TOPICAL REVIEW

Serum laboratory markers for noninvasive diagnosis and monitoring of liver fibrogenesis in patients with chronic liver diseases

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Abstract

Progressive hepatic fibrosis with the development of cirrhosis is a feature of almost all chronic liver diseases. Liver biopsy remains to be the gold standard for the diagnosis and staging of liver fibrosis. Laboratory tests for noninvasive diagnosis of liver fibrosis have been studied extensively in the past years. Many different parameters including circulating products of collagen synthesis or degradation, enzymes involved in collagen biosynthesis, extracellular matrix glycoproteins and proteoglycans or matrix-degrading enzymes and their inhibitors have been evaluated. Unfortunately, none of the currently used serum markers of liver fibrosis are sensitive and specific enough for accurate staging and monitoring of liver fibrogenesis. The review summarizes current information on laboratory tests used for noninvasive diagnosis and monitoring of liver fibrosis. ***(Tab. 1, Ref. 29.)***

Key words: liver fibrogenesis, fibrosis markers, procollagen peptides, hyaluronate, alpha-2-macroglobulin.

The liver is of vital importance in intermediary metabolism, detoxification and elimination of toxic substances. Damage of this organ does not have to obviously affect its activity since the liver has a considerable functional reserve and, as a consequence, the sensitivity of simple liver function tests (e.g. plasma bilirubin and albumin concentrations) in coincidence with liver diseases is low.

The mechanisms underlying hepatic diseases can be divided into four main groups. These often coexist, but one usually predominates in any conditions: (a) liver-cell damage, (b) cholestasis, (c) a considerably reduced mass of functioning cells and (d) fibrogenesis. Laboratory tests used in detecting the presence of liver disease and in following its progress reflect one of these main pathological processes.

Liver fibrosis, a common sequel of chronic inflammatory liver diseases is a complex dynamic process that includes an increase in extracellular matrix components, activation of cells producing matrix material, cytokine release and tissue remodelling. It is well established that the changes are brought about by an active process (fibrogenesis) initiated by parenchymal cell damage of various etiologies and consecutive inflammation (Gressner, 1998). The target extracellular matrix-producing cell type has been identified as cells referred to as hepatic stellate cells, lipocytes or Ito cells. This non-parenchymal liver cell type is located in the perisinusoidal space of Disse. The activation of hepatic stellate cells to myofibroblasts is thought to be the core pathogenetic event in fibrogenesis since it results in a largely expanded pool of myofibroblasts capable of the production of virtually all matrix components found in the fibrotic extracellular matrix (Gressner, 1998).

The extracellular matrix is a complex network within which the defined molecules are precisely organized. Under normal conditions, the composition of extracellular matrix within the liver is not homogenous. The connective tissue of the fibrous external capsule, septa, periductal and perivascular areas and portal tracts are of fibrillar and interstitial types that are particularly rich in collagen of types I, III and V and fibronectin (Schuppan, 1990), while collagen of types IV and VI together with laminin and fibronectin are the major matrix proteins in the space of Disse.

Collagen III is the first type to be increased due to liver damage (Ballardini et al, 1985), later on it is largely substituted by

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Acknowledgements. This work was supported by VEGA grant 01/0549/03.

collagen of type I. The excessive accumulation of collagen of types III and I, which can be easily detected in all fibrotic biopsies by in situ hybridisation (Milani et al, 1995) in the portal tracts, fibrous septa and central vein walls, appears to be the consequence of hyperactivation of fibroblasts and myofibroblasts. An increased accumulation of fibrotic tissue in Disse's space, where the fibrogenesis is initiated at the beginning, is not beneficial. It deteriorates the supply of oxygen and nutritients toward hepatocytes, and reduces the removal of toxins absorbed from the gastrointestinal tract leading to damage and necrosis of hepatocytes, which in turn enhances again the process of fibrogenesis.

The possibility to follow the extent and activity of liver fibrogenesis in patients with chronic hepatopathies would be very helpfull. From the practical standpoint it would be ideal to have a noninvasive fibromarker that can (1) stage the degree of liver fibrosis, (2) reflect the rate of matrix deposition or its removal, (3) monitor the impact of therapy. It should be (4) readily available, (5) reproducible and (6) inexpensive. Unfortunately so far, none of the known and clinically used liver fibromarkers can meet all these requirements.

Despite its invasive character liver biopsy remains to be the gold standard in the diagnosis and staging of liver fibrosis. For both, the physician and the patient the decision to proceed with liver biopsy is not a trivial one. Significant complications occurs in 1–5 % of patients with a reported mortality rate of between 1:1000 and 1:10000 (Afdhal and Nunes, 2004). Pain occurs in an estimated 30 % of patients (Bravo et al, 2001). Many patients are reluctant to overgo repeated biopsies, the fact of which limits our ability to monitor the disease progression and the effects of treatment. Furthermore, liver biopsy is expensive. It is a procedure that requires a physician, a supporting staff and an experienced histopathologist to interprete the slides. The procedure becomes even more expensive if the biopsy is gained via laparoscopy.

Due to the above mentioned limitations of liver biopsy, much effort was invested to establish a reliable serum liver fibromarker that would be a useful adjunct to clinical assessment and would reduce the need of repeated liver biopsy. Many different parameters were studied in connection with liver fibrosis in patients with various chronic hepatopathies. In the following short review it is our objective to describe those with best clinical results.

The markers of liver fibrosis include a number of serum or urinary metabolites or enzymes coinciding with extracellular matrix metabolic turnover (Tab. 1).

Tab. 1. Potential markers of liver fibrogenesis (according to Afdhal and Nunes (2004) and Gressner (1987).

Markers of matrix deposition
- COOH-terminal propeptide of procollagen I
- NH_2-terminal propeptide of procollagen III
- tenascin
- tissue inhibitors of metalloproteinases (TMP-1, TMP-2)
Markers of matrix removal
- COOH-terminal peptide of procollagen IV
- NH_2-terminal peptide of procollagen IV
- collagen IV
- matrix metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-9)
- undulin
- hydroxyproline excretion
Uncertain
- hyaluronate
- laminin
- N-acetylglucosaminidase
- Monoaminooxidase

Hyaluronic acid (HA)

Among the glycosaminoglycans, it is the circulating hyaluronic acid, the levels of which have been studied most intensively in coincidence with chronic liver diseases. It is an unbranched high-molecular-weight polysaccharide that is widely distributed in the extracellular space. Part of HA enters the general circulation via the lymphatic system and is rapidly cleared and degraded mainly in the hepatic endothelial cells. Its concentration in normal liver is low but fibrotic liver shows both relative and absolute increases (Murata et al, 1985). Elevated serum HA levels reflect an increased expression in hepatic stellate cells and/or a decreased clearance by hepatic endothelial cells in the diseased liver. It has been proposed that circulating HA levels may be helpful in recognizing cirrhosis in liver in order to monitor liver functions and evaluate the extent of liver fibrosis. Several authors showed that serum HA is a useful marker of liver fibrosis in patients with chronic HCV infection (Wong et al, 1998; Kupčova et al 2002). It was demonstrated that HA levels decrease during interferon-alpha therapy parallel to improved fibrosis staging (Guechot et al, 1995; Kupčova et al, 2004). The greatest clinical utility of HA may reside in its ability to exclude patients with significant fibrosis and cirrhosis (McHutchinson et al, 2000).

NH_2-terminal propeptide of procollagen type III (PIIINP)

Healthy liver contains only a small amount of fibrillar collagen, which increases several times in cirrhotic liver tissue. Collagen, which is the main protein constituent of connective tissue, is synthesized as a precursor, procollagen, containing both NH_2- and COOH-terminal extensions. These extensions or propeptides are cleaved from procollagen molecules during fiber formation and circulate in the blood. PIIINP is produced by activated hepatic stellate cells and released into the serum during matrix deposition and remodelling. An increase in serum PIIINP may result also from decreased clearance by hepatic sinusoidal endothelial cells, which is disturbed in advanced liver diseases. Serum PIIINP levels in chronic liver diseases have been studied extensively during the past several years. The association with hepatic fibrogenesis was variable with diagnostic sensitivities ranging from 24 to 89 % and specificities from 38 to 88 % (Oberti et al, 1997; Lichtinghagen and Bahr, 2004). In the comparative study of Oberti et al (1997) HA performed best with a diagnostic accuracy of 86 %, whereas the performance of PIIINP

was less impressive (74 %). Other comparative studies have also supported the superiority of HA over PIIINP for the diagnosis of cirrhosis (Nyberg et al, 1992; Murawaki et al, 1995). One explanation of the difference in performance may be related to the observation that PIIINP levels correlate more closely with histological and serum markers of hepatic inflammation than HA (Ramadori et al, 1991; Murawaki et al, 1995; Holomáň et al, 2002). Therefore the determination of HA serum concentration is of greater diagnostic value, especially when inflammatory activity is low.

Laminin

Laminin together with collagen type IV are the major components of the newly formed basement membranes in the fibrotic liver. The levels of circulating basement membrane peptides exhibit a close correlation to fibrosis (Walsh et al, 2000). Increased levels of laminin may be predictive of portal hypertension and severity of alcoholic liver disease (Afdhal and Nunes, 2004).

Type IV collagen

Type IV collagen is a major component of basement membrane. This type of collagen is composed of the major collagenous NH_2-terminal triple helical domain and COOH-terminal noncollagenous globular domain, and is organized in a network fashion. The deposition of type IV collagen with laminin becomes so prominent in fibrotic liver that it develops real basement membranes, a process known as capillarization of the sinusoid (Schaffner and Popper, 1963). Takamatsu et al (1997) in their study demonstrated that serum levels of 7S domain of type IV collagen and levels of type IV collagen increased in parallel with the progression of hepatic fibrosis. The correlation coefficients of 7S-collagen and IV-collagen with the amounts of collagen in liver were 0.771 and 0.705, respectively, showing that both fibromarkers, particularly the 7S domain, are highly useful indicators for detecting the degree of hepatic fibrosis.

Metalloproteinases and their inhibitors

Metalloproteinases (MMPs) are enzymes which participate in the degradation of hepatic extracellular matrix. The activity of MMPs is controlled at different levels including inhibition by a group of antagonists – the tissue inhibitors of metalloproteinases (TIMPs). In several clinical studies MMP-2 and TIMP-1 showed close correlation between circulating levels and histological staging of liver fibrosis (Lichtinghagen et al, 2001; Boeker et al, 2002)

FibroTest

Recently, the FibroTest described by the group of Poynard (Imbert-Bismut et al, 2001) raised much attention. The test is based on the estimation of five relatively simple biochemical parameters – namely alpha$_2$-macroglobulin, haptoglobin, gamma-glutamyltransferase, total bilirubin and apolipoprotein A. Published results showed very good correlation between the FibroTest score and the histologic evaluation of liver fibrosis stage in patients with chronic hepatitis type C, type B and in patients with HCV/HIV coinfection (Poynard et al, 2002; Myers et al, 2003). Alpha-2-macroglobulin (AMG) is a glycoprotein produced and released locally by hepatocytes and stellate cells at sites of inflammation and liver fibrosis. Alpha$_2$-macroglobulin is also well-known as a protease inhibitor. Increased synthesis of this protein may enhance hepatic fibrosis by inhibiting catabolism of other proteins in the extracellular matrix (Kawser et al, 1998). Haptoglobin decreases when fibrosis increases. Different changes in levels of AMG and haptoglobin could be explained by the differences in the roles of cytokines and hepatocyte growth factor (HGF) during fibrogenesis. HGF stimulates the synthesis of AMG and decreases the synthesis of haptoglobin (Poynard et al, 2002). The group of Turecky deals with alpha$_2$-macroglobulin and its role of a possible marker of liver fibrosis. They showed a very good correlation between AMG levels and histological staging of fibrosis in the group of patients with chronic hepatitis of types B and C (Turecký et al, 2003, 2004 a, b). Kupčova et al (2004) reported about the effect of interferon alpha therapy on AMG levels in patients with chronic hepatitis C. Their study showed that serum AMG levels were related to the therapeutic outcome of interferon alpha and ribavirine in patients with chronic hepatitis C.

N-Acetylglucosaminidase

N-acetyl-ß-D-glucosaminidase (2-acetamido-2-deoxy-ß-D-glucoside acetamidodeoxy-glucohydrolase, EC 3.2.1.30., NAG) is a lysosomal enzyme widely distributed throughout the body with highest activities in kidney and liver tissue. NAG is an enzyme involved in the glycoprotein and glycosaminoglycans metabolism. It was reported that the serum activity of NAG could be a potential fibromarker in patients with liver fibrosis (Gressner and Greiling, 1991). Turecky et al (1985) found increased serum activities of NAG in patients with chronic liver diseases. The activity of NAG was higher in the group of liver cirrhosis than in the group of patients with chronic active hepatitis. The comparison of NAG activities with HA levels in patients with chronic hepatitis B and C showed significant correlation ($r = 0.55$, $p<0.001$). The levels of HA were well correlated with histological staging, but NAG activities were not significantly different in groups of patients with mild fibrosis (Turecky et al, 2002). One of the possible explanations for the relatively weak correlation between NAG activity and stage of fibrosis in patients with mild fibrosis is the fact, that NAG shows a close relation to inflammatory activity of the disease. This limits the usefulness of NAG activity in correct staging of liver fibrosis. The monitoring of NAG activities during interferon alpha therapy showed a positive correlation with therapeutic effects in the group of patients with chronic hepatitis (Uhlikova et al, 2004).

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Received January 15, 2005.
Accepted February 20, 2005.