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Two Specific Immunoassays of Cyclosporine Compared in Liver Transplant Recipients

To the Editor:

Cyclosporine (Cs A) measurement in blood has been used to guide dosing and reduce the risk of nephro- or hepatotoxicity in patients while maintaining adequate immunosuppression (1, 2). Abbott Laboratories (Abbott Park, IL) has recently developed a nonradioactive monoclonal immunoassay for Cs A in whole blood, based on fluorescent polarization (FPIA). Yatscoff et al. (3) reported a good correlation between the FPIA assay and the Sandimmune selective RIA in blood specimens from renal transplant recipients.

Because the metabolism of Cs A and the excretion of metabolites are strongly influenced by hepatic disease, we compared the Cs A results obtained with two specific assays, FPIA (Abbott) and RIA Cyclo-Trac SP (Instar, Stillwater, MN), which involve the use of two different monoclonal antibodies and 52 blood samples from four liver transplant recipients (average post-transplant time, 89 days; range, 1–335 days). Both assays were performed according to the manufacturers' instructions (4, 5). A multicrystal gamma counter (LB 2104; Berthold, München, F.R.G.) measured 125I activity (counting time per sample, 120 s). The FPIA response was measured with Tdx instrumentation (Abbott).

We also analyzed the serum specimens for bilirubin (Hitachi 717; Boehringer, Mannheim, F.R.G.), aspartate aminotransferase (EC 2.6.1.1; AST), and alanine aminotransferase (EC 2.6.1.2; ALT) to determine the extent of liver damage in these patients. The mean (and range) for bilirubin, AST, and ALT concentrations were 86.1 (21.7–240.2) mg/L, 91.97 (6–2085) U/L, and 137.8 (10–1495) U/L, respectively.

The average overall CVs were 4.63% and 7.99% for the FPIA and RIA methods, respectively. The regression analysis for Cs A concentrations determined with the Cyclo-Trac (x) and Tdx (y) assays was y = 0.978x + 134.13 (r = 0.89, n = 52, S_yx = 56.08 µg/L).

To see whether the discrepant Cs A results we found between methods could be attributed to liver damage, we investigated the relationships between bilirubin, AST, and ALT concentrations and the corresponding Tdx/Cyclo-Trac ratios. The comparison among all patients showed no significant correlations. The correlations for individual patients are given in Table 1.

Cs A values measured by the two methods show a weak correlation, the Tdx values being higher than those of Cyclo-Trac. This is probably due to the greater cross-reactivity of the antibody used in the Tdx assay with the primary Cs A metabolites: M-17 (8.2%), M-1 (15.3%), and M-21 (3.7%) (3). In contrast, the cross-reactivity of the Cyclo-Trac antibody with these metabolites is <2% (6). The metabolites that produce the greatest cross-reactivity in the Tdx assay are usually in high concentrations in the blood of transplant patients; moreover, the relative concentrations of Cs A and each metabolite differ considerably among patients (7).

In our group, bilirubin concentrations were always above normal (mean 86 mg/L). Because excretion in the bile is the major pathway for eliminating Cs A metabolites (8), perhaps the higher Cs A results in the Tdx assay are explained by the impaired excretion of Cs A metabolites through the biliary system.

In summary, caution seems to be warranted in monitoring Cs A by different specific methods in liver transplant recipients; the differing cross-reactivity of the antibodies may result in substantial disagreement in Cs A values between methods.

References

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Seum Fructosamine and Obesity

To the Editor:

Serum fructosamine is widely used as an indicator of the short-term control of diabetes mellitus. Its value is influenced by the mean blood glucose concentration in the previous two to three weeks as well as by the half-life of serum proteins. Repeatedly observed low concentrations of serum fructosamine, despite poor control in obese Type 2 diabetic patients, prompted us to quantify fructosamine in obese healthy persons.

We measured the serum fructosamine concentrations according to Johnson et al. (1) in 25 healthy nonobese persons (mean age 36, range 20–45 years) and in 25 obese subjects (mean age 33, range 19–50 years). A significant difference in the mean (±SD) body mass index was observed

Table 1. Inpatient Correlations of Tdx Results with Cyclo-Trac Results and of the Tdx/Cyclo-Trac Ratio with Various Measures of Liver Function

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tdx vs Cyclo-Trac</th>
<th>Tdx/Cyclo-Trac vs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tdx vs Cyclo-Trac</td>
<td>BT AST ALT</td>
</tr>
<tr>
<td></td>
<td>Tdx vs Cyclo-Trac</td>
<td>BT AST ALT</td>
</tr>
<tr>
<td>1</td>
<td>0.91</td>
<td>-0.39</td>
</tr>
<tr>
<td>2</td>
<td>0.96</td>
<td>0.18</td>
</tr>
<tr>
<td>3</td>
<td>0.44</td>
<td>0.99</td>
</tr>
<tr>
<td>4</td>
<td>0.88</td>
<td>0.41</td>
</tr>
</tbody>
</table>

BT, total bilirubin.
Adenosine Deaminase Increased in Serum in Toxoplasmosis

To the Editor:

Adenosine deaminase (ADA; EC 3.5.4.4), an enzyme essential for differentiation of lymphoid cells, has been used for monitoring several diseases in which immunity is altered (1-3). Toxoplasmosis is a parasitic disease of worldwide distribution caused by *Toxoplasma gondii*. As in many parasitic infections, cell-mediated immunity is involved in protecting the organism against this parasite (4, 5).

We measured catalytic concentrations of ADA in serum from a group of healthy controls (n = 22) and from a group of patients presenting with IgM antibodies to *T. gondii* (n = 20). Fast- ing blood samples were obtained by venipuncture. All variables were controlled according to IFCC recommendations (6). In all cases, the enzyme activities in serum exhibited a gaussian distribution. Results for the groups were compared by using Student’s t-test.

Catalytic concentrations of serum ADA differed significantly (P = 0.031) between groups: 14.7 (SD 3) U/L for the controls vs 24.4 (SD 18.5) U/L for the toxoplasmosis-infected patients. In nine patients (45%), serum ADA concentrations were >20 U/L.

As has been pointed out, IgM antibodies to *T. gondii* can be persistently increased in the clinical course of disease (7). Accordingly, in some patients, acute-phase diagnosis and management are very difficult. According to our data, measuring serum ADA in patients infected with this parasite could be helpful in monitoring the course of disease. However, further studies will be necessary to establish the role of ADA as a marker in *T. gondii* infection.

References

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Simple, Quantitative Enzymic Assay of Galactose in Urine

To the Editor:

Galactosemia is a rare inborn error of galactose metabolism, with an incidence of 1:68 000 live births in New Zealand. Despite the introduction of regional neonatal screening programs for the disorder based on whole-blood analysis, the routine laboratory is often asked to test for reducing substances in urine before the results of the blood test are available. In the past, we have performed the urine test with Clinistest tablets (Ames Division, Miles Labs., Mulgrave, Vic., Australia), with positive urine samples then being specifically tested for glucose, in keeping with the recommendations of others (1). However, the simple interpretation of these results can be erroneous because of reducing substances other than galactose (2). Therefore, in an attempt to prevent unnecessary interruptions to infant feeding, we have developed a simple assay for the rapid estimation of galactose in urine.

The urine assay is based on that for whole blood (3, 4), D-galactose being oxidized to D-galactonolactone with concomitant reduction of NAD⁺ in the presence of galactose dehydrogenase.

References


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