

The Predictive Value of Fibrotest vs. APRI for the Diagnosis of Fibrosis in Chronic Hepatitis C

To the Editor:

Wai et al.¹ developed indexes that identified significant fibrosis in patients with chronic hepatitis C. These indexes, including aspartate aminotransferase (AST) and platelets, had good discriminative power, as shown by areas under the receiver operating characteristic curve (AUROC) of 0.82 for formula 1 predicting fibrosis, 0.92 for formula 2 predicting cirrhosis, 0.83 for AST-Platelets Ratio Index (APRI) for fibrosis, and 0.90 for cirrhosis. We also validated a fibrosis index (Fibrotest; Biopredictive, Houilles, France, US Patent Application Serial No. 09/687,459) with high predictive values.²⁻⁴

We sought to compare these indices by using data collected retrospectively in 323 patients from our original population with complete biochemical data.² The prevalence of significant fibrosis (F2-F4) was 41%, and cirrhosis (F4) was 13%. The mean value (SE) for AST expressed in ULN was 1.71 (0.10) and platelets count 192 (3) $10^9/L$. AUROCs for significant fibrosis were 0.75 (0.03) for formula 1 and 0.74 (0.03) for APRI versus 0.83 (0.02) for Fibrotest ($P = 0.03$ vs. formula 1 and $P = 0.02$ vs. APRI),⁵ suggesting that the latter has greater discriminative power. The AUROCs for cirrhosis were 0.82 (0.04) for formula 2 and 0.80 (0.04) for APRI versus 0.92 (0.03) for Fibrotest ($P = 0.04$ vs. formula 2 and $P = 0.02$ vs. APRI), suggesting that the latter has greater discriminative power. There was a continuous, almost linear, relationship between Fibrotest and fibrosis stage, with significant differences between stages that were not observed for the Wai¹ indexes (Fig. 1).

In addition to superior diagnostic power, Fibrotest has several other advantages. First, Fibrotest is not transaminase-dependent. AST has poor sensitivity for fibrosis detection. When we apply the sensitive cut-off recommended (1.00),¹ we observe a 27% false-negative rate for cirrhosis: 11 out of 41 patients with cirrhosis had an APRI below 1.00. Among these 11 patients, 6 had normal AST values. We observed 25 fibrotic patients out of 131 (19% false-negative rate) who had APRI of 0.50 or below, the cut-off recommended for excluding significant fibrosis. Among these 25 patients, 22 had normal AST. The so-called standardization of transaminases using the upper normal limit given by laboratories is hazardous.⁶ To explain the differences in sensitivities observed in our population versus the Wai population, we wonder if, in their center, they were performing biopsies routinely in patients with normal transaminases, as we did in our center. This type of selection could have overestimated the sensitivity of AST.

Second, we did not include platelet count in Fibrotest to develop an index easily automated using minimal equipment. The components of Fibrotest can be measured using a single autoanalyzer with minimal variability.⁶

Third, Fibrotest has been validated in patients with human immunodeficiency virus/HCV-coinfection,⁴ who often have thrombocytopenia. Fibrotest is responsive to changes in fibrosis attributable to interferon-based therapy.³ Finally, a combination of alanine aminotransferase levels and Fibrotest (Actitest) accurately predicts the severity of necroinflammatory activity.^{2,3} In conclusion, these comparisons suggest that Fibrotest provides a more accurate estimate of HCV-related fibrosis than the Wai indexes.¹

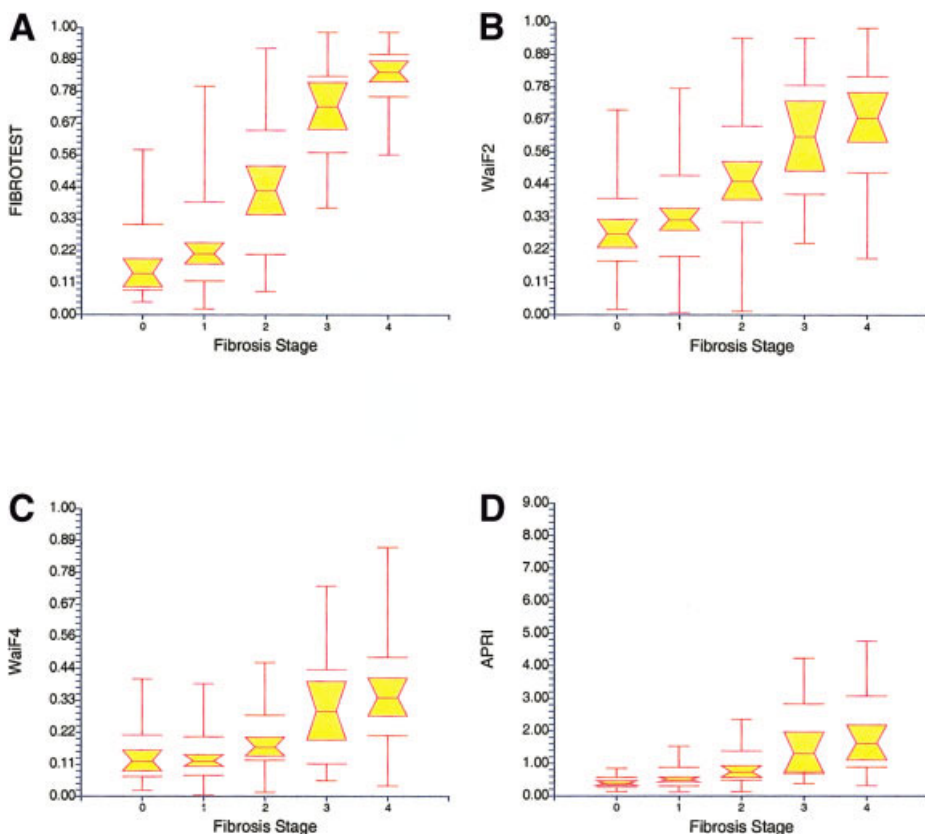


Fig. 1. Notched box plots showing the relationship between the stage of fibrosis and (A) Fibrotest and (B) Wai et al.¹ formula index for significant fibrosis (WaiF2) and (C) formula for cirrhosis (WaiF4) and (D) ASAT-to-platelets ratio index (APRI). The horizontal line inside each box represents the median and the width of each box and the 95% confidence interval. Failure of the shaded boxes to overlap signifies statistical significance ($P < 0.05$) between group medians. The horizontal lines above and below each box encompass the interquartile range, and the vertical lines from the ends of the box encompass the adjacent values.

Prospective comparisons in additional centers are warranted to confirm this finding.

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T.P. is a consultant and has a capital interest in Biopredictive, the company marketing FibroTest-Actitest.

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Reply:

We appreciate the interests expressed by Calvez and colleagues on the use of the aspartate aminotransferase to platelet ratio index (APRI) in predicting fibrosis in patients with chronic hepatitis C.¹

We welcome external validation of APRI, because the ultimate utility of any noninvasive model for prediction of hepatic fibrosis depends on its practicality and validation by other investigators in a wide range of patients. Several factors may account for the lower accuracy of APRI in the cohort of French patients studied. First, we used an Ishak fibrosis score, not a Metavir score. Second, there may have been differences between the patient populations in the two studies. Our study included consecutive treatment-naïve chronic hepatitis C patients who underwent liver biopsy at our center. Also, 26% of our patients were non-Caucasians. The French study did not specify if any of the patients had prior antiviral therapy, how many patients declined to participate, or the ethnicity of their patients.² Third, the proportion of patients

biopsied who had normal aminotransferases may be different. Patients in our study had higher aspartate aminotransferase than those in the study by Calvez and colleagues (mean: 2.3 vs. 1.7 times the upper limit of normal). At our center, we recognize the risks of liver biopsy and the generally benign course of patients with persistently normal aminotransferases; consequently, liver biopsy is performed only on selected chronic hepatitis C patients who have normal aminotransferases. Fifty-one (19%) patients in our study had normal aminotransferases at the time of biopsy. We are surprised that Calvez and colleagues routinely perform liver biopsies in patients with normal aminotransferases when their group emphasizes the risks of liver biopsies and recommends that liver biopsy should not be mandatory anymore.³

We recognize that the FibroTest has been validated in various patient populations by Poynard and colleagues,^{2,3} but a recent study in Australia using the FibroTest score computed from the same biochemical analytes, age, and gender found a lower area under receiver operating characteristic curve (AUROC) of 0.739 and lower positive and negative predictive values⁴ compared with other studies reported by the French investigators. This highlights the importance of external validation by other investigators in different patient populations with test results from other laboratories.

We do not claim that APRI is the most accurate model for prediction of significant fibrosis or cirrhosis in patients with chronic hepatitis C. In fact, our study showed that APRI was slightly less accurate than other models using complex formulas with more variables.¹ The advantages of APRI include the use of a simple formula that is amenable to mental calculation in the clinic, and the use of laboratory test results that are routinely available to every clinician managing chronic hepatitis C patients. Thus, no additional blood collection or costs are needed. We acknowledge that one limitation of APRI is the uncertainty regarding the appropriate definition of the upper limit of normal for aspartate aminotransferase. We agree that this is an important question that needs to be resolved, not only for the application of APRI but also for the evaluation of all forms of liver diseases.

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used with diaminobenzidine as chromogen for revelation. Quantification of CD8 and GzB were performed as follows: for each liver biopsy slide, 8 randomly selected $\times 400$ magnification fields (0.2 mm^2) out of portal tracts were analyzed for the number of CD8- and GzB-positive cells. Mean and standard deviation of the number of GzB- and CD8-positive cells per field for each biopsy and the ratio of the number of GzB+ to CD8+ cells was calculated for each case.

The number of CD8+ lymphocyte was not statistically different in ALPS cases compared to control cases (21 ± 10 vs. 24 ± 14 ; Wilcoxon rank test: $P > 0.4$). A 10 ± 5 -fold increase in the GzB-to-CD8 ratio was found (Wilcoxon rank test: $P < 0.05$). Details of the results are presented in Figure 1C.

We show that when viral hepatitis occurs in a patient with Fas deficiency, GzB-positive cells are overrepresented in the liver lymphocytic infiltrate compared to a control population, while there was no difference in the number of CD8+ lymphocytes in the infiltrate.

These observations show that:

- 1) Even if Fas is essential in the development of hepatitis,⁵ hepatitis can occur in human even in the case of a defect in the Fas pathway.
- 2) The hyperactivation of GzB may counterbalance Fas deficiency and explain, at least partially, the mechanism of viral hepatitis in patients with ALPS.

Nevertheless, GzB is activated during T-cell activation, and is therefore an excellent marker of an activated T-cell phenotype. Thus, other quantitative methods of GzB activity in the liver but also in circulating lymphocytes are required to confirm the functional role of GzB, and whether or not GzB overexpression is constitutive or depends on certain stimuli such as viral infections. Further studies are also required to confirm that overexpression of GzB can be met in patients with other mutations than the one we describe here.

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Systemic, Renal, and Hepatic Hemodynamic Derangement in Cirrhotic Patients With Spontaneous Bacterial Peritonitis

To the Editor:

We read with great interest the article by Ruiz-del-Arbol and colleagues.¹ They report a lower cardiac index and a higher systemic vascular resistance in patients with cirrhosis and spontaneous bacterial peritonitis (SBP) who developed renal failure when compared with SBP patients who did not develop renal failure. This difference in cardiovascular function was accompanied by a significant increase in plasma norepinephrine (NE) and plasma renin activity (PRA). A combination of cirrhotic and septic cardiomyopathy was proposed as one of the factors leading to circulatory dysfunction, where tumor necrosis factor may play an important pathogenic role.

A decrease in left ventricular function using the Sarnoff curves² has been found in cirrhotic patients exposed to sepsis.³ Because the systemic inflammatory response syndrome could be found more often in patients with renal failure, the decreased left ventricular stroke work is quite logical. The argument against this concept is the increase in systemic vascular resistance (SVR) found in patients with renal failure; this is in contrast to findings in septic patients

with cirrhosis, where an extreme decrease in SVR could be found.⁴ Increases in SVR, NE, and PRA, as well as a decrease in renal function, are common findings in the setting of elevated intra-abdominal pressures,⁵ which may have been relevant in the patients who were reported to have renal failure.

In addition, hypovolemia induces an increase in SVR, NE, and PRA, decreases stroke volume, and leads to prerenal failure. Hypovolemia must have played a relevant role in the patients reported, because the right atrial pressure ($3 + 3 \text{ mm Hg}$) as well as the pulmonary capillary wedge pressure (PCWP; $5.7 + 4 \text{ mm Hg}$), an indirect parameter of left ventricular filling pressure, were reduced. The relevance of hypovolemia for prognosis in sepsis was recently shown by Rivers and colleagues.⁶ With an early, goal-directed hemodynamic therapy—consisting of infusion of crystalloids and/or colloids to normalize central venous pressure to above 8 mm Hg ; application of vasoconstrictors to increase mean arterial pressure above 90 mm Hg ; transfusion of red cells until hematocrit is above 30%; and administration of inotropic agents until a central venous oxygen saturation of above 80% is achieved—mortality in septic patients could be significantly reduced.

More confusing is the fact that examination of right atrial pressure and PCWP revealed that patients who were not developing renal failure were also in a hypovolemic state. This might be caused by the high variability in the interpretation of PCWP curves. Trottier and Raylor⁷ recently demonstrated high intra- and interobserver variabilities in the measurement of PCWP, even in measurements made by physician "experts" and physicians who commonly use the pulmonary artery catheter. Therefore, because of the small numbers of patients studied, hypovolemia with comparable ventricular compliance cannot be excluded totally as the explanation for the hemodynamic findings. In addition, an elevation of intra-abdominal pressure may increase intrathoracic pressure; therefore, measured atrial pressures will not accurately reflect transmural pressures.

In conclusion, the interesting findings of Ruiz-del-Arbol and colleagues may be caused by a combination of septic and cirrhotic cardiomyopathy with hypovolemia combined with systemic inflammatory response syndrome (SIRS) and increased intra-abdominal pressure. The finding of high mortality is a further argument for an early, goal-directed therapy as proposed by Rivers and colleagues⁶ in patients with spontaneous bacterial peritonitis.

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Reply:

We are grateful to Dr. Lenz and coworkers for their interest in our study.¹ They remark on the complexity of circulatory dysfunction leading to hepatorenal syndrome in patients with cirrhosis and spontaneous bacterial peritonitis (SBP). They suggest that hypovolemia is a major event, since patients with hepatorenal syndrome showed low

right-atrial pressure and wedged capillary pulmonary pressure. We did not observe an increase in peripheral vascular resistance in these patients, as Lenz et al. pointed out in their letter. In fact, no significant changes in this parameter were observed in patients who did and did not develop hepatorenal syndrome after SBP. Nevertheless, we agree with their suggestion. As indicated in the discussion of the article, a reduction in cardiac output in the absence of a significant increase in cardiopulmonary pressure is consistent with a decreased venous return to the heart. Also, the lack of increase in peripheral vascular resistance in the setting of a decrease in cardiac output and a marked stimulation of the renin-angiotensin system is consistent with an increase in arterial vasodilation, probably in the splanchnic circulation, since it is well known that in cirrhosis the vascular resistance in the renal,² cutaneous, muscular,³ and cerebral⁴ territories is directly related to the plasma levels of renin. Therefore, the mechanism of circulatory dysfunction associated with hepatorenal syndrome in patients with SBP is probably the result of the simultaneous occurrence of progression of the arterial vasodilation already present in nonazotemic patients with decompensated cirrhosis together with impairment in cardiac function. This contention is further supported by the high efficacy of the simultaneous treatment with plasma volume expansion and vasoconstrictors in reversing hepatorenal syndrome,⁵ an effect rarely observed when these measures are provided alone.

However, the suggestion by Lenz et al. that the decrease in cardiac function is mainly related to hypovolemia is, in our opinion, an oversimplification of the problem. The lack of increase in heart rate observed in our study in patients developing type-1 hepatorenal syndrome is striking, since there was an intense stimulation of the sympathetic nervous system. This indicates a profound deterioration of the chronotropic function of the heart. The effect of SBP on cardiac inotropic function has never been explored.

We have previously shown that at the time of diagnosis of SBP, plasma volume expansion with albumin reduces the incidence of hepatorenal syndrome and hospital mortality by approximately 60%.⁶ The next step is to assess whether this figure could be improved by the simultaneous administration of vasoconstrictors and albumin or by the multiple treatments proposed by Lenz et al. in their letter.

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Pre-emptive Use of Lamivudine in Bone Marrow Transplantation With Chronic Hepatitis B Virus Infection

To the Editor:

We read with much interest the article by Lau and colleagues.¹ They reported on the pre-emptive use of lamivudine in bone marrow transplant recipients to reduce the risk of hepatitis due to exacerbation of hepatitis B. In their study, only one of 20 HbsAg-positive patients had HBV-related hepatitis following transplantation. This patient received 100 mg of lamivudine daily at least 1 week before allogeneic hematopoietic cell transplantation, and this dosage was continued for 52 weeks after transplantation.

We recently encountered a 62-year-old Canadian woman of Filipino origin with acute myelogenous leukemia who was in need of urgent life-saving bone marrow transplantation. The only possible match identified for donation was her brother, a 34-year-old man with known chronic hepatitis B infection: aspartate aminotransferase 28 U/L; alanine aminotransferase 56 U/L; bilirubin 10 μ mol/L; albumin 41 g/L; INR 0.93; HbsAg positive, HbeAg positive; HBV DNA 4262 pg/mL (Digene Hybrid Capture System). His liver biopsy showed inflammation grade 1/4 and fibrosis stage 1/4.²

After the brother agreed to become a bone marrow donor, therapy with 300 mg of lamivudine daily was started to decrease his HBV viral load as rapidly as possible³ prior to bone marrow donation. Table 1 shows his serum HBV DNA levels sampled on a weekly basis while on lamivudine therapy until his bone marrow was harvested on week 6.

To our surprise, the HBV DNA on the harvested tissue (peripheral blood stem cells) after 6 weeks of lamivudine therapy was 1330 pg/mL when it was negative in the serum. The recipient was HbsAg negative, HBcAb positive, and HbsAb positive prior to bone marrow transplant induction therapy, with no evidence of recurrent infection 6 months following the transplantation. She has received prophylaxis with lamivudine and hepatitis B–immune globulins. While still on lamivudine, the donor had a reappearance of HBV viremia 3 months after the donation. Lamivudine was then discontinued, followed by a return to baseline liver enzymes (alanine aminotransferase 54 U/L) and HBV DNA (HBV DNA 3828 pg/mL) levels.

HBV exacerbation is a serious cause of morbidity and mortality in patients receiving immunosuppression. We assumed that decreasing the viral load in the donor tissue would decrease the risk of transmitting the infection to the recipient. To our surprise, at a time when the donor had a very low serum HBV DNA level, the cells harvested from the bone marrow actually had a high viral load. We are unaware of any data regarding changes in viremia in this cellular compartment in patients receiving antiviral therapy. Although not tested, because of the short duration we doubt the high viral load detected in the harvested tissue was due to a lamivudine-resistant mutation. However, in retro-

spect one has to be concerned that the previous exposure to lamivudine offered to the donor may help the selection of drug-resistant mutations⁴ and lead to failure of future prophylaxis with lamivudine in the recipient.

Fourteen of the donors (group 1 and 2; see Table 1) in the study by Lau and colleagues were HbsAg-positive. We would be interested to know if the authors compared serum and bone marrow HBV DNA levels in some of their donors or if they have had a similar experience in treating a donor with lamivudine.

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Reply:

Deschenes et al. raise several important questions relating to the use of hepatitis B surface antigen (HBsAg) positive donor for allogeneic bone marrow transplantation. First, the use of HBsAg positive donors, particularly those with a high pretransplant serum hepatitis B virus (HBV) DNA level, has been associated with the development of post-bone marrow transplant HBV-related hepatitis.¹ To this end, it is highly justifiable to reduce the HBV viral load in the donor in an attempt to reduce the subsequent risk of posttransplant hepatitis. In addition, our center has adopted the policy of treating all bone marrow transplant recipients of HBsAg positive donors with preemptive lamivudine to prevent posttransplant HBV-related liver diseases, irrespective of the recipients' pretransplant HBV serological status. This has

Table 1.

	Week of Lamivudine Therapy						
	Baseline	1	2	3	4	5	6
HBV DNA (pg/ml)	5319	419	66	20	13	12	Negative

restricted us from studying the impact of the HBV DNA level in donor marrow and subsequent posttransplant HBV-related liver diseases.

In our previous study, we did not test donor marrow samples for HBV DNA.² In our ongoing study, we have tested 10 pairs of serum and mononuclear cells (derived from bone marrow) from the HBsAg positive donors for HBV DNA level, using our in-house quantitative assay based on polymerase chain reaction. Similar to the experience of Deschenes et al., we have found a positive correlation in HBV DNA level between serum and mononuclear cells. However, from the follow-up paired serum-peripheral blood mononuclear cells from these HBsAg positive donors treated with lamivudine, our preliminary results showed that the response to antiviral therapy is different in these 2 compartments, with a less well-defined response in the mononuclear cell compartment. Specifically, in 4 HBsAg positive donors treated with lamivudine for 12 weeks, despite the lowering of serum HBV DNA to more than 3 log, the HBV DNA level in the paired PBMC remain unchanged, suggesting that mononuclear cells from these treated HBsAg positive donor may still be infectious. Our study is investigating whether the HBV DNA level in the peripheral blood mononuclear cells will be lowered with prolonged lamivudine therapy. The discrepancy of the antiviral response observed in the serum and mononuclear cells' HBV DNA level might be related to the replication capacity of the HBV in the liver and mononuclear cells. Indeed, the biological behavior of HBV within the mononuclear cells compartment is still largely unknown. In keeping with this, the presence of covalently closed circular DNA molecules, the key replicative intermediate in the synthesis of the viral pregenome and transcripts during productive HBV infection, has not been convincingly demonstrated in peripheral mononuclear cells.³ Hence, it is not surprising to observe a discrepancy between the reduction of HBV DNA

level in the serum, which reflects mainly active and productive HBV replication in the liver, and in the mononuclear cells compartment.

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Serum Laminin-2 and Hyaluronan Predict Severe Liver Fibrosis in Children With Chronic Hepatitis B

To the Editor:

Liver fibrosis and cirrhosis result from an imbalance of fibrogenesis and fibrolysis. Serum levels of matrix components may be useful for assessing hepatic matrix turnover or to predict fibrosis stage,¹ which is a problem in children in whom fibrosis markers are influenced by body growth.^{1,2}

We therefore determined serum levels of tenascin, laminin-2, hyaluronan, collagen IV and VI, procollagen III N propeptide (PII-*INP*), matrix metalloproteinase-2 (MMP-2), tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), and the MMP-9/TIMP-1 complex after an overnight fast in 47 children (mean age 8 years; range 4–16) with chronic HBe-Ag positive hepatitis B prior to interferon- α therapy. Measurements were performed on the Bayer Immuno 1 Analyzer (Bayer AG, Leverkusen, Germany) using fluorescein and alkaline phosphatase-labeled monoclonal antibodies to the protein antigens.^{3,4} Liver biopsies were obtained at the time of serum sampling, and the protocol was approved by the ethics committee of the Medical University of Białystok. Fibrosis and inflammation were assessed by a single-blinded pathologist (M. S. Ł). Staging was according to Ishak and colleagues (early fibrosis: stage 1–3 vs. advanced fibrosis: stage 4–6),⁵ METAVIR,⁶ and Batts and Ludwig (stage 1–2 vs. 3–4).⁷ Receiver operating characteristics (ROC, ACCUROC, Canada) and area under curve analysis were measured to calculate the power of the markers to detect advanced fibrosis.

Seven,⁵ nine,⁶ or eight⁷ children had advanced fibrosis. Using the Batts and Ludwig score, serum hyaluronan above 27 ng/mL had

a sensitivity of 100% and a specificity of 50%, and serum laminin-2 above 34.9 ng/mL had a sensitivity of 62.5% and a specificity of 92.1% to predict advanced fibrosis. The combination of both markers was superior. With laminin-2 above 34.9 ng/mL and/or hyaluronan above 27 ng/mL, the area under curve was 0.8429 ($P = .003$), the sensitivity was 87.5%, and the specificity was 73%. Similar results for single and combined markers were obtained for the Ishak and METAVIR scores (Fig. 1). Using the combined markers and the Batts and Ludwig score, 28 and 6 out of the 45/47 children (for two children only one of the two parameters was determined) could be allocated with 100% confidence either to the group with mild or severe fibrosis, respectively, potentially avoiding biopsy in 34 (75.6%) of children. All other serum markers and their combinations yielded a lower predictive power. None of the markers was a good predictor of histologic inflammation.

Liver biopsy remains the gold standard for the grading and staging of liver disease. However, this risky procedure is dispensable for children who have only minor fibrosis. Notably, among a broad panel of serum fibrosis markers, only hyaluronan and laminin-2 accurately predicted the presence of advanced fibrosis. Other serum fibrosis markers were not deemed useful in children because they are influenced by body growth (PIIINP and procollagen IV) or bone metabolism (procollagen I).^{1,2} The predictive power of hyaluronan and laminin-2 may be due to their short biologic half life (hyaluronan)⁸ or their selective expression in fibrosing sinusoidal basement membranes (laminin-2),⁹ which precludes a major contribution by (growing) bones or ubiquitous interstitial tissues. The usefulness of these parameters for children with other chronic liver

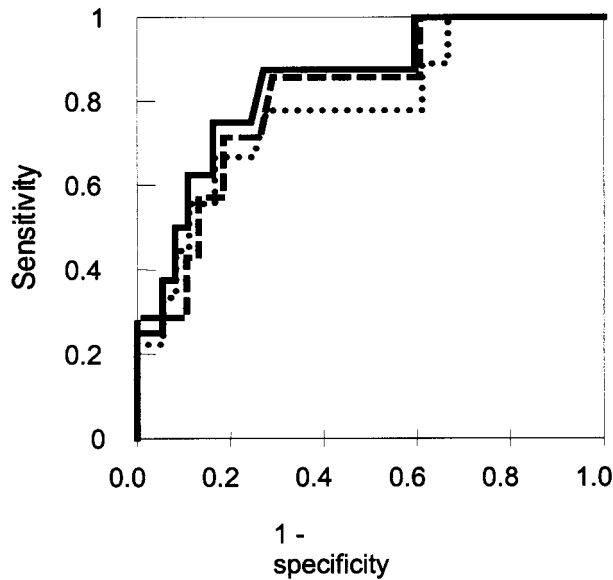


Fig. 1. ROC analysis for the combination of serum hyaluronan and serum laminin-2 to predict severe fibrosis, according to the three staging systems described in the text. **Solid line**, HA+Lam (Batts); **broken line**, HA+Lam (Ishak); **dotted line**, HA+Lam (Metavir).

diseases (e.g., chronic hepatitis C, biliary atresia) has yet to be demonstrated.

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