Intestinal origin alkaline phosphatase activity in plasma for differential diagnosis of jaundice

T Kuwana, S B Rosalki

Abstract
Intestinal origin alkaline phosphatase activity (ALP) in plasma was measured by a sensitive immunocapture assay in 104 jaundiced patients—84 with intrahepatic and 20 with post-hepatic jaundice. Increased enzyme activities were observed in those with intrahepatic disease and subnormal values in those with post-hepatic disease. At a discriminant level intestinal origin ALP showed a diagnostic sensitivity of 77% for intrahepatic cholestasis, with a diagnostic accuracy of 75% for its differentiation from post-hepatic jaundice. This diagnostic accuracy is not as good as that derived with other techniques—for example, imaging—and the technique is therefore not recommended as a supplement or replacement.

Intestinal origin alkaline phosphatase (ALP; EC 3.1.3.1) may be increased in plasma in intrahepatic disease, but is unusual in post-hepatic cholestasis.1-3 Using polyacrylamide gel electrophoresis, Warnes, Hine, and Kay found intestinal (β-globulin mobility) ALP in the plasma of 45% of jaundiced patients with intrahepatic disease, but not in those with post-hepatic lesions.4 They suggested that in jaundiced patients its presence was indicative of an intra-hepatic aetiology and was a useful differential diagnostic aid. They did not, however, study the effect of blood group, a factor that is known to affect the activity of this isoenzyme.5-7

Studies from our laboratory, using both polyacrylamide gel and cellulose acetate membrane electrophoresis, indicated poor diagnostic sensitivity of intestinal ALP for intrahepatic jaundice, especially in patients not of blood group O, and incomplete specificity for post-hepatic disease, so that overall efficiency for the differentiation of jaundice was poor.8 This poor differentiation by these qualitative procedures was supported by quantitative studies of activity remaining after inhibition of p-bromotetramisole.3 These showed substantial overlap in intestinal origin ALP activity between the two diagnostic groups.

The development of more sensitive measurements of intestinal origin ALP activity by immunoassay has increased interest in possible differences between the two forms of jaundice. Using such an assay, Domar et al noted a pronounced increase in intestinal origin ALP in patients with non-biliary or primary biliary cirrhosis but no increase in patients with other causes of cholestasis.9 The numbers studied were small, however, and again blood group was not considered.

In addition to high analytical sensitivity, immunological measurement of intestinal origin ALP has the advantage of measuring both intestinal (β-electrophoretic mobility) ALP and the so-called intestinal variant fraction.10,11 This latter fraction is normally obscured by liver and bone isoforms on electrophoresis. It is thought to represent intestinal ALP that is released with attached membrane-binding domain.11 Though p-bromotetramisole inhibition also quantifies both fractions,12 immunoassay provides greater sensitivity and precision at low activity.

We therefore re-evaluated the measurement of intestinal origin ALP in the plasma for the differentiation of intra- and post-hepatic jaundice using a highly specific and sensitive immunoassay13 that reacts with both intestinal and intestinal variant fractions, which together comprise intestinal origin ALP in plasma.

Methods
Non-fasting plasma samples were studied from 104 consecutive patients admitted to a specialist liver unit, with total bilirubin greater than 50 μmol/l: 84 had intrahepatic and 20 post-hepatic jaundice. Diagnosis (table 1) was based on clinical findings, together with liver function tests and serological tests either alone (n = 17) or supplemented by ultrasonography (n = 46), radiological examination (n = 65), or liver biopsy.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical diagnosis in jaundiced patients studied</th>
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| Intrahepatic jaundice | Non-biliary cirrhosis: alcoholic 27  
  Other 13  
  Hepatitis: alcoholic drug 8  
  Other 5  
  Primary biliary cirrhosis 9  
  Carcinoma 4  
  Miscellaneous 15  
  Total 84 |
| Post-hepatic jaundice | Gallstones 3  
  Benign strictures 3  
  Carcinoma: pancreas 9  
  Bile duct 1  
  Miscellaneous 4  
  Total 20 |

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for jaundice.\(^8\)\(^1\) G0 C-i

Blood tP < 0

**Table 2** Intestinal origin ALP in healthy subjects and in patients with intra- and post-hepatic jaundice

<table>
<thead>
<tr>
<th>Intestinal origin ALP (IU/l)</th>
<th>Healthy subjects</th>
<th>Jaundiced patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Upper reference limits</td>
</tr>
<tr>
<td>Blood group O</td>
<td>2-9 (n = 36)</td>
<td>12-0</td>
</tr>
<tr>
<td>A</td>
<td>0-6 (n = 19)</td>
<td>2-0</td>
</tr>
<tr>
<td>All</td>
<td>1-5 (n = 61)</td>
<td>12-0</td>
</tr>
</tbody>
</table>

*p < 0-05, **p < 0-005 compared with healthy subjects by Mann-Whitney U test.

The distribution of intestinal origin ALP values in both patients and students was non-Gaussian (figure). Median activities in the patients for all blood groups considered together, and for blood groups O and A considered separately, are shown in table 2. Median values on the student samples are also shown, together with their upper reference limits (98th percentile).

Compared with the reference population, intestinal origin ALP was increased in the patients with intrahepatic jaundice (p < 0-005, overall, by Mann-Whitney U testing) and decreased (p < 0-05) in post-hepatic jaundice, resulting in a significant (p < 0-005) difference in activity between those with intrahepatic and those with post-hepatic jaundice.

The clinical sensitivity and specificity of intestinal origin ALP measurement for the diagnosis of intrahepatic jaundice were calculated (table 3) using the blood group related upper reference limits (12 IU/l for blood group O, 2 IU/l for group A). A discriminant value was also calculated to give the fewest number of misclassifications between intra- and extra-hepatic disease. This level was IU/l for all patients considered together and for the individual blood groups O and A. Using this discriminant, clinical sensitivities and specificities were calculated again (table 3).

**Discussion**

Our findings confirm the finding of higher intestinal origin ALP in plasma in healthy subjects and in patients with liver disease of blood group O compared with blood group A.\(^5\)\(^7\) With such differences taken into account, increased intestinal origin ALP was seen in plasma in intrahepatic jaundice and reduced activity in post-hepatic disease. This agrees with a previous suggestion that post-hepatic biliary obstruction may interfere with the absorption of intestinal ALP; hepatocyte damage may decrease its plasma clearance.\(^1\)\(^4\)

To assess the diagnostic value of the measurement we first considered activity in relation to the relevant blood group upper reference limit, 12 IU/l for blood group O and 2 IU/l for group A, respectively. Other blood groups were insufficiently represented in the reference population to derive reference values. The diagnostic sensitivity for intrahepatic jaundice of increased intestinal origin ALP activity was poor (45%), as was diagnostic accuracy (54%), though specificity (89%) and the predictive value of a positive test (94%) in the population (prevalence of intrahepatic jaundice 81%) were good.

The need to identify increased activity by referring to the patients’ blood group was a disadvantage. Fortunately, the activity value giving the fewest misclassifications between intra- and post-hepatic jaundice (1 IU/l) was the same for the blood groups A and O as for the total patient population, permitting the use of a single diagnostic discriminant value. Using this discriminant, diagnostic sensitivity improved (to 77%), as did accuracy (75%), though at the expense of reduced specificity (65%).

This diagnostic accuracy of 75% is a considerable improvement on the less than 60% value previously obtained by electrophoretic demonstration of intestinal ALP.\(^*\) As it was

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\(^*\)p < 0-05, **p < 0-005 compared with healthy subjects by Mann-Whitney U test.

\(^\dagger\)p < 0-005 compared with intra-hepatic jaundiced patients by Mann-Whitney U test.
obtained using a quantitative immunoassay of excellent analytical performance further improvement of diagnostic accuracy is unlikely.

In conclusion, an overall diagnostic accuracy of (at best) 75% was obtained by a specific and sensitive measurement of intestinal origin ALP. As this method remains inferior to accuracies close to 90% that can be obtained with other techniques used for the differential diagnosis of jaundice such as ultrasonography, computed tomography, and liver biopsy, we do not recommend that these procedures be replaced or supplemented by the measurement.

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