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EDITORIAL

Non-invasive biomarkers for monitoring the fibrogenic process in liver: A short survey

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Abstract

The clinical course of chronic liver diseases is significantly dependent on the progression rate and the extent of fibrosis, i.e. the non-structured replacement of necrotic parenchyma by extracellular matrix. Fibrogenesis, i.e. the development of fibrosis can be regarded as an unlimited wound healing process, which is based on matrix (connective tissue) synthesis in activated hepatic stellate cells, fibroblasts (fibrocytes), hepatocytes and biliary epithelial cells, which are converted to matrix-producing (myo-)fibroblasts by a process defined as epithelial-mesenchymal transition. Blood (non-invasive) biomarkers of fibrogenesis and fibrosis can be divided into class Ⅰ and class Ⅱ analytes. Class Ⅰ biomarkers are those single tests, which are based on the pathophysiology of fibrosis, whereas class Ⅱ biomarkers are mostly multiparametric algorithms, which have been statistically evaluated with regard to the detection and activity of ongoing fibrosis. Currently available markers fulfil the criteria of ideal clinical-chemical tests only partially, but increased understanding of the complex pathogenesis of fibrosis offers additional ways for pathophysiologically well based serum (plasma) biomarkers. They include TGF-β-driven marker proteins, bone marrow-derived cells (fibrocytes), and cytokines, which govern pro- and anti-fibrotic activities. Proteomic and glycomic approaches of serum are under investigation to set up specific protein or carbohydrate profiles in patients with liver fibrosis. These and other novel parameters will supplement or eventually replace liver biopsy/histology, high resolution imaging analysis, and elastography for the detection and monitoring of patients at risk of developing liver fibrosis.

Key words: Biochemical markers; Diagnostic validity; Liver fibrosis; Monitoring; Multiparametric algorithms; Non-invasive diagnostic tools

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INTRODUCTION

Tissue fibrosis is characterized by the excess deposition of extracellular matrix (ECM) involving molecular and histological re-arrangement of various types of collagens, proteoglycans, structural glycoproteins and hyaluronan (Figure 1). It is a hallmark of liver cirrhosis and contributes significantly to the deleterious outcome of chronic liver diseases[1]. The deposition of ECM in the space of Disse (perisinusoidal fibrosis) between the sinusoidal surface of hepatocytes and the endothelial cell layer of liver sinusoids, the generation of (incomplete) subendothelial basement membranes, and the strangulation of hepatocytes by surrounding matrix impair not only the blood flow through the organ, but also the biosynthetic function of hepatocytes and the clearance capability of these and other cell types[2].

The molecular pathogenesis of the fibrotic transition of liver parenchyma turns out to be a muti-faceted process largely due to the activation of resting, vitamin A-storing stellate cells to matrix-producing myofibroblasts[3,4] in the immediate neighbourhood of hepatocytes, to the phenotypic switch of hepatocytes and bile duct epithelial

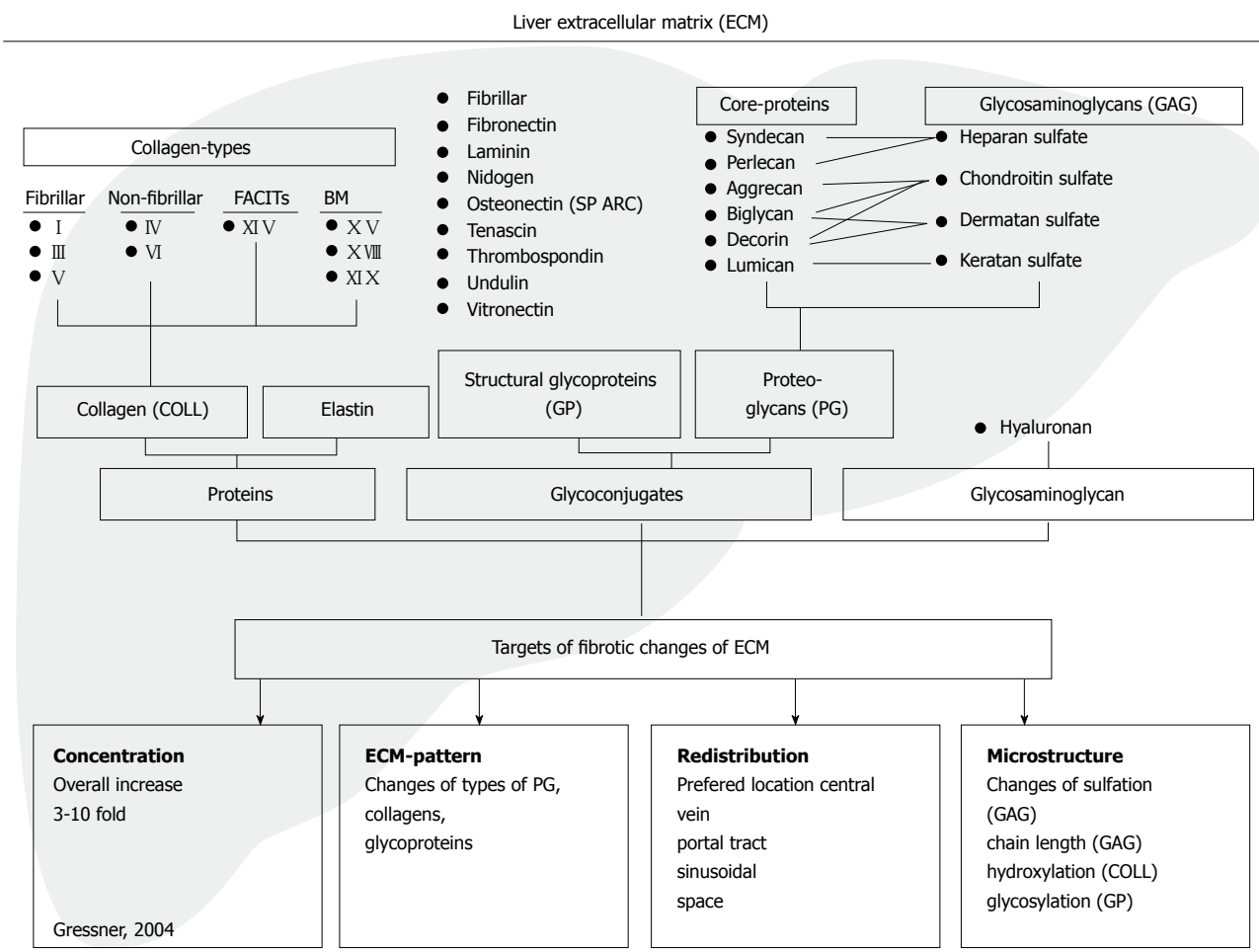

Figure 1 Components of the extracellular matrix (connective tissue) of the fibrotic liver and their major changes. The binding of glycosaminoglycans (GAG) to the respective core proteins (CP) of proteoglycans (PG) are shown. BM: Basement membranes; FACIT: Fibril-associated collagens with interrupted triple-helices.

cells to fibroblasts termed epithelial-mesenchymal transition (EMT)[5-7], and to the influx of bone marrow-derived cells (fibrocytes) reaching the liver via the systemic circulation[8,9] (Figure 2). The fractional contribution of these pathways to fibrosis depends on the underlying disease and probably on the stage of the fibrotic transition[2]. The activation of stellate cells results from interaction with damaged hepatocytes, activated Kupffer cells, disintegrated platelets and various subfractions of leucocytes. Among the cytokines involved in the pathogenetic processes, TGF-β plays a dominant role, but PDGF, endothelin-1, VEGF, and others also contribute significantly. Antagonistic (antifibrotic) mediators might also exist among which bone morphogenetic protein (BMP)-7 plays an important role, e.g. in the inhibition of EMT-derived fibroblasts[10].

The pathogenetic complexity is mirrored by multiple approaches of a clinical diagnosis and a follow-up of ongoing liver fibrosis.

The widely used diagnostic "gold standard" of liver biopsy has many draw-backs besides its invasiveness such as sampling error (around 1/50 000th of liver mass is obtained), irreproducible sample quality depending on length and size of the tissue specimen (coefficient of variation 45%-35%) and a histological evaluation strictly dependent on the experience of the pathologist (observer error)[11]. Therefore, the development of non-invasive, objective and quantitative serum- or plasma-based biomarkers of fibrogenesis is an important goal, which can be approached by the assessment of two, principally different lines of blood-borne (non-invasive) analytes: Class Ⅰ and class Ⅱ serum fibrosis markers.

CLASSIFICATION OF CIRCULATING BIOMARKERS OF FIBROSIS

Class Ⅰ fibrosis biomarkers are pathophysiologically derived from ECM turnover and/or from changes of the fibrogenic cell types, in particular hepatic stellate cells (HSC) and (myo-)fibroblasts[3]. They should reflect the activity of the fibrogenic and/or fibrolytic process and, thus, remodelling of ECM. These biomarkers do not indicate the extent of connective tissue deposition, i.e. the stage of fibrotic transition of the organ. Frequently, they involve costly laboratory tests and are the result of translation of fibrogenic mechanisms into clinical application. Thus, their selection is hypothesis-driven.

Class Ⅱ fibrosis biomarkers mostly estimate the degree of fibrosis (extent of ECM deposition). In general, they comprise common clinical-chemical tests (enzymes, proteins, coagulation factors), which do not necessarily

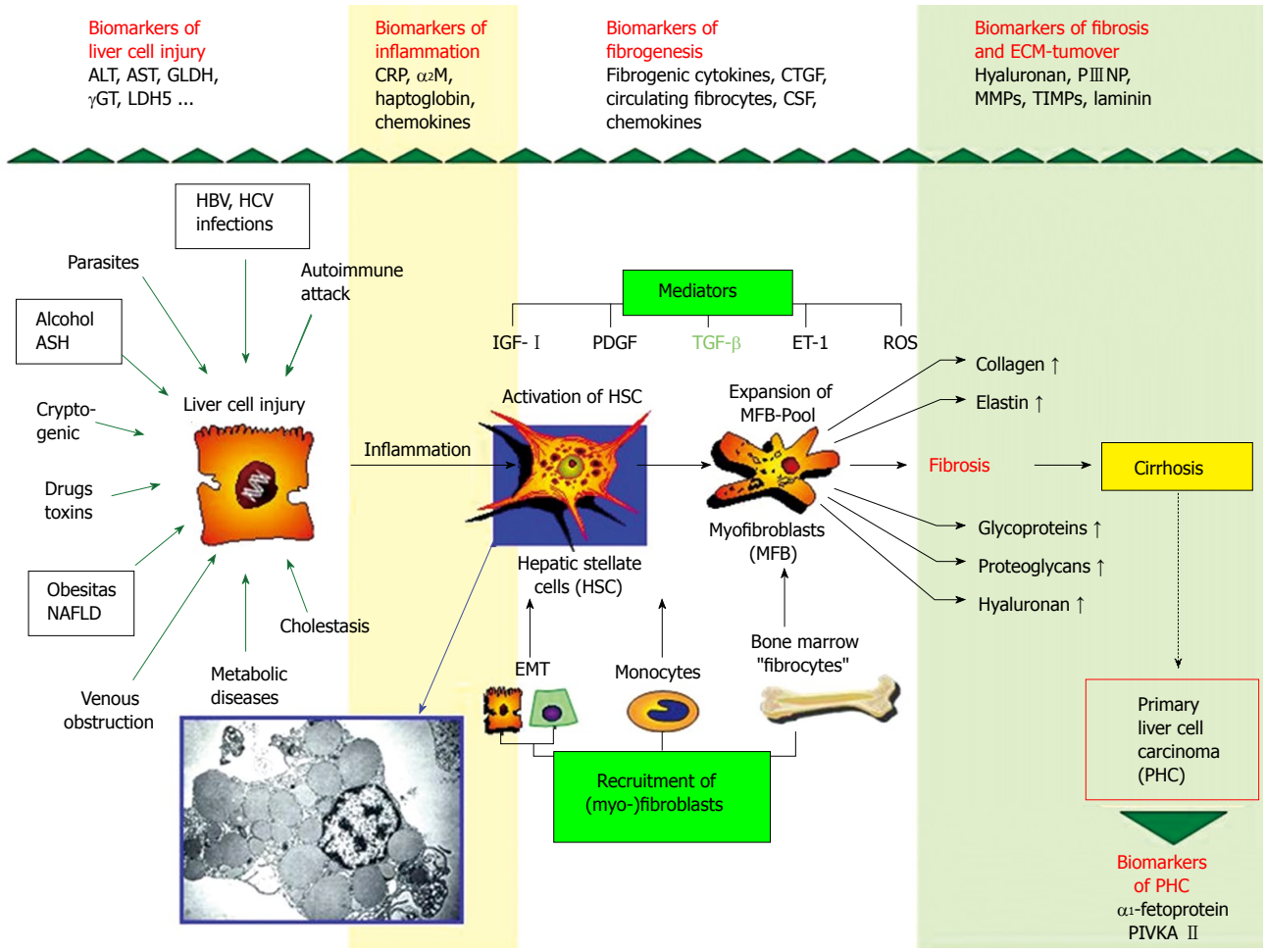

Figure 2 Synopsis of pathogenetic mechanisms of liver fibrosis (fibrogenesis). The cells produce an increase in extracellular matrix derived from activated hepatic stellate cells (HSC)/expended pool of myofibroblasts (MFB) produce various components of the extracellular matrix (fibrosis) leading to cirrhosis. Newly recognized pathogenetic mechanisms point to the (i) influx of bone marrow-derived cells (fibrocytes) to the liver, (ii) to circulating monocytes and to their TGF-β-driven differentiation to fibroblasts and (iii) to the epithelial-mesenchymal transition (EMT) of hepatocytes and bile duct epithelial cells to fibroblasts. All three complementary mechanisms enlarge the pool of matrix-synthesizing (myo-)fibroblasts. Some important fibrogenic mediators are transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), insulin-like growth factor I (IGF- I), endothelin-1 (ET-1), and reactive oxygen metabolites (ROS). Abbreviations: ASH: Alcoholic steatohepatitis; NAFLD: Non-alcoholic fatty liver disease. The insert shows an electron micrograph of hepatic stellate cells containing numerous lipid droplets.

reflect ECM metabolism or fibrogenic cell changes. Their pathobiochemical relationship with fibrogenesis is indirect if at all. Thus, their selection is not hypothesis-driven, but empiric. The markers are standard laboratory tests and are integrated into multiparametric panels.

In general, both types of serum biomarkers follow different pathophysiological concepts. Class I markers inform about "what is going on" (grade of fibrogenic activity), class II markers indicate "where fibrosis is" (stage of fibrosis).

Class I fibrosis biomarkers

These biomarkers are components of the connective tissue (matrix) increasingly expressed by activated hepatic stellate cells (HSC) and (myo-)fibroblasts[12], have a delayed clearance by Kupffer cells or sinusoidal endothelial cells in the liver due to metabolic dysfunction and/or hemodynamic bypasses, or are increasingly expressed mediators of fibrogenesis such as TGF-β. Taken together, of the several procollagen and collagen fragments proposed, only the N-terminal propeptide of type III procollagen (PIIINP) has reached a limited clinical application, but not widespread acceptance[13]. Sensitivities of about 76%-78% and specificities of 71%-81% have been reported, which can be increased up to 88%, if combined with additional collagen fragment markers. It should be emphasized that PIIINP is not a liver-specific biomarker. Similarly, structural glycoproteins (e.g. undulin, tenascin), biosynthetic (e.g. prolyl hydroxylase) or catabolic enzymes (e.g. matrix metalloproteinases) of collagen and other ECM-components have not been convincing in the detection, grading, and staging of fibrosis (Table 1). Several studies have shown that hyaluronic acid (hyaluronan) is currently the best class I biomarker of fibrosis having an area under the receiver operating characteristics (AUROC) of 0.97, a sensitivity of 86%-100%, and a specificity of about 88% in a recent investigation of cirrhosis due to non-alcoholic fatty liver disease[14] and other aetiologies. Since the negative predictive value of hyaluronan at a cut off value of 60 μg/L is much higher (98%-100%) than the positive predictive value (61%), the main utility of serum hyaluronan lies in its ability to exclude advanced fibrosis and cirrhosis. Its

Table 1 Class I biomarkers of liver fibrogenesis

	Specimen			Method
	Serum	Urine	Liver biopsy	
Extracellular matrix-related enzymes				
Enzyme				
Prolyl hydroxylase	+	-	+	Radioenzymatic, RIA
Monoamine-oxidase	+	-	(+)	Enzymatic
Lysyl oxidase	+	-	+	RIA
Lysyl hydroxylase	+	-	-	RIA
Galactosylhydroxylysyl-glucosyltransferase	+	-	+	RIA
Collagen peptidase	+	-	+	Enzymatic
N-Acetyl-β-D-glucosaminidase	+	+	+	Enzymatic
Collagen fragments and split products				
Type of collagen				
Type Ⅰ-procollagen				
N-terminal propeptide (PINP)	+	-	+	ELISA
C-terminal propeptide (PICP)	+	-	+	RIA
Type Ⅲ-Procollagen				
Intact Procollagen	+	-	-	RIA
N-terminal propeptide (PⅢNP)				
Complete propeptide (Col 1-3)	+	-	-	RIA
Globular domain of Propeptide (Col-1)	+	-	-	RIA
Type Ⅳ-Collagen				
NC1-fragment (C-terminal)				
crosslinking domain (PIVP)	+	+	-	ELISA, RIA
7S domain ("7S Collagen")	+	+	-	RIA
Type Ⅵ-Collagen	+	+	+	RIA
Glycoproteins and matrix-metalloproteinase (inhibitors)				
Marker				
Laminin, P1-fragment	+	-	-	RIA, EIA
Undulin	+	-	-	EIA
Vitronectin	+	-	-	EIA
Tenascin	+	-	-	ELISA
YKL-40	+	-	+	RIA/ELISA
(pro)matrix metalloproteinase (MMP-2)	+	-	-	ELISA
Tissue inhibitor of metalloproteinases (TIMP-1, TIMP-2)	+	-	-	ELISA
sICAM-1 (soluble intercellular adhesion molecule, sCD54)				
sVCAM-1 (soluble vascular cell adhesion molecule, sCD106)	+	-	-	ELISA
Glycosaminoglycans				
Marker				
Hyaluronic acid (Hyaluronan)	+	-	-	Radioligand assay ELISA
Molecular mediators				
Marker				
Transforming growth factor β (TGF-β)	+	-	+	ELISA
Connective tissue growth factor (CTGF/CCN2)	+	?	+	ELISA

stimulated synthesis in activated HSC, secretion into the sinusoidal blood stream, and short half life of 2-9 min in the circulation are good suppositions for a valid fibrosis biomarker. Laminin was reported to be a predictor of portal hypertension since significantly elevated concentrations were found under these conditions[15]. TGF-β concentration in plasma is elevated in and correlates with the severity of liver disease and is suggested to be a non-invasive biomarker of fibrosis. However, the significant correlation with AST and ALT activity[16] and the pathobiochemical finding that substantial amounts of TGF-β are localized in hepatocytes and released into the medium if hepatocytes are permeabilized[17] suggest that the elevation of TGF-β is a marker of necrosis instead of fibrogenesis.

Preliminary studies point to connective tissue growth factor (CTGF/CCN2) in serum as an innovative class Ⅰ biomarker of fibrogenesis[18]. This 38 kDa protein is synthesized not only in HSC, but also in hepatocytes where the expression and secretion is strongly dependent on TGF-β[19,20]. Accordingly, the expression of the TGF-β down-stream mediator CTGF in fibrotic liver tissue is up-regulated and its concentration in blood is elevated if fibrogenesis is occurring. There is a correlation between CTGF levels and fibrogenesis, because the levels decrease in fully developed, end-stage cirrhosis, compared to fibrosis. The AUCs for fibrosis *vs* control and cirrhosis *vs* control were calculated to be 0.955 and 0.887, respectively, the sensitivities 100% and 84%, respectively, the specificities 89% and 85%, respectively[18]. These criteria suggest that CTGF is a potentially valuable class Ⅰ biomarker of active fibrogenesis.

Recently, the glycoprotein YKL-40 ("chondrex", molecular mass 40 kDa), which is likely a growth factor for fibroblasts and endothelial cells, was shown to be

Table 2 Class Ⅱ biomarkers of liver fibrogenesis

Index	Parameters	Chronic liver disease	Sensitivity (%)	Specificity (%)
PGAA-Index	Prothrombin time, γGT, apolipoprotein A1, α2-macroglobulin	Alcohol	79	89
Bonacini-Index	ALT/AST-ratio, INR, platelet count	HCV	46	98
Sheth-Index	AST/ALT (De Ritis)	HCV	53	100
Park-Index		HCV	47	96
PGA-Index	Prothrombin time, γGT, apolipoprotein A1	Mixed	91	81
Fortunato-Score	Fibronectin, prothrombin time, PCHE, ALT, Mn-SOD, β-NAG	HCV		94
Fibrotest (Fibro-Score)	Haptoglobin, α2-macroglobulin, apolipoprotein A1, γGT, bilirubin	HCV HBV	75	85
Pohl-Score	AST/ALT-ratio, platelet count	HCV	41	99
Actitest	Fibrotest + ALT	HCV		
Forns-Index	Age, platelet count, γGT, cholesterol	HCV	94	51
Wai-Index (APRI)	AST, platelet count	HCV	89	75
Rosenberg-Score (ELF-Score)	PⅢNP, hyaluronan, TIMP-1	Mixed	90	41
Patel-Index (FibroSpect)	Hyaluronan, TIMP-1, α2-macroglobulin	HCV	77	73
Sud-Index (fibrosis probability-index, FPI)	Age, AST, cholesterol, insulin resistance (HOMA), past alcohol intake	HCV	96	44
Leroy-Score	PⅢNP, MMP-1	HCV	60	92
Fibrometer test	Platelet count, prothrombin index, AST, α2-macro-globulin, hyaluronan, urea, age	Mixed	81	84
Hepascore	Bilirubin, γGT, hyaluronan, α2-macroglobulin, age, gender	HCV	63	89
Testa-Index	Platelet count/spleen diameter-ratio	HCV	78	79
FIB-4	Platelet count, AST, ALT, age	HCV/HIV	70	74
FibroIndex	Platelet count, AST, γ-globulin	HCV	38	97

GGT: γ-glutamyltransferase; PⅢNP: N-terminal propeptide of type Ⅲ procollagen; TIMP: Tissue inhibitors of metalloproteinases; MMP: Matrix metalloproteinases; β-NAG: N-acetyl-β-glucosaminidase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; INR: International normalized ratio.

strongly expressed in human liver tissue[21]. In particular, HSC contain YKL-40 mRNA. Several studies have found elevated YKL-40 concentrations in sera of patients with liver diseases. A sensitivity and specificity of around 80% and an AUC of 0.81 for fibrosis have been reported for HCV-patients[22], for those with alcoholic liver disease, a specificity of 88% and a low sensitivity of 51% were calculated[23]. Serum concentrations of this protein correlated with other ECM products secreted by HSC and fibroblasts, e.g. PⅢNP, hyaluronan, MMP-2, and TIMP-1. It is claimed that YKL-40 concentrations reflect the degree of liver fibrosis but extensive clinical evaluation is still required and other inflammatory diseases as potential causes of YKL-40 elevations have to be excluded. In addition, the expression of this protein is not restricted to the liver, but occurs in chondrocytes (synovial fluid), bone cells, vascular smooth muscle cells and therefore non-specific to the liver[21].

Class Ⅱ fibrosis biomarkers

This category comprises a rapidly increasing, wide variety of biochemical scores and multiparameter combinations (biomarker panels), which are selected by various statistical models and mathematical algorithms, e.g. multiple logistic regression analysis. They fulfil the most appropriate diagnostic criteria for the detection and staging of fibrosis and to a lesser extent for grading of fibrogenesis. In general, the panels consist of rather simple (standard) laboratory tests, which are subject to changes in the serum or plasma of fibrotic and cirrhotic patients (Table 2). Several of the parameters included in the more than 20 scores currently available have no pathophysiological relation to fibrogenesis. Some of them have an indirect relation, and only a few parameters can be regarded as being directly related to fibrogenesis. The parameters measured comprise those of necrosis such as ALT and AST, coagulation-dependent tests, transport proteins, bilirubin and some ECM-parameters. Frequently, the reduction of platelet counts in cirrhotic patients is included. Most prevalent are the Fibrotest™ and for necro-inflammatory activity the Actitest™ (Biopredictive, Paris, France)[24]. These tests are based on γ-glutamyl-transferase (γ-GT), total bilirubin, haptoglobin, α2-macroglobulin, apolipoprotein A1, and for the Actitest additionally on alanine-aminotransferase (ALT)[25]. The data of Fibrotest and Actitest are calculated with a patented artificial intelligence algorithm to give measures of fibrosis stage and necro-inflammatory grade (activity), respectively. The Wai-score based on aspartate-aminotransferase (AST), alkaline phosphatase and platelet count[26], the ELF-test based on TIMP-1, PⅢNP, hyaluronan[27], and the Hepascore based on bilirubin, γ-GT, hyaluronan, α2-macroglobulin, age and gender[28] are further scores with up to now limited clinical application. The Fibrotest was shown to be a better predictor than biopsy staging for HCV complications and death[25]. Recently, Fibrotest™ and Actitest™ were included to detect biomarkers for the prediction of liver steatosis (Steato-test™), alcoholic steato-hepatitis (ASH-test™), and non-alcoholic steato-hepatitis (NASH-test™) by supplementation with serum cholesterol, triglycerides, glucose (and AST for NASH-test) adjusted for age,

Table 3 Future candidate biomarkers of non-invasive diagnosis and follow-up of liver fibrogenesis

Biomarker	Specimen	Assay technology	Pathobiochemical basis
CTGF	Serum	Immunoassay	TGF-β induced expression in and secretion by hepatocytes and hepatic stellate cells
Fibrocytes	Blood, buffy coat	Flow cytometry of $CD34^+$, $CD45^+$, Coll I^+ cells qPCR	Supplementation of local fibroblasts at site of liver injury by bone-marrow derived fibrocytes
BMP-7	Serum	Immunoassay	Antagonist of TGF-β, inhibitor of EMT
G-CSF GM-CSF M-CSF	Blood	Immunoassays	Mobilization of bone-marrow derived fibrocytes
Proteomics	Serum	Mass spectrometry (MS)	Fibrosis-specific serum protein profiles
Glycomics	Serum	Adaptation of DNA-sequencer/fragment analyzer technology to profiling of desialylated N-linked oligo-saccharides	Fibrosis-specific profiles of desialylated serum protein linked oligosaccharides (N-glycans)
Xylosyl-transferase (EC 2.4.2.26)	Serum	LC-MS/MS	Key enzyme in the biosynthesis of glycosaminoglycan chains in proteoglycans, e.g. in hepatic stellate cells and hepatocytes

gender, and body mass index (BMI)[29]. The diagnostic criteria elaborated in a large cohort of patients suggested that the Steato-test was a simple and non-invasive quantitative measure of liver steatosis and the NASH-test was a useful screening procedure for advanced fibrosis and NASH in patients with various metabolic syndromes[29]. FibroMax™ (Biopredictive) was recently developed as a method of combining calculations of these fibrosis-related tests in a single procedure. Comparative evaluation of class Ⅱ serum biomarker panels, however, did not highlight their clinical superiority if liver biopsy was used as the reference method[30]. Since only about 40% of the results were assigned to be correct, a fraction of about 50%-70% was inaccurate with regard to the staging of fibrosis severity and a small fraction of results was even incorrect[30]. Thus, currently suggested multi-parameter approaches with class Ⅱ fibrosis biomarker panels have to be used with caution in clinical practice. A successful approach to improve the diagnostic accuracy of the panel markers in chronic hepatitis C might be their stepwise combination[31]. By combining the sequential algorithms of APRI, Forns' index and Fibrotest (Table 2) the diagnostic performance could be significantly improved resulting in a 50%-70% reduction in the need for liver biopsy[31]. Recently, a comparison of the diagnostic power of up to five class Ⅱ biomarkers led to suggestions to strongly increase their overall accuracy which would, thus, reduce the need for a liver biopsy from 56% to 0% in chronic hepatitis C[32]. Additionally, an algorithm based on the AST-to-platelet-ratio-index (APRI) and liver surface ultrasound nodularity showed a strong diagnostic power making liver biopsy unnecessary[33].

It should be emphasized that the combination of individually assessed parameters necessarily creates a relatively high variance due to the imprecision of each separate measurement[34]. Coefficients of variation range from series to series and are usually between 3% and 6% for common clinical-chemical parameters and from 4% to more than 12% for hyaluronan, PⅢNP, and other matrix parameters. Furthermore, and even more important is the lack of standardized assays for many of these parameters, which excludes the general use of cut-offs and algorithms[34].

DEVELOPMENTS OF INNOVATIVE BIOMARKERS

A growing understanding of the pathogenesis of hepatic fibrosis has indicated potentially powerful non-invasive (blood) biomarkers of hepatic fibrogenesis and fibrosis (Table 3). CTGF/CCN2 was already mentioned as a pluripotent downstream modulator of TGF-β, and was found to be up-regulated by TGF-β in hepatocytes. Although most CTGF will only have a defined paracrine function in fibrogenic tissue, a certain fraction spills over into the circulation, resulting in elevated serum concentrations during active fibrogenesis[18]. The circulating level of CTGF might be an objective and sensitive measure of ongoing fibrogenesis in necro-inflammatory liver tissue.

Bone-marrow-derived fibrocytes might offer new approaches not only for understanding the pathogenesis, but also for the diagnosis of liver fibrosis. Fibrocytes are circulating progenitor cells (CD34 positive) of hematopoietic origin (CD45 positive) capable of differentiating into diverse mesenchymal cell types[35]. The additional markers of fibrocytes, i.e. positivity of type Ⅰ collagen and the CXCR4 chemokine expression can be used to quantitate this special sub-population of circulating leucocytes applying quantitative PCR and/or flow cytometry. The determination of the colony stimulating factors M-CSF, G-CSF, and GM-CSF, which are increasingly expressed in fibrotic liver tissue and elevated in serum[36], are possibly involved in the mobilisation of fibrocytes from the bone marrow and their homing in the liver during fibrogenesis. These factors may be further candidates for diagnostic evaluation.

A new, but currently still controversial aspect of fibrogenesis is epithelial-mesenchymal transition (EMT) of hepatocytes and biliary epithelial cells, respectively, to (myo-)fibroblasts[2]. EMT is governed by the balance of TGF-β (pro-EMT) and its antagonist, i.e. BMP-7 (anti-EMT). In addition to its anti-EMT effect, BMP-7 was shown to have anti-apoptotic and anti-inflammatory activities. Thus, the measurement of BMP-7 alone or even in relation to TGF-β in serum might reflect the activity of fibrogenesis and, hence, the velocity of

fibrotic organ transition[37].

Xylosyltransferase (XT), a key enzyme in the biosynthesis of glycosaminoglycans in proteoglycans, was shown to have increased activities in the serum of patients with connective tissue diseases. With HPLC-tandem mass spectrometry, measurements in large cohorts of liver fibrotic patients may to be possible[38]. Since HSC in fibrotic liver tissue (myofibroblasts) have a greatly stimulated proteoglycan synthesis[39], XT activity in serum might be a promising class I biomarker of fibrogenesis.

Further successful developments could emerge from serum proteome profiling[40] and from total serum protein glycomics, i.e. the pattern of N-glycans[41]. It was reported that a unique serum proteomic fingerprint is powerful enough (accuracy > 90%) to differentiate between various stages of fibrosis and to allow prediction of fibrosis and cirrhosis in patients with a chronic hepatitis B infection[40]. Specificities, sensitivities and accuracy of prediction of cirrhosis are around 89%. Similarly, N-glycan profiling can distinguish between compensated cirrhosis from non-cirrhotic chronic liver diseases with a sensitivity and specificity of 79% and 86%, respectively[41].

Supplementation of all these laboratory tests by modern high resolution or molecular imaging analyses would be extremely helpful in the consolidation of objective and valid non-invasive biomarkers of diagnosis and follow-up of fibrogenic (liver) diseases. In conclusion, currently available type I and II serum biomarkers should be used with caution, because neither single nor panel markers fulfil the requirements of an ideal non-invasive biomarker of fibrosis[42], i.e. analytical simplicity allowing performance in any laboratory, standardization of the test system and calibrators allowing comparison between laboratories over a long period, cost effectiveness, specificity for the liver and the disease, clear association with the stage of fibrosis or grade of fibrogenesis and independency of the aetiology of fibrosis. Even the best and most extensively evaluated type I (i.e. hyaluronan) and type II (i.e. Fibrotest, Actitest) serum biomarkers do not meet the criteria of an ideal marker. Further detailed insight into the mechanism of liver fibrosis and improvements in analytical techniques will result in new approaches for the non-invasive assessment of fibrosis with biochemical or physical means.

In addition, genetic markers linked with the progression rate of fibrosis will become important diagnostic and prognostic tools for patients with liver fibrosis.

CONCLUSION

Non-invasive evaluation of the fibrogenic response of the chronically injured liver has made considerable progress over the past few years, in particular over the last three years multiple algorithms based on a combination of more or less routine parameters have been suggested frequently. A rigorous, independent and widespread evaluation of the utility of these panels in the diagnosis and follow-up of chronic liver diseases is still needed for a final decision and the recommendation for use in routine clinical practice. Novel single biochemical markers have been suggested, but there putative diagnostic value in clinical practice is far from defined. The fundamental problem in the evaluation of existing and novel non-invasive parameters lies in the limited validity of the present diagnostic "gold standard", i.e. histology of liver biopsy specimens. Perhaps new developments in highly sensitive and tissue-specific scanning techniques of the liver will solve this problem. These procedures will then be suitable for the correct validation of effective antifibrotic treatments.

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