations of cyclosporine and its metabolites that are found in the transplant indications studied and are consistent with previously reported data comparing selective HPLC measurements with those produced by the original RIA (*3, 4*).

For blood samples from patients receiving kidney or bone-marrow transplants the specific assay produced median results that were about half as great as those given by the original assay; the median results for plasma samples collected from such patients were about 70% as great as those produced by the original assay. For both matrices, the difference between the original assay and the specific assay was smaller for samples from patients receiving bone-marrow transplants than for samples from renal-transplant patients, suggesting that there is a smaller proportion of cyclosporine metabolites in samples collected from recipients of bone-marrow transplants.

The difference between the specific and the original assays was even larger after heart and liver transplantation. For blood samples, the median results with the specific antibody were about 33% as large as those given by the original assay; the comparable figure for plasma samples was between 40 and 45%. Several authors have noted a relatively large difference between the original RIA and a selective measurement of cyclosporine in samples from these two transplant groups, especially early after liver transplantation. In our group of liver-transplant patients the distribution of the ratio between the original and specific assays was almost identical with that described by Burckart et al. (*3*), who used the original RIA and HPLC for blood samples collected from pediatric liver-transplant patients.

Almost all the measurements made by the specific assay were lower than the corresponding measurements made by the original assay. Only 15 of 1320 were higher by the specific assay and, on re-examination of the results, it was noted that for seven of these the original assay result was below the limit of accurate measurement (<62.5 μg/L). Because the specific assay has a lower limit of sensitivity than the original assay, this resulted in an unreliable calculation of the ratio. For all the transplant indications studied, the median ratio between the specific and original

assays was higher for plasma than for blood. This observation is consistent with the relatively smaller proportion of cyclosporine metabolites found in plasma than in whole blood (*15*). As a result there is a smaller difference between a specific and a nonspecific measurement when plasma is the sample than when the corresponding whole blood is the mple.

The nonspecific and original assays correlated better than the specific and the original assays, because both the nonspecific antibodies cross-react with the cyclosporine metabolites commonly found in man. However, the nonspecific assay gave even higher results than those produced by the original assay. This observation is in line with the broader spectrum of cross-reactivity possessed by the monoclonal nonspecific antibody, especially with respect to metabolite 17 (*10*), which is found in relatively high concentration in man (*16*). For blood samples the median results with the nonspecific assay were 40% to 140% higher than those produced by the original assay, depending upon transplant indication; median results for plasma samples were 10% to 90% higher. For both matrices samples from liver- and heart-transplant patients gave the largest difference when the nonspecific assay was compared with the original assay.

The discrepancy between the two nonspecific measurements of cyclosporine was particularly noticeable for samples from liver-transplant patients. The range of ratios between the original and specific assays was very similar to the previously reported value comparing the original RIA and HPLC (*3*), but the range of ratios was even greater when the results for the nonspecific and specific assays were compared (range 1.7 to 51.5). This increase in the range of ratios, and the higher measured concentration, reflected the broader spectrum of cross-reactivity of the nonspecific antibody. Previous studies have shown high proportions of cyclosporine metabolites in the plasma or blood of patients o have received heart or liver transplants, especially soon ter the transplantation (*3, 4, 17*). The marked within-patient variation in the ratio between a specific and nonspecific measurement at various times after transplantation was demonstrated well in an individual patient by both the specific and nonspecific RIA assays (Figure 5).

The original Sandoz RIA is to be withdrawn from most markets this year, to be replaced by the Sandimmun®-Kit. In some countries this has already taken place. In view of the marked differences we have noted in the values obtained by the nonspecific RIA assay as compared with the specific measurement, transfer to the specific assay seems to be more appropriate than transfer to the nonspecific assay. This conclusion is also in accord with one of the principal findings of the American Task Force on Cyclosporine Monitoring (*8*).

The clinical value of the new monoclonal nonspecific assay has yet to be established, but its availability, with a guaranteed spectrum of cross-reactivity, could prove to be a valuable research tool. Preliminary studies have been undertaken to determine the significance of the specific and nonspecific assay measurements (*18*). Further studies will be needed to establish whether there is a role for the nonspecific RIA in patient monitoring.

Our experience suggests that, for recipients of renal transplants or bone-marrow transplants, the results for the specific RIA will be about 45% to 55% as great as those obtained by the original RIA when the measurement is made in blood; for plasma samples the results will be about % as great as those obtained with the original assay. However, to transfer from the original assay to the specific assay we suggest that centers should establish their own within-house correlation between the two assays. Although the overall correlation between the assay results is highly significant, the difference between the original and specific assays can vary widely, even for patients with these indications. For the measurement of cyclosporine in samples from patients who have received heart or liver transplants, the difference between the original and specific assays varies so widely that it is inappropriate to equate specific measurements with original assay results by means of multiplication factors.

Our data using the nonspecific assay suggest that the monoclonal nonspecific antibody produces results that are, in some instances, substantially higher than those produced by the original RIA; the clinical significance of these results has yet to be established. The data obtained for samples collected from liver-transplant patients indicate that this assay should not be used alone for the measurement of cyclosporine in this transplant indication, because spuriously high results may be obtained. Similarly, its use alone after heart transplantation should be avoided. Whether monitoring within-patient changes in the ratio between the specific and nonspecific assays is of clinical value requires further investigation.

In conclusion, we welcome the availability of a specific RIA for the measurement of cyclosporine. Its use could help unify the data accumulating in the various transplant indications and provide a technically less-demanding method than HPLC for the study of cyclosporine pharmacokinetics. In addition, kits based on semi-automated, isotopic, and non-isotopic techniques may incorporate the monoclonal specific antibody. Thus, those centers at which a routine service for the measurement of the compound by HPLC was impractical can now assess the clinical utility of specific measurements with ease.

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Atypical Patterns of Lactate Dehydrogenase Isoenzymes in Acute Myocardial Infarction

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Total lactate dehydrogenase (LD; EC 1.1.1.27) activity in serum and LD isoenzymes were quantified in 190 patients with acute myocardial infarction (AMI) 24, 48, and 72 h after admission. In 90% of the 570 blood specimens an LD isoenzyme pattern typical of AMI (LD-1/LD-2 >0.76) was found. The other 56 blood specimens showed an LD isoenzyme pattern atypical of AMI (LD-1/LD-2 <0.76). They were divided into three groups: 28 specimens with isomorphic pattern (relative increase in all five LD isoenzymes); 18 with relatively increased LD-3 proportion (>35%); and 10 specimens with increased LD-5 proportion (>10%). No difference was found in mean total LD activity in serum between the typical isoenzyme group and the three atypical groups. The LD isomorphic pattern was found in 60% of AMI patients complicated by cardiogenic shock. Fifty percent of AMI patients admitted with pulmonary edema showed increased LD-3 proportion and half of the patients with AMI and congestive heart failure, predominant right, demonstrated increased LD-5 proportion. We conclude that although most patients with AMI present at diagnosis with a typical LD isoenzyme pattern, it is important to recognize that some may present with atypical LD isoenzyme patterns, which may be associated with specific AMI complications.

Additional Keyphrases: *cardiogenic shock · pulmonary edema · "flipped" LD pattern · congestive heart failure*

It is routine to assess myocardial damage and to diagnose acute myocardial infarction (AMI) by determining creatine kinase (CK; EC 2.7.3.2.) and lactate dehydrogenase (LD; EC 1.1.1.27) in serum (*1*).[3] The correlation between the LD-1/LD-2 ratio and AMI is well established (*1–3*), and LD-1>LD-2 ("flipped" LD pattern) may be found in about 80% of AMI patients during the first 72 h (*1, 4*). Others (*5, 6*) found that an LD-1/LD-2 ratio >0.76 is more sensitive for diagnosing AMI: it may be found in almost all patients with AMI and has a specificity of 91%. However, only few studies (*7–9*) deal with the atypical patterns of LD isoenzymes that may be found in patients with AMI, and their significance.

The aim of our study was to try to characterize atypical patterns of LD isoenzymes obtained in patients with AMI during the first 72 h after admission and to find whether they had any clinical significance.

Patients and Methods

One hundred and ninety patients with AMI admitted to the coronary-care unit and internal-medicine department were studied, 128 men, mean age 64 years (range 41–65) and 62 women, mean age 56 years (range 42–73). The diagnosis of AMI was based on WHO criteria, which included a typical clinical history, typical electrocardiographic pattern, definite enzyme results (increase in total serum CK activity, with MB fraction 4% or more of the total), or postmortem confirmation. The LD data were not used in making the diagnosis.

Blood specimens were obtained 24, 48, and 72 h after admission. Each specimen was assessed for LD activity in serum, LD isoenzymes, CK activity in serum, and CK isoenzymes. Blood samples for LD activity and isoenzyme analyses were kept at 4 to 8 °C until assay (no longer than 24 h).

Total LD activity in serum was measured at 37 °C by the method of Wacker et al. (*10*), with a centrifugal analyzer (Electro-Nucleonics, Fairfield, NJ). The normal reference interval in our laboratory is 100 to 225 U/L, and the CVs for normal and abnormal serum LD values are 3.3 to 4.2%. The proportions of the LD isoenzymes were determined by electrophoresis on cellulose acetate with the Helena kit (cat. no. 5451) and instrumentation (Helena Laboratories, Beaumont, TX). The normal reference interval for the LD isoenzymes, expressed in percent of total LD, are: LD-1 10–31%,

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[3] Nonstandard abbreviations: AMI, acute myocardial infarction; LD, lactate dehydrogenase; CK, creatine kinase.

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