METHODS FOR DIAGNOSING ADVANCED LIVER FIBROSIS OR LIVER CIRRHOSIS

The present invention relates to a method for the diagnosis of advanced liver fibrosis or liver cirrhosis, for prognosing or monitoring the progression of liver fibrosis in a subject or for assessing the efficacy of an anti-fibrotic agent. The invention also relates to the compounds for use in the treatment of liver fibrosis, wherein the subject to be treated is identified according to the method of the invention.

BACKGROUND

Liver fibrosis, common to liver damage and liver diseases, may have many, chronic or not, etiologies including viral Hepatitis B and C (HBV and HCV), Human Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV) co-infection, Drug-Induced Liver Injury (DILI), cholestatic liver diseases including Primary Biliary Cholangitis (PBC) and Primary Sclerosing Cholangitis (PSC), AIH (AutoImmune Hepatitis), biliary atresia, acute liver disease (ALD), Acute Liver Failure (ALF), cirrhosis, Acute on Chronic Liver Failure (ACLF), Wilson disease, Metabolic dysfunction-associated fatty liver disease (MAFLD), Metabolic dysfunction-associated steatohepatitis (MASH), Alcohol Related Liver Disease (ARLD), alcoholic liver disease and hemochromatosis.

Liver problems can be caused by a variety of factors that damage the liver, such as viruses, immune system abnormality, inherited abnormal genes, cancer, alcohol use and obesity. Over time, conditions that damage the liver can lead to scarring (cirrhosis), which can lead to liver failure, a life-threatening condition that demands urgent medical care. Liver failure occurs when large parts of the liver become damaged beyond repair.

Liver fibrosis is an abnormal wound repair process and is characterized by an excessive accumulation of extracellular matrix protein. It is stimulated by chronic inflammation and occurs as a result of the liver healing process when the liver becomes scarred.

The prediction of liver fibrosis is a key step in the assessment and management of patients with liver damage and/or liver disease. Therefore, since the early and precise evaluation of severity and status of liver fibrosis is essential for diagnosis, monitoring and prognosis, a quantitative measurement is crucial to assess disease progression.

Most forms of liver diseases are without symptoms until the disease has progressed to a later stage. The early detection of the disease is therefore challenging. The risk of liver-related mortality increases exponentially with increase in fibrosis stage and mortality and morbidity rates increase exponentially once cirrhosis develops. Cirrhosis is the point where the liver is completely scarred and is beyond the self-healing ability.

Cirrhosis is a leading cause of mortality and morbidity across the world. It is the 11th leading cause of death and 15th leading cause of morbidity, accounting for 2.2% of deaths and 1.5% of disability-adjusted life years worldwide in 2016. Chronic liver diseases caused 1.32 million deaths in 2017 in the world (Sepanlou, *et al.* The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2020;5:245-266.). Cirrhosis is said compensated when patients do not have any visible symptoms of the disease, and cirrhosis is decompensated when cirrhosis has progressed to the point that the liver is having trouble functioning and with the occurrence of symptoms of the disease. Although the clinical features of cirrhosis decompensation are well described (i.e., ascites, spider naevi, jaundice, signs of hepatic encephalopathy), patients who have compensated cirrhosis often have no clinical signs and might be entirely asymptomatic.

Hepatologists and healthcare providers have proposed scoring systems for staging liver fibrosis like the BRUNT/KLEINER system, wherein:

F0 refers to a subject with an absence of liver fibrosis;

F1 refers to a subject with portal or perisinusoidal fibrosis;

F2 refers to a subject with portal/periportal and perisinusoidal fibrosis;

F3 refers to a subject with septal or bridging liver fibrosis;

F4 refers to a subject with liver cirrhosis.

Since severe (fibrosis stage F>2) liver diseases can progress to hepatocellular carcinoma, the accurate staging of liver fibrosis in these liver diseases, especially the early diagnosis of advanced liver fibrosis (Fibrosis stage F3 or F4) and liver cirrhosis (Fibrosis stage F4) is crucial.

Furthermore, the rate of fibrosis progression evolves over time and the diagnostic assay has to be performed several times. In consequence, this test must be repeatable and without risk for the patients, reliable and accurate. Therefore, non-invasive assays are needed for the diagnosis of advanced liver fibrosis (F3 or F4) and liver cirrhosis (F4). It is also important to specifically distinguish patients with cirrhosis (F= 4) among patients with severe liver disease, because these F4 patients require an emergency treatment.

So far, invasive liver biopsy remains the Gold Standard for the assessment of liver fibrosis.

Nevertheless, liver biopsy has several recognized limitations including sampling errors, inter-observer variability, and hospitalization. The main disadvantage is the significant risk of complications including bleeding, pain and even death. Furthermore, a biopsy does not reflect the changes in the whole liver and does not differentiate early cirrhosis from progressed cirrhosis and therefore does not constitute a reliable prognostic predictor (Sumida Y *et al.* Limitations of liver biopsy and non-invasive diagnostic

tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* 2014; 20: 475-485).

To avoid above detailed life-threatening risks and diagnostic weakness, *in vitro* non-invasive diagnostic methods using biomarkers, scores, and physical methods have been developed. By contrast to biopsy which cannot be repeated without inconvenience, these methods can capture the dynamic process of fibrosis resulting from progression and regression since the measures are repeatable. These methods are based on readily available biochemical data and clinical features, such as the FIB4 test (Sterling RK *et al.* Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; 43:1317-1325), or assays that directly measure markers of fibrogenesis and fibrolysis, such as the Enhanced Liver Fibrosis (ELF) test (Guha, I.N., *et al.,* Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European Liver Fibrosis Panel and exploring simple markers. *Hepatology* 2008; 47: 455-460).

However, the substitution of these methods for liver biopsy still remains controversial and is not generally accepted due to insufficient diagnostic performance. The main issue of these two biochemical assays is that they cannot reliably differentiate in individual patients the advanced stages of liver fibrosis. Moreover, ELF test is expensive, which constitutes a drawback in case of repetition of the tests. Additionally, FIB4 has a poor performance in patients of less than 35 years old and is less specific in patients over 65.

Physical methods include imaging based on high frequency sound waves (ultrasound and echocardiography), computed tomography (CT), magnetic resonance imaging (MRI), transient elastography (TE, FibroScan) as well as scintigraphy. The main drawbacks of physical measures are the high cost, the low availability of equipment and the complexity of the method, that limit the daily clinical practice.

Moreover, obesity, ascites, acute inflammation, liver congestion, and elevated portal vein pressure may for example reduce ultrasound TE (Fibroscan) accuracy by influencing the velocity of shear wave. Furthermore, a falsely increased liver stiffness, due to postprandial increase in portal vein pressure, has been observed with this method.

Therefore, there is still an unmet medical need to develop new non-invasive diagnostic methods having an optimal diagnostic accuracy compared to biopsy, but also useful in monitoring the time course of liver fibrosis and/or showing whether there is a response to a given medication. In addition, it is important to provide a new method giving the best predictivity at the lowest cost and the easiest feasibility.

SUMMARY OF THE INVENTION

The Inventors have conducted several very fine and complete analysis of a cohort of 3875 patients with different stage of fibrosis to provide novel and highly sensitive non-invasive diagnostic and monitoring methods of advanced liver fibrosis (F3 or F4) and liver cirrhosis (F4). WO 2024/013229 described a non-invasive diagnostic method of these diseases by measuring the circulating levels of two biomarkers, i.e. Soluble Vascular Cell Adhesion Molecule-1 (sVCAM) and Thrombospondin 2 (TSP-2). Said method demonstrates an accurate diagnosis of advanced liver fibrosis and liver cirrhosis compared to liver biopsy and existing solutions. However, it was recently observed by the inventors that the diagnostic performance of this method may not be homogeneous among subpopulations of different ages. Working on this problematic, the inventors have successfully developed a new mathematical function which allows to correct the impact of age on diagnostic performance by combining, in a mathematic function, the circulating levels of these biological markers measured in a biological fluid sample isolated from a subject with the age of said subject. This mathematical function provides an improved diagnosis of advanced liver fibrosis (F3 or F4) and liver cirrhosis (F4) compared to existing solutions . Most importantly, compared to the method only combining the levels of sVCAM and TSP-2, the method of the invention provides consistent high clinical performances independently of the age of the patients, which allows a reliable follow up of the liver fibrosis.

Accordingly, a first aspect of the invention relates to an *in vitro* method for diagnosing advanced liver fibrosis (F3 or F4) or liver cirrhosis (F4) in a subject, comprising:

a) providing (i) the circulating levels of Soluble Vascular Cell Adhesion Molecule-1 (sVCAM) and Thrombospondin 2 (TSP-2) in a biological fluid sample isolated from said subject, and (ii) the age of the subject;
b) combining the provided levels of sVCAM and TSP-2 and the age in a mathematical function to assign a score; and
c) comparing said score with cut-off values to diagnose or prognose advanced liver fibrosis or liver cirrhosis in said subject.

In a particular embodiment, the score is a score SA obtained through the following mathematical function:

$$\mathrm{SA} = \frac{1}{1 + \exp\left(-y\right)}$$

with

$y = \beta0 + \beta1 * \log_{10}(TSP2\ (ng/mL)) + \beta2 * \log_{10}(sVCAM\ (ng/mL)) + \beta3 * (Age\ (years)^3)$

wherein:

β0 is comprised between -40 and -14, in particular between -38 and -17;

β1 is comprised between 1.7 and 6.7, in particular between 2.2 and 6.3;

β2 is comprised between 3.0 and 12, in particular between 3.5 and 11.5;

β3 is comprised between -6 e^{-06} and 2.5 e^{-6}, in particular between -5 e^{-6} and 2 e^{-6}.

In a more particular embodiment, a score SA higher than a cut-off value co1 is indicative of advanced liver fibrosis, particularly co1 being comprised between 0.2 and 0.7, more particularly co1 being comprised between 0.25 and 0.63.

In another particular embodiment, a score SA higher than a cut-off value co2 is indicative of a liver cirrhosis, particularly co2 being comprised between 0.4 and 0.9, more particularly co2 being comprised between 0.5 and 0.85.

Another aspect of the invention relates to an *in vitro* method for monitoring the progression of liver fibrosis in a subject, comprising the steps of:

a) providing (i) the circulating levels of sVCAM and TSP-2 in a biological fluid sample isolated from said subject, and (ii) the age of the subject;

b) combining the provided levels of sVCAM and TSP-2 and the age in a mathematical function to assign a score SC; and

c) comparing the score SC with a score SB, which is obtained by combining levels of sVCAM, TSP-2 and the age previously measured in the same subject and with the same mathematical function.

In a particular embodiment, an increase of SC compared to SB indicates the progression of liver fibrosis. In another particular embodiment, a decrease of SC compared to SB indicates the regression of liver fibrosis. In a specific embodiment, no significant change between SC and SB measured in a certain lapse of time in a same subject means that liver fibrosis is stable.

In a particular embodiment of said method, step (a) of said method for monitoring the progression of liver fibrosis is implemented at least 3 months after a previous measurement of the levels of sVCAM

and TSP-, particularly in a period between 3 months and 10 years, more particularly in a period between 3 months and 2 years, after a previous measurement of the levels of sVCAM and TSP-2.

In a specific embodiment of said method, one event linked to the evolution of pathological state occurs during the monitoring, between SB and SC. In a particular embodiment, said event is selected from liver transplantation, acute on chronic liver fibrosis, compensated cirrhosis, decompensated cirrhosis, episode of ascites and presence of esophageal varices at endoscopy.

In a preferred embodiment, said SB and SC are calculated through the above defined mathematical function.

A third aspect of the invention concerns a method for assessing the efficacy of an anti-fibrotic agent in treating advanced liver fibrosis or liver cirrhosis, comprising:

a) providing (i) the circulating levels of sVCAM and TSP-2 in a biological fluid sample isolated from a subject suffering from advanced liver fibrosis, wherein said subject has been administered an anti-fibrotic agent before the isolation of the biological fluid sample, and (ii) the age of the subject;

b) combining the provided levels of sVCAM and TSP-2 and the age in a mathematical function to assign a score SE; and

c) comparing the score SE with a score SD, which is obtained by combining the levels of sVCAM and TSP-2 and the age, previously measured before administration of the anti-fibrotic agent to the same subject and with the same mathematical function.

Particularly, a decrease of SE compared to SD indicates the efficacy of the anti-fibrotic agent; an increase of SE compared to SD indicates the non-efficacy of the anti-fibrotic agent, and/or the non-responsiveness of the patient.

In a preferred embodiment, said SE and SD are calculated through the above defined mathematical function.

In a preferred embodiment, the biological fluid sample of the subject used in the methods of the present invention is an interstitial fluid, saliva, urine or whole blood sample. In a particular embodiment, the biological fluid sample of the subject used in the methods of the present invention

is a blood sample, a plasma sample or a serum sample. Preferably, the biological fluid sample is cell-free. More preferably, the blood sample is a serum sample from a subject.

Another aspect of the invention concerns a method for prognosing the evolution of liver fibrosis in a subject with biopsy-proven stage 1, 2 or 3 liver fibrosis, comprising

a) providing (i) the circulating levels of sVCAM and TSP-2 in a biological fluid sample of said subject, and (ii) the age of the subject;
b) combining the provided levels of sVCAM and TSP-2 and the age in a mathematical function to assign a score SF; and
c) comparing said score with a cut-off value to prognose the evolution of liver fibrosis in said subject.

In a particular embodiment, the SF score higher than a cut-off value is indicative of that the subject's liver fibrosis will progress (i.e. the subject will be a liver fibrosis worsener); the SF score lower than the cut-off value is indicative of that the subject's liver fibrosis will regress (i.e. the subject will be a liver fibrosis improver); the SF score neither higher nor lower than the cut-off value is indicative of that the subject's liver fibrosis will be stable.

Another aspect of the invention is also to provide anti-fibrotic agents for use in the treatment of advanced liver fibrosis or liver cirrhosis in a subject, wherein said subject is diagnosed as suffering from advanced liver fibrosis or liver cirrhosis according to the method of the present invention, wherein said agent is selected in the group consisting of pegbelfermin, cenicriviroc, dapagliflozin, dulaglutide, empagliflozin, fenofibrate, lanifibranor, liraglutide, obeticholic acid, pioglitazone, resmetirom, saroglitazar magnesium, seladelpar, semaglutide, sitagliptin, TERN-101, TERN-201, tropifexor, ambrisentan, BMS-963272, BMS-986251, BMS-986263, HepaStem, LYS006, MET409, MET642 and orlistat.

In a particular embodiment, said agent is resmetirom.

FIGURES

Figure 1A represents Mean scores of modelization ST for patients younger than 50 (0) and older than 60 years old (1) in a homogenized subpopulation (n=339 patients per category). ****: $p < 0.0001$.

Figure 1B represents Mean scores of modelization ST II for patients younger than 50 (0) and older than 60 years old (1) in a homogenized subpopulation (n=339 patients per category). “ns”: no significant difference between the scores obtained from the two groups of patients.

Figure 2A represents clinical performances of modelization ST for the detection of F3. “Spe”: specificity; “Sen”: sensitivity.
Figure 2B represents clinical performances of modelization ST II for the detection of F3. “Spe”: specificity; “Sen”: sensitivity.

Figure 3 represents Roc curves for detection of advanced liver fibrosis of the modelization ST II and other usual non-invasive tests (FIB-4, ELF, NFS and APRI) in a validation cohort of 1850 subjects.

Figure 4 represents Roc curves for detection of liver cirrhosis of the modelization ST II and other usual non-invasive tests (FIB-4, ELF, NFS and APRI) in a validation cohort of 1850 subjects.

Figure 5 represents boxplot of the SF score calculated at screening visit (V_0) for the categories “improver”, “stable”, “worsener” of patients with stage 1, 2 or 3 biopsy-proven liver fibrosis at V_0. Improver: patients who had a lower fibrosis stage at visit 7 (81 weeks later) compared to V_0; Stable: patients who had the same fibrosis stage at visit 7 compared to V_0; Worsener: patients who had a higher fibrosis stage at visit 7 compared to V_0. The lines above the boxplots represent the p values (Student test) by pairs of boxplots values.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Fibrosis scoring/staging

According to the present invention, the term "fibrosis” or “liver fibrosis” denotes a pathological condition of excessive deposition of fibrous connective tissue in the liver. More specifically, fibrosis is a pathological process, which includes a persistent fibrotic scar formation and overproduction of extracellular matrix by the connective tissue, as a response to tissue damage. Physiologically, the deposit of connective tissue can obliterate the architecture and function of liver.

The different stages of liver fibrosis are defined by the Kleiner scoring system (Kleiner *et al,* Hepatology 2005, Vol 41, Issue 6, 1313-1321) wherein:

F0 refers to a subject with an absence of liver fibrosis;

F1 refers to a subject with portal or perisinusoidal fibrosis;

F2 refers to a subject with portal/periportal and perisinusoidal fibrosis;

F3 refers to a subject with septal or bridging liver fibrosis;

F4 refers to a subject with liver cirrhosis.

The stage of F0-2 is assigned to subjects having early liver fibrosis, F3 or F4 is assigned to subjects having advanced liver fibrosis, and the stage F4 is assigned to subjects having liver cirrhosis.

Using this fibrosis staging system, patients with no or minimal fibrosis (F=0 or 1) are generally not considered at risk of cirrhosis, liver failure, HCC (hepatocellular carcinoma) or liver-related death. Patients with severe (F>2) liver fibrosis are at risk of developing cirrhosis, liver failure, HCC and liver-related death. Patients with compensated cirrhosis (F=4) are at high risk of liver failure (decompensated cirrhosis), HCC and liver-related deaths.

In the context of the present invention, the term "advanced fibrosis" or “advanced liver fibrosis” refers to a fibrosis stage of F≥3, i.e. a fibrosis stage F3 or a fibrosis stage F4.

In the context of the present invention, the term “cirrhosis” or “liver cirrhosis” refers to a fibrosis stage of F4.

The terms "subject" and "patient" may be used interchangeably herein and refer to a human subject.

Within the context of the present invention, the terms “biomarker”, “marker”, “biological marker” are interchangeable.

Within the scope of the present invention, any range must be considered as including the upper and lower limits.

<u>Concurrent Noninvasive tests</u>

The Fibrosis-4 (FIB-4) index is calculated as age (years) x AST (U/L) /platelet (x 10^9/L) /VALT(U/L), where AST is aspartate aminotransferase and ALT is alanine aminotransferase.

The Enhanced Liver Fibrosis panel (ELF, Siemens Healthcare GmbH, Eriangen, Germany) is a test to predict fibrosis based on three fibrosis biomarkers: hyaluronic acid (HA), tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) and amino-terminal propeptide of procollagen type III (PIIINP). This test uses the following equation to calculate the ELF score (2.278 + 0.851 $\ln(_{CHA})$ + 0.751 $\ln(_{CPIIINP})$ + 0.394 $\ln(_{CTIMP\text{-}1})$), wherein C is the concentration of the biomarker.

NFS score is calculated as follows: NFS = − 1.675 + 0.037 x age (years) + 0.094 x BMI (kg/m^2) + 1.13 * IGF/diabetes (yes = 1, no = 0) + 0.99 x AST/ALT ratio – 0.013 x platelet (× 10^9/l) – 0.66 x albumin (g/dl)

APRI score is calculated as follows:

APRI=[(AST Level (IU/L)/upper limit of the normal AST range(IU/L)) X 100]/Platelet Count (× 10^9/L).

Diagnostic performance analysis

Sensitivity is the ability of a test to correctly identify those who have the disease. The sensitivity measures the proportion of positive ("disease") cases in a population of sick patients evaluated using a reference method ("gold standard"), or as reliable as possible considering biopsy profiling. Sensitivity (Se) is the proportion of positive results (True Positive = TP) divided by the total number of sick patients (TP + False Negative = FN): Se = TP / (TP + FN). Sensitivity is usually expressed as a percentage (%), from 0 to 100%.

True Positive (TP) subjects are subjects with the disease with the value of a parameter of interest above a cut-off.

False Negative (FN) subjects are subjects with the disease with the value of a parameter of interest below a cut-off.

Specificity is the ability of a test to correctly identify those who do not have the disease. The specificity measures the proportion of negative ("healthy") cases in a population of healthy patients evaluated using a reference method ("gold standard"). Specificity (Sp) is the proportion of negative results (True Negative = TN) divided by the total number of healthy patients (TN + False Positive = FP): Sp = TN / (TN + FP).

True Negative (TN) subjects are subjects without the disease with the value of a parameter of interest below a cut-off.

False Positive (FP) subjects are subjects without the disease with the value of a parameter of interest above a cut-off.

The prevalence of a given population is the number of cases of the disease within the population.

The Positive Predictive Value (PPV) is the probability to have the disease when a test is positive. PPV = number of True Positives / (number of True Positives + number of False Positives). PPV = TP/ (TP+FP). The negative Predictive Value (NPV) is the probability of not having the disease when a test is negative. NPV = number of True Negatives / (number of True Negatives + number of False Negatives). NPV = TN/ (TN+FN).

Prevalence affects PPV and NPV differently. PPV is increasing, while NPV decreases with the increase of the prevalence of the disease in a population. Whereas the change in PPV is more substantial, NPV is somewhat less influenced by disease prevalence. For low prevalence (5-10%), PPV value is low. In parallel, NPV is high. For high prevalence (80-90 %), PPV value is high and vice versa for the NPV.

Likelihood ratio is defined as the ratio of expected test result in subjects with a certain state/disease to the subjects without the disease.

Likelihood ratio for positive test results (LR+) represents the ratio of the probability that the positive test result is to occur in subjects with the disease compared to those without the disease. LR+ = sensitivity / (1-specificity). LR+ is the best indicator for ruling-in diagnosis. The higher the LR+, the more the test is indicative of a disease. LR+ > 5 indicates a moderate to large increase evidence that the disease is present. Good diagnostic tests have LR+ > 10 and their positive result has a significant contribution to the diagnosis.

Likelihood ratio for negative test result (LR-) represents the ratio of the probability that a negative result will occur in subjects with the disease to the probability that the same result will occur in subjects without the disease. LR- = (1-sensitivity) / specificity. LR- is a good indicator for ruling-out the diagnosis.

LR- between 0.1 and 0.2 indicates a moderate probability to well diagnose patients without the studied condition. Good diagnostic test of patients that are without the studied condition has LR- < 0,1.

There is a pair of diagnostic sensitivity and specificity values for every individual cut-off. To construct a receiver operating characteristic (ROC) graph, these pairs of values are plotted on the graph with the 1-specificity on the x-axis and sensitivity on the y-axis. The shape of a ROC curve and the area under the receiver operating characteristic curve (AUROC) shows how high is the discriminative power of a test. The closer the curve is located to upper-left hand corner and the larger the area under the curve, the better the test is at discriminating between diseased and non-diseased subjects. The area under the curve can have any value between 0.5 and 1 and it is a good indicator of the goodness of the test. A perfect diagnostic test has an AUROC of 1.0. whereas a non-discriminating test has an AUROC of 0.5. AUROC is a global measure of diagnostic accuracy.

The “mean score” is the average of the results of the test calculated in a group.

METHOD OF THE INVENTION

As mentioned above, advanced liver fibrosis and cirrhosis are associated with liver-related death and easy detection of advanced fibrotic subjects and cirrhotic subjects is thus of outmost importance. The present invention provides a solution to these unmet needs.

In the methods of the present invention, the levels of two circulating markers measured from a biological fluid sample isolated from a subject and the age of the subject are combined in a mathematical function to assign a score. Said circulating markers are: Soluble Vascular Cell Adhesion Molecule-1 (sVCAM) and Thrombospondin 2 (TSP-2). sVCAM is also known as VCAM 1, INCAM-100, CD106 and registered in database UniProt under the number P19320. TSP-2 is also known as THBS2 and registered in database UniProt under the number P35442.

According to the invention, the circulating levels of sVCAM and TSP-2 are measured in a biological fluid sample of a subject. In all the methods and embodiments presented herein, the biological fluid sample may be a sample of blood or of a blood-derived fluid such as serum and plasma, of saliva, of interstitial fluid or of urine. In a particular embodiment, the biological fluid sample of the subject is a blood,

serum, or plasma sample. In a more particular embodiment, the sample is a serum sample. In another particular embodiment, the biological fluid sample is a cell-free sample.

According to the present invention, a biological fluid sample may be analyzed immediately after collection or be stored in cold environments, for example in freezers, for further analysis.

The circulating levels of sVCAM and TSP-2 may be measured by any conventional methodology well known in the art, such as immunoassays (e.g. ELISA (enzyme-linked immunosorbent assay), immunoturbidimetry, immuno-nephelometry, immune cytometry, protein array). For example, the levels of sVCAM and TSP-2 can be determined by antibodies, aptamers or peptides respectively directed against said markers.

In some methods of the invention, the circulating levels of sVCAM and TSP-2 are measured from multiple blood-derived samples isolated from a subject on the same time or at different time-points during a period of from some months to some years. In that case, the same kind of sample is used each time when a measure has to be done. For the sake of clarity, this means that if a previous measure was done from a serum sample, the subsequent measures are done from serum samples of the same subject. Likewise, if the previous measure was done from a blood or plasma sample, the subsequent measures are done from blood or plasma samples, respectively, of the same subject.

According to the methods of the invention, the levels of the two biomarkers measured in a biological fluid sample isolated from a subject and the age of the subject are combined in a mathematical function to assign a score. For the sake of clarity, the age of the subject to be used in the mathematical function is the age when the biological fluid sample was isolated from said subject for the measurement of the levels of said two biomarkers.
Said score is correlated with fibrosis status of a subject. Said score can be used for the diagnosis or the prognosis of advanced liver fibrosis or liver cirrhosis in a subject. In addition, said score is also correlated with the evolution of liver fibrosis, which allows not only to diagnose but also to monitor liver fibrosis progression or to assess the efficacy of an anti-fibrotic agent in treating advanced liver fibrosis or liver cirrhosis.

Within the context of the present invention, one skilled in the art is aware of numerous suitable methods for developing mathematical function, and all of these are within the scope of the present

invention. In a particular embodiment, the mathematical function includes a logistic regression equation.

In a further embodiment, the score is assigned by the following mathematical function:

$$\text{Score} = \frac{1}{1 + \exp(-y)}$$

with

$y = \beta 0 + \beta 1 * \log_{10}(\text{TSP2 (ng/mL)}) + \beta 2 * \log_{10}(\text{sVCAM (ng/mL)}) + \beta 3 * (\text{Age (years)}^3)$

wherein:

β0 is comprised between -40 and -14, in particular between -38 and -17;

β1 is comprised between 1.7 and 6.7, in particular between 2.2 and 6.3;

β2 is comprised between 3.0 and 12, in particular between 3.5 and 11.5;

β3 is comprised between -6 e^{-06} and 2.5 e^{-6}, in particular between -5 e^{-6} and 2 e^{-6}.

In a particular embodiment, the invention relates to an *in vitro* method for diagnosing or prognosing advanced liver fibrosis or liver cirrhosis in a subject, comprising:

a)providing (i) the circulating levels of sVCAM and TSP-2 in a biological fluid sample isolated from said subject, and (ii) the age of the subject;

b)combining the provided levels of sVCAM and TSP-2 and the age in a mathematical function to assign a score; and

c) comparing said score with cut-off values to diagnose or prognose advanced liver fibrosis or liver cirrhosis in said subject.

By being compared to a specific cut-off value, the score obtained can be used to discriminate subjects having advanced liver fibrosis or liver cirrhosis from subjects not having advanced liver fibrosis, or not having liver cirrhosis, or be used to predict the evolution of a subject's liver fibrosis.

According to a particular embodiment, the invention relates to an *in vitro* method for diagnosing advanced liver fibrosis or liver cirrhosis in a subject, wherein the score is a score SA obtained through the above-defined mathematical function.

According to an embodiment of the invention, SA value is compared to a cut-off value (co). Said value may be calculated from Youden' statistical analysis of SA scores which are obtained on a training population. "Training population" refers to a population consisted of a given number of subjects, wherein the fibrosis stage of each subject is already determined by a method of prior art, like liver biopsy.

Particularly, said SA and cut-off value are calculated using a mathematical function to differentiate patients having advanced liver fibrosis from patients without advanced liver fibrosis.
Alternatively, said SA and cut-off values are calculated using a mathematical function to differentiate patients having liver cirrhosis from patients without liver cirrhosis.

A cut-off value co1 may be determined to be used to indicate the presence or absence of advanced liver fibrosis. A cut-off value co2 may be determined to be used to indicate the presence or absence of liver cirrhosis.

In a more particular embodiment, the invention relates to an *in vitro* method for diagnosing advanced liver fibrosis in a human subject, comprising:

a) providing (i) the circulating levels of sVCAM and TSP-2 in a biological fluid sample isolated from said subject, and (ii) the age of the subject,
b) calculating a score SA based on step (a) by using a mathematical function,
c) comparing this SA to a cut-off value co1,
d) wherein a SA greater than the cut-off value co1 is indicative of a subject with advanced liver fibrosis.

In contrast, a calculated SA value lower than the cut-off value co1 is indicative of a subject not having advanced liver fibrosis.

In another more particular embodiment, the invention relates to an *in vitro* method for diagnosing liver cirrhosis in a human subject, comprising

a) providing (i) the circulating levels of sVCAM and TSP-2 in a biological fluid sample isolated from said subject, and (ii) the age of the subject,
b) calculating a score SA based on step (a) by using a mathematical function,
c) comparing this SA to a cut-off value co2,
d) wherein a SA greater than the cut-off value co2 is indicative of a subject with liver cirrhosis.

In contrast, a calculated SA value lower than the cut-off value co2 is indicative of a subject not having liver cirrhosis.

In a particular embodiment, the score A (SA), the cut-off value co1 and the cut-off value co2 used for the diagnosis of advanced liver fibrosis or liver cirrhosis are calculated according to above defined mathematical function on a training population.

In a particular embodiment, SA is compared with the cut-off value co1, which is indicative of an advanced liver fibrosis. Particularly, co1 is comprised between 0.2 and 0.7, more particularly between 0.25 and 0.63, in particular equal to 0.3358.

In another specific embodiment, SA is compared with the cut-off value co2, which is indicative of a liver cirrhosis. Particularly, co2 is comprised between 0.4 and 0.9, more particularly between 0.5 and 0.85, in particular equal to 0.5829.

According to another aspect, the invention relates to a computer program comprising instructions that, when executed by a processor/processing means, cause the processor/processing means to:
- receive levels of sVCAM and TSP-2 measured in a biological fluid sample isolated from a subject and the age of a subject;
- calculate SA from these measured levels and the age, from a mathematical function as described herein; and
- assign the subject into the group of subjects having advanced liver fibrosis or liver cirrhosis upon the calculated score compared to predetermined cut-off values.

The present invention further provides a computer readable medium comprising the computer program described therein. According to a particular embodiment, the computer readable medium is non-transitory medium or a storage medium.

The invention also relates to an *in vitro* method for prognosing the evolution of liver fibrosis in a subject with biopsy-proven stage 1, 2 or 3 liver fibrosis, comprising

a) providing (i) the circulating levels of sVCAM and TSP-2 in a biological fluid sample of said subject, and (ii) the age of the subject;
b) combining the provided levels of sVCAM and TSP-2 and the age in a mathematical function to assign a score SF; and
c) comparing said score with a cut-off value to prognose the evolution of liver fibrosis in said subject.

According to this embodiment, the score SF of the subject higher than a cut-off value is indicative of that the subject's liver fibrosis will progress; the score SF of the subject lower than the cut-off value is indicative of that the subject's liver fibrosis will regress; and the SF score of the subject neither higher nor lower than the cut-off value is indicative of that the subject's liver fibrosis will be stable.

The cut-off value to be used in this method depends on the liver fibrosis stage of the subject. According to this embodiment, the SF score of a subject with biopsy-proven stage 1 liver fibrosis is compared with a cut-off value CO-F1; SF score of a subject with biopsy-proven stage 2 liver fibrosis is compared with a cut-off value CO-F2; SF score of a subject with biopsy-proven stage 3 liver fibrosis is compared with a cut-off value CO-F3.

The cut-off values CO-F1, CO-F2 and CO-F3 may be determined in training populations with stable biopsy-proven stage 1, 2 or 3 liver fibrosis, respectively. For instance, the cut-off value CO-F1 is determined in a training population with stable biopsy-proven stage 1 liver fibrosis. A patient is considered to have stable liver fibrosis if the patient's liver fibrosis stage remained unchanged at the screening visit and 18 months after the screening visit. For example, a patient with biopsy-proven stage 1 liver fibrosis is considered to have stable liver fibrosis if the patient's liver fibrosis stage remained at stage 1 18 months after the screening visit.

According to an embodiment, the cut-off values of the SF score may be a range of values. According to a particular embodiment, the cut-off value CO-F1 is a range comprised between 0.09 and 0.27; the cut-off value CO-F2 is a range comprised between 0.29 and 0.45; and the cut-off value CO-F3 is a range comprised between 0.45 and 0.83.

In a specific embodiment, when the SF score of a subject with biopsy-proven stage 1 liver fibrosis is higher than a range comprised between 0.09 and 0.27, this indicates that the subject's liver fibrosis will progress; when the score SF of the subject is lower than said range, this indicates that the subject's liver fibrosis will regress; when the SF score of the subject is comprised in said range, this indicates that the subject's liver fibrosis will be stable.

In another specific embodiment, when the SF score of a subject with biopsy-proven stage 2 liver fibrosis is higher than a range comprised between 0.29 and 0.45, this indicates that the subject's liver fibrosis will progress; when the score SF of the subject is lower than said range, this indicates that the subject's liver fibrosis will regress; when the SF score of the subject is comprised in said range, this indicates that the subject's liver fibrosis will be stable.

In another specific embodiment, when the SF score of a subject with biopsy-proven stage 3 liver fibrosis is higher than a range comprised between 00.45 and 0.83, this indicates that the subject's liver fibrosis will progress; when the score SF of the subject is lower than said range, this indicates that the subject's liver fibrosis will regress; when the SF score of the subject is comprised in said range, this indicates that the subject's liver fibrosis will be stable.

According to another particular embodiment, the cut-off values of the SF score may be specific values. According to a more particular embodiment, the cut-off value CO-F1 is a value selected in a range comprised between 0.09 and 0.27; the cut-off value CO-F2 is a value selected in a range comprised between 0.29 and 0.45; the cut-off value CO-F3 is a value selected in a range comprised between 0.45 and 0.83. More particularly, the cut-off value CO-F1 may be 0.16; the cut-off value CO-F2 may be 0.37; and the cut-off value CO-F3 may be 0.63.

In a specific embodiment, when the SF score of a subject with biopsy-proven stage 1 liver fibrosis is higher than a CO-F1 value selected in a range comprised between 0.09 and 0.27, this indicates that the subject's liver fibrosis will progress; when the SF score of the subject is lower than said selected CO-F1 value, this indicates that the subject's liver fibrosis will regress; when the SF score of the subject is identical to said selected CO-F1 value, this indicates that the subject's liver fibrosis will be stable.

In another specific embodiment, when the SF score of a subject with biopsy-proven stage 2 liver fibrosis is higher than a CO-F2 value selected in a range comprised between 0.29 and 0.45, this indicates that the subject's liver fibrosis will progress; when the SF score of the subject is lower than said selected CO-F2 value, this indicates that the subject's liver fibrosis will regress; when the SF score of the subject is identical to said selected CO-F2 value, this indicates that the subject's liver fibrosis will be stable.

In another specific embodiment, when the SF score of a subject with biopsy-proven stage 3 liver fibrosis is higher than a CO-F3 value selected in a range comprised between 0.45 and 0.83, this indicates that the subject's liver fibrosis will progress; when the SF score of the subject is lower than said selected CO-F3 value, this indicates that the subject's liver fibrosis will regress; when the SF score of the subject is identical to said selected CO-F3 value, this indicates that the subject's liver fibrosis will be stable.

The invention also relates to a computer program comprising instructions that, when executed by a processor/processing means, cause the processor/processing means to:

- receive (i) levels of sVCAM and TSP-2 measured in a biological fluid sample of a subject with biopsy-proven stage 1, 2 or 3 liver fibrosis; and (ii) the age of the subject
- calculate a SF score from these measured levels, through a mathematical function;
- compare the score SF with a predetermined cutoff value of SF score, and
- assign the subject as:

a subject whose liver fibrosis will progress, when the SF score is higher than the cutoff value;

a subject whose liver fibrosis will regress when the SF score is lower than the cutoff value:

a subject whose liver fibrosis will be stable when the SF score is neither higher nor lower than the cutoff value.

The present invention also provides an *in vitro* method for monitoring the progression of liver fibrosis in a subject by measuring the levels of two circulating markers, i.e. sVCAM and TSP-2.

Particularly, said method comprises the steps of:

a) providing (i) the circulating levels of sVCAM and TSP-2 in a biological fluid sample isolated from said subject, and (ii) the age of the subject;

b) combining the provided levels of sVCAM and TSP-2 and the age in a mathematical function to assign a score SC; and

c) comparing the score SC with a score SB, which is obtained by combining levels of sVCAM and TSP-2 and the age previously measured in the same subject and with the same mathematical function.

As used herein, the term "monitoring" refers to the tracking of the evolution of a disease or condition. Monitoring is the ongoing, systematic collection and analysis of data as a protocol or condition progresses, for example during a clinical study or a treatment protocol.

Since the score SC is calculated by using a non-invasive method and correlated with the evolution of liver fibrosis, it allows not only to diagnose but also to monitor easily liver fibrosis progression, by repeated measures.

In a particular embodiment, the circulating levels of sVCAM and TSP-2 are measured from at least two blood-derived samples from the subject.

The collection over time of at least two samples from the same subject allows to assess longitudinal changes in the score. For instance, a first score, named SB, may be calculated from the levels of sVCAM and TSP-2 previously measured in a biological fluid sample of a subject and the corresponding age, and a second score, named SC, may be calculated from the levels sVCAM and TSP-2 subsequently measured

in a biological fluid sample of the same subject and the corresponding age. SB and SC may be obtained through the mathematical function as defined above. If the score increases with time in the same subject, i.e. SC is higher than SB, it means that liver fibrosis worsens, whereas if the SC decreases with time in the same subject, i.e. SC is lower than SB, it means that liver fibrosis decreases. No significant change between SC and SB measured in a certain lapse of time in a same subject means that liver fibrosis is stable.

Since the score calculated according to the mathematical function as defined above is a linear value, by comparing the score C (SC) with the score B (SB), the method of the invention also allows to determine the likelihood of a subject with advanced liver fibrosis to progress towards cirrhosis or towards worsening cirrhosis. During a follow-up, the change in the value between SC and SB is therefore an indicator of liver fibrosis progression or liver fibrosis regression.

In a particular embodiment, SC is measured while an event linked to the evolution of pathological state occurs. Said events comprise liver transplantation, acute on chronic liver fibrosis (ACLF), compensated cirrhosis and decompensated cirrhosis, episode of ascites and presence of esophageal varices at endoscopy. Preferably, the events are acute on chronic liver fibrosis, compensated and decompensated cirrhosis.

In a particular embodiment, SC is measured at least 3 months after the measurement of SB, particularly in a period between 3 months and 10 years, preferably in a period between 3 months and 2 years, more preferably between 1 and 2 years, after the measurement of SB.

In a more particular embodiment, if the subject is diagnosed as having liver cirrhosis, e.g. by the method of the invention as described above, then SC is measured in a period between 3 months and 2 years, and preferentially 3 months, after the measurement of SB. The suitable moment for the measurement of SC may depend on comorbidities.
Comorbidities comprise malignancy, type 2 diabetes, overweight and obesity, heart disease and kidney diseases.

In another particular embodiment, if the subject is diagnosed as having advanced liver fibrosis, e.g. by the method of the invention as described above, then SC is measured in a period between 1 and 10

years after the measurement of SB. The suitable moment for the measurement of SC depends on comorbidities.

Thanks to the methods of the invention, a decision may be taken to give life-style recommendations to a subject (such as a food regimen or providing physical activity recommendations), to medically take care of a subject (e.g. by setting regular visits to a physician or regular examinations, for example for regularly monitoring markers of liver damage), or to administer at least one liver fibrosis therapy to the patient, to treat advanced liver fibrosis or liver cirrhosis. Particularly, a decision may be taken to give life-style recommendations to a subject or to administer at least one liver fibrosis therapy. The invention thus further relates to an anti-fibrotic compound for use in a method for treating advanced liver fibrosis or liver cirrhosis in a subject in need thereof, wherein the subject has been identified thanks to a method according to the invention.

The invention also relates to a computer program comprising instructions that, when executed by a processor/processing means, cause the processor/processing means to:

- receive levels of sVCAM and TSP-2 measured in a biological fluid sample isolated from a subject and the age of the subject;
- calculate a score SC from these measured levels and the age, from a mathematical function as described above;
- compare the score SC with a score SB, which is obtained by combining levels of sVCAM and TSP-2 and the age previously measured in the same subject with the same mathematical function and
- assign the subject as :
    having the progression of liver fibrosis when the score SC is higher than SB;
    having the regression of liver fibrosis when the score SC is lower than SB; or
    having stable liver fibrosis when there is no significant change between SC and SB.

The invention thus further relates to an anti-fibrotic compound for use in a method for treating advanced liver fibrosis or liver cirrhosis in a subject in need thereof, wherein the subject has been identified thanks to a method according to the invention.

The term “treatment”, as used herein, relates to both therapeutic measures and prophylactic or preventive measures, wherein the goal is to prevent or slow down (lessen) an undesired physiological change or disorder. Beneficial or desired clinical results include, but are not limited to, alleviation of

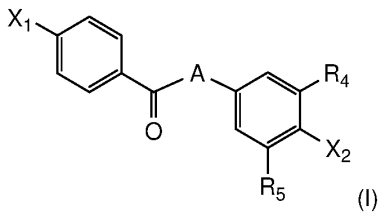
symptoms, stabilizing pathological state (specifically not worsening), slowing down or stopping the progression of the disease, improving or mitigating the pathological condition. Particularly, for the purpose of the present invention, treatment is directed to slow the progression of fibrosis and reduce the risk of further complications. It can also involve prolonging survival in comparison with the expected survival if the treatment is not received.

The anti-fibrotic agent is administered in a therapeutically effective amount. As used herein, the expression "therapeutically effective amount" refers to an amount of the drug effective to achieve a desired therapeutic result. A therapeutically effective amount of a drug may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of drug to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of agent are outweighed by the therapeutically beneficial effects. The effective dosages and dosage regimens for drug depend on the disease or condition to be treated and may be determined by the persons skilled in the art. A physician having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician could start doses of drug employed in the pharmaceutical composition at levels lower than that required to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable dose of a composition of the present invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect according to a particular dosage regimen. Such an effective dose will generally depend upon the factors described above.

The invention further relates to an anti-fibrotic compound for use in a method for treating liver fibrosis in a F3 or F4 patient, wherein the patient is classified as having advanced liver fibrosis or liver cirrhosis according to the method of the invention. The invention also relates to an anti-fibrotic compound for use in a method for treating liver fibrosis, wherein the subject, diagnosed or classified as having advanced liver fibrosis or liver cirrhosis, is treated with an anti-fibrotic compound as defined herebelow, thanks to a method according to the invention.

Anti-fibrotic compounds comprise:

- a compound of formula (I) or a pharmaceutically acceptable salt thereof:



(I)

wherein:

X1 represents a halogen atom, a R1 group or G1-R1 group;

A represents a CH=CH or CH2-CH2 group;

X2 represents a G2-R2 group;

G1 represents an atom of oxygen;

G2 represents an atom of oxygen or sulfur;

R1 represents a hydrogen atom, an unsubstituted alkyl group, an aryl group or an alkyl group that is substituted by one or more substituents selected from halogen atoms, alkoxy groups, alkylthio groups, cycloalkyl groups, cycloalkylthio groups and heterocyclic groups;

R2 represents an alkyl group substituted by a –COOR3 group, wherein R3 represents a hydrogen atom or an alkyl group that is substituted or not by one or more substituents selected from halogen atoms, cycloalkyl groups and heterocyclic groups.

R4 and R5, identical or different, represent an alkyl group that is substituted or not by one or more substituent selected from halogen atoms, cycloalkyl groups and heterocyclic groups;

- AMP activated protein kinase stimulators such as PXL-770, MB-11055, Debio-0930B, metformin, CNX-012, O-304, mangiferin calcium salt, eltrombopag, carotuximab, and imeglimin;

- Bile acids such as obeticholic acid (OCA), ursodeoxycholic acid (UDCA), norursodeoxycholic acid, and ursodiol;

- CCR antagonists such as cenicriviroc (CCR2/5 antagonist), PG-092, RAP-310, INCB-10820, RAP-103, PF-04634817, and CCX-872;

- Dipeptidyl peptidase IV (DPP4) inhibitors such as evogliptin, vidagliptin, fotagliptin, alogliptin, saxagliptin, tilogliptin, anagliptin, sitagliptin, retagliptin, melogliptin, gosogliptin, trelagliptin, teneligliptin, dutogliptin, linagliptin, gemigliptin, yogliptin, betagliptin, imigliptin, omarigliptin, vidagliptin, and denagliptin;

- Farnesoid X receptor (FXR) agonists such as obeticholic acid (OCA), tropifexor (LJN452), cilofexor (GS9674), Nidufexor (LMB763), EDP-305, AKN-083, INT-767, GNF-5120, LY2562175, INV-33, NTX-023-1, EP-024297, Px-103, SR-45023, TERN-101 (6-{4-[5-Cyclopropyl-3-(2,6-dichloro-phenyl)-isoxazol-4-ylmethoxy]-piperidin-1-yl}-1-methyl-1H-indole-3 carboxylic acid), TERN-201, TERN-501 and TERN-301;

- Fibroblast Growth Factor 19 (FGF-19) receptor ligand or functional engineered variant of FGF-19;

- Fibroblast Growth Factor 21 (FGF-21) agonists such as PEG-FGF21 (pegbelfermin, formely BMS-986036), YH-25348, BMS-986171, YH-25723, LY-3025876, and NNC-0194-0499;
- engineered Fibroblast Growth Factor 19 (FGF-19) analogues such as NGM-282 (aldafermin);
- Glucagon-like peptide-1 (GLP-1) analogs such as semaglutide, liraglutide, exenatide, albiglutide, dulaglutide, lixisenatide, loxenatide, efpeglenatide, taspoglutide, MKC-253, DLP-205, and ORMD-0901;
- Nicotinic acid such as Niacin and Vitamin B3;
- nitazoxanide (NTZ), its active metabolite tizoxanide (TZ) or other prodrugs of TZ such as RM-5061;
- PPAR alpha agonists such as fenofibrate, ciprofibrate, pemafibrate, gemfibrozil, clofibrate, binifibrate, clinofibrate, clofibric acid, nicofibrate, pirifibrate, plafibride, ronifibrate, theofibrate, tocofibrate, and SR10171;
- PPAR gamma agonists such as pioglitazone, deuterated pioglitazone, rosiglitazone, efatutazone, ATx08-001, OMS-405, CHS-131, THR-0921, SER-150-DN, KDT-501, GED-0507-34-Levo, CLC-3001, and ALL-4;
- PPAR delta agonists such as GW501516 (Endurabol or ({4-[({4-methyl-2-[4-(trifluoromethyl)phenyl]-1,3-thiazol-5-yl}methyl)sulfanyl]-2-methylphenoxy}acetic acid)), MBX8025 (Seladelpar or {2-methyl-4-[5-methyl-2-(4-trifluoromethyl- phenyl)-2H-[l,2,3]triazol-4-ylmethylsylfanyl]-phenoxy}-acetic acid), GW0742 ([4-[[[2-[3-fluoro-4-(trifluoromethyl)phenyl]-4-methyl-5-thiazolyl]methyl]thio]-2-methyl phenoxy]acetic acid), L165041, HPP-593, and NCP-1046;
- PPAR alpha/gamma dual agonists (also named glitazars) such as saroglitazar, aleglitazar, muraglitazar, tesaglitazar, and DSP-8658;
- PPAR gamma/delta dual agonists such as conjugated linoleic acid (CLA), and T3D-959;
- PPAR alpha/gamma/delta pan agonists or PPARpan agonists such as IVA337, TTA (tetradecylthioacetic acid), bavachinin, GW4148, GW9135, bezafibrate, lanifibranor, lobeglitazone, and CS038;
- Sodium-glucose transport (SGLT) 2 inhibitors such as licoglifozin, remogliflozin, dapagliflozin, empagliflozin, ertugliflozin, sotagliflozin, ipragliflozin, tianagliflozin, canagliflozin, tofogliflozin, janagliflozin, bexagliflozin, luseogliflozin, sergliflozin, HEC-44616, AST-1935, and PLD-101.
- stearoyl CoA desaturase-1 inhibitors/fatty acid bile acid conjugates such as aramchol, GRC-9332, steamchol, TSN-2998, GSK-1940029, and XEN-801;
- thyroid receptor β (THR β) agonists such as VK-2809, resmetirom (MGL-3196), MGL-3745, SKL-14763, sobetirome, BCT-304, ZYT-1, MB-07811 and eprotirome;
- Vitamin E and isoforms; vitamin E combined with vitamin C and atorvastatin.

In the present invention, anti-fibrotic compounds are preferably selected in the group consisting of pegbelfermin, cenicriviroc, dapagliflozin, dulaglutide, empagliflozin, fenofibrate, lanifibranor, liraglutide, obeticholic acid, pioglitazone, resmetirom, saroglitazar magnesium, seladelpar, semaglutide, sitagliptin, TERN-101, TERN-201, tropifexor, ambrisentan, BMS-963272, BMS-986251, BMS-986263, HepaStem, LYS006, MET409, MET642, and orlistat (Xenical).

More preferably, the anti-fibrotic agent is selected from pegbelfermin, cenicriviroc, dapagliflozin, dulaglutide, empagliflozin, fenofibrate, lanifibranor, liraglutide, obeticholic acid, pioglitazone, resmetirom, saroglitazar magnesium, seladelpar, semaglutide, sitagliptin, TERN-101, TERN-201 and tropifexor.

In a more particular embodiment, the anti-fibrotic agent is resmetirom.

The invention further relates to a method for assessing the efficacy of an anti-fibrotic agent in a subject suffering from advanced liver fibrosis or liver cirrhosis, comprising:

a) providing (i) the circulating levels of sVCAM and TSP-2 in a biological fluid sample isolated from a subject suffering from advanced liver fibrosis, wherein said subject has been administered an anti-fibrotic agent before the isolation of the biological fluid sample, and (ii) the age of the subject;

b) combining the provided levels of sVCAM and TSP-2 and the age in a mathematical function to assign a score SE; and

c) comparing the score SE with a score SD, which is obtained by combining the levels of sVCAM and TSP-2 and the age, previously measured before administration of the anti-fibrotic agent to the same subject and with the same mathematical function.

According to said method, the levels of sVCAM and TSP-2 are respectively measured in a sample isolated before administration of an anti-fibrotic agent and a sample isolated after said administration to obtain a first score named SD (before the administration) and a second score named SE (after the administration).

Said SD and SE may be obtained through a same mathematical function, especially the mathematical function as defined above.

If SE is higher than SD, then liver fibrosis worsens, meaning either that the subject does not respond to the treatment with the anti-fibrotic agent or the treatment is not effective.

If SE is lower than SD, then liver fibrosis regresses, meaning that the subject responds to the treatment with the anti-fibrotic agent and the treatment is effective.
If SD is equal to SE, then liver fibrosis is stable.

The invention also relates to a computer program comprising instructions that, when executed by a processor/processing means, cause the processor/processing means to:
- receive (i) levels of sVCAM and TSP-2 measured in a biological fluid sample isolated from a subject suffering from advanced liver fibrosis, wherein said subject has been administered an anti-fibrotic agent before the isolation of the biological fluid sample and (ii) the age of the subject;
- calculate SE from these measured levels and the age, from a mathematical function as described above;
- compare the score SC with a score SD, which is obtained by combining the levels of sVCAM and TSP-2 and the age, previously measured before administration of the anti-fibrotic agent to the same subject and with the same mathematical function; and
- assign the subject as:
a subject who does not respond to the treatment with the anti-fibrotic agent, when the score SE is higher than SD;
a subject who responds to the treatment with the anti-fibrotic agent, when the score SE is lower than SD ;.or
having stable liver fibrosis when there is no significant change between SE and SD.

The present invention also relates to a data-processing device comprising means for carrying one of the methods of the present invention as described above.

The present invention provides also a computer program product or a computer-readable storage medium comprising instructions which, when executed by a computer, cause the computer to carry out one of the methods of the present invention as described above.

The invention is further described with reference to the following, non-limiting, examples.

EXAMPLES

1. Datasets

The study population consists of patients that were screened for potential inclusion in the Resolve-It phase3 clinical trial. All patients with biopsie results available, and blood samples used for biomarkers measurements with less than 90days between biopsies and blood collections dates were selected as of potential utility for this study.

- **Training dataset**

Selected patients are those with full data on numerous biomarkers, as well as on the usual demographical and clinical parameters (age, sex, Type-2 Diab, Dyslipidemia, Arterial Hypertension [HT], BMI) to maximize both the number of patients and of biomarkers. This selection led to a total of N=1640 patients with full data on 40 biomarkers. To control for potential confounding factors when processing the modelization and the selection of relevant biomarkers, two propensity score matching algorithms (PSM) are applied to homogenize the following parameters firstly among patients with F<3 (F0F2) and F=4 (F4) and secondly previously matched F=4 with F=3 (F3).

To balance the fibrosis stages distribution in the training dataset, in the first PSM, two patients from the F0F2 group were matched with each patient from the F4 group. This process led to a selection of n=272 patients, 136 from the F0F2 groups, 68 from the F3 group and 68 from the F4 group, well-balanced for the parameters indicated in the list above (Table 1). In this training cohort, the mean age of patients was 58 years, 60% of them were male and 53% had T2D (type 2 diabetes).

- **Validation dataset**

The training process led to the selection of a biomarker panel comprising two biomarkers: sVCAM and TSP-2.

Based on this result, we selected all patients with available measures of these biomarkers, fulfilling the condition regarding biopsies results availability and ≤ 90 days between blood and biopsy dates, and who were not part of the training dataset. This led to an independent validation dataset comprising N=1850 patients. Principal characteristics are reported in Table 1 below.

- **Statistical analyses**

Descriptive statistics were generated for baseline characteristics and reported as means ± sd or %(n), and patients without and with advanced fibrosis or cirrhosis were compared using appropriate 2-sample tests (Student t test for comparison of means and $\chi 2$ test for comparison of percentages).

The modelization process was performed using a Stepwise algorithm, comparing sequentially binomial logistic regression models for the detection of advanced fibrosis or cirrhosis based on their associated BIC (Bayesian Information Criterion), starting with an empty model and sequentially inserting the variables that minimize this criterion. The process stopped when none of the remaining variables led to a decrease of the BIC value. For this, the stat R package (step function) was used. The regression coefficients of the selected model were extracted and associated 95% CI were reported.

Specific cutoffs values were derived using the training dataset. For the detection of F≥3 a low cutoff to achieve 85% sensitivity (Sen) and a high cutoff to achieve 90% specificity (Spe) were extracted, and diagnostic performances (Sen, Spe, PPV, NPV, Acc) associated to these cutoffs were computed in both the training and validation datasets. For the detection of F=4 a low cutoff to achieve 85% Sen and a high cutoff to achieve 95% Spe were extracted, and diagnostic performances (Sen, Spe, PPV, NPV, Acc) associated to these cutoffs were computed in both the training and validation datasets. Also, a Youden cutoff that maximize Sen + Spe is reported for each endpoint.

The overall diagnostic performance of non-invasive tests and biomarkers are estimated using AUROC values, and these statistics are tested for significant differences using Delong tests. AUROC values are reported with 95%CI estimated using 1000 bootstrap samples.

Boxplots are presented with associated Student t-tests for mean comparisons, and p values are graphically reported as follow :

**** if $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ and NS if $p \geqslant 0.05$

All propensity score matching algorithms were executed using the matchit function from the MatchIt R package, and all statistical analyses were performed using R version 4.3.0.

Table 1: Cohorts descriptive features

	Training cohort							Validation cohort						
	F0F2		F3		F4			F0F2		F3		F4		
	n	value	n	value	n	value	p value	n	value	n	value	n	value	p value
Demographics														
Gender, Male	136	60.29% (82)	68	60.29% (41)	68	60.29% (41)	1	1202	63.64% (765)	575	59.13% (340)	73	52.05% (38)	0.407
Age (years)	136	58.38±9.31	68	57.68±9.74	68	57.87±9.43	0.8667	1202	51.8±12	575	56.86±11.3	73	59.79±7.97	<0.0001
Clinics														
Type 2 Diabetes	136	52.94% (72)	68	54.41% (37)	68	52.94% (36)	0.9781	1202	33.69% (405)	575	53.91% (310)	73	68.49% (50)	<0.0001
Dyslipidemia	136	40.44% (55)	68	41.18% (28)	68	38.24% (26)	0.9334	1202	48.34% (581)	575	53.74% (309)	73	34.25% (25)	0.0031
Arterial hypertension	136	63.97% (87)	68	66.18% (45)	68	63.24% (43)	0.9304	1202	51.83% (623)	575	66.09% (380)	73	67.12% (49)	<0.0001
BMI (kg/m²)	136	33.81±5.38	68	34.26±5.94	68	33.91±6.36	0.8712	1202	33.64±6.21	575	33.51±5.97	73	32.83±4.84	0.3901
Laboratory Measures														
Ast (IU/L)	136	36.86±24.48	68	44.31±21.69	68	51.59±28.3	0.0011	1202	35.46±23.63	575	51.18±32.48	73	52.79±36.03	<0.0001
Alt (IU/L)	136	49.75±35.6	68	53.04±33.2	68	58.01±37.2	0.3175	1202	52.31±39.41	575	66.11±47.07	73	59.47±42.96	<0.0001
GGT (IU/L)	136	57.85±49.02	68	70.28±61.3	68	127.49±182.32	2e-04	1202	59.23±74	575	97.27±117.45	73	115.49±118.71	<0.0001
ALP (IU/L)	136	83.15±24.84	68	80.25±28.45	68	94.44±38.77	0.0477	1201	83.07±29.07	575	87.62±32.81	73	97.3±40.93	7e-04
FPG (mmol/L)	136	6.29±1.9	68	6.49±1.82	68	6.94±2.82	0.2253	1201	5.76±1.63	575	6.44±2.07	73	6.59±1.87	<0.0001
HbA1c (%)	136	6.37±1.01	68	6.34±0.94	68	6.61±1.19	0.293	1193	6.08±1	572	6.4±1.06	73	6.59±1.07	<0.0001
Triglycerides (mmol/L)	136	2.02±1.24	68	1.93±0.99	68	1.76±0.74	0.1543	1202	1.94±1.27	575	2±1.28	73	1.7±0.89	0.0346
Platelet (10e9/L)	136	241.41±62.8	68	211.18±68.43	68	201.12±67.25	1e-04	1202	249.05±65.51	575	223.22±64.03	73	197.75±71.21	<0.0001
Histology														
At-risk MASH	136	47.79% (65)	68	76.47% (52)	68	67.65% (46)	1e-04	1202	31.7% (381)	575	78.78% (453)	73	65.75% (48)	<0.0001
Fibrosis	136	1.36±0.77	68	3±0	68	4±0	NA	1202	1.17±0.76	575	3±0	73	4±0	NA
Fibrosis by stage, 0/1/2/3/4	136	17.6%/ 28.7%/ 53.7%/ 0%/ 0%	68	0%/ 0%/ 0%/ 100%/ 0%	68	0%/ 0%/ 0%/ 0%/ 100%	NA	1202	21.5%/ 40%/ 38.4%/ 0%/ 0%	575	0%/ 0%/ 0%/ 100%/ 0%	73	0%/ 0%/ 0%/ 0%/ 100%	NA
NAS	136	4.43±1.9	68	4.54±1.77	68	4.5±1.86	0.9154	1202	3.77±1.95	575	5.01±1.88	73	4.14±2.28	<0.0001
NAS by category, 0-1, 2-3, 4-5, 6-8	136	7.4%/ 19.1%/ 39%/ 34.6%	68	8.8%/ 14.7%/ 41.2%/ 35.3%	68	7.4%/ 22.1%/ 33.8%/ 36.8%	NA	1202	16.5%/ 24.5%/ 38.3%/ 20.7%	575	6.4%/ 13.2%/ 33.6%/ 46.8%	73	17.8%/ 15.1%/ 34.2%/ 32.9%	NA

2. Results

2.1 Modelization development

When executing the stepwise algorithm, the combination of TSP2 and SVCAM was found as the best biomarker combination, as it returned the lowest BIC, the addition of further biomarker did not lead to a sufficient decrease to be considered significant.

In addition, it is important to guarantee that any diagnostic test could be interpreted irrespective of the patients' characteristics. Thus, the impact of different potential confounding factors, i.e. age, sex, prevalence of Type-2 Diabetes (T2D), dyslipidemia, arterial hypertension (HT), Body mass index (BMI), fibrosis, steatosis, ballooning and lobular inflammation by class, was analyzed by using a modelization STA:

$$\mathrm{ST} = \frac{1}{1 + \exp(-y)}$$

with

y= -30.001 + 8.64 × log10 (sVCAM (g/L)) + 5.750 x log10 (TSP-2 (ng/mL))

This process led to the creation of 2 subpopulations for each parameter of interest (age, sex, gender, hypertension, dyslipidemia and T2D), each time these subpopulations being well-balanced for the other parameters listed above. To be noted, regarding age, we created 2 subpopulations, one comprising patients aged 50 and the second with patients aged 60 to ensure a clear separation of age between both subpopulations as well as a second PSM for extreme case study with patients ages $\leqslant$ 45 and patients ages$\geqslant$ 65. For BMI, two groups were created with patients with a BMI $\leqslant$ 29 and a second with patients with a BMI $\geqslant$ 31. For gender, age, T2D, HT, BMI and Dyslipidemia, we extracted a total of 1124 patients (562 in each category), 678 (339 in each category), 1048 (524 in each category), 1084 patients (542 in each category), 750 patients (375 in each category) and 1052 patients (526 in each category) respectively.

Out of the six confounding factors, age was the only one which had a significant impact on the mean scores of the modelization ST, with a significantly higher score for patient older than 60 (p values <0.0001, see Fig. 1A).

A new mathematical modelization was developed to obtain a score which is not impacted by the age of a subject.

The following equation (named ST II) was obtained using a binomial logistic regression model:

$$\text{ST II} = \frac{1}{1 + \exp(-y)}$$

with

$y = \beta 0 + \beta 1 * \log_{10}(\text{TSP2 (ng/mL)}) + \beta 2 * \log_{10}(\text{sVCAM (ng/mL)}) + \beta 3 * (\text{Age (years)}^3)$

Coefficients of this model are reported in Table 2 with associated 95%CI, all of them reaching high significance with associated p values <0.0001.

Table 2: coefficients summary

	$\beta 0$	β_{TSP2}	β_{sVCAM}	β_{Age}
Coefficient	-28.52 (****)	3.769 (****)	7.634 (****)	-1.361e-0 6(NS)
CI (95%)	(-37.3003, -20.7272)	(2.3453, 5.3394)	(4.9392, 10.6129)	(-4.6333e-06, 1.8808e-06)

The impact of age on scores and mean values of biomarkers, Gpe 0 (younger than 50) and Gpe 1 (older than 60) is shown in Fig. 1B.

This result shows that compared to mean scores of the modelization ST, the modelization ST II displayed consistent clinical performances independently of the age classes of the patients. To illustrate this, the inventors extracted a youden cutoff (respectively 0.3831 for ST and 0.3358 for ST II) for the detection of F3 in the validation dataset and calculated the clinical performances for the modelizations ST and ST II. We can observe that the specificity of the modelization ST decreased with the increase of the age of the patients and the sensitivity increased with increasing age (Fig. 2A). This result shows that the clinical performance of modelization ST is not homogeneous across all groups of age. On the contrary, the specificity and the sensitivity of the modelization ST II are homogeneous and constant across all groups of age (Fig. 2B).

Based on the modelization ST II and the training dataset, the inventors extracted the following cutoff values to achieve a 85% sensitivity and 90% specificity for the detection of F≥3 in Table 3 and to achieve a 85% sensitivity and 95% specificity for the detection of F=4 in Table 4.

Table 3: Low (Lc, 85%Sen), Youden and high (Hc, 90%Spe) cut-offs for the detection of F3

	Low (Lc)	Youden	High (Hc)
ST II	0.2746	0.3358	0.5942

Table 4: Low (Lc, 85%Sen), Youden and high (Hc, 95%Spe) cut-offs for the detection of F4

	Low (Lc)	Youden	High (Hc)
ST II	0.5564	0.5829	0.7925

2.2 ST II diagnostic performances validation

The inventors then computed the diagnostic performances of the modelization ST II obtained in both the training and validation datasets for both endpoints. The low and high cutoffs were used to derive these statistics, which are reported in Table 5 for the detection of F⩾3 and in Table 6 for the detection of F=4.

Table 5: ST II performances validation for the detection of advanced fibrosis

	Training	**Validation**
N	272	1850
Prev	50	35
AUC	0.8707(0.78, 0.88)	0.828 (0.81, 0.85)
Rule-out		
Lc	0.2746	0.2746
AccLc	0.71	0.71
SenLc	0.91	0.85
SpeLc	0.51	0.63
NPV	0.85	0.89
IRZ	0.32	0.29
Rule-in		
Hc	0.5942	0.5942
AccHc	0.76	0.77
SenHc	0.64	0.52
SpeHc	0.88	0.9
PPV	0.84	0.74

Table 6 : ST II performances validation for the detection of cirrhosis

	Training	**Validation**
N	272	1850
Prev	25	3.9
AUC	0.8242 (0.77, 0.87)	0.863 (0.82, 0.9)
Rule-out		
Lc	0.5564	0.5564
AccLc	0.74	0.76

SenLc	0.78	0.86
SpeLc	0.72	0.76
NPV	0.91	0.99
IRZ	0.28	0.2
Rule-in		
Hc	0.7925	0.7925
AccHc	0.8	0.89
SenHc	0.54	0.56
SpeHc	0.88	0.9
PPV	0.61	0.19

- **Detection of F≥ 3**

In the validation cohort, at low cutoff, modelization ST II achieved a sensitivity of 0.85 and a specificity of 0.63, while the NPV was 0.89. Similarly, at high cutoff, ST II achieved a specificity of 0.9 combined with a sensitivity of 0.52 and a PPV of 0.74. Interestingly, only 29% of patients relied in the indeterminate risk zone (IRZ). Overall, ST II and its associated performances were validated.

- **Detection of F=4**

In the validation cohort, at low cutoff, ST II achieved a sensitivity of 0.86 and a specificity of 0.76, while the NPV was 0.99. Similarly, at high cutoff, ST II achieved a specificity of 0.9 combined with a sensitivity of 0.56 and a PPV of 0.19. Interestingly, only 15% of patients relied in the indeterminate risk zone (IRZ), meaning that almost 4/5 had clinically actionable test results with ST II. Overall, ST II and its associated performances were validated.

2.3 ST II performances comparison vs other NITs

The overall performances of ST II for the detection of F≥3 are also compared with other usual non-invasive tests: FIB-4, ELF, NFS and APRI.

We calculated the AUROC values and associated Delong tests toc test for AUROC difference between ST II and other NITs. Results for the detection of F≥3 are summarized in Table 7 and results for the detection of F=4 are summarized in Table 8.

Table 7: AUROC values for advanced fibrosis detection

	N	n Advanced fibrosis	AUC (95% CI)	p value
ST II	1850	648	0.83 (0.81, 0.85)	NA
FIB4	1850	648	0.75 (0.72, 0.77)	<0.0001
ELF	1850	648	0.77 (0.74, 0.79)	<0.0001
NFS	1850	648	0.68 (0.66, 0.71)	<0.0001
APRI	1850	648	0.73 (0.7, 0.75)	<0.0001

Table 8: AUROC values for cirrhosis detection

	N	n cirrhosis	AUC (95% CI)	p value
ST II	1850	73	0.86 (0.82, 0.9)	NA
FIB4	1850	73	0.78 (0.72, 0.83)	0.0019
ELF	1850	73	0.83 (0.78, 0.88)	0.0540
NFS	1850	73	0.73 (0.66, 0.78)	0.0000
APRI	1850	73	0.71 (0.65, 0.77)	0.0000

The ROC curves for the detection of F≥3 are graphically reported in Fig. 3 and the ROC curves for the detection of F=4 are graphically reported in Fig. 4.

For the detection of F≥3, ST II achieved an AUROC of 0.83, significantly higher than those achieved by the other NITs, all p values <0.0001. For the detection of F=4, ST II achieved an AUROC of 0.86, significantly higher than those achieved by the other NITs. These results confirm the high performance of the methods of the invention and notably the gain of using this new diagnostic test over the usual ones.

2.4 Prognostic performance of SF score

In order to evaluate the prognostic performance of the method, 612 patients included in the clinical trial have been used. These patients had available information for sVCAM and TSP2 and biopsy results both at screening visit (V_0) and visit 7 (V_7, 81 weeks later) of the clinical trial and had biopsy-proven stage 1, 2 or 3 liver fibrosis at V_0. The SF scores of these patients were calculated at V_0. For each group of patients with initial stage 1, 2 or 3 fibrosis, three categories of fibrosis evolution were defined:

- Improver: patients who had a lower fibrosis stage at V_7 compared to V_0
- Stable: patients who had the same fibrosis stage at V_7 compared to V_0
- Worsener: patients who had higher fibrosis stage at V_7 compared to V_0

There are 9 subgroups of patients in total. The numbers of patients in each sub-group are shown in Table 9 below.

Table 9:

		Fibrosis stage evolution		
		Improver	Stable	Worsener
Fibrosis stage at V_0	F1	15	32	41
	F2	91	103	51
	F3	79	171	29

The comparison of the SF scores of each sub-group shows that a low SF score at V_0 is indicative of that the subject's liver fibrosis will regress and a high score SF at V_0 is indicative of that the subject's liver fibrosis will progress (Figure 5).

2.5 Validation of prognostic performance of SF score

In order to verify the prognostic performance of SF score, 27 patients with biopsy-proven stage 1, 2 or 3 liver fibrosis were classified at V_0 according to their fibrosis stage based on a biopsy. A blood sample of these patients was taken concomitantly. Patient's SF score was calculated according to the method of the invention and was compared with the cut-off value range according to their original fibrosis stage at V0 (ie CO-F1: a range comprised from 0.09 and 0.27; CO-F2: a range comprised between 0.29 and 0.45; CO-F3 : a range comprised between 0.45 and 0.83). After the comparison, the patients were prognosed as either improving, staying stable or worsening. The course of the disease assessed by the score was verified with a biopsy at V7. The SF score of each patient and the disease course assessed at V7 are summarized in Table 10. The results show the performance of SF score for prognosing the evolution of liver fibrosis in a subject with biopsy-proven stage 1, 2 or 3 liver fibrosis.

Table 10:

	Biopsy V0	STage	Progression V7
Patient 1	F1	0.33158665	Worsener
Patient 2	F1	0.38108108	Worsener
Patient 3	F1	0.51762242	Worsener
Patient 4	F1	0.15870065	Stable
Patient 5	F1	0.19743121	Stable
Patient 6	F1	0.23166407	Stable
Patient 7	F1	0.03504990	Improver
Patient 8	F1	0.07527025	Improver
Patient 9	F1	0.03682796	Improver
Patient 10	F2	0.6242051	Worsener
Patient 11	F2	0.5256547	Worsener
Patient 12	F2	0.5542208	Worsener
Patient 13	F2	0.43621682	Stable
Patient 14	F2	0.27770849	Stable
Patient 15	F2	0.33181941	Stable

Patient 16	F2	0.17911948	Improver
Patient 17	F2	0.27448721	Improver
Patient 18	F2	0.19784296	Improver
Patient 19	F3	0.8850492	Worsener
Patient 20	F3	0.9287135	Worsener
Patient 21	F3	0.9188243	Worsener
Patient 22	F3	0.56233008	Stable
Patient 23	F3	0.63385321	Stable
Patient 24	F3	0.77330685	Stable
Patient 25	F3	0.28399722	Improver
Patient 26	F3	0.35713031	Improver
Patient 27	F3	0.31039014	Improver

Claims

1. An *in vitro* method for diagnosing or prognosing advanced liver fibrosis or liver cirrhosis in a subject, comprising

a) providing (i) the circulating levels of Soluble Vascular Cell Adhesion Molecule-1 (sVCAM) and Thrombospondin 2 (TSP-2) in a biological fluid sample isolated from said subject, and (ii) the age of the subject;

b) combining the provided levels of sVCAM and TSP-2 and the age in a mathematical function to assign a score; and

c) comparing said score with cut-off values to diagnose or prognose advanced liver fibrosis or liver cirrhosis in said subject.

2. The method according to claim 1, wherein the score is a score SA obtained through the following mathematical function:

$$SA = \frac{1}{1 + \exp(-y)}$$

with

$y = \beta0 + \beta1 * \log_{10}(\text{TSP2 (ng/mL)}) + \beta2 * \log_{10}(\text{sVCAM (ng/mL)}) + \beta3 * (\text{Age (years)}^3)$

wherein:

β0 is comprised between -40 and -14, in particular between -38 and -17;

β1 is comprised between 1.7 and 6.7, in particular between 2.2 and 6.3;

β2 is comprised between 3.0 and 12, in particular between 3.5 and 11.5;

β3 is comprised between -6 e^{-06} and 2.5 e^{-6}, in particular between -5 e^{-6} and 2 e^{-6}.

3. The method according to claim 1 or 2, wherein a score SA higher than a cut-off value co1 is indicative of advanced liver fibrosis, particularly co1 being comprised between 0.2 and 0.7, more particularly co1 being comprised between 0.25 and 0.63.

4. The method according to claim 1 or 2, wherein a score SA higher than a cut-off value co2 is indicative of a liver cirrhosis, particularly co2 being comprised between 0.4 and 0.9, more particularly co2 being comprised between 0.5 and 0.85.

5. An *in vitro* method for monitoring the progression of liver fibrosis in a subject, comprising the steps of:

a) providing (i) the circulating levels of sVCAM and TSP-2 in a biological fluid sample isolated from said subject, and (ii) the age of the subject;

b) combining the provided levels of sVCAM and TSP-2 and the age in a mathematical function to assign a score SC; and

c) comparing the score SC with a score SB, which is obtained by combining levels of sVCAM, TSP-2 and the age previously measured in the same subject and with the same mathematical function.

6. The method according to claim 5, wherein:

- an increase of SC compared to SB indicates the progression of liver fibrosis;
- a decrease of SC compared to SB indicates the regression of liver fibrosis;
- no difference between SC and SB indicates a stable liver fibrosis.

7. The method according to claim 6, wherein SC is measured at least 3 months after the measurement of SB, particularly in a period between 3 months and 10 years, preferably in a period between 3 months and 2 years.

8. The method according to any one of claims 5 to 7, wherein SC or SB are calculated through the following mathematical function:

$$\text{SC or SB} = \frac{1}{1 + \exp(-y)}$$

with

$y = \beta0 + \beta1 * \log_{10}(\text{TSP2 (ng/mL)}) + \beta2 * \log_{10}(\text{sVCAM (ng/mL)}) + \beta3 * (\text{Age (years)}^3)$

wherein:

β0 is comprised between -40 and -14, in particular between -38 and -17;

β1 is comprised between 1.7 and 6.7, in particular between 2.2 and 6.3;

β2 is comprised between 3.0 and 12, in particular between 3.5 and 11.5;

β3 is comprised between $-6\ e^{-06}$ and $2.5\ e^{-6}$, in particular between $-5\ e^{-6}$ and $2\ e^{-6}$.

9. A method for assessing the efficacy of an anti-fibrotic agent in treating advanced liver fibrosis or liver cirrhosis, comprising:

a) providing (i) the circulating levels of sVCAM and TSP-2 in a biological fluid sample isolated from a subject suffering from advanced liver fibrosis, wherein said subject has been administered an anti-fibrotic agent before said measure, and (ii) the age of the subject;

b) combining the provided levels of sVCAM and TSP-2 and the age in a mathematical function to assign a score SE; and

c) comparing the score SE with a score SD, which is obtained by combining the levels of sVCAM and TSP-2 and the age, previously measured before administration of the anti-fibrotic agent to the same subject and with the same mathematical function.

10. The method according claim 9, wherein a decrease of SE compared to SD indicates the efficacy of the anti-fibrotic agent.

11. The method according claim 9 or 10, wherein SE and SD are calculated through the following mathematical function:

$$\text{SE or SD} = \frac{1}{1 + \exp(-y)}$$

with

$y = \beta0 + \beta1 * \log_{10}(\text{TSP2 (ng/mL)}) + \beta2 * \log_{10}(\text{sVCAM (ng/mL)}) + \beta3 * (\text{Age (years)}^3)$

wherein:

β0 is comprised between -40 and -14, in particular between -38 and -17;

β1 is comprised between 1.7 and 6.7, in particular between 2.2 and 6.3;

β2 is comprised between 3.0 and 12, in particular between 3.5 and 11.5;

β3 is comprised between $-6\ e^{-06}$ and $2.5\ e^{-6}$, in particular between $-5\ e^{-6}$ and $2\ e^{-6}$.

12. The method according to any one of claims 1 to 11, wherein the biological fluid sample is a saliva sample, an interstitial liquid sample, a urine sample, a blood sample, a plasma sample or a serum sample.

13. The method according to claim 12, wherein the biological fluid sample is a serum sample.

14. An anti-fibrotic agent for use in the treatment of advanced liver fibrosis or liver cirrhosis in a subject, wherein said subject is diagnosed as suffering from advanced liver fibrosis or liver cirrhosis according to the method of any one of claims 1 to 4, wherein said agent is selected from the group consisting of pegbelfermin, Cenicriviroc, Dapagliflozin, Dulaglutide, Empagliflozin, Fenofibrate, Lanifibranor, Liraglutide, obeticholic acid, Pioglitazone, Resmetirom, saroglitazar magnesium, Seladelpar, Semaglutide, Sitagliptin, TERN-101, TERN-201, Tropifexor, Ambrisentan, BMS-963272, BMS-986251, BMS-986263, HepaStem, LYS006, MET409, MET642 and orlistat.

15. An anti-fibrotic agent for use in the treatment of advanced liver fibrosis or liver cirrhosis in a subject, wherein said subject is diagnosed as suffering from advanced liver fibrosis or liver cirrhosis according to the method of any one of claims 1 to 4, wherein said agent is Resmetirom.

16. An *in vitro* method for prognosing the evolution of liver fibrosis in a subject with biopsy-proven stage 1, 2 or 3 liver fibrosis, comprising

a) providing (i) the circulating levels of sVCAM and TSP-2 in a biological fluid sample of said subject, and (ii) the age of the subject;

b) combining the provided levels of sVCAM and TSP-2 and the age in a mathematical function to assign a score SF; and

c) comparing said score with a cut-off value to prognose the evolution of liver fibrosis in said subject.

17. The method according to claim 16, wherein the score SF is obtained through the following mathematical function:

$$\mathrm{SF} = \frac{1}{1 + \exp(-y)}$$

with

$y = \beta 0 + \beta 1 * \log_{10}(\text{TSP2 (ng/mL)}) + \beta 2 * \log_{10}(\text{sVCAM (ng/mL)}) + \beta 3 * (\text{Age (years)}^3)$

wherein:

β0 is comprised between -40 and -14, in particular between -38 and -17;

β1 is comprised between 1.7 and 6.7, in particular between 2.2 and 6.3;

β2 is comprised between 3.0 and 12, in particular between 3.5 and 11.5;

β3 is comprised between -6 e^{-06} and 2.5 e^{-6}, in particular between -5 e^{-6} and 2 e^{-6}.

18. The method according to claim 16 or 17, wherein the SF score higher than a cut-off value is indicative of that the subject's liver fibrosis will progress; the SF score lower than the cut-off value is indicative of that the subject's liver fibrosis will regress; the SF score neither higher nor lower than the cut-off value is indicative of that the subject's liver fibrosis will be stable.

19. A computer assisted program comprising instructions that, when executed by a processor/processing means, cause the processor/processing means to:

- receive (i) levels of sVCAM, TSP-2 measured in a biological fluid sample isolated from a subject; and (ii) the age of the subject

- calculate a SA score from these measured levels, through a mathematical function; and
- assign the subject into the group of subjects having advanced liver fibrosis or liver cirrhosis upon the calculated score compared to predetermined cutoff values.

20. a computer program comprising instructions that, when executed by a processor/processing means, cause the processor/processing means to:
- receive (i) levels of sVCAM and TSP-2 measured in a biological fluid sample isolated from a subject and (ii) the age of the subject;
- calculate SC from these measured levels and the age, from a mathematical function as described above;
- compare the score SC with a score SB, which is obtained by combining levels of sVCAM and TSP-2 and the age previously measured in the same subject in the same mathematical function and
- assign the subject as :

having the progression of liver fibrosis when the score SC is higher than SB;

having the regression of liver fibrosis when the score SC is lower than SB; or

having stable liver fibrosis when there is no significant change between SC and SB.

21. A computer program comprising instructions that, when executed by a processor/processing means, cause the processor/processing means to:
- receive (i) levels of sVCAM and TSP-2 measured in a biological fluid sample isolated from a subject suffering from advanced liver fibrosis, wherein said subject has been administered an anti-fibrotic agent before the isolation of the biological fluid sample and (ii) the age of the subject;
- calculate a SE score from these measured levels and the age, from a mathematical function as described above;
- compare the score SC with a score SD, which is obtained by combining the levels of sVCAM and TSP-2 and the age, previously measured before administration of the anti-fibrotic agent to the same subject in the same mathematical function; and
- -assign the subject as :

a subject who does not respond to the treatment with the anti-fibrotic agent, when the score SE is higher than SD;

a subject who responds to the treatment with the anti-fibrotic agent, when the score SE is lower than SD ;.or

having stable liver fibrosis when there is no significant change between SE and SD.

22. A computer assisted program comprising instructions that, when executed by a processor/processing means, cause the processor/processing means to:

- receive (i) levels of sVCAM and TSP-2 measured in a biological fluid sample of a subject with biopsy-proven stage 1, 2 or 3 liver fibrosis; and (ii) the age of the subject
- calculate a SF score from these measured levels, through a mathematical function;
- compare the score SF with a predetermined cutoff value for score SF, and
- assign the subject as:

a subject whose liver fibrosis will progress when the SF score is higher than the cutoff value;

a subject whose liver fibrosis will regress when the SF score is lower than the cutoff value;

a subject whose liver fibrosis will be stable when the SF score is neither higher nor lower than the cutoff value.

23. The computer assisted program according to any one of claims 19 to 22, wherein the scores are calculated through the mathematical function as defined in claims 2, 8, 11 and 17.

24. A data-processing device comprising means for carrying the method of any one of claims 1 to 13, 16-18.

25. A computer program product comprising instructions which, when the program is executed by a computer, cause the computer to carry out the method of any one of claims 1 to 13, 16-18.

26. A computer-readable storage medium comprising instructions which, when executed by a computer, cause the computer to carry out the method of any one of claims 1 to 13, 16-18.