data, including the pathology and the serum creatinine concentration; and finally the current dosage regimen with the accurate time schedule of the infusion and blood sampling. Obtaining such information may require frequent clinical seminars to emphasize the usefulness of a correct monitoring and consequently of the fundamental limitations involved.

Aminoglycosides monitoring has been successfully performed in our hospital for more than 10 years. The PHARMONITOR program has been routinely used since August 1989 to monitor >7000 patients' analyses, with a daily average of 25 patients.

In conclusion, once the limitations inherent in the pharmacokinetic model are well understood, this computer program is a useful tool for individual adjustment of aminoglycosides in a clinical environment, but obviously should not substitute for the clinician's judgment in the final decision, especially for critically ill and unstable patients. This program is designed to optimize the well-known method of pharmacokinetic forecasting of Sawchuk and Zaake (7), by adapting this to the computer and by enhancing its safety by including recommendation messages based on population statistics. Both the kinetic model selected and the data-handling contribute to the speed and reliability of the analysis. We expect that the PHARMONITOR program could be used as the basic framework for other drug monitoring developments.

References

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Serum Lactate Dehydrogenase Isoenzyme 4/5 Ratio Discriminates between Hepatocarcinoma and Secondary Liver Neoplasia

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Total lactate dehydrogenase (LD; EC 1.1.1.27) and its five isoenzymes were determined in sera from (a) 98 cases of cirrhosis at various stages classified according to Child and Turcotte; (b) 37 cases of hepatocarcinoma (HC) at different stages of the Okuda classification; (c) 17 patients with secondary liver neoplasia (SLN), mainly from an abdominal primary site; and (d) 19 cases of abdominal neoplasia without liver metastasis, in an attempt to contribute to the differential diagnosis between these conditions. LD-4 was enhanced in SLN and LD-5 in HC, thus indicating the LD-4/LD-5 ratio as a potential index with which to differentiate between HC and SLN patients. At a cutoff value of 1.05, 91% of these patients were correctly classified (82% for SLN and 95% for HC). Consequently, this biochemical index appears to be an efficient and rapid indicator to distinguish HC from SLN. On the other hand, the LD isoenzymes are unable to discriminate between HC and cirrhosis or between abdominal neoplasia with and without liver metastases.

Additional Keyphrases: cancer • cirrhosis • hepatobiliary disease

Recent studies in clinical enzymology have improved the capacity to differentiate among various chronic liver diseases, including neoplasia (1-6). We have previously focused our attention on discriminating between hepatocarcinoma (HC) and cirrhosis (4-6), which is one of the most frequently encountered differential diagnosticians in the field of hepatobiliary diseases. Various patterns of serum γ-glutamyltransferase isofoms have been proposed as specific indicators of these diseases; in addition, a faster anodic γ-glutamyltransferase isofom has been identified as a specific serum signal of HC.

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To date, clinical biochemistry has contributed little to the distinction between HC and secondary liver neoplasia (SLN). This combination is a relatively frequent differential diagnosis in cases where the first clinical sign is the presence of a focal hepatic lesion that can be due either to HC proper or to an SLN from an asymptomatic gastrointestinal primary site. In addition, it is important to distinguish cases of abdominal neoplasia (AN) with hepatic metastases from those without metastases, for making timely therapeutic decisions.

We designed a study to investigate the activity concentrations of total lactate dehydrogenase (LD; EC 1.1.1.27) and its isoenzymes in sera of patients affected by cirrhosis, HC, SLN mainly from an abdominal site, and AN without liver metastases, to try to contribute to the above-mentioned differential diagnoses.

**Patients and Methods**

**Patients**

We studied all the new patients admitted to the Hepatology Division of our Hospital between 1987 and 1989. In all patients, the preliminary diagnosis, based on clinical and instrumental findings, was histologically or cytologically proven. Four homogeneous groups of patients were selected for this study: (a) 98 cirrhotic patients at different stages of the disease according to the classification of Child and Turcotte (7), which is based on serum bilirubin and albumin concentration, severity of ascites and encephalopathy, and the state of nutrition (each criterion on a three-point scale): 55 patients were at stage A, 37 at stage B, and six at stage C (advanced disease); (b) 37 affected by HC, staged according to Okuda et al. (8), a three-stage scheme based on the presence or absence of ascites, serum albumin above or below 30 g/L, serum bilirubin above or below 30 mg/dL (51.3 μmol/L), and tumor size (smaller or greater than 50% of the whole liver area, measured planimetrically by computerized tomography scans): 15 patients were at Okuda stage 1, 14 at stage 2, seven at stage 3, and one was not classified; (c) 17 patients affected by SLN (two also with extrapathic diffusion) from an abdominal primary site (except two of pulmonary origin) not clinically evident at admission. These patients were free from other diseases that could alter serum LD isoenzyme results; and (d) 19 patients with AN (in the ovary, colon-rectum, stomach, or extrapathic biliary tract) with no liver metastases.

The number of cases studied does not reflect the exact prior prevalence of each disease in the Naples area, but it does reflect the prevalence of admission to a specialized medical (nonsurgical) Hepatology Division.

**Methods**

Sera from the patients were collected and immediately processed for LD and LD isoenzyme analysis together with a routine battery of hematochemical indices: urea, glucose, aspartate aminotransferase (EC 2.6.1.1), alanine aminotransferase (EC 2.6.1.2), alkaline phosphatase (EC 3.1.3.1), γ-glutamyltransferase (EC 2.3.2.2), creatine kinase (EC 2.7.3.2), α-fetoprotein, carcinoembryonic antigen, bilirubin, etc.

We evaluated total LD serum activity with a Hitachi 737 automatic analyzer (Boehringer Biochemia, Mannheim, F.R.G.), using reagents from the same company; the reference values for healthy subjects were 227–450 U/L at 37 °C.

LD isoenzymes were analyzed by a cellulose acetate electrophoretic procedure (procedure and materials from Helena Laboratories, Beaumont, TX). The reference intervals for the LD isoenzymes in healthy subjects were as follows: LD-1, 17–31%; LD-2, 30–39%; LD-3, 19–29%; LD-4, 4–12%; LD-5, 3–11%. Although this method is not absolutely accurate, it is one of the most widely used in routine practice (see 9), and the extent of its variability could not affect the validity of the results obtained in this study.

We performed one-way analysis of variance (ANOVA) with multiple comparisons between groups (Scheffé test) (11), using the SPSS PC program (SPSS Inc., Chicago, IL). The discriminatory power of the test, which in our case is the percentages of subjects correctly classified between two diseases, is equivalent to the diagnostic efficiency calculated according to Galen and Gambino (12).

**Results**

Table 1 shows the ranges and the mean serum values of total LD and of the five LD isoenzymes, expressed both in percentage and U/L. The findings for total LD

| Table 1. Mean Values (and Ranges) of LD Isoenzymes in Four Groups of Patients |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Cirrhosis       | HC              | SLN             | AN              |
| Total LD, U/L                  | 346 (127–776)   | 904 (258–9685)  | 199 (312–2680)  | 347 (181–674)   |
| LD-1, %                        | 26.3 (13.3–43.2)| 23.2 (13.5–32.6)| 19.1 (10.2–28.5)| 23.3 (12–34.2)  |
| LD-1, U/L                      | 92 (28.1–221.2)| 184 (57–2033)   | 177 (74–488)    | 82 (26–139)     |
| LD-2, %                        | 35.4 (30.3–40.5)| 32.9 (20.1–39.3)| 30.9 (20–36.8)  | 33.2 (23.8–39.8) |
| LD-2, U/L                      | 122 (44–295)   | 279 (82–3806)   | 300 (107–731)   | 118 (51–245)    |
| LD-3, %                        | 21.5 (12.4–31.0)| 21.3 (15.9–26.7)| 24.2 (16.2–31.1)| 23.3 (17.8–30.2) |
| LD-3, U/L                      | 74 (23–149)    | 176 (55–2343)   | 244 (73–731)    | 83 (43–180)     |
| LD-4, %                        | 7.0 (3.8–12.8) | 8.1 (5.2–14.1)  | 13.7 (7.4–21.4) | 9.9 (5.4–17)    |
| LD-4, U/L                      | 24 (5–49)      | 72 (17–1036)    | 146 (31–566)    | 36 (11–79)      |
| LD-5, %                        | 9.7 (3.5–22.5) | 14.3 (4.8–40.2) | 12.0 (5.3–27.8) | 10.3 (4.7–28.2) |
| LD-5, U/L                      | 34 (9–86)      | 94 (20–464)     | 121 (27–380)    | 37 (9–76)       |
were high in both HC and SLN, the greatest increase being found in the latter group. The most striking results were a remarkable increase of LD-4 in SLN patients, and an increase of LD-5 in HC patients with respect to the reference intervals for normal subjects, expressed as percentage of total activity. Thus, to enhance the discriminatory power of LD-4 and LD-5 in differentiating between HC and SLN, we calculated the LD-4/LD-5 ratio. Table 2 shows the ANOVA statistical analysis of the LD-4 and the LD-5 isoenzymes, both as U/L and as percentage of total activity, and the LD-4/ LD-5 ratio calculated among the various groups of patients.

At a significance of \( P < 0.01 \), total LD and isoenzyme fractions 1, 2, and 3 did not contribute to the differential diagnosis of any of the pairs of groups studied; therefore, we have not reported these values. The results of the statistical analysis obtained for cirrhosis patients vs AN and for HC vs AN are not reported because a clinical differential diagnosis is never required in such pairs. Some of the analytes shown in Table 2 clearly differentiated between cirrhosis and SLN. However, again, the results are not reported because these findings cannot be exploited in the clinical situation: cirrhosis is rarely confused with SLN, and SLN is hardly ever found in cirrhotic patients (13).

As shown in Table 2, some analytes showed a highly significant difference \( (P < 0.001) \) for some analytes between cirrhosis vs HC and HC vs SLN. The mean value of LD-5 (in U/L) was significantly different in the cirrhosis vs HC comparison but, as shown in the scatterplot (Figure 1), the values widely overlap in the two groups of patients, so that no effective discriminating cutoff value can be selected. The same results were obtained when percentage LD-5 values were used for the scatterplot (data not shown). The highly significant difference between the two groups can probably be attributed to the very large increase of serum LD-5 in some HC patients, a situation that leads to an asymmetric distribution of the data.

The most interesting result to emerge from the ANOVA analysis is the highly significant difference in the mean values of LD-4 and of the LD-4/LD-5 ratio in HC vs SLN patients. Because the LD-4 values greatly overlapped in HC and SLN, we were unable to select a discriminant cutoff (data not shown). In contrast, as shown in Figure 2A, the LD-4/LD-5 ratio values are almost completely separated in the two diseases, and the value of 1.05, selected on the basis of results of receiver operating characteristic (ROC) curve analysis (Figure 2B), is the best discriminant cutoff. In Table 2 (SLN vs AN) only the LD-4 isoenzyme expressed as a percentage was significantly different. However, again the scatterplot showed a wide overlap of the two groups of values (data not shown).

Table 3 shows the discriminatory power of the serum LD-4/LD-5 ratio in distinguishing HC from SLN. At the cutoff value of 1.05 previously selected (Figure 2B), 95% of HC cases and 82% of SLN cases were correctly diagnosed. The overall discriminatory power was 91%, which also indicates a diagnostic sensitivity of 82% for patients with SLN in a mixed population of HC and SLN subjects, and a diagnostic specificity of 95% in identifying within the same population the subjects not affected by SLN but affected by HC (see ref. 12). However, as the ROC curve of Figure 2B clearly shows, the clinical use of the LD-4/LD-5 ratio is not to emphasize either one of the two diagnostic characteristics, but to contribute to the discrimination between the two situations. Simulating prevalences of 1:1 or 1:2 for HC vs SLN, which are closer to the real prior prevalence of the diseases in the general population to which the patients belonged, did not significantly reduce the discriminatory power of the test for HC and SLN: 89% and 87%, respectively. The LD-4/ LD-5 ratio was not statistically correlated to total serum LD activity. In addition, the behavior of the LD-4/LD-5 ratio was the same in patients with normal (27 of 54 patients) and above-normal total LD activity in serum.

**Discussion**

Biochemical studies performed so far on the differential diagnosis between HC and SLN have been rather
disappointing (2). The distinction between HC and SLN is required mainly in cases in which physical examination of the liver reveals an irregular surface or edge, or in which ultrasound or roentgenographic scans show single or multiple nodules in liver parenchyma. This type of evidence suggests either a primary liver cancer, or a secondary liver neoplasia from a primary localization in the abdomen, usually in the large bowel. In these patients the differential diagnosis is usually based on invasive procedures, namely, laparoscopy or liver biopsy, followed by histological assessment. In addition, in

patients with an abdominal neoplasia, particularly when the neoplasia is located in the gastrointestinal system, the liver is the first site of metastases. Thus, early recognition of liver metastases is important because surgical therapy of the primary site in such cases is usually not beneficial.

Our results show that the LD-4/LD-5 ratio can contribute with a high discriminatory power to the differential diagnosis between HC and SLN, whereas neither total LD nor its isoenzymes appear useful in the discrimination between cirrhosis and HC, or between AN with and AN without liver metastases. These data concur with unpublished findings (see ref. 2) that suggest that the LD-4/LD-5 ratio could be useful in distinguishing HC from SLN. Rotenberg et al. (9) reported increases of all serum LD isoenzymes in some patients with secondary liver diseases, whereas in others only LD-4 and LD-5 were increased; however, their study contained no data on the LD-4/LD-5 ratio. An extra LD band has been reported in serum from liver cancer patients (14), but this finding cannot be compared with our results because it was obtained by agarose gel electrophoresis, whereas our study involved the cellulose acetate procedure.

The serum concentrations of the two currently used markers for HC and SLN, namely, carcinoembryonic antigen and α-fetoprotein, respectively, in the two groups of patients (data not reported) showed that carcinoembryonic antigen values almost completely overlapped in the two populations, whereas α-fetoprotein separated HC and SLN patients with a high discriminatory power at a cutoff lower than that used conventionally for normal subjects. Thus, further studies should be conducted to establish whether α-fetoprotein in fact reliably distinguishes between HC and SLN.

Of the laboratory tests used—serum total bilirubin, γ-glutamyltransferase, and other cholestatic enzymes, namely, alkaline phosphatase, 5′-nucleotidase (EC 3.1.3.5), and leucine-aminopeptidase (EC 3.4.11.1)—none distinguished HC from SLN. Moreover, the presence of jaundice was not a discriminating factor. Neither did medical records help separate the two groups: most of the hepatocellular carcinomas developed on a pre-existing chronic liver disease; however, some SLN patients also had a history of this type of disease. Finally, viral hepatitis B serologic markers, i.e., HBsAg and HBcAb-IgG, did not separate the HC group from the SLN patients.

The biochemical basis of the increase of LD-5 in HC and LD-4 in SLN has yet to be clarified. The increase of

Table 3. Classification of HC and SLN Patients on the Basis of the Serum LD-4/LD-5 Ratio

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>&lt;1.05 (and %)</th>
<th>&gt;1.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>37</td>
<td>35 (95)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>SLN</td>
<td>17</td>
<td>3 (18)</td>
<td>14 (82)</td>
</tr>
</tbody>
</table>
LD-5 in sera from HC patients can be explained by the homotetrameric structure of LD-5, whose monomer, the so-called M subunit, is actively synthesized in hepatocytes, particularly during neoplastic transformation (14). Why large amounts of LD-4 appear in SLN sera is obscure. The increase of LD-4 seen in sera from SLN patients cannot be attributed to high production of the isoenzyme by the primary tumor, because our AN patients without liver metastases did not show increased concentrations of LD-4. Moreover, clinical and laboratory data excluded the possibility that the enhanced LD-4 as well as LD-5 activity resulted from other diseases. Muscle damage was also excluded because creatine kinase activity in serum was within the reference values in all SLN and HC patients we examined.

In conclusion, whatever the mechanism, the LD-4/LD-5 serum ratio may be used as a rapid, simple, noninvasive procedure when HC must be differentiated from SLN, even when total LD serum activity is within normal range.

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References

Free and Bound Malondialdehyde Measured as Thiobarbituric Acid Adduct by HPLC in Serum and Plasma

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Assay of free and total malondialdehyde (MDA) in human serum and plasma from healthy subjects and from patients with high risk of lipoperoxidation was performed as follows: (a) acidic (HClO4, pH 1, at 20 °C) or basic (NaOH, pH 13, at 60 °C) treatments for 30 min; (b) reaction of the protein-free extract (obtained by acid precipitation) with thiobarbituric acid (TBA); (c) HPLC separation on C18 columns with an eluting solution of methanol/phosphate buffer, 10 mmol/L, pH 5.8 (40/60, by vol), at a flow rate of 1.5 mL/min. Free MDA averaged 0.042 (SEM 0.008) and 0.043 (SEM 0.007) μmol/L, respectively, in serum and plasma from healthy subjects. Free (±SEM) MDA increased significantly in the plasma from cancer patients (0.270 ± 0.047 μmol/L) and from hemodialyzed patients (0.214 ± 0.035 μmol/L). In serum of hemodialyzed patients, analyses for total MDA were unsuitable because of interfering peaks. MDA bound to NH2 groups constituted 83.2% and 83.5% of total MDA in serum and plasma of healthy subjects, respectively, and only 58% in plasma of hemodialyzed patients.

Additional Keyphrases: cancer · hemodialysis patients

Lipid peroxidation, a general mechanism of tissue damage by free radicals, is known to be responsible for cell damage and may induce pathological events (1). Clinical chemistry tests to assess lipid peroxidation include the following techniques: fluorometry of lipofuscin-like substances in serum (2); spectrophotometry of conjugated dienes in lipid extracts of plasma and microsomes (3, 4); gas chromatography of ethane or pen-