

# **SERUM PROGNOSTICS IN LIVER DISEASES**

**Pierre Bedossa**

**Hôpital Beaujon, Université Paris 7**

**France**

Prognostic predictors are crucial to estimate the outcome of a liver disease in a given individual and the potential impact of conventional or investigational treatments, as well as to design prospective trials. Beside liver biopsy that provides direct insight into the damages to the liver, non invasive approaches such as imaging techniques and blood tests contribute both to diagnosis and prognostication. Because the liver has an exceptionally abundant blood supply, it is anticipated that blood component analysis should provide valuable insight into liver disease evaluation. With more than 10.000 of different proteins, a large variety of carbohydrates, lipid particles and pathogens, changes in blood tests might be a major source of information both for diagnosis and prognosis providing that the appropriate component is scrutinized.

## **SERUM FOR PROGNOSTICATION IN SEVERE LIVER DISEASES: PROGNOSTIC INDEX**

Not only diagnosis but also prognosis can be assessed by blood sample analysis. Considering the major topics in hepatology, there is no one liver disease where a prognostic index combining several liver-related blood factors have been proposed. Interestingly, these indexes are more often composite including serum measurements and other clinical or pathological factors. Here are some prognostic index routinely used in hepatology

### **Prognostication in Hepatocellular Carcinoma:**

Faced with a patient with an established diagnosis of hepatocellular carcinoma, the first question that the physician faces is, as with most cancers, "What is the prognosis?" At the outset it must be recognized that the overall prognosis is almost always universally poor, with perhaps less than 5% of patients being cured. Nevertheless, there is a fairly wide variation in survival times. This "heterogeneity of survival" is important to patients and obliges physicians to attempt accurate prognostication. Prognostic scores can be divided into two groups: those based on expert opinion, such as TNM staging, and those developed through regression analysis of actual data. It is now recognized that the prognosis of cancer patients is not solely related to tumor stage but cirrhosis that underlies the tumor should be considered when modeling HCC prognosis. With this last approach in mind, several studies have developed models that combine the assessment of tumor stage and liver function impairment. The Okuda staging system is based on tumour volume, presence of ascites and serum bilirubin and albumin. Unfortunately, it provides only a rough estimation of the outcome. More recent prognostic index has been proposed (CLIP-Italy, Barcelona, France, Tokyo). Recently there has been much debate regarding

which prognostic staging system is the best. Although they differ in several criteria they all mix pathological data with serum markers of liver function

### **Prognostication in end stage chronic liver diseases:**

The Child-Pugh classification is used to assess the prognosis of chronic liver disease, mainly cirrhosis. Although it was originally used to predict mortality during surgery, it is now used to determine the prognosis, as well as the required strength of treatment and the necessity of liver transplantation. The score includes five items; Bilirubin (total); serum albumin; INR; ascites; encephalopathy. Each measure is scored 1-3, with 3 indicating most severe derangement defining 3 classes: Class A / points 5-6, Class B / points 7-9, Class C / points 10-15

The model for end stage liver disease (MELD) is also suitable for assessing the prognosis of patients with severely impaired liver function. Because of the ability of MELD to accurately stratify patients according to mortality risk, it has now replaced the Child-Pugh score to prioritize and rank organ allocation of cadaveric livers for transplantation. The model was originally derived from a cohort of 231 patients to assess the short-term prognosis of patients with cirrhosis undergoing elective transjugular intrahepatic portosystemic shunt at four centers in the United States. This model was subsequently validated as an independent predictor of survival in several independent cohorts of patients with cirrhosis. The success of the MELD score is based not only on its accuracy in predicting prognosis, but also on the fact that it is objective, reproducible, and readily available in all settings. The variables in MELD include PT, bilirubin and serum creatinine. Its superiority over other existing prognostic indexes is likely due to the fact that it includes a variable that estimates the degree of renal dysfunction (serum creatinine), because renal function is known to accurately predict prognosis in patients with cirrhosis.

### **Prognostication in cholestatic liver diseases:**

One of the most difficult questions in the management of patients with primary biliary cirrhosis (PBC) is when to intervene with transplantation to optimize quality and length of life. Several prognostic indicators have been proposed and prognostic indexes developed to predict survival. Serum bilirubin is surely the best predictor of survival in PBC, and elevated serum bilirubin levels have been used to decide when to refer patients for transplantation. Prognostic indexes that combine bilirubin with several other variables to define a predictive score have also been developed. The European model uses serum bilirubin, serum albumin, age, the presence of cirrhosis and cholestasis on liver biopsy, and azathioprine use. So far, the survival model for PBC patients developed in the Mayo Clinic has gained the greatest popularity. The model employs bilirubin, serum albumin, age, prothrombin time, and the presence of edema. The Mayo model was based on data from a group of 312 patients involved in a trial of D-penicillamine and has been externally cross-validated. It was developed to predict survival probability of patients with PBC at initial diagnosis. Subsequently, it has also been used as a guide to help predict the appropriate time to intervene with transplantation.

### **Prognostication in severe acute alcoholic hepatitis:**

Alcoholic hepatitis, in its severe form, is associated with a high risk of short-term mortality. The Maddrey discriminant function (DF) ( $DF \sim 4.6 \sim [\text{prothrombin time (PT) in seconds} \sim \text{control PT}] \sim \text{serum bilirubin in mg/dL}$ ) was introduced in 1978 as a tool for predicting risk for mortality in AH and thereby identifying a subset of patients that may benefit from intervention with corticosteroids. Based on these analyses, corticosteroid treatment is advocated by many clinicians for patients with a DF score of more than 32, because these patients appear to have a mortality rate exceeding 50% in the absence of treatment. This criterion was also used in a recent clinical trial evaluating the potential clinical efficacy of pentoxifylline in AH.

### **IS SERUM ABLE TO PREDICT MILD LIVER DISEASES? THE PARADIGM OF LIVER FIBROSIS SERUM MARKERS**

Whether it is clear that serum with simple liver parameters contributes strongly to prognostication in severe liver diseases, the next raising question is whether serum should help to predict milder form of chronic liver diseases. Since fibrosis is the hallmark of all chronic liver diseases and because fibrosis is the main determinant of clinical outcome, several groups investigate whether serum might predict the stage of liver fibrosis and help to follow its progression. For these investigations, chronic hepatitis C offers to hepatologists a large field of investigation. In addition, limits of liver biopsy such as potential morbidity and mortality, cost, observer variation and sampling variation, a major concern for fibrosis evaluation in liver biopsies, pushed forward clinical research on serum biomarkers of liver fibrosis.

Liver fibrosis biomarkers have been investigated using two approaches. The first was to correlate routine liver serum parameters commonly available in clinical practice and fibrosis stage as evaluated on liver biopsy using usual semi-quantitative scoring systems. Some of these parameters were reported to predict the presence of bridging fibrosis, or cirrhosis with considerable diagnostic accuracies. Among those, decreased platelet count, increase in the ratio of aspartate to alanine aminotransferase (AST/ALT), and prolonged prothrombin time are the best indicators of cirrhosis. However, they have limited accuracy in predicting earlier hepatic fibrosis.

To increase the sensitivity, several groups have developed algorithms through regression analysis that combine the measurement of several blood components. Biomarker of liver function, platelet counts or biomarkers of liver cell necrosis or inflammation are the most commonly used. After several years of development, it seems clear that these serum markers are efficient to discriminate between mild and severe fibrosis. However, these indexes were designed to detect the presence of clinically significant fibrosis and have a low negative predictive value for mild fibrosis. Furthermore, because of strong overlap, the performance of fibrosis biomarkers to differentiate between adjacent scores of hepatic

fibrosis is limited. All these drawbacks limit their use for individual patients. The potential of more sophisticated extracellular matrix derived serum components has also been assessed. They include hyaluronic acid, products of collagen synthesis or degradation, enzymes involved in matrix biosynthesis or degradation; extracellular matrix glycoproteins, and proteoglycans/glycosaminoglycans. Although this approach is more hypothesis-driven, it was no more successful since the diagnostic accuracy of these extracellular matrix components in prediction of liver fibrosis is also limited. Furthermore, for these markers to accurately reflect hepatic fibrogenesis or fibrosis, they should be organ-specific and the biological half-life should be independent of urinary and biliary excretion as well as sinusoidal endothelial uptake. Unfortunately, none of the available markers fulfill all these criteria. Therefore, biochemical blood tests have only modest value in predicting fibrosis on liver biopsy. Several studies have concordantly shown that their use may render liver biopsy unnecessary only in a minority of patients with chronic HCV.

Improved serum fibrosis markers with greater sensitivity (if exist) are needed. Whether imaging will provide a more accurate approach to investigate liver fibrosis is under investigation.

### **SERUM PROTEOMIC: A NEW TOOL FOR PROGNOSTICATION IN LIVER DISEASES?**

Prognostic index have gained large popularity in clinical hepatology because they rely on simple, easy-to-access and reliable biochemical tests. However, blood can tell much more than a rough evaluation of the global liver function. Blood plasma is a complex body fluid, which contains a large diversity of compounds. Intact as well as partially degraded proteins or protein fragments circulate in the blood. Over 10.000 different proteins have been estimated to be present in the plasma, most of which are at very low relative abundance with only 1500 different proteins being identified so far. Thus, blood provides an enormous source of information that lies beyond the classical liver parameters.

The “Proteome” describe ideally the whole pool of proteins expressed in a biological milieu at a given time. There are some differences between traditional protein biochemistry and proteomics. Both proteomics and protein biochemistry involve protein identification, but proteomics is the study of multi-protein systems in which the focus is on the interplay of multiple, distinct proteins in their roles as part of a larger system or network. By comparison to the previously described approach, mining the proteome does not need an a-priori hypothesis on the physiopathology of the liver disease.

Therefore, the challenge of clinical proteomic studies is to link protein expression profile variations to specific disease phenotypes and to find out relevant biomarkers in order to develop diagnostic or prognosis tools. However, there are several hurdles to jump. One of the major technical difficulties is that the concentrations range of different protein species in the blood extend over at least 15 orders of magnitude, resulting in an enormous range that precludes full analysis of all protein species especially low abundance

peptide/protein entities. As a matter of fact, there is no similar amplification techniques such as PCR, when dealing with proteins.

Therefore, the task of characterizing the serum/plasma proteome requires accurate and high throughput analytical methods for detecting and quantifying proteins. It is based on the development and integration of four essential tools: (1) The database. Protein, expressed sequence tag, and complete genome-sequence databases collectively provide a complete catalogue of proteins expressed in organisms for which databases are available; (2) Mass spectrometry (MS); (3) An increasing number of software that can match MS data with specific protein sequences in databases; and (4) Improvements and simplification in analytical protein-separation technology. Current proteomic technology has been provided by the integration of these four tools.

Currently, a prevalent approach to clinical proteomic appears to be based on SELDI profiling. The Surface-Enhanced Laser Desorption Ionization (SELDI) technology, arising from the MALDI technique, refers to the process of affinity capture on special chemical surfaces, followed by precise mass analysis using laser desorption/ionization based detection. Differences in protein patterns (profiling) between different conditions can then be detected. The possibility to obtain rapidly and to compare profiles, directly from the original source material and without laborious sample preparation, makes this technique a promising possibility for clinical applications. However, SELDI/ MS is a partial analysis, not giving sequence data in many cases. Other methods, combining different separation techniques (liquid chromatography, multidimensional chromatography, capillary electrophoresis) followed by MS, are currently more and more employed.

Although, the reproducibility of SELDI-TOF MS approaches have been questioned, interesting results have been obtained in liver pathology. Using artificial intelligence based on pattern recognition algorithms, it was possible to find patterns distinguishing cirrhotic patients with or without hepatocellular carcinomas. Recently, SELDI-TOF MS allowed the identification of a cleavage fragment of vitronectin, a major serum protein, as a new marker of HCC with higher sensitivity and specificity than alpha-foeto protein. SELDI-TOF also provided insight into the potential for serum to predict liver fibrosis. By comparing serum proteome of patients with mild fibrosis (F0 to F2), no significant peak differences were observed according to the different scores. By contrast, when serum profiles of patients with more advanced fibrosis were analyzed and compared, significant modifications of peaks were observed. This large-scale approach raises some doubt for the future discovery of sensitive blood test that should accurately monitor or predict early liver fibrosis.

Beside diagnostic biomarkers, there are oncoming data about the predictive value of serum proteome in liver diseases. There are encouraging pilot studies showing specific serum profiles that could predict response to antiviral therapy in HCV patients or HCC in cirrhotic patients. These results have to be validated in prospective studies.

In conclusion, because serum is a rich milieu and since blood-liver exchanges are extremely abundant, serum has shown to be a valuable source of information for diagnosis and prognosis in severe liver diseases. Whether, it is also helpful for prediction of milder changes needs some confirmations. With the development of new technologies such as high throughput proteomic methods, some progress might be expected in a near future.