Differential diagnosis between hepatocellular carcinoma and cirrhosis through a discriminant function based on results for serum analytes

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We applied a multivariate analysis to a large series of serum biochemical tests in an attempt to identify a function that could efficiently discriminate cirrhosis from hepatocellular carcinoma (HC). We analyzed two successive temporal cohorts (1987–90; 1991–94) of HC and cirrhotic patients, all histologically classified (first cohort: 69 cirrhosis and 39 HC; second cohort: 66 cirrhosis and 38 HC). Using data from the first temporal cohort of patients, we obtained a discriminant function based on seven serum analytes: α-fetoprotein, the hepatic isoenzyme of alkaline phosphatase, lactate dehydrogenase isoenzyme 5, total γ-glutamyltransferase (GGT), GGT isoforms complexed with low-density lipoprotein, aspartate aminotransferase, and copper. The same panel of analytes emerged when the second cohort was tested and also when both cohorts were tested together. In the two successive cohorts (total, 212 patients) with a prevalence of cirrhosis vs HC of $\approx 2:1$, the discriminant function correctly classified 93% of cases, the highest percentage of correct classification of the two diseases obtained so far by laboratory approaches. Validation with the jackknife reallocation statistical algorithm confirmed these results. In addition, of six patients with liver cirrhosis for whom we had the opportunity of following up and observing the evolution to HC, five were classified as HC at diagnosis by the multivariate discriminant analysis; i.e., discriminant analysis provided a diagnostic lead time of 6–12 months over histology. This discriminant function, based on easy-to-perform serum biochemical tests, may help solve a fundamental problem of differential diagnosis in the evolution of chronic liver diseases from cirrhosis to HC.

INDEXING TERMS: α-fetoprotein • alkaline phosphatase • lactate dehydrogenase • γ-glutamyltransferase • aspartate aminotransferase • isoenzymes • copper • statistics

Hepatocellular carcinoma (HC), one of the most frequent human tumors [1], commonly (in $\approx 95\%$ of cases) evolves from cirrhosis, particularly in areas such as Southern Italy that have a high incidence of hepatitis C and B viruses [2]. Hepatitis B virus is carcinogenic [3], and the C virus has been implicated in the pathogenesis of liver cancer [3, 4]. Because of the evolution from chronic hepatitis of various origins to, in most cases, cirrhosis and then liver cancer, the need often arises to differentiate liver cirrhosis from HC. Furthermore, because HC is frequently superimposed on preexisting cirrhosis, monitoring cirrhotic patients for early recognition of neoplasia is essential for optimum treatment of HC. Several diagnostic procedures used thus far have proved to be less than satisfactory. Computed tomography has a low sensitivity in detecting neoplastic evolution in cirrhotic patients [5]; similarly, magnetic resonance imaging has low diagnostic efficiency for correctly classifying malignant vs nonmalignant liver diseases [6, 7]. In other cases, the instrumental approaches may be more sensitive but are based on invasive procedures, such as ultrasound scanning-guided fine-needle aspiration [8]. Finally, the clotting disorders typical of advanced cirrhosis often preclude liver biopsy via laparoscopy.

Within the realm of biochemical signals, high serum concentrations of α-fetoprotein (AFP) are claimed to be strongly predictive of HC [9], but AFP has a low diagnostic sensitivity ($\approx 50\%$) and is thus not a great aid in differential and early

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3 Nonstandard abbreviations: HC, hepatocellular carcinoma; AFP, α-fetoprotein; MDA, multivariate discriminant analysis; LD, lactate dehydrogenase; AP, alkaline phosphatase; and GGT, γ-glutamyltransferase.
diagnosis. Similar conclusions can be drawn for isoforms of AFP [10, 11] and for various other biochemical signals [12-15].

After a long-term study, we recently obtained good results in differentiating between malignant and nonmalignant ascites in patients with ascites of unknown origin [16] and between primary and secondary liver cancer [17]. In both cases we used associations of biochemical analytes selected via multivariate discriminant analysis (MDA).

Well before undertaking these studies, we started to analyze a large variety of biochemical analytes and subjected the results to MDA to select the variables that could best differentiate between cirrhosis and HC. We wanted to avoid recourse to liver biopsy, which is considered the "gold standard" for differentiating between these two conditions. Because in a high percentage of cases HC has been described as a multiclonal neoplasia [18], we used MDA also to select a panel of biochemical tests that, by assessing various different biochemical abnormalities, could discriminate HC from cirrhosis.

**Patients and Methods**

**Patients**

All the new patients admitted to the Hepatology Division of our Medical School between 1987 and 1994 (first cohort, 1987-90; second cohort, 1991-94) were enrolled in the study. The procedures used in our study were approved by the Ethics Committee of our Medical School. Histological diagnoses were obtained for all patients, as were the results of other conventional clinical and instrumental approaches. Two homogeneous groups of patients were successively enrolled. The first cohort of patients included 39 with HC and 69 with cirrhosis. The HC cases were staged according to Okuda et al. [19], a three-stage classification based on the presence of ascites, serum albumin (> or <30 g/L), serum bilirubin (> or <30 mg/L), and tumor size (< or >50% of the whole liver area). Seventeen of these patients were at Okuda stage 1, 16 at stage 2, and 6 at stage 3. The 69 cirrhotic patients were staged according to Child and Turcotte [20], i.e., a three-stage classification based on the presence of encephalopathy and ascites and various values for serum bilirubin, albumin, and prothrombin activity (the Quick test). Of these patients, 37 were at Child stage A, 27 at stage B, and 5 at stage C. The second cohort recruited consisted of 38 HC patients (Okuda 1, 17; Okuda 2, 18; and Okuda 3, 3) and 66 cirrhotic patients (Child A, 44; Child B, 19; Child C, 3). Of the total of 77 HC, 55 (73%) were hepatitis C-positive and 14 (18%) were hepatitis B surface antigen-positive.

All 77 cases of HC (total of both cohorts) were superimposed on liver cirrhosis. Most of the HC were also classified on the basis of the number of liver lesions (1 lesion, 35 HC; 2 lesions, 10 HC; 3 or 4 lesions, 6 HC; diffuse HC, 13 cases) and the diameter (or the sum of diameters for multiple-lesion HC) of the neoplastic lesion (<5 cm, 12 HC; 5-10 cm, 16 HC; >10 cm, 16 HC). The number of cases included in this study does not reflect the prevalence of the two diseases in our geographical area, but does reflect the prior prevalence of cirrhosis and HC (~2:1) seen in a highly specialized Hepatology Division in the Naples area.

**Analytical Methods**

Serum from each patient was collected and processed within 2 h for the analysis of lactate dehydrogenase (LD) and LD isoenzymes, AFP, total alkaline phosphatase (AP), AP isoenzymes (bone, hepatic, biliary, and intestinal), total ϕ-glutamyltransferase (GGT), GGT isoenzymes, ϕ-nucleotidase, leucine aminopeptidase, cholinesterase, iron, ferritin, and copper. In addition, a panel of other hematocultural indices was also examined: aspartate aminotransferase, alanine aminotransferase, total bilirubin, cholesterol, triglycerides, glucose, and urea. Iron, total serum enzyme activities (U/L), and the other hematocultural indices were evaluated at 37 °C with a Hitachi 737 automated analyzer (Boehringer Mannheim, Mannheim, Germany) and Boehringer reagents. Serum AFP and ferritin were evaluated with an ELISA method on the Hitachi ES 300 automated analyzer with reagents from the same company. Serum LD and AP isoenzymes were analyzed after zone electrophoresis with materials from Helena Labs. (Beaumont, TX).

The same methods were used to analyze the various biochemical indices during the whole period of the study. Inaccuracy, checked by using both intra- and interlaboratory quality-control systems, was practically constant: CVs were <5.0% for all analytes except the GGT, AP, and LD isoenzymes, for which the CVs were always <10%.

**Statistical Analyses**

We applied the statistical procedure previously used to select a panel of ascitic biochemical indices to discriminate malignant ascites from ascites that was due to liver cirrhosis or to HC [16]. We have also used this procedure to select a panel of serum biochemical indices that discriminates HC from secondary liver cancer [17]. The distribution of all the analyte values in HC and cirrhosis patients were compared by the nonparametric Mann-Whitney test. After evaluating the diagnostic sensitivity—i.e., true positive/(true positive + false negative), diagnostic specificity—i.e., true negative/(true negative + false positive) and diagnostic efficiency—(true positives + true negatives)/grand total of tested subjects—as described by Galen and Gambino [25], we used receiver-operating characteristic (ROC) plots [26] to determine the best cutoff values for the analytes and for the discriminant function (see below) [26]. To compare the ROC plots, we compared the areas under the curves [26].

The fitting of all the analytes to a gaussian distribution was checked with the Shapiro–Wilks method. Most of the variables deviated significantly from gaussian distribution in original scale; in subsequent procedures, therefore, we used the natural logarithm (ln) of the results for all the analytes. The ln transformation of the results yielded a gaussian distribution for all the analytes except AFP. In any case, however, MDA is reasonably robust against deviations from gaussian distribution.
The first cohort of patients was used to select, among all the variables, the linear combination that best discriminated HC from cirrhosis. To avoid introducing into the model any variables that were correlated among themselves [27], we checked the correlation between all pairs of analytes by calculating the Pearson r coefficient. Of the highly correlated variables (r > 0.50), only the most significant one was used for further analysis [28]. The MDA was carried out stepwise with use of the minimum Wilks' lambda (ratio between within-groups sum of squares and the total sum of squares) to evaluate the group discrimination. At the first step, the variable with the greatest discriminant power was selected; at each of the following steps, the selection criteria were reevaluated for all variables not in the model, and the one with the largest acceptable criterion value was included. The variables previously selected were reevaluated to check if they met the removal criterion. Any variable that met this criterion was removed, and the variable selection was concluded when no more variables met entry or removal criteria.

The discriminant score, calculated for each patient on the basis of a linear combination of variables selected by MDA, was used to classify the patients into one of two groups, according to Bayes' rule [29], which takes into account not only the probability that a case belongs to one of two groups on the basis of the discriminant score (conditional probability) but also the inherent probability that the case belongs to one of the two groups in the absence of any information (prior probability). In our case, the observed proportions of cases in each group were used as estimates of the prior probabilities because we considered our sample to be representative of the patient population. The diagnostic sensitivity and specificity of the discrimination function were evaluated by taking into account the rate of misclassification of this reallocation method.

We used two statistical techniques to cross-validate the results of the MDA on the first cohort of patients. First, we used the jackknife procedure, in which one patient at a time is excluded, the rule is rederived from the remaining data, and the rederived rule is used to classify the excluded patient [29]. This algorithm removes much of the bias of the simple reallocation method [27]. Second, we tested the validity of the discriminant function prospectively with 104 patients (38 HC and 66 cirrhosis) recruited later (1991–1994).

After the positive check of the validity of the first discriminant function, we calculated a second function to cover the overall population, i.e., a pool of the two cohorts studied. The validity of this second MDA was also verified by the jackknife reallocation method, which confirmed the correct classification rate of the MDA.

### Results

In this long-term study of cirrhotic and HC cases we measured a large variety of biochemical tests, including all the major markers used so far, to differentiate between the two phases of chronic liver disease. Table 1 shows the analytes that were significantly different between the two disorders (P < 0.01, nonparametric Mann–Whitney U-test) for the first cohort of patients. Notwithstanding the high significance of the differences in the mean values for all these analytes, there was always some degree of overlap between the cases of the two clinical situations, so that no cutoff value efficiently discriminated HC from cirrhotic patients. To enhance the discrimination power, we entered all of the analytes into the SPSS computer program for MDA, after having log-transformed their measured values; we also specified a prevalence of 2:1 for cirrhosis vs HC. Total protein, bilirubin, alanine aminotransferase, cholinesterase, iron, LD isoenzymes 2–4, cholesterol, and triglycerides were excluded from the Wilks analysis of the MDA because they were correlated to other, more significant, variables [28].

#### Table 1. Serum analytes with a significantly different distribution (P <0.01, Mann–Whitney U test) between patients affected by cirrhosis and those affected by hepatocarcinoma (HC) in the first cohort (1987–90).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>Mean *</th>
<th>SD</th>
<th>P</th>
<th>n</th>
<th>Mean *</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>g/L</td>
<td>38.5 e</td>
<td>4.6</td>
<td>0.0008</td>
<td>38</td>
<td>34.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Copper</td>
<td>µmol/L</td>
<td>103.5</td>
<td>29.6</td>
<td>0.0011</td>
<td>37</td>
<td>122.9</td>
<td>34.7</td>
</tr>
<tr>
<td>5'-NT</td>
<td>U/L</td>
<td>16.8 e</td>
<td>17.5</td>
<td>&lt;0.0001</td>
<td>37</td>
<td>27.9 e</td>
<td>22.1</td>
</tr>
<tr>
<td>LAP</td>
<td>U/L</td>
<td>47.4</td>
<td>40.6</td>
<td>0.0006</td>
<td>38</td>
<td>57.4</td>
<td>24.3</td>
</tr>
<tr>
<td>AST</td>
<td>U/L</td>
<td>87.7</td>
<td>63.0</td>
<td>0.0036</td>
<td>39</td>
<td>218.0</td>
<td>468.4</td>
</tr>
<tr>
<td>LD</td>
<td>U/L</td>
<td>170</td>
<td>58.4</td>
<td>&lt;0.0001</td>
<td>39</td>
<td>422.6</td>
<td>734.6</td>
</tr>
<tr>
<td>LD5</td>
<td>U/L</td>
<td>16.6</td>
<td>12.1</td>
<td>&lt;0.0001</td>
<td>39</td>
<td>54.2</td>
<td>50.3</td>
</tr>
<tr>
<td>AP</td>
<td>U/L</td>
<td>287.0</td>
<td>154.5</td>
<td>&lt;0.0001</td>
<td>38</td>
<td>505.0</td>
<td>336.1</td>
</tr>
<tr>
<td>Hepatic AP</td>
<td>U/L</td>
<td>42.0</td>
<td>44.8</td>
<td>&lt;0.0001</td>
<td>37</td>
<td>133.0</td>
<td>101.8</td>
</tr>
<tr>
<td>AFP</td>
<td>µg/L</td>
<td>34.0 e</td>
<td>63.4</td>
<td>&lt;0.0001</td>
<td>39</td>
<td>22537.0</td>
<td>77789.9</td>
</tr>
<tr>
<td>CEA</td>
<td>µg/L</td>
<td>3.3 e</td>
<td>2.9</td>
<td>0.0045</td>
<td>38</td>
<td>8.0</td>
<td>14.8</td>
</tr>
<tr>
<td>GGT</td>
<td>U/L</td>
<td>120.0</td>
<td>289.9</td>
<td>&lt;0.0001</td>
<td>39</td>
<td>171.0</td>
<td>134.2</td>
</tr>
<tr>
<td>GGT–LDL</td>
<td>U/L</td>
<td>15.7</td>
<td>35.2</td>
<td>&lt;0.0001</td>
<td>35</td>
<td>51.9</td>
<td>70.8</td>
</tr>
<tr>
<td>LD4/LD5</td>
<td></td>
<td>0.83</td>
<td>0.32</td>
<td>0.0034</td>
<td>39</td>
<td>0.67</td>
<td>0.34</td>
</tr>
</tbody>
</table>

5'-NT, 5'-nucleotidase; LAP, leucine aminopeptidase; AST, aspartate aminotransferase; CEA, carcinoembryonic antigen.

* Geometric mean.

# n = 68.
Table 2. Differential diagnosis between cirrhosis and HC by serum biochemical indices selected by MDA in two consecutive cohorts of patients.

<table>
<thead>
<tr>
<th></th>
<th>1987-90 cohort</th>
<th></th>
<th>1991-94 cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n*</td>
<td>Cases correctly classified, no. (%)</td>
<td>n*</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>68</td>
<td>67 (98.5)</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>HC</td>
<td>31</td>
<td>3 (9.7)</td>
<td>28 (90.3)</td>
</tr>
<tr>
<td>Overall diagnostic efficiency, %*</td>
<td>96.0</td>
<td>92.3</td>
<td></td>
</tr>
</tbody>
</table>

Discriminant score: 0.31 × In copper – 0.23 × In AST + 0.30 × In AFP – 0.50 × In GGT + 0.15 × In GGT isoforms complexed to low- and very-low-density lipoproteins + 0.81 × In LD5 + 0.69 × In Hep AP – 4.53.

* Only cases with no missing values were entered into the MDA.

* Cutoff = 0.86 (<0.86 = cirrhosis; >0.86 = HC).

Among the variables inserted, the statistical procedure selected serum AFP (μg/L), liver AP isoenzyme, LD5, GGT, GGT isoforms complexed with low- and very-low-density lipoprotein, aspartate aminotransferase, and copper (μmol/L) for a discriminant function that correctly classified 90.3% of the HC and 98.5% of cirrhotic patients (96.0% diagnostic efficiency). Table 2 shows the numerical values for the discriminant equation, the cutoff selected, and the cases correctly classified for the two diseases in the first temporal cohort of patients (left panel). Applying this discriminant function to the second cohort (right panel) of patients admitted to the study yielded a diagnostic efficiency of 92.3%, confirming the results obtained in the first cohort. Finally, we pooled the data of both cohorts, and again confirmed that the overall MDA selected with very high diagnostic efficiency the same analytes previously chosen in the two separate cohorts of patients. The final equation is reported in Table 3.

Table 3. Differential diagnosis between cirrhosis and HC by MDA in the overall population (both cohorts) of patients.

<table>
<thead>
<tr>
<th></th>
<th>1987-94 population</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n*</td>
<td>Cases correctly classified, no. (%)</td>
<td>n*</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>128</td>
<td>124 (97)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>HC</td>
<td>62</td>
<td>9 (14.5)</td>
<td>53 (85.5)</td>
</tr>
<tr>
<td>Overall diagnostic efficiency, %*</td>
<td>93.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discriminant score: 0.45 × In copper – 0.50 × In AST + 0.24 × In AFP – 0.48 × In GGT + 0.27 × In GGT isoforms complexed to low- and very-low-density lipoproteins + 1.13 × In LD5 + 0.50 × In Hep AP – 4.54.

* Only cases with no missing values were entered into the MDA.

* Cutoff = 0.70 (<0.70 = cirrhosis; >0.70 = HC).

Among the variables inserted, the statistical procedure selected serum AFP (μg/L), liver AP isoenzyme, LD5, GGT, GGT isoforms complexed with low- and very-low-density lipoprotein, aspartate aminotransferase, and copper (μmol/L) for a discriminant function that correctly classified 90.3% of the HC and 98.5% of cirrhotic patients (96.0% diagnostic efficiency). Table 2 shows the numerical values for the discriminant equation, the cutoff selected, and the cases correctly classified for the two diseases in the first temporal cohort of patients (left panel). Applying this discriminant function to the second cohort (right panel) of patients admitted to the study yielded a diagnostic efficiency of 92.3%, confirming the results obtained in the first cohort. Finally, we pooled the data of both cohorts, and again confirmed that the overall MDA selected with very high diagnostic efficiency the same analytes previously chosen in the two separate cohorts of patients. The final equation is reported in Table 3.

To further validate the final discriminant equation, we checked it by the jackknife reallocation algorithm and again confirmed its high discriminatory power: diagnostic sensitivity 85.5%; diagnostic specificity 96.9%; and overall diagnostic efficiency 93.2%.

The scattergram in Fig. 1 shows the numerical MDA score for each patient, classified as cirrhosis or HC on the basis of histology. At the cutoff chosen by the MDA (0.70 as reported in Table 3), 9 cases of HC and 4 cases of cirrhosis were incorrectly classified. To evaluate the comparative power of the discriminant function in differentiating between HC and cirrhosis, we plotted a ROC curve for the discriminant function and for other analytes that had previously been found to be significantly different between HC and cirrhosis (see Fig. 2). The best curve in terms of diagnostic efficiency is clearly the one calculated with the discriminant function reported above. The difference between the area under the curve of this ROC plot and the values obtained with all the other analytes listed in Table 1 was always highly significant (P <0.001), demonstrating that the MDA is more efficient than any univariate analyte.

The diagnostic sensitivity of the MDA score for HC detection is 100% in the HC subgroup with >2 lesions and in the HC subgroup with total lesion diameter >10 cm. However, the diagnostic sensitivity for single-lesion HC was 69.2%, and for HC with a total lesion diameter <5 cm was 75%.

Figure 3 shows the probability for each patient of being...
affected by either of the two diseases on the basis of the individual discriminant scores. Thus, by consulting the MDA score, one may predict the probability for each individual patient of being affected by HC.

During the study, we had the opportunity of observing six cases of liver cirrhosis that evolved to HC. The MDA of these patients calculated at diagnosis showed that five of them were classified as HC and not as cirrhosis; at that time, however, all the other clinical, histological, and instrumental indicators favored a diagnosis of liver cirrhosis. Only after 6–12 months did histology show a clear picture of HC in these five patients.

Table 4 shows the values of the MDA in these six patients at diagnosis, together with the probability each patient at that time had of being affected by HC, as the Bayesian curve clearly shows (Fig. 3).

**Discussion**

The incidence of HC is increasing in most countries [1], but also treatment of neoplasia is becoming increasingly efficient. The effects are obviously related to a timely diagnosis. Because most HC cases develop after cirrhosis, there is often the need to discriminate between these clinically confounding disorders. Imaging and other instrumental techniques are usually invasive or insufficiently sensitive, particularly for small lesions [30, 31]; for example, one must sample the nodules, particularly HC nodules, by needle biopsy, which can be difficult because very small nodules can elude the imaging technology used. Moreover, the diagnostic efficiency of the individual biochemical indices described above ranges over a wide spectrum but never completely discriminates between HC and cirrhosis. The GGT isoforms complexed with low- and very-low-density lipoproteins (GGTL) as compared with the discriminant function obtained after the MDA described for the discrimination between HC and cirrhosis.

Histological examination of liver biopsy specimens (gold standard) was used for diagnosis in all cases.

![ROC plot of AFP and GGT isoforms compared to low- and very-low-density lipoproteins (GGT) versus the discriminant function obtained after the MDA described for the discrimination between HC and cirrhosis.](image)

**Fig. 2.** ROC plot of AFP and GGT isoforms complexed to low- and very-low-density lipoproteins (GGT) as compared with the discriminant function obtained after the MDA described for the discrimination between HC and cirrhosis.

Table 4. MDA score and probability of being affected by hepatocarcinoma in six cirrhotic patients who evolved to HC 6–12 months after MDA.

<table>
<thead>
<tr>
<th>Case</th>
<th>MDA score</th>
<th>Probability of being affected by HC at time of MDA, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.88</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>0.52</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>0.81</td>
<td>56</td>
</tr>
<tr>
<td>4</td>
<td>1.38</td>
<td>84</td>
</tr>
<tr>
<td>5</td>
<td>2.37</td>
<td>98</td>
</tr>
<tr>
<td>6</td>
<td>1.22</td>
<td>77</td>
</tr>
</tbody>
</table>

Table 4 shows the values of the MDA in these six patients at diagnosis, together with the probability each patient at that time had of being affected by HC, as the Bayesian curve clearly shows (Fig. 3).

![Relation between the probability of being affected by HC and the score obtained from the MDA for each patient.](image)

**Fig. 3.** Relation between the probability of being affected by HC and the score obtained from the MDA for each patient.
The panel selected via MDA showed a diagnostic sensitivity for HC of 85%, higher than or comparable with that of other, sometimes invasive tools, including instrumental approaches. For example, for approximately the same number of fewer cases, the diagnostic sensitivity of computed tomography for HC was 70% [5], that of magnetic resonance imaging ranged between 80% and 90% [6], and that of cytology by fine-needle aspiration biopsy for HC was <85% [34].

An additional result of our study is the probability plot, which allows calculation on the basis of the MDA score of each patient's probability for being affected by HC. This can help physicians to plan the best therapeutic strategy for each patient, e.g., invasive diagnostic approaches, further monitoring, therapy, and so on.

The MDA panel we present also indicated the presence of HC in five of the six patients affected by cirrhosis that during the study evolved to neoplasia. The lead time for this was ~6–12 months ahead of the other diagnostic approaches, including histology. Combined with an acceptable diagnostic sensitivity (75%) of the MDA for HC with a diameter <5 cm, this result is relevant for screening for early diagnosis of HC. Although ultrasound and AFP have been proposed for the screening of general populations [9], their cost in a population screening for HC would be very high [35]. Given that the risk categories for HC have been clearly identified, a screening program should be limited to these high-risk groups, i.e., subjects with cirrhosis and chronic hepatitis from hepatitis B and C viruses. The relatively low cost of using this noninvasive procedure involving a panel of biochemical tests could make it a valid contributory tool for HC screening.

In conclusion, with all the caution accorded to generalizations, the combination of unrelated biochemical tests selected by the MDA as reported here is an efficient tool for the differential diagnosis of cirrhosis and HC, and perhaps also for the monitoring of high-risk groups who may be evolving to HC, i.e., for the early identification of the neoplasia.

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