Clinical Implications of Differences between Two Recommended Procedures for Determination of Aspartate Aminotransferase

Jan L. S. Dols1 and Anton P. van Zanten2

We compared two officially recommended methods for determination of aspartate aminotransferase (EC 2.6.1.1): that of the International Federation of Clinical Chemistry (IFCC) and that of the Deutsche Gesellschaft für Klinische Chemie (DGKC). We used automated enzyme analyzers, initiating the reactions with 2-oxoglutarate. Normal values, 10–30 U/L (IFCC) and 7–18 U/L (DGKC), were apparently insensitive to intra-individual variations. Samples obtained from patients with heart disease showed a markedly different amount of activation with pyridoxal phosphate as compared with samples from other patient categories. Ratios for aspartate aminotransferase/alanine aminotransferase, as used in the differentiation of liver disease, can still be used with either method for determination of aspartate aminotransferase.

Additional Keyphrases: aminotransferase ratios • heart disease • liver disease

We compared the standard method of the Deutsche Gesellschaft für Klinische Chemie (DGKC) for the determination of aspartate aminotransferase (ASAT) (1) with a method based on the use of optimized reagents according to the International Federation of Clinical Chemistry (IFCC) (2, 3).

The IFCC method differs from the DGKC method in the temperature at which activity is measured, the buffer system, the volume fraction of sample, the addition of pyridoxal phosphate, and the use of sample and reagent blanks.

Before introducing the IFCC method in our routine laboratories, we investigated the influence of using the optimized reagents on enzyme activities from normal and from pathological samples.

Materials and Methods

Materials

Reagents. L-Aspartic acid, L-alanine, Tris, disodium EDTA, and all inorganic substances were obtained from E. Merck, Darmstadt, F.R.G. Sodium 2-oxoglutarate, disodium NADH, pyridoxal-5-phosphoric acid monohydrate, lactate dehydrogenase (LDH) from hog muscle, and malate dehydrogenase (MDH) from pig heart (both in 500 mL/glycerol solution) were obtained from Boehringer, Mannheim, F.R.G. All chemicals were of analytical grade. LDH and MDH contained <0.005% ASAT and ALAT, and <0.003% glutamate dehydrogenase (GLDH). All contaminant catalytic activities were measured as stipulated in references 2 and 3.

Apparatus. For spectrophotometric measurements we used automated Eppendorf 5020 and 5040 enzyme analyzers (Eppendorf Gerätebau, Netheler & Hinz, Hamburg, F.R.G.). pH was measured at 25 and 30 °C with an IL 305 pH meter (Instrumentation Laboratory, S.P.A., Dugano, Italy) and an ElKay OHP-1433-U combined pH reference electrode (El-Kay Products Inc., Worcester, MA 01613).

Samples. We analyzed 968 serum and plasma samples: 223 samples from apparently healthy hospital workers and 745 patients' samples, submitted to our laboratories for routine diagnostic investigation.

The patients' samples consisted of 225 samples from patients admitted to the coronary-care unit, 101 from patients suffering from liver disease, 61 from patients suffering from chronic renal disease, and 358 that could not be classified in one of the above-specified categories; the last three categories included both in- and outpatients.

Blood was collected into evacuated blood-collection tubes (Venoject; Terumo Europe N.V., Haasrode, Belgium). Serum and heparinized plasma were obtained by centrifugation, and were stored at −20 °C for no longer than five weeks.

Procedures

DGKC procedure. ASAT and ALAT activities were measured at 25 °C with the Eppendorf 5040 enzyme analyzer as follows: to 250 μL of reagent, add 50 μL of plasma or serum and preincubate for 7.5 min. Start the reaction by adding 50 μL of 2-oxoglutarate to the pre-incubation mixture; after 80 s measure the absorbance at 334 nm (mercury line) for 20 s.

The final composition of the ASAT incubation mixture was: phosphate 80 mmol/L (pH 7.4 at 25 °C), L-aspartate 200 mmol/L, 2-oxoglutarate 12 mmol/L, β-NADH 0.18 mmol/L, LDH 1200 U/L (at 25 °C), and MDH 600 U/L (at 25 °C).

The final composition of the ALAT incubation mixture was: phosphate 80 mmol/L (pH 7.4 at 25 °C), L-alanine 800 mmol/L, 2-oxoglutarate 18 mmol/L, β-NADH 0.18 mmol/L, and LDH 1200 U/L (at 25 °C).

IFCC and Bergmeyer et al. (4) procedures. ASAT and ALAT activities were measured at 30 °C with the Eppendorf 5020 analyzer as follows:

To 500 μL of reagent add 50 μL of plasma or serum and pre-incubate for 15 min. Start the reaction by adding 50 μL of 2-oxoglutarate to the pre-incubation mixture including pyridoxal phosphate. After 26 s, measure the absorbance at 334 nm (mercury line) for 154 s. No corrections for reagent and sample blanks were made.

The final composition of the ASAT incubation mixture was: Tris 80 mmol/L (pH 7.8 at 30 °C), L-aspartate 240 mmol/L, 2-oxoglutarate 12 mmol/L, β-NADH 0.18 mmol/L, pyridoxal phosphate 0.10 mmol/L, LDH 600 U/L (at 30 °C), and MDH 420 U/L (at 30 °C).

The final composition of the ALAT incubation mixture (4) was: Tris 100 mmol/L (pH 7.5 at 30 °C), L-alanine 500 mmol/L.
Table 1. Precision Studies

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>( \bar{x} ) and (SD), U/L</th>
<th>CV,%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pooled serum</td>
<td>15</td>
<td>8.1 (0.99)</td>
<td>12.1</td>
</tr>
<tr>
<td>Pooled serum</td>
<td>15</td>
<td>16.5 (0.99)</td>
<td>6.0</td>
</tr>
<tr>
<td>Animal ASAT in albumin</td>
<td>15</td>
<td>27.2 (0.56)</td>
<td>2.0</td>
</tr>
<tr>
<td>Animal ASAT in albumin</td>
<td>15</td>
<td>60.3 (0.98)</td>
<td>1.6</td>
</tr>
<tr>
<td>Day-to-day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal ASAT in albumin</td>
<td>18</td>
<td>60.8 (1.79)</td>
<td>2.9</td>
</tr>
<tr>
<td>Animal ASAT in albumin</td>
<td>51</td>
<td>26.2 (1.63)</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Table 2. Intra- and Interindividual Variability of ASAT

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>( \bar{x} ) and (SD), U/L</th>
<th>CV,%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGKC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-individual variability</td>
<td>Av ( s_{\bar{x}} ) ( (U/L)^2 )</td>
<td>1.75</td>
<td>7.4</td>
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<tr>
<td>Mean ASAT</td>
<td></td>
<td>10.2</td>
<td>19.3</td>
</tr>
<tr>
<td>Av CV</td>
<td></td>
<td>9.7</td>
<td>13.3</td>
</tr>
<tr>
<td>Range CV</td>
<td></td>
<td>5-30</td>
<td>5-30</td>
</tr>
<tr>
<td>Interindividual variability</td>
<td>( s_{x} ) ( (U/L)^2 )</td>
<td>4.18</td>
<td>14.93</td>
</tr>
<tr>
<td>( s_{y} ) ( (U/L)^2 )</td>
<td></td>
<td>2.45</td>
<td>8.44</td>
</tr>
<tr>
<td>( s_{o} ) ( (U/L)^2 )</td>
<td></td>
<td>3.69</td>
<td>13.24</td>
</tr>
<tr>
<td>Index of intra- vs interindividual variability</td>
<td>( r )</td>
<td>0.70</td>
<td>0.75</td>
</tr>
</tbody>
</table>

*See text for definition of terms (from ref. 6).
*bCorrected for the analytical component of variance.

d, 2-oxoglutarate 15 mmol/L, \( \beta \)-NADH 0.18 mmol/L, and LDH 1200 U/L (at 30 °C).

To evaluate the effect of pyridoxal phosphate in the IFCC reagent, we used the above ASAT incubation mixture, but omitted the pyridoxal phosphate.

Statistics. For statistical analyses we used Biomedical Computer Programs (5).

Results

We observed no difference in ASAT results between 111 paired serum (y) and heparinized-plasma (x) samples, by either the IFCC method or the DGKC method. The respective regression equations and statistics are: \( y = 1.001x + 0.05, \bar{x} = 31.07, \bar{y} = 31.52, r^2 = 0.970; \) and \( y = 0.991x - 0.07, \bar{x} = 27.26, \bar{y} = 26.94, r^2 = 0.985. \)

Applying Student's \( t \)-test (considering the serum and plasma enzyme activity determinations as duplicates), we found \( t = 10.05, \nu = 109, p << 0.001 \) for the DGKC method.

Figure 1 shows the correlation between results for all 968 samples, determined by both methods: \( y = 2.312x - 1.13, \bar{x} = 26.95, \bar{y} = 61.07, r^2 = 0.821. \)

Results for samples from 223 apparently healthy hospital workers are shown in Figure 2. They showed no gaussian distribution.

We established the normal reference interval for ASAT by using nonparametric statistics, in particular, the 95th percentile for normal values. The reference interval for the DGKC method was 7–18 U/L and 10–30 U/L for the IFCC.
based method. We observed no sex-related difference in normal values.

Statistical Analysis
We evaluated imprecision by repeated assay of two pooled sera and two commercial preparations of ASAT of animal origin in an albumin matrix (Table 1).

To assess the effect of the different methods on intra- and interindividual variability, we determined ASAT in samples from 16 apparently healthy volunteers.

Blood was sampled five times, once weekly, at 0800 hours; samples were promptly frozen and kept at -20 °C until analyzed one to five weeks later. All samples were analyzed in one run. Results of these determinations are presented in Table 2 and Figure 3. Terms are defined as in reference 6. 

$s_A^2$, the estimated analytical variance, by the present procedure consisted only of the within-run component of variance. In this study we assumed this to be equal to the variance, as determined by analyzing control materials (from human and animal origin). In the calculations with $s_A$, the values of $s_A$ are based on the CV for a particular range. We used the following CVs, determined from data on within-run precision in control materials: By DGKC: for ASAT < 10 U/L, CV = 12%; 10 U/L ≤ ASAT < 12 U/L, CV = 8%; ASAT ≥ 12 U/L, CV = 5%. By IFCC: for ASAT < 15 U/L, CV = 10%; 15 ≤ ASAT < 25 U/L, CV = 5%; ASAT ≥ 25 U/L, CV = 3%.

$s_p^2$ is the estimated intra-individual biological variance.

$s_p^2$ is the estimated interindividual biological variance:

$s_p^2 = s_A^2 - s_a^2$

$s_p^2$ and $s_a^2$ equal the average of variances estimated for each person, $(x_i^2 - s_p^2/16)$ and $(x_i^2 - s_p^2/16)$, respectively.

$s_G^2$ is the estimated apparent interindividual (group) variance.

$s_G^2$ is the estimated interindividual (group) variance, with five determinations for each person:

$s_G^2 = s_a^2 - (s_p^2/5)$

$s_B^2$ is the total biological variability:

$s_B^2 = s_G^2 + s_p^2$

$r$ is the index of intra- vs interindividual variability:

$r = s_p^2/(s_B^2)100$

Thirty of 745 results for patients' samples were identified as abnormal by the IFCC-based method but normal by the DGKC-method; 13 were classified as normal by the IFCC-based method but abnormal by the DGKC method.

Figure 4 shows results for all patients' samples having ASAT activities <70 U/L as determined by the IFCC-based method. Results for samples from different patient categories, as obtained with both methods, are shown in Figure 5.

Discussion
In comparing the DGKC method for determination of ASAT with the IFCC-based procedure, several points must be considered. In the DGKC method the buffer used inhibits the activation with pyridoxal phosphate (7). On the other hand, the IFCC-based method includes pyridoxal phosphate to increase enzyme activity. Consequently and because of the higher temperature used, the IFCC-based method showed a higher upper limit of normal. Because ASAT activities in the IFCC-based method are presumed to be independent of circulating and tissue concentrations of pyridoxal phosphate, one would expect a narrowing of the normal range. To our surprise, this was not the case (Figures 2 and 3). The indices of intra- vs interindividual variability showed that normal ranges in both methods are insensitive to intra-individual variations in serum ASAT activities, in agreement with results published by Høder and Bowers (6).

As Figure 5 shows, the correlation between ASAT values by each method for samples obtained from heart patients is worse than for other patient categories. This phenomenon apparently is produced by the large spread in pyridoxal phosphate activation for samples from patients with heart disease (Figure 6). This has also been observed by others (8, 9) not using IFCC-based methodology. Analyzing a rather limited number of samples, they obtained a much smaller range of pyridoxal phosphate activation than we did.

Therefore, a change from the DGKC method to the IFCC-based method may unpredictably result in increased apparent ASAT activities in serum or plasma obtained from patients with heart disease.
Determination of ASAT/ALAT ratios has been advocated in the differentiation of liver diseases (10). However, Lustig (11) states that ASAT/ALAT ratios are invalid after sample supplementation with pyridoxal phosphate. Figure 7 shows the correlation of ASAT/ALAT ratios for 101 patients suffering from biopsy-proven liver disease. The ALAT activities were determined by the DGKC procedure and by a procedure described by Bergmeyer et al. (4). The latter procedure resembles the proposed IFCC procedure for ALAT (12), but NaHCO₃ is included in the reagent. Clearly, ASAT/ALAT ratios obtained by the IFCC-based method are still usable.

In conclusion, the IFCC-based method for determination of ASAT showed no impressive advantages over the established DGKC method. If a changeover is planned, clinicians should be properly informed, not only about changes in normal ranges but also about changes to be expected in data on pathological samples.

References


