Matrix-derived serum markers in monitoring liver fibrosis in children with chronic hepatitis B treated with interferon alpha

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Abstract

AIM: To evaluate prospectively 4 selected serum fibrosis markers (tenascin, hyaluronan, collagen VI, TIMP-1) before, during and 12 mo after IFN treatment of children with chronic hepatitis B.

METHODS: Forty-seven consecutive patients with chronic hepatitis B (range 4-16 years, mean 8 years) underwent IFN treatment (3 MU twi for 20 wk). Fibrosis stage and inflammation grade were assessed in a blinded fashion before and 12 mo after end of treatment. Serum fibrosis markers were determined using automated assays.

RESULTS: IFN treatment improved histological inflammation but did not change fibrosis in the whole group or in subgroups. Only hyaluronan correlated significantly with histological fibrosis ($r = 0.3383$, $P = 0.0211$). Basal fibrosis markers did not differ between responders (42.5%) and nonresponders (57.5%). During IFN treatment only serum tenascin decreased significantly in the whole group and in nonresponders. When pretreatment values were compared to values 12 mo after therapy, TIMP-1 increased in all patients and in nonresponders, and hyaluronan decreased in all patients and in responders.

CONCLUSION: Tenascin reflects hepatic fibrogenesis and inflammation which decreases during IFN treatment of children with chronic hepatitis B. TIMP-1 correlates with nonresponse and hyaluronan with histological fibrosis.
allowing repeated assessment of progression or therapeutic interventions, especially in children with chronic hepatitis B or C who are treated with IFN or other antiviral or potential antifibrotic agents[17-19].

The aim of this study was to investigate the clinical usefulness of selected matrix-derived serum markers (tenascin, hyaluronan, collagen VI, tissue inhibitor of metalloproteinase 1 or TIMP-1) in a long-term follow-up of children with chronic hepatitis B treated with IFN α.

MATERIALS AND METHODS

Patients

The study was carried out prospectively in 47 children (mean age 8 years, range 4-16, 31 boys and 16 girls) with serologically and biopsy-verified chronic hepatitis B. The children were positive for HBs and HBe antigens and had increased serum activity of HBV DNA polymerase for at least 1 year. Patients with autoimmune hepatitis or HCV coinfection were excluded from the study. None of the children was treated with antiviral and immunomodulating drugs during the 12-month period before inclusion into the study. Informed consent was obtained from all patients’ parents and the protocol was approved by the local ethical committee of the Medical University of Bialystok. Serum samples were evaluated at three time points: at the start and the end (5 mo) of IFN α treatment, and 12 mo after end of treatment. Serum samples were stored at -70 °C until use. Standard liver tests were measured by validated automated methods and included total bilirubin, albumin, alanine transaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (gamma-GT). HBsAg and HBeAg were determined by MEIA (IMx, Abbott).

IFN treatment and definition of response

IFN (IFN α 2a: 30 children or IFN α 2b: 17 children) was applied at the dose of 3 MU tw subcutaneously for 20 wk according to the schedule approved by the Polish Interferon Study Group[20]. HBeAg/antiHBe seroconversion and lack of HBV DNA polymerase activity 1 year after end of treatment was considered as the criterion of treatment response.

Measurement of serum fibrosis markers

Monoclonal antibodies were used to detect tenascin, collagen VI and TIMP-1 in sandwich immunoassays performed in an automated analyzer employing fluoresceine-labelled capture antibodies and alkaline phosphatase labeled detection antibodies. Hyaluronan was determined using biotinylated cartilage link protein. The immune complexes were separated from serum using magnetic particles covered with monoclonal anti-fluoresceine (anti-biotin in case of hyaluronan). The assays were developed for the BAYER IMMUNO 1 immunoassay system and validated in several cohorts of liver patients and healthy individuals[21-23].

Histological analysis

Percutaneous liver biopsies were obtained before treatment and 12 mo after IFN α discontinuation. The liver specimens were fixed in buffered formalin and embed-
There were no significant differences in mean serum levels of tenascin, collagen VI and TIMP-1 between children with mild and advanced liver fibrosis, while levels of hyaluronan were higher in the latter group (30.3±15.8 μg/L vs 53.1±34.3 μg/L; P = 0.0266). There was a trend for increased tenascin and TIMP-1 in children with advanced fibrosis (Figure 1, Figure 2). We also arbitrarily defined mild inflammation as grade 1 (n = 20) and severe inflammation as grade ≥2 (n = 27) according to Batts and Ludwig. There were no significant differences in mean concentrations of all serum fibrosis markers between children with mild and severe hepatic inflammation.

**Effect of IFN on serum fibrosis markers**

At end of treatment there were no significant changes in serum fibrosis markers in responders, while in nonresponders only tenascin decreased significantly (P < 0.05). Twelve months after end of treatment serum hyaluronan was significantly lower than before treatment (P = 0.0076), while serum TIMP-1 was increased (P = 0.0072). In responders only hyaluronan decreased significantly (P = 0.0304), while in nonresponders the level of TIMP-1 increased (P = 0.0064, Table 2). Tenascin reached pretreatment levels in both responders and nonresponders.

**Effect of IFN on liver histology**

There were no significant changes in fibrosis stage after IFN therapy in the whole cohort, 2.0 ± 0.6 vs 2.1 ± 0.6, according to Batts and Ludwig and in subgroups, responders: 2.3 ± 0.5 vs 2.2 ± 0.7; nonresponders: 1.8 ± 0.6 vs 2.0 ± 0.6. Histological inflammation improved significantly in the whole group, 1.6 ± 0.7 vs 1.2 ± 0.7, P = 0.0373.

**Correlation between serum fibrosis markers, histology and biochemical parameters**

There were no significant correlations between baseline levels of the 4 serum fibrosis markers with liver fibrosis or inflammation according to Batts and Ludwig, or with AST, ALT, GGT, albumin or bilirubin. Only hyaluronan correlated significantly with histological fibrosis (r = 0.3383, P = 0.021).

**DISCUSSION**

Liver biopsy has been considered the gold standard for the assessment of hepatic fibrosis. Current recommen-
ations suggest that this procedure precede antiviral treatment in most patients with chronic hepatitis B or C. However, liver biopsy is invasive with the potential for complications, such as bleeding which occurrence ranges from 0.3% to 0.5%[26-27], and mortality up to 0.1%[26-28]. In addition, since the biopsy core only represents 1/20 000 to 1/50 000 of the liver, biopsy is prone to sampling error, and variations in fibrosis staging may be high among different pathologists[33,34,21,29]. For these reasons, especially in children, non-invasive detection of histological liver damage, particularly of fibrosis, is needed. Ideally serum markers of fibrosis should be applicable to patients with chronic hepatitis to either diagnose the stage of liver fibrosis, potentially replacing liver biopsy for this purpose, or to monitor progression of fibrosis or fibrogenesis, particularly during treatment[17,30]. Markers of the dynamics of fibrogenesis and fibrolysis are urgently needed, e.g. for short-term assessment of antifibrotic drug effects, but difficult to validate.

In this study we evaluated the changes of 4 serum fibrosis markers derived from the extracellular matrix (tenascin, hyaluronan, collagen VI and TIMP-1) before, at the end of and 12 mo after treatment of children with chronic hepatitis B with IFN. Our results showed a significant decrease of hyaluronan in responders and increased TIMP-1 in nonresponders, when levels before and 12 mo after interferon α treatment were compared. While falling during treatment, serum tenascin reached pretreatment levels in both responders and nonresponders. There were no significant changes in histological liver fibrosis 12 mo after the 5-mo course of IFN in all patients or in the subgroups of responders and nonresponders. This was expected, since the rate of fibrosis progression or regression in patients with chronic hepatitis B or C was usually slow. Assuming that IFN has at least some antifibrotic activity, as suggested before in large retrospective analyses of patients with chronic hepatitis C[31,32], the histological scoring systems are obviously not sensitive enough to detect small changes in liver fibrosis and (modest) antifibrotic treatment effects. Nonetheless, the course of serum fibrosis (fibrogenesis) markers in our small but well defined group of children suggests that IFN indeed has antifibrogenic activity, especially in responders. This antifibrotic effect seems to be transient, as exemplified by serum tenascin which was depressed only during IFN treatment.

Prior to our study there had been no longitudinal, prospective studies of serum fibrosis markers in children with chronic hepatitis B, and only few studies analysed the effect of IFN therapy on the stage of liver fibrosis in children. Our findings are consistent with our previous study[33] and with those of others, who did not observe improvement of liver fibrosis by antiviral treatment in children, when biopsy was performed before and immediately after[18] or 9-12 mo after end of treatment[19,34], while Gregorio et al[22] found significant improvement in staging in responders. However, these reports included small numbers of patients (<24). It has been demonstrated that fibrosis stage changes more slowly than inflammation grade[35,36]. This explains why we did not observe a significant improvement in fibrosis, while inflammation was clearly suppressed by IFN treatment.

We found that hyaluronan was the best serum marker to predict advanced liver fibrosis, since its level correlated significantly with histological fibrosis and was significantly higher in children with advanced vs mild/moderate liver fibrosis. These data are in keeping with previous results in patients with chronic viral hepatitis[37-39]. Thus the ability of this test to differentiate patients with extensive liver fibrosis from those with mild liver fibrosis was stronger than that of other markers, i.e., PIIINP, collagen IV, MMP-1, MMP-2 and TIMP-1[39,40] and laminin, collagen IV, PIIINP and TGF β[45].

In children, up to now serum hyaluronan had only been studied in biliary atresia and cystic fibrosis[46-48], and there had been no data on collagen VI in chronic viral hepatitis or on TIMP-1 or tenascin in childhood liver diseases in general. Previous studies indicated that most serum fibrosis markers are influenced by body growth, especially PIIINP[49,50]. Thus healthy children have higher PIIINP levels than adults, excluding its use as reliable fibrosis marker for pediatric patients. Hyaluronan appears to be a useful marker of fibrosis stage also in children due to its short biological half life of only a few minutes and a prominent uptake by sinusoidal endothelial cells[51]. Similarly, tenascin and TIMP-1 are applicable to children with chronic hepatitis B as markers of fibrogenesis/inflammation and of fibrogenesis, respectively.

Our data suggest that serum hyaluronan, tenascin and TIMP-1 could be useful fibrosis markers in future studies of children with chronic viral hepatitis.

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