Biochemical Markers for Hepatic Fibrosis Preview

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Abstract: The liver is an organ essential for functioning of the organism. One of the liver pathologies, the leading cause of mortality and morbidity occurs by fibrosis and cirrhosis. The current diagnosis and subsequent treatment standard of excellence for fibrosis is the biopsy, however, the fibrotic process is not accompanied by dynamically and liver physiology is not demonstrated, in addition to persist intra-observatory errors. This study sought to prove by means of a review, the importance of the use of serological tests that feature a noninvasively, generating the patient a treatment faster and less painful. Non-invasive methods applied jointly establish indexes correlated to a histopathological study, so the metabolic, morphological and functional changes better evaluated within the framework of reducing liver disease by up to 70% the need for biopsies. So non-invasive markers offer, through technological advancement, better standardization of measurement of hepatic fibrosis histological preparation and advanced mode. Keywords: Hepatic fibrosis serum markers, liver.

1. INTRODUCTION

The leading cause of mortality and morbidity related to diseases arising from liver fibrosis which evolves with desquamation of cells and buildup of scar tissue, even after the regeneration of the liver parenchyma. There is considerable tissue damage which remains impossible, invariably, the regeneration. Several diseases can affect the liver: viral hepatitis, metabolic diseases, autoimmune diseases, exposure to alcohol and drugs, bile diseases and steatohepatitis-hepatitis; for reasons both non-alcoholic and alcoholic drinks, such as metabolic syndrome. Fibrosis occurs due to chronic and leucocyte infiltration assault; the organism can cause an immune response to wound healing and tissue regeneration simultaneously. The most advanced stage of fibrosis is of great rides to cirrhosis (OLIVEIRA, 2009).

Current medicine treatment gold "Gold standard" for diagnosis, severity and possible progression is the liver biopsy, invasive process that does not allow show the pathological process dynamically and necessary for complete evaluation of the patient. Despite being safe, is subject to complications, has high cost and around 10 to 20% of observers have any discrepant information between themselves (BAES, 2008). The risk/benefit of the biopsy demonstrates a coefficient of variation of 40%, with 0.3% of serious events and 3/10,000 deaths. It is still used as first line investigation of hepatic fibrosis (POYNARD, 2007).

There is, obviously, the interest for a diagnostic methodology involving minor scratches and efficiency guaranteed. There is also a way to extend the treatment protocol for patients with diseases that have great potential oncogenic, since one of its complications is cirrhosis. A way to minimize or even avoid the need of liver biopsy in some cases is the use of protocols in which the dosage of laboratory analytes that, together, and through calculations, would result in an index, or parameter, regression or progression related hepatic frame. These protocols also feature a noninvasively, that assessment does not generate discomfort or higher risks to the patient. Non-invasive methods applied jointly establish indexes correlated to a histopathological study, providing that the metabolic, morphological and functional changes be best evaluated within the framework of liver disease.

Studies dating from 1998 show those non-invasive tests has become source of research – even if indirectly and somewhat expensive. Some stand out when the result variables and comfort of the patient are applied in practice: Fibroscan (Transient Hepatic Elastography), APRI® (AST to platelet ratio index), ELF ® (Enhanced Liver Fibrosis Score) and Fibrotest® or Fibromax®.

So serum indirect markers and markers of dynamic process algorithms present hepatic solutions for the rapid diagnosis of the development of certain hepatic pathology to a fibrosis, liver histological activity related and the usual facility in a clinical laboratory.

It is important to note that the biopsy suffers interference from sampling, and the process is technical-dependent, so its results have 10 to 20% variance between observers. Liver biopsies do not demonstrate the potential of liver dynamically and physiological soon such potential within the pathology in the liver can be minimized or masked. Thus deter-
mining signals for the early diagnosis of fibrosis can be ignored inadvertent form.

According to studies already carried out to obtain biopsy samples is expensive and includes risks to the patient as pain, hemorrhage and even death. The processing of specimens is time consuming and laborious. For such reasons the frequent repetition of liver biopsies is considered unacceptable for patients and doctors, even for monitoring the progression if you do need a new biopsy (ROSENBERG et al., 2004). Faced with a complex scenario this study aimed to report and analyze the non-invasive liver tests in order to contribute to the minimization of interference and histological errors in this specific field.

2. PANORAMA OF HEPATIC FIBROSIS

The applications of markers discriminate phase’s determinants of fibrosis, reducing 50% the number of biopsies, but currently only are used with precision in the fibrotic stage previously known, offering diagnostic security. Concomitant to the tests and ultrasound image direct or indirect markers are important for risk monitoring and the development of better patient treatment strategy (AFDHAL et al. 2015).

The ELF® test, in studies, has as main objective the detection of sinusoidal fibrosis, since his Panel of markers is noticeable to the continuous fibrogenesis with greater emphasis in patients of non-alcoholic fatty liver disease (PINZANI, 2010).

In studies applying the FibroTest® and Fibromax® showed the use of transient elastography that measures liver stiffness. This technique consists of applying an ultrasound probe with a specific vibration providing a shock wave or elastic wave penetrates in liver by measuring the elasticity. From these evidences, the FibroTest® has been validated as an important marker for monitoring and the staging progression of fibrosis. (Marine, 2007). The junction necroinflammatoty activity monitoring with fibrotic activity presented the FibroTest® as an essential marker to be reduced occurrences of biopsies in patients, mostly with fibrosis from hepatitis C.

Already the APRI® was the most simple and accurate index studied, easily accessible and low cost, especially in studies of HCV and HIV, since these AST debugging infections is decreased (MANNING, 2008).

Although APRI® and other indirect markers of fibrosis as "Forns score" and "FIB-4" showed similar performance, especially in tables of HIV and HCV, for advanced fibrosis detection, the accuracy of these need to be studied concurrently to other techniques, since your variables are too simple and may suffer some interference. (MERLI, et al., 2016).

It is known that the serum markers can differentiate between the beginning and the degree of involvement of the pathology guiding treatment decisions, as well as decreasing the number of biopsies performed. The inherent errors of the biopsy are substantially resolved. However, studies showed controversies in the detection of intermediary stages of liver disease especially regarding FibroTest®. Therefore, it is urgent to deepen in further studies, since the gold standard – the liver biopsy – has its limitations. We must also follow all the clinical data and get to know the possibilities of false positives or negatives due to syndromes or inflammation. (POYNARD, 2007).

3. THE LIVER

The liver is the largest organ in the human body and can reach 1.5 kg of body weight of an adult (GUY-TON et al., 2006). The liver is an organ essential for functioning of the organism, it is fundamental proteins like bile for digestion and absorption of fats, coagulation proteins and immune system contributing to protecting the body against bacteria and germs, excretes substances several mainly in the processes of distribution of drugs and blood circulation, in its Constitution found four wolves: right, left, square and caudate. In its histology can be seen parenchymal cells such as hepatocytes and biliary cells; and parenchymal cells as Kupffer cells, endothelial cells and cells of hepatic stellate cells or fat rich in vitamin a. With great capacity for filtering your endothelium, the liver is responsible for the metabolic activity, secretion and production of cytokines, immune system defense that is demonstrated in inducing response to inflammation (OLIVEIRA, 2009) (FRIEDRICH-RUST, 2010).

Biliary secretion is responsible for the digestive function of the liver, as this is essential in regulating the metabolism of carbohydrates, proteins and lipids, storage, degradation and excretion of substances and hormones, hemostasis, aid the immune response. Therefore possible to understand the hepatic Physiology it is possible to analyze the pathologies that affect the liver (SCHINONI, 2006).

The liver has a capacity of restructuring after hepatic tissue loss, either partial or hepatectomia for liver damage provided there are no viral infection or inflammation. In case of partial hepatectomia, where up to 70% of liver is removed, the wolves intact increase and restore to original size. In approximate seven days, that restructuring is complete. The explanation is due to the estimate of the hepatocytes to replicate at up to two times and after the wolves and hepatocytes reach the size and volume of the liver
hepatocytes replicated revert to its State at rest (GUYTON et al., 2006).

The little knowledge acquired around the liver regenerative process, it is known that hepatic growth is closely regulated by a sign related to body size, keeping the proportion to metabolic functioning between body weight and liver, but that according to the pathology involved the degree of fibrosis of liver regenerative process compromises and liver function deteriorates (GUYTON et al., 2006).

By having a metabolic function of large proportions, various pathologies get injured the liver directly and indirectly. Even though belatedly, metabolic disorders, protein and replacement deficiencies, insufficient mechanisms of cell production take the liver to work inefficiently. Hematogenic systemic infections or the abdominal cavity; viral hepatitis, Protozoan hepatitis, drugs, fungi, and bacteria are examples of autoimmune disorders (ORTEGA, 2004).

Fibrosis is the result of deleterious leading cause of mortality and morbidity. The extracellular material leads to impairment of hepatic microcirculation, excretion of substances and analytes, secretion of substances, functions of the immune response and homeostatic (POYNARD, 2000). There is an increased liver stromal, or may be systemic in the injured area, but lead to functional modification of the organ. In short, the process is similar to that of cirrhosis of the liver, but there is no change in the architecture of the organ, with hepatic stellate cells activation or Ito cells (perissinusoidal cells) phenotypic changes in fibroblasts and myofibroblasts making them proliferative and fibrogenic, since through the storage of fat cells perform organ conjunctivization next to myofibroblasts. In situations of normality stellate cells are the principal structures responsible for liver homeostasis and in response to injury, in addition to storing fat and vitamin A, with an injury that role is reduced or modified (SON, 2011; BARRETO, 2011).

4. JAUNDICE

The free and conjugated to copathological correlation in excess by body fluids body a yellowish tint gives both the skin and deep tissues. The normal concentration of bilirubin is on average of 0.5 mg/dL in plasma free form almost entirely. In approximate concentrations to 1.5 mg/dL, it is possible signs of jaundice in the skin. The most common causes of jaundice are the destruction of red blood cells-the hemolytic; and obstruction of the bile ducts, or liver cells injury preventing excretion through the gastrointestinal tract of normal levels of bilirubin, being called obstructive jaundice (GUYTON et al., 2006).

Hemolytic jaundice occurs at time of excretion of bilirubin and hemoglobin formation in bilirubin, i.e. red blood cells undergo hemolysis prematurely preventing liver cells to excrete bilirubin that is already formed and absorb new groupings of globin, soon the concentration of free plasma bilirubin rises, but also can occur increasing urobilinogen in the feces and urine (GUYTON et al., 2006).

Obstructive jaundice is opportunist since the obstruction of the bile ducts occurs in most common situations such as cancer, biliary calculus or injury of hepatocytes and hepatitis. The formation of bilirubin is normal, but does not pass to the intestines, so free bilirubin is directed to the hepatocytes and is conjugated normally, soon accumulates conjugated bilirubin and its buildup helps rupture of hepatic ducts that drains into the lymph and bile. Consequently, the highest concentration in plasma of conjugated bilirubin (GUYTON et al., 2006).

5. HEPATITIS

Infectious disease, acute or chronic, caused by hepatotropic virus, affects the liver and secondarily other organs causing inflammation and necrosis of hepatocytes (ORTEGA, 2004).

In times of war was possible in humans a search in order to search the route of transmission of the disease; These studies have designated that the disease may have the forms of oral-fecal contagious or infectious hepatitis and parenteral transmission or homologous serum hepatitis (ORTEGA, 2004).

The viral hepatitis, even after numerous studies developed along with vaccines and cleared in molecular biology techniques, are public health cases and develop into millions of people all over the world so acute and chronic and often asymptomatic. (ORTEGA, 2004; MINISTRY OF HEALTH, 2005).

6. ALCOHOLIC LIVER DISEASE

Alcoholic liver disease (DHA) is a multifactorial disease, complex, modulated and triggered by the man, from the uncontrolled intake of ethanol and its metabolite, which converge with environmental factors, social, economic and biological. The DHA is one of the leading liver diseases of perinatal transmission. The hepatotoxicity of ethanol is of major proportions involving in the progression of the disease, in addition to other factors such as dose, duration and type of alcohol intake, sex, ethnicity, obesity, malnutrition, concomitant infections the viral hepatitis and genetic factors; Although it is still large number of people who develop liver disease culminating in cirrhosis and DHA (MINCIS, 2010; BUCHO, 2012).
The DHA occurs for a liver infiltration by inflammatory cells and hepatocellular lesions associated with progressive fibrosis. The aggression through oxidative stress of ethanol and its metabolite generate interactions with amino acids that alter the function and intestinal morphology. Nevertheless, there is also the favor of endotoxin absorption and intestinal flora products acting on Kupffer cells produce cytokines such as TNF-α, IL-1, IL-6 and IL-8, but this production promotes inflammation, hepatocyte aggression and stimulates stellate cells that induce fibrosis (BUCHO, 2012).

The symptoms largely presents paintings insidious and nonspecific like nausea and vomiting, but more severe clinical pictures can be striking, ranging from fever, leukocytosis hepatomegaly, jaundice, liver failure and may even the latter lead to death. Even with the use of ethanol, the wounds in the architecture of the liver heal and can progress to scarring (BUCHO, 2012).

7. NON-ALCOHOLIC FATTY LIVER DISEASE

Variable pathology, on or off the DHA with steatosis, steatohepatitis, fibrosis and in some cases with high potential of developing into cirrhosis and hepatocellular carcinoma. Several metabolic disorders and eating habits such as obesity and diabetes, for example, cause NAFLD, but all are associated with insulin resistance. The chronicity of the condition may have causes by habits such as alcoholism, or increase of hormones and cytokines (MINCIS, 2010).

8. HEPATIC STEATOSIS

Early onset, frequent and less severe alcoholic liver disease, affects the liver parenchyma and fat accumulation in hepatocytes by chronic consumption and exacerbated ethanol. Chronic alcohol consumption changes the NADH / NAD ratio, increasing the proportion of dinucleotide in addition, nicotinamide adenine in reduced form and the oxidized form in the hepatocytes, as a result mitochondrial β-oxidation of fatty acids is altered leading to the accumulation of these in developing hepatocytes steatosis (Maw, 2012).

Under normal conditions, the free fatty acids are lipids that penetrate hepatocytes and are esterified into triglycerides, but may also be oxidized and produce ATP and ketones, balancing cycle fatty acids between adipose tissue and liver. In steatosis mitochondrial function is affected by increasing the synthesis and absorption of fatty acids and decreasing oxidation, causing accumulation of lipids occurs. Much of the affected individuals are asymptomatic, but when there are big fat infiltration occurs hepatomegaly, pain in the right upper quadrant and liver failure. The architectural hepatic steatosis caused by the condition may be reversible as the alcohol consumption is reduced, unlike the liver manifestations advance so that develop hepatitis, cirrhosis and hepatic fibrosis (Maw, 2012).
ciscely tumor suppressors and activation of oncogenic pathways. From manifestations seen by researchers the metabolism of ethanol and mechanisms facilitate the development of hyperplasia, leading to worsening disease dysplasia with genomic instability. The latter in turn compounded and added the lack of tumor suppressor gene (P53) and the reactivation of telomerase, formed hepatocellular carcinoma (BU-CHO, 2012).

**Figure 3 - Hepatocarcinogenesis. Source: Paraskevi et al., 2006.**

### 10. CIRRHOSIS

In the extracellular matrix (ECM) of the liver there are different characteristics, to take into account the different spaces of the liver - lobed, vein space centrilobular and portal spaces - can be found proteoglycans, glycosaminoglycans, laminin, fibronectin, and entactin ondulina. In each room there are different collagen concentrations ranging from type I to VI (SON, 2011).

Each matrix component participates in the production of components, but necrosis situations, especially focal, there is an accumulation of hepatocytes - that have little of matrix components production activity - along with necrotic cells, but the array is still intact. Still, hepatocytes are the reticulum or reticular net for alignment, and when there is no such alignment reticular stroma is reduced, indicating very extensive necrosis and even with regenerated hepatocytes no fibers of the stroma to their alignment, so the accumulation hepatocyte nodules that develop form wrapped with the matrix deposition of collagen fibrous septa. Thus, there is a hepatic fibrosis with conjunctival neoformation, nodular regeneration of hepatocytes and modification of liver architecture so denonminating liver cirrhosis (SON, 2011).

Fibrosis can result from scarring processes, traumatic or inflammatory lesions and systematic injuries in the latter, the production of MEC should be increased to compensation in the injured and bodies that do not participate in the production of MEC (SON, 2011).

![Fibrotic stage Score Metavir Class](image)

<table>
<thead>
<tr>
<th>Fibrotic stage</th>
<th>Score Metavir</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fibrosis</td>
<td>F0</td>
<td></td>
</tr>
<tr>
<td>Portal and periportal fibrosis without septa</td>
<td>F1</td>
<td></td>
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<tr>
<td>Portal and periportal fibrosis with rare septa</td>
<td>F2</td>
<td></td>
</tr>
<tr>
<td>Portal and periportal fibrosis with many septa</td>
<td>F3</td>
<td></td>
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<tr>
<td>Cirrhosis</td>
<td>F4</td>
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**Figure 4 - Fibrosis scoring systems in the liver. Adapted from Asselah 2014.**

The most advanced degree of fibrosis is cirrhosis, damage to and destruction of hepatic parenchyma, mainly viral hepatitis B and C as the stages of fibrosis are classified according to the Metavir score, which evaluates fibrosis and necroinflammatory activity, according to the basic pathological features biopsy (Friedrich-Rust, 2010).

The fibrotic activity is evaluated in five stages:
- F0: no fibrosis;
- F1: portal and periportal fibrosis without septa;
- F2: portal and periportal fibrosis with rare septa;
- F3: portal and periportal fibrosis with many septa;
- F4: cirrhosis.

The necroinflammatory activity estimates the degree of activity of portals injury and hepatocellular necrosis in four stages:
- A0: no activity;
- A1: light;
- A2: moderate;
- A3: severe.

### 11. SERUM MARKERS

#### 11.1 THE IMPORTANCE OF ENZYMATIC TESTS

The chronicity of various liver diseases is the result of accumulation of ECM components from collagens, elastin, proteoglycans, and other components, culminating in hepatic fibrosis. In a chronic process, there are approximately six times the amount of collagens, fibronecinct, elastin, proteoglycans and...
hyaluronic acid on hepatic stellate cells in fibrotic process (Barreto, 2011).

The detection of the presence and severity of disease and possibly liver fibrosis is extremely important in order to have proper treatment along with strategies for patient response, prognostic, monitoring, and risks involving patients develop complications. Surveillance strategies should be taken, since it is not possible to tailor treatment effective drugs and conducting frequent biopsies are non-specific for precise monitoring (FRIEDRICH-RUST, 2010; BARRETO, 2011).

Thus, the assessment of fibrosis through a noninvasive marker must necessarily be through an accurate predictor for the presence or significant lack of fibrosis, being sensitive and specific, low cost, rapid assessment and reproducibility tend to cancel errors laboratory observers, then this marker has its broad applicability to cases of fibrosis arising from various diseases and treatment with sensitive (Morais, 2007).

Noninvasive methods may be indirect, reflecting liver function and its derivations based on mathematical algorithms, which reflect or direct the extracellular matrix or fibrogenic changes at the cellular level. The latter in turn involves different molecules and cytokines in liver fibrogenesis (Sharma, 2014; CHIN et al., 2016).

Biomarkers need to be readily available markers in clinical practice in a safe, inexpensive and reproducible. They also need to apply to the progression or regression of the disease, to adapt to the natural history of the disease and its treatment regimen. Present reliable predictive positive results and not be susceptible to false positive or negative results are also required. (Baranova et al., 2011).

The difficulties and errors in treatment biopsies were instrumental in the development of non-invasive methods, reproducible and high accuracy in the evaluation of fibrosis. Noteworthy is the index: aspartate aminotransferase (AST) + alanine aminotransferase (ALT) + gamma-glutamyl transferase (GGT) and platelet count. Hyaluronic acid (HA), although not show common clinical routine, but present significant (PIIIINP) (FONTANA, 2010).

11.2 DIRECT MARKERS

Direct markers reflect the molecular pathogenesis and effects on the liver extracellular membrane. The liver injury leads to production of cytokines and activation of hepatic stellate cells causing inflammation and scarring at the potential of liver tissue matrix architecture. Direct markers may be divided into subcategories: Enzymatic markers of collagen, glycosaminoglycans, glycoproteins and metallloproteins matrix (CHIN et al., 2016).

According to Chin et al., hyaluronic acid in this class, it has been more widely used both for clinical practice, and to develop new markers.

Aspartate aminotransferase (AST) or glutamic-oxaloacetic transaminase (GOT)

Enzyme responsible for catalyzing the reversible transfer of amino groups of an amino acid to α-ketoglutarate to form keto acid and glutamic acid. In these reactions oxaloacetate is reduced by NADH in reaction catalyzed by malate dehydrogenase. The reactions catalyzed aminotransferase plays a central role in the synthesis and degradation of amino acids such as bridges and the metabolism of amino acids and carbohydrates (Motta, 2003).

Aspartate + α-ketoglutarate ↔ oxaloacetate + glutamic acid

The highest level of AST activity is beyond the liver, cardiac and skeletal muscle; addition of small amounts kidney, pancreas, spleen, brain, lung and erythrocytes (Motta, 2003).

Alanine aminotransferase (ALT) or glutamic-pyruvic transaminase (GPT)

Aminotransferase responsible for catalyzing the interconversion analogous AST amino acids.

L-Alanine + ↔ α-ketoglutarate, pyruvate + L-glutamate

It is primarily found in liver and kidney, their forms are most cytoplasmic. It is effective marker for the detection of liver damage in its activity is detectable lesions and elevated longer in relation AST. In general, their concentration is greater than AST, liver disease but in both concentrations are high (Tietz, 2008).

Concentrations changed the aminotransferases (ALT and AST) in liver diseases has its value varied reference up to five times its baseline level, with the ratio AST / ALT > 1.0, so can this ratio reflected in the fibrosis degree and reducing production by the liver ALT (Tietz, 2008).

In diagnostic determinations aminotransferase match the sensitivity and specificity of 48-68% and 81 to 83% respectively (MORLING, 2016).
**Gamma-glutamyl transferase (GGT)**

Enzyme responsible for regulating the transport of amino acids through cell membranes, catalyzing the transfer of glutamyl glutathione to free amino acid. It is present in the proximal tubule kidney, liver, pancreas and intestine. Cytoplasmic enzyme, but largely located at the cell membrane which functions as a carrier for amino acids and peptides. It is a sensitive indicator of hepatobiliary disease, but little specific. In liver or biliary diseases, their activity can be five to fifteen times the upper limit (MCPHERSON et al, 2012; Tietz, 2008).

**Platelets**

Thin discs, which act in homeostasis, the maintenance of vascular integrity and blood clotting process. The in vitro platelet volume increases up to an hour, until their third hour of collection and stabilizes after this tends to increase. Usually your score is performed by automated equipment detected an anomaly in the volume you need a quick and careful evaluation of blood smears in order to specify the reason for an abnormal count. Platelet aggregation may suffer agglutinin incipient spontaneous coagulation and aggregation (MCPHERSON et al., 2012).

**Albumin**

Main protein produced by the liver and the most abundant protein in plasma isolated because of this is the main balance of substance of intravascular oncotic pressure and intravascular colloid osmotically active. It is also a transport protein of exogenous and endogenous substances. It has a half-life of 17 days. Low levels of serum albumin in liver diseases are caused by massive destruction of hepatic tissue, which is important prognosis of patients with cirrhosis, the low concentration, as well as the drop in total protein. When there is, hypoalbuminemia may occur edema, or ascites, which is a common finding in cirrhosis by high hydrostatic pressure in the portal system, due to increased resistance to blood flow, combined with reduced osmotic pressure of colloids, since there is less concentration of albumin (MCPHERSON et al., 2012).

**Alpha-fetoprotein (AFP)**

With synthesis in embryonic and fetal yolk sac hepatocytes, the α-fetoprotein has specific functions, but may be immunosuppressive and assist in the prevention of maternal antibodies to be one of the first globulins embryonic detection and due to that is dominant in the embryonic stage. It is also important marker of hepatocellular carcinoma. High levels of AFP mean liver tumor or disease is already disseminated form, but its detection varies according to the age of the patient, the extent and degree of differentiation of the disease (MCPHERSON et al, 2012; Tietz, 2008).

**Alkaline phosphatase**

Responsible for catalyzing the alkaline hydrolysis of natural and synthetic substrates. It is present throughout the body and especially associated with membranes and cell surfaces, especially to the small intestine, proximal tubule kidney, bone, liver, and placenta. Its main function is not well defined, but is associated with the transport of intestinal lipids and bone calcification. Its isoenzymes originating in the liver and bones have major clinical importance as they are most commonly measured in serum dosage (Tietz, 2008).

In hepatobiliary diseases synthesis by hepatocytes is induced and directed circulation increasing enzyme activity in serum, which may reach 10 to 12 times the upper limit (Tietz, 2008). Alkaline Phosphatase assess hepatobiliary function, but for this, the dosage is needed in conjunction with the GGT (Jesus, 2014).

**Bilirubin**

Product catabolism of red blood cells by which gives rise to indirect bilirubin carried from the albumin to the liver is transformed into direct bilirubin; its high plasma levels denote damage in metabolizing or hepatic excretion (Jesus, 2014).

**α2-macroglobulin**

Important plasma proteinase inhibitors, such as serine, cysteine, and metal ions, is a large molecule and is not present in significant quantities in the extracellular fluids. It is synthesized by hepatic parenchymal cells. There is an increased synthesis when renal loss of protein is high as compensating system, together with haptoglobin is the zone α2-globulin neonatal post-serum electrophoresis, which is the present (Tietz, 2008).

**Apolipoprotein A1**

Protein component of lipoproteins, and apolipoprotein A in HDL main, it modulates the activity of enzymes that act on lipoproteins, maintains the structural integrity of the lipoprotein complex and facilitates the uptake of the lipoprotein binding to specific cell surface receptors. The apolipoproteins have easily interact with lipids in an aqueous environment (Tietz, 2008).
Haptoglobin
It is an α2-glycoprotein which is connected, in situations of hemolysis irreversibly free hemoglobin (Hb) having a configuration similar to the hemoglobin peptide, that connection avoiding the loss of iron and Hb on renal function, the formed complex is then synthesized by the liver in order to be separated into iron and amino acids. Haptoglobin is important natural bacteriostatic agent and agent hemolysis important indicator. The altered metabolism of estrogen and lysis of red blood cells in hepatitis or jaundice is the main source of decreased concentrations of haptoglobin.

Hyaluronic Acid (HA)
Mucopolysaccharide major component of the extracellular matrix is found in most tissues and body fluids and abundant in loose connective tissue. It is synthesized in the cytoplasmic membrane of fibroblasts and other cells like liver stellate cells with central role in the extracellular matrix. Much reaches the bloodstream via lymphatics and is cleared by the liver via binding to the CD44 adhesion molecule sinusoidal endothelial cells subsequently brought to hepatocytes. In fibrosis sinusoidal endothelial cell becomes less permeable leading to failure in eliminating HA, raising serum levels. The AGO has a negative predictive value of 98 to 100% in cirrhosis (ROCHA, 2007; BARRETO, 2011).

Tissue inhibitor of metalloproteinase-1 (TIMP-1)
The metalloproteinases are zinc-dependent endopeptidases capable of degrading components of the extracellular matrix, these are activated and TIMP-1 is a tissue inhibitor, which has an effect on activation ofzymogens of metalloproteinases, as these have remodeling function of tissues and wound healing, besides being associated with tumor growth, invasion and metastasis. The inhibition is an important therapeutic strategy in tumor conditions (Tietz, 2008).

YKL-40
Glicohidrolases member of the family, growth factor for connective tissue cells and endothelial cells. It is also migration factor for endothelial cells. It is prevalent in areas with fibrogenesis. It is secreted by the liver stellate cells, which are the primary effector cells of hepatic fibrogenesis, by macrophages and neutrophils (Marquez, 2005).

N-terminal peptide of collagen type 3 (PIIINP)
Amino propeptide procollagen type III terminal is a component of connective tissue, while at higher concentrations in the basal membrane during hepatic fibrogenesis, after increasing serum. It has a sensitivity and specificity medians, but is related to aminotransferases to help determine the degree of fibrosis (CEQUERA, 2013).

11.3 INDIRECT MARKERS

ELF® (Enhanced Liver Fibrosis/ optimized assay of hepatic fibrosis)
Index multivariable obtained by combining a hyaluronic acid algorithm, pro-peptide amino-terminal pro-collagen III and tissue inhibitor of metalloproteinase-1. Useful score in hepatic fibrosis in chronic liver disease, fibrosis monitoring changes over time, with optimal therapy aid and determining prognosis (SIEMENS, 2011).

The ELF test demonstrates studies, a sensitivity rate of 90% and specificity of 69%. Being accurate in hepatitis B and C (Sharma, 2014).

FIBROTEST®
Correlation six laboratory tests that determines the degree of hepatic impairment, along with Actitest® is related to fibrotic activity or detection and necroinflammatory activity. The dosage of α2-macroglobulin is required, haptoglobin, apolipoprotein A1; gamma-glutamyl transpeptidase; total bilirubin and ALT (BIOPREDECTIVE, 2016).

With this data, through a licensed software, it is generated correlative score with METAVIR fibrosis stages, with values from 0.0 to 1.0. The sensitivity reaches 80% and the specificity 85%. The Fibrotest® has a high positive predictive value, reaching 90%, it is estimated that there is reduced need for biopsy in approximately 46% (CEQUERA, 2013; Sharma, 2014; Motola, 2014)

Testing in patients with HCV, HBV and alcoholic disease had a five-year survival prognosis (POYNARD, et al., 2011).

FIBROMAX®
Correlation of 10 α2-macroglobulin laboratory tests, haptoglobin, apolipoprotein A1, gamma-glutamyl transpeptidase (GGT) Total bilirubin, ALT, AST, Total cholesterol, triglycerides and fasting glucose levels. It is also related to age, sex, height and weight. (BIOPREDECTIVE, 2016).

The Fibromax is a combination of tests allowing evaluating the degree of fibrosis arising from chronic hepatitis B and C, alcoholic liver disease, metabolic or alcoholic steatosis. Including cases of coinfection
with HIV, hepatic diseases from diabetes, overweight, hypertriglyceridemia, hypercholesterolemia and hypertension. (BIOPREDECTIVE, 2016)

**APRI® (AST to platelet ratio index / AST ratio platelet Index)**

Aspartate aminotransferase level of relationship and platelets, easy to apply formula with simple and usual laboratory indices, high accuracy as a predictor of fibrosis, mostly in cases of chronic hepatitis C, and therapy monitoring. The APRI® test has shown great sensitivity, low cost and diagnostic facility, as it helps significantly in fibrosis and cirrhosis prognosis as well as the therapeutic monitoring and the possibility of regression (AMARAL et al., 2009).

Developed by Wai et al., the results of this method are determined by cut-offs, lower and upper, of which it is possible to define the presence of cirrhosis and fibrosis in significant degree, especially in cases of HCV and co-infection of HIV. They are used values less than 0.5 and greater than 1.5. The sensitivity can reach 80% and specificity reaches approximately 94% depending on the aetiology (Gonçales, 2008; CEQUERA, 2013; Sharma, 2014).

\[ \text{APRI} = \frac{\text{AST} \times 100}{\text{PLATES}} \]

**HepaScore®**

Correlative index age, bilirubin, gamma - glutamyl transferase, Hyaluronic acid and α2-macroglobulin. It shows high specificity for the detection of fibrosis and cirrhosis. It has cut-off 1.00, a single automated analyzer can be sufficient and necessary to test run (Baranova, 2011; CEQUERA, 2013).

**FIB4®**

Panel using age, AST, ALT and platelet count. This marker has been validated for the diagnosis of cirrhosis in patients coinfected with HIV and HCV. It has a higher sensitivity 70% and specificity depend on the cause of 85% or higher. Regarding values, this index can be compared to Fibrotest® (Sharma, 2014).

Easily calculated index has a cutoff value of 1.45, with sensitivity 70%, specificity 97%, positive predictive value of 65%. It is consistent with the Fibrotest® in 92% of cases, but unlike the latter, does not suffer interference by hemolysis, bilirubin elevation or biliary obstruction. Approximately 70% of fibrosis frames are classified in their corresponding stages (Motola, 2014).

\[ \text{FIB4} = \frac{(\text{AGE} \times \text{AST})}{(\text{platelet} \sqrt{\text{ALT}})} \]

12. MATERIAL AND METHODS

It conducted literature of journal articles and theses and dissertations national, indexed in the following electronic databases: PubMed, Scopus, Web of Science, SciELO, Lilacs and Capes Thesis Portal, from Jan / 1998 to Jul / 2015. Combinations were used key words and descriptors such as liver fibrosis, hepatic markers, hepatitis, liver disease, liver.

Was used as an inclusion criterion articles the development of fibrosis in some pathology, markers reliable that contribute to the understanding of pathological dynamic and articles with supporting and statistical studies on liver fibrosis and its diagnostic analysis. Articles were excluded veterinary studies and diagnostic studies for purposes in favor of the usual positive demonstration of biopsy as the primary factor.

13. PERSPECTIVES

The main cause of death from liver diseases is by fibrosis and thus cirrhosis, and for its current diagnostic treatment gold standard is made by biopsy. However, this does not present a dynamic operation, which is observed gravity and fibrotic progression. Thus from serological tests of clinical practice correlates indexes to study and dynamic monitoring and diagnosis of fibrosis.

From the survey data, we observed the growing interest in recent years, clinical and reference studies on various indices, but these are not shown in broadly so that its use is properly known diagnostic and practice range, enabling clinical monitoring and possible staging of the pathological picture.

It can be seen in Table 1 and compared, using noninvasive biomarker for diagnosis of fibrosis, avoiding the need for invasive techniques, as well as the aid of imagenology relating the progression or regression of fibrosis and biomarkers techniques.

Much of the index is patented and marketed to large laboratories, but they are made from markers used in clinical practice, favoring the emergence of more viable rates and low cost and can thus facilitate the use and knowledge of the target audience.

The Fibrotest® index is one of the largest clinical study and more time marketing, having advanced specificity of demonstration tests for fibrosis; ELF® index shows specific and sensitive markers for fibrosis, making diagnosis and proving phases crucial to preventing the fibrotic progression.

It is necessary to demonstrate in clinical practice the potential of indirect and direct serum markers for diagnosis and monitoring of fibrosis to be offered higher quality diagnostic service and remedying
analytical errors in biopsies, thus improving the treatment time and the life expectancy of the patient.

The direct and indirect serological markers have yet to be extensively studied and documented; already it makes available much of these specific form and practice, thus demonstrating that the fibrotic treatment can be improved.

One of the major challenges to clinical trials is made by the statistical validation through a large number of samples and controls, since there is a range of physiological significance of biomarkers large scale and comparing their predictive values of noninvasive biomarker panels.

<table>
<thead>
<tr>
<th>BIOPSY</th>
<th>INDIRECT MARKERS</th>
<th>DIRECT MARKERS</th>
<th>IMAGE EXAMINATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Useful in defining the fibrotic phase</td>
<td>Spectrum broad, but not physiological</td>
<td>advanced fibrosis</td>
<td>advanced fibrosis</td>
</tr>
<tr>
<td>Prediction of clinical outcomes</td>
<td>hepatocellular carcinoma</td>
<td>hepatocellular carcinoma</td>
<td>hepatocellular carcinoma</td>
</tr>
<tr>
<td>Access and evaluation of the usefulness</td>
<td>It is not practical due to the invasive nature.</td>
<td>Easily accessible.</td>
<td>Easily accessible.</td>
</tr>
<tr>
<td>Costs US$ (approximate)</td>
<td>4,800.00 per procedure.</td>
<td>4.00 to 33.00 per measured analyte.</td>
<td>227.00 to 648.00 per measured analyte.</td>
</tr>
<tr>
<td>Reliability</td>
<td>Sampling error intra- server</td>
<td>Variable for instructions and equipment</td>
<td>Operator variation, body and equipment conditions.</td>
</tr>
<tr>
<td>Execution</td>
<td>Hospital network</td>
<td>Hospital network</td>
<td>Hospital network</td>
</tr>
</tbody>
</table>

Table 1: Comparison of tests and in liver fibrosis markers. Adapted from Morling, 2016.

Direct markers have limitations that need not analyzed into account the natural history of disease and every patient’s medical history. For example, biliary or renal dysfunction and endothelial cells can influence the rate of release of these serum markers. Just as some concomitant inflammation sites interfere with the deposition of the matrix, then revealing disparate absolute values trending to false positives and false negatives for fibrogenic or fibrinolytic activity.

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