

METHODS FOR THE SCREENING OF PATIENTS WITH AT-RISK METABOLIC DYSFUNCTION-ASSOCIATED
STEATOHEPATITIS (MASH)

The present invention relates to a method for the screening of patients with at-risk MASH.

BACKGROUND

Metabolic dysfunction-associated steatohepatitis (MASH), formerly named nonalcoholic steatohepatitis or NASH, is a progressive condition that may lead to cirrhosis, hepatocellular carcinoma, and end-stage liver disease. It has now become a major indication for liver transplantation. Active steatohepatitis, defined histologically by a Metabolic dysfunction-associated steatotic liver disease (MASLD) Activity Score (MAS) ≥ 4 and significant fibrosis (stage F ≥ 2), is commonly referred to as at-risk MASH as it is associated with a higher risk of liver-related mortality, liver cancer, and progression to cirrhosis (Harrison SA, *et al.* 2020;5(11):970-985). The risk of liver-related mortality increases exponentially with increase in fibrosis stage and mortality and morbidity rates increase exponentially once cirrhosis develops.

In the absence of approved therapies, drug development for the treatment of at-risk MASH is an active area of research. Therefore, patients with at-risk MASH have to be acutely selected for clinical trials with histological endpoints. However, those with cirrhosis (F4) are excluded, as cirrhosis trials have specific designs and endpoints (Noureddin M, *et al.*, *Gastroenterology*. 2020;159:422-427).

Currently, inclusion of patients with at-risk MASH can only be performed through histopathological examination of a liver biopsy (LB) specimen. 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 since it is a needle biopsy and the liver tissue obtained from the subcapsular region is not representative of the whole liver. Therefore, a biopsy does not constitute a reliable prognostic predictor. In addition, the use of LB in clinical trials poses significant logistical challenges such as the cost of the procedure, and the important screening failures rate (Sumida Y *et al.*, *World J Gastroenterol* 2014; 20: 475-485). Therefore, a screening method is carried out to select patients to be elected to liver biopsy. Stringent inclusion and exclusion criteria of clinical trials

may lead to narrow enrollment of patients and to limit the number of patients sent to subsequent liver biopsy while improving the inclusion rate.

Indeed, one of the biggest challenges of MASH trials is the very high rate of screening failures on histological grounds, with LBs not confirming the clinical suspicion of at-risk MASH (Ratziu V, *et al.*, *J Hepatol.* 2022;76:1263-1278). Quite often, these results in LB do not confirm at-risk MASH histologically, with a consequent increase in the screening failure rate. The high screening failure rate does not only mean unnecessarily exposing many patients to the risks associated with LB, but also increases the number of patients to be screened and the overall cost of the trial. Furthermore, if a screening failure rate is consistently high, it also carries the risk of demobilizing the investigative screening sites for a particular trial. An important amount of liver biopsies to perform also requires the mobilization of on-site specialists. Consequently, when conducting MASH clinical trials, it is essential to identify accurately patients with non-cirrhotic at-risk MASH, who will then undergo a liver biopsy (LB) for histological confirmation. So far, MASH clinical trials do not have non-invasive strategy for selecting patients to refer for LB other than relying on clinical and biological features that are neither sensitive nor specific. Indeed, during Clinical Trials Screening, there is an important number of patients who are excluded after carrying out a Liver Biopsy. This scenario highlights a need to reduce liver biopsy failure rate (LBFR) to avoid associated risk and cost. There is thus a need to maximize the accuracy of the screening method to select subjects who are non-cirrhotic at-risk MASH to optimize referrals for LB.

In addition, since cirrhotic MASH patients are not the patients targeted by at-risk MASH treatments in development, it is also important and necessary to accurately diagnose non-cirrhotic at-risk MASH patients who will be receivers and responders of these treatments in real world setting, outside of clinical trial frame.

Therefore, there is still an unmet medical need to develop a method to identify non cirrhotic at-risk MASH patients, for referral to liver biopsy in MASH clinical trials aiming to reduce the number of unnecessary procedures, to reduce the liver biopsy failure rate (LBFR), to reduce the number of patients with cirrhosis referred for liver biopsy, to reduce the cost of the screening process without inflating the Number Needed to Screen (NNS), and more generally to provide a method to diagnose more accurately MASH patients with fibrosis stage F2 or F3.

SUMMARY OF THE INVENTION

By analyzing a cohort of patients suffering from MASH and liver fibrosis, the Inventors have showed that the combination of levels of five circulating markers, i.e. alanine aminotransferase (ALAT), aspartate transaminase (ASAT), platelets, hsa-miR-34a and YKL-40 (also named
5 CHI3L1) in a subject, and eventually the age and the gender of said subject, allows to accurately identify non-cirrhotic at-risk MASH.

The information obtained from the levels of these markers also allows to screen a subject as being eligible to liver biopsy, for example in the enrollment of MASH clinical trials, and/or to classify a subject as a receiver or non-receiver of a treatment of MASH, and/or to classify a
10 subject as a responder or non-responder of a treatment of MASH.

Therefore, a first aspect of the present invention relates to a method for the diagnosis of a subject with non-cirrhotic at-risk MASH, and/or for the screening of a subject as being eligible to liver biopsy, and/or for the classification of a subject as a receiver or non-receiver of a treatment of MASH, and/or the classification of a subject as a responder or non-responder of a treatment of
15 MASH.

The method of the present invention is based on the levels of ALAT, ASAT, platelets, hsa-miR-34a and YKL-40 in a biological fluid sample of a subject, and eventually the age and the gender of the subject.

More specifically, the method of the invention may comprise:

- 20 - providing the levels of ALAT, ASAT, platelets, hsa-miR-34a and YKL-40 in a biological fluid sample of a subject,
 - comparing (i) the levels of ALAT, ASAT, and platelets with reference levels obtained in samples from cirrhotic at-risk MASH subjects, and (ii) the levels of hsa-miR-34a and YKL-40 with reference levels obtained in samples from subjects without at-risk MASH.
- 25 In an embodiment, the method of the invention further comprises the determination of the age and the gender of the subject.

In a particular embodiment, the method of the invention comprises:

- providing the levels of ALAT, ASAT, platelets, hsa-miR-34a and YKL-40, in a biological fluid sample of said subject,

- comparing (i) the provided levels of ALAT, ASAT, and platelets with reference levels obtained in samples from cirrhotic at risk MASH subjects, and (ii) the provided levels of hsa-miR-34a and YKL-40 with reference levels obtained in samples from subjects without at-risk MASH,

5 wherein the provided levels of ALAT, ASAT, and platelets in the subject are combined with the age of the subject in a first mathematical function to calculate a first score, and

wherein the provided levels of hsa-miR-34a and YKL-40 in the subject are combined with the gender of the subject in a second mathematical function to calculate a second score.

In a particular embodiment of the method, the first score (S1) is compared with a first cut-off value (co1) and the second score (S2) is compared with a second cut-off value (co2),

10 wherein the co1 is calculated from the reference levels of ALAT, AST and platelets obtained in samples from cirrhotic at risk MASH subjects and the ages of said subjects, and the co2 is calculated from the reference levels of hsa-miR-34a and YKL-40 obtained in samples from subjects without at-risk MASH, and the genders of said subjects,

15 wherein a subject having the S1 lower than the co1 and the S2 higher than the co2 is identified as a subject with non-cirrhotic at-risk MASH, and/or a subject as being eligible to liver biopsy, and/or a subject as a receiver of a treatment for MASH; and/or a subject as a responder of a treatment for MASH.

In a more particular embodiment, the first score S1 is obtained from a mathematical function

20 defined as $S1 = \text{age (years)} \times \text{ASAT (U/L)} / \text{platelet (x } 10^9/\text{L)} / \sqrt{\text{ALT (U/L)}}$.

In a still more particular embodiment, the first cut-off value (co1) is comprised between 2 and 3, particularly comprised between 2.4 and 2.8, more particularly equal to 2.48 or 2.67. In a particular embodiment, co1 is equal to 2.48.

25 In another more particular embodiment, the second score S2 is obtained from a logistic function:

$$S2 = \frac{1}{1 + \exp(-y)}$$

wherein

$$y = \beta_0 + \beta_1 * \log_{10}(\text{miR-34a-5p (Fold)}) + \beta_2 * \log_{10}(\text{YKL-40 (ng/ml)}) + \beta_3 * \text{Gender} + \beta_4 * \log_{10}(\text{miR-34a-5p (Fold)}) * \text{Gender}$$

wherein

Gender is 0 if the subject is a female, or 1 if the subject is a male.

5 β_0 is comprised between -3 and 3, in particular between -2 and 2.

β_1 is comprised between 1 and 5, in particular between 2 and 4.

β_2 is comprised between 0 and 4.5, in particular between 0.5 and 3.

β_3 is comprised between -2 and 2, in particular between -1 and 1.

β_4 is comprised between -1 and 2, in particular between 0 and 2.

10 In a still more particular embodiment, the second cut-off value 2 (co2) is comprised between 0.4 and 0.8, particularly comprised between 0.5 and 0.7, more particularly equal to 0.53 or 0.68. In a particular embodiment, co2 is equal to 0.53.

In an embodiment, the biological fluid sample is a sample of blood or a blood-derived sample such as serum or plasma.

15 In another embodiment, the hsa-miR-34a measured in the method of the present invention is hsa-miR-34a-5p.

The invention also relates to a diagnostic kit, in particular a kit for diagnosing non-cirrhotic at-risk MASH subjects, and/or a subject as being eligible to liver biopsy, and/or for classifying a
20 subject as a receiver of a treatment for MASH; and/or for classifying a subject as a responder of a treatment for MASH. Said kit comprises means for determining the levels of ALAT, ASAT, Platelets, hsa-miR-34a-5p and YKL-40. In a particular embodiment, said kit comprises enzymatic reagents for measuring ALAT and ASAT, cell counters for measuring Platelets, antibodies directed against YKL-40 and primers and/or probes specific to hsa-miR-34a-5p.

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

- a) receive levels of ALAT, ASAT, Platelets, hsa-miR-34a, YKL-40, the age and the gender of the subject,

- b) calculate (i) a first score from the levels of ALAT, ASAT, Platelets and the age, and (ii) a second score from the levels of hsa-miR-34a and YKL-40 and the gender,
- c) compare (i) the calculated first score to a first cutoff value and (ii) the calculated second score to a second cutoff value ,
- 5 d) if the first score is lower than the co1 and the second score is higher than the co2, assign the subject into a group of non-cirrhotic at-risk MASH subjects, and/or a patient as being eligible to liver biopsy, and/or a subject classified as a receiver of a treatment for MASH, and/or a subject classified as a responder of a treatment for MASH; if the first score is higher than the co1 and/or the second score is lower than
- 10 the co2, the subject is not assigned into a group of non-cirrhotic at-risk MASH subjects, and/or is not a patient eligible to liver biopsy, and/or a subject classified as a receiver of a treatment for MASH, and/or a subject classified as a responder of a treatment for MASH.

15 FIGURES

Figure 1:

S1 score and S2 score were calculated in 1929 patients from Resolve-It® study according to the mathematical functions described above. “Cirrhotic” was defined as cirrhotic subjects (F=4). “At-risk MASH” was defined as non-cirrhotic MASH with MAS ≥ 4 and F =2 or 3. “Not-

20 at-risk MASH” was defined as subjects who are not diagnosed as at-risk MASH nor cirrhotic. Each point in the figure was defined by a S1 score and a S2 score obtained from a same subject. A cut-off value 0.53 was applied for S2 score . A cut-off value 2.48 was applied for S1 score. The position of each endpoint in the figure is defined by a S1 score and a S2 score. The distribution of the endpoint shows that the points corresponding to “at-risk MASH” are mainly

25 situated in the zone defined by S2 score ≥ 0.53 and S1 score ≤ 2.48 .

DETAILED DESCRIPTION OF THE INVENTION

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-

30 alcoholic fatty liver disease (NAFLD), is defined as a condition characterized by liver fat

accumulation in the presence of at least one of the following three metabolic conditions: overweight/obesity, T2DM, or at least two of seven metabolic risk abnormalities in those subjects who do not have T2DM and are lean by ethnic-specific body mass index (BMI) criteria: (1) waist circumference $\geq 102/88$ cm in Caucasian men and women (or $\geq 90/80$ cm in Asian men and women); (2) blood pressure $\geq 130/85$ mmHg or specific drug treatment; (3) plasma triglycerides ≥ 150 mg/dl (≥ 1.70 mmol/L) or specific drug treatment; (4) plasma high-density lipoprotein (HDL)-cholesterol < 40 mg/dl (< 1.0 mmol/L) for men and < 50 mg/dl (< 1.3 mmol/L) for women or specific drug treatment; (5) prediabetes (i.e., fasting glucose levels 100 to 125 mg/dl [5.6 to 6.9 mmol/L], or 2-h post-load glucose levels 140 to 199 mg/dl [7.8 to 11.0 mmol] or HbA1c 5.7% to 6.4% [39 to 47 mmol/mol]); (6) Homeostasis model assessment (HOMA) of insulin resistance score ≥ 2.5 ; and (7) plasma high-sensitivity C-reactive protein (CRP) level > 2 mg/L.

Histological scoring/staging systems have been developed to assess MASLD activity level and fibrosis stage and to estimate the risk of evolution to clinical liver outcomes. The MASLD-Activity-Score (MAS) has been developed to assess the severity of MASLD. MAS is the sum of three histological scores determined from liver biopsy slices:

- S: Steatosis score: 0: $<5\%$; 1: 5-33%; 2: 34-66% and 3: $>66\%$;
- LI: Lobular Inflammation score (foci per 20x field): 0: none; 1: <2 foci; 2: 2-4 foci and 3: >4 foci; and
- HB: Ballooning degeneration score: 0: none; 1: few; 2: many cells/prominent ballooning.

Using this scoring system, a "patient with MASH" has $MAS \geq 3$, with at least 1 point in steatosis, at least 1 point in lobular inflammation and at least 1 point in hepatocyte ballooning. A "non-MASH" patient is a patient having either (i) a $MAS \geq 3$ with at least one of steatosis, lobular inflammation and hepatocyte ballooning scores equal to 0; or (ii) a $MAS < 3$. In addition, in the context of the present invention, a patient is excluded as being a MASH patient if said patient has viral hepatitis, autoimmune liver disease, alcohol-related liver disease, drug-induced liver disease or congenital causes of chronic liver disease such as hereditary hemochromatosis, Wilson's disease, alpha-1-antitrypsin deficiency and polycystic ovary syndrome.

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.

5 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;
- 10 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.

Using this fibrosis staging system, patients with no or minimal fibrosis (F=0 or 1) are generally

15 not considered at risk of cirrhosis, liver failure, HCC (hepatocellular carcinoma) or liver-related death. Patients with significant (F=2) or severe (F=3) 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 "cirrhosis" or "liver cirrhosis" refers to a

20 fibrosis stage of F4.

In the context of the present invention, the terms "a subject with at-risk MASH", otherwise referred to as "an at-risk subject" or as "a subject at risk of hepatic outcome", is a patient with a MAS higher or equal to 4, a S score higher or equal to 1, a LI score higher or equal to 1, a HB score higher or equal to 1 and a F score of higher or equal to 2. It defines a subgroup of MASH

25 patients have a high risk of developing at least one life-threatening liver outcome such as cirrhosis for MASH patients with fibrosis F2 or F3, or even liver failure, HCC and liver-related death for MASH patients with fibrosis F4.

The term “a not at-risk MASH” refer to a subject with MASH who has a MAS lower than 4, and/or at least a S score, a LI score or a HB score lower than 1, and/or a F score equal to 0 or 1.

5 The terms “a subject with non-cirrhotic at-risk MASH”, “a non-cirrhotic at-risk MASH subject” and “a subject as being eligible to liver biopsy” both refer to a subject with a MAS higher or equal to 4, a S score higher or equal to 1, a LI score higher or equal to 1, a HB score higher or equal to 1 and a F score equal to 2 or 3.

The term “a cirrhotic at-risk MASH subject” refers to an at-risk MASH subject with liver cirrhosis (F4).

10 The terms “subject” and “patient” may be used interchangeably herein and refer to a human subject.

The expression “screening”, as used herein, refers to the selection of a patient to be referred to liver biopsy or not be referred to liver biopsy in a cohort or in a clinical study. The screening of patients may imply a diagnostic step and then the distribution of the patient in a group to be referred to biopsy for confirmation or not to be referred.

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

Within the context of the present invention, the term “Liver Biopsy Failure Rate (LBFR)” refers to the proportion of patients carrying out a negative (not-At-Risk MASH or cirrhotic) Liver Biopsy among patients who underwent a Liver Biopsy.

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The term “Liver Biopsy Failure Rate global (LBFR global)” corresponds to the proportion of Liver Biopsy Failure among all screened patients ($LBFR_{global} = LBF / NNS$).

The term “Number Needed to Screen (NNS)” corresponds to the number of patients to Screen in order to include 1000 patients in the Clinical Trial.

25 The term “Liver Biopsy Failure number (LBFn)” is the number of patients carrying out a negative (not-At-RiskMASH or cirrhotic) Liver Biopsy.

The term “Liver Biopsy Failure avoided” is the number of patients excluded with a Non-Invasive Test who would have carried out a negative (not-At-Risk MASH or cirrhotic) Liver Biopsy with Usual Screening pathway.

The term “Positive Liver Biopsy” refers to a liver biopsy that confirms the at-risk MASH phenotype.

METHOD OF THE INVENTION

It is herein shown that the levels of five circulating markers provide information for accurate
5 diagnosis of non-cirrhotic at-risk MASH patients. The information provided by these five
circulating markers also allows to accurately identify the MASH patients eligible to biopsy, for
example in the context of MASH clinical trials and thus decrease liver biopsy failure rate. Said
information can also be used to assign the patients in a group of patients who will receive a
treatment targeting non-cirrhotic at-risk MASH or in a group of patients who will not receive
10 said treatment. Based on said information, the MASH patients who will respond to a treatment
targeting non-cirrhotic at-risk MASH or who will not respond to said treatment can also be
more accurately predicted.

More specifically, by comparing the levels of these 5 markers with reference levels of these
markers obtained from subjects diagnosed as cirrhotic at-risk MASH subjects or not at-risk
15 subjects, the method of the invention distinguishes non-cirrhotic at-risk MASH subjects from
cirrhotic at-risk MASH subjects and not at-risk subjects.

The measure of ALAT, ASAT, Platelets, hsa-miR-34a and YKL-40 can be conducted thanks
to methods well-known in the art.

Illustrative methods for measuring circulating ALAT and ASAT include without limitation,
20 clinical measurement, cell culture methods, and enzymatic assays. For example, the circulating
levels of ALAT and ASAT may be measured by a blood test, such as a liver function test or
liver enzyme test.

The circulating levels of platelets may be measured by medical imaging or a blood test, such as
a complete blood count.

25 The circulating levels of YKL-40 may be measured by any conventional method 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 YKL-40 can be determined by antibodies, aptamers or peptides respectively
directed against said markers from the serum. The level of YKL-40 can be measured using a
30 YKL-40-specific binding agent, such as an anti- YKL-40 antibody. For example, we can cite

methods based on immunodetection, such as ELISA (enzyme-linked immunosorbent assay). In this respect, commercial kits for YKL-40 level determination are readily available, such as the Quantikine® Chitinase 3-like 1 Immunoassay (ref.: DC3L10, R&D Systems, Inc.).

5 The circulating hsa-miR-34a may be measured by common technique used in molecular biology such as RNA extraction, reverse transcription and quantitative PCR with specific primers and/or probes from the serum.

The measure of the levels of these markers is conducted in a biological fluid sample of the subject, such as a sample of blood or a blood-derived sample, e.g. serum or plasma, or a sample of saliva, interstitial fluid or urine. In a more particular embodiment, the biological fluid sample
10 of the subject is a sample of blood or a blood-derived sample such as serum or plasma.

In an embodiment, (i) the levels of ALAT, ASAT and platelets in a biological fluid sample of a subject are compared with reference levels obtained in samples from cirrhotic at risk MASH subjects and (ii) the levels of hsa-miR-34a and YKL-40 in the same sample are compared with reference levels obtained in samples from subjects without at-risk MASH. It should be
15 understood that comparison (i) may be performed before, after or simultaneously with comparison (ii).

In a particular embodiment, the reference levels of ALAT, ASAT and platelets are measured in samples from cirrhotic at-risk MASH subjects who are diagnosed by a conventional method and are confirmed by liver biopsy. The reference levels of hsa-miR-34a and YKL-40 are
20 measured in samples from subjects without at-risk MASH who are diagnosed by a conventional method.

It should be understood that in all embodiments and variants disclosed herein, hsa-miR-34a can be more particularly hsa-miR-34a-5p.

In a particular embodiment, the method of the invention further comprises the determination of
25 the age and the gender of the subject.

The levels of these markers and the age and/or the gender of the subject can be used to develop mathematical functions.

In a particular embodiment, the method comprises:

- the provided levels of ALAT, ASAT and platelets are combined with the age in a first mathematical function to calculate a first score, and

- the provided levels of hsa-miR-34a and YKL-40 are combined with the gender in a second mathematical function to calculate a second score.

- 5 In an embodiment of the invention, the first score (S1) is compared with a first cut-off value (co1), and the second score (S2) is compared with a second cut-off value (co2), wherein the co1 is calculated from the reference levels of ALAT, ASAT and platelets obtained in samples from cirrhotic at risk MASH subjects and the ages of said subjects, and the co2 is calculated from the reference levels of hsa-miR-34a and YKL-40 obtained in samples from subjects without at-risk MASH, and the genders of said subjects.
- 10

The first cut-off (co1) is calculated by combining the reference levels of ALAT, ASAT, platelets and the age in the first mathematical function. The second cut-off is calculated by combining the reference levels of hsa-miR-34a and YKL-40, and the gender in the second mathematical function.

- 15 The co1 is the reference value for determining if a subject is cirrhotic. By comparing the S1 with co1, a subject can be assigned in a group of cirrhotic subjects or a group of non-cirrhotic subjects. Specifically, a subject with the first score higher than the first cut-off is determined as a cirrhotic subject and/or not eligible to liver biopsy, while a subject with the first score lower than the first score is determined as a non-cirrhotic subject.

- 20 The co2 is the reference value for determining if a subject has at-risk MASH. By comparing the S2 with co2, a subject can be assigned in a group of at-risk MASH subjects or a group of not at-risk MASH subject. A subject with the second score higher than the second cut-off is determined as an at risk MASH subject, while a subject with the second score lower than the second cut-off is determined as a not at risk subject.

- 25 In a particular embodiment, a subject having the S1 lower than the co1 and the S2 higher than the co2 is identified as a non-cirrhotic at-risk MASH subject, and/or as a subject being eligible to liver biopsy, and/or as a receiver of a treatment for MASH; and/or as a responder of a treatment for MASH.

- When a subject having the S1 higher than the co1 or the S2 lower than the co2, said subject is
- 30 excluded.

In a more particular embodiment, the S1 is a score obtained by combining the levels of ASAT, ALAT and platelet measured in a biological fluid sample of a subject in a mathematical function. In another more particular embodiment, the S1 is a score obtained by combining the levels of ASAT, ALAT and platelet measured in a biological fluid sample of a subject and the age of said subject in a mathematical function.

In a still more particular embodiment, the S1 is obtained from a mathematical function defined as $S1 = \text{age (years)} \times \text{ASAT (U/L)} / \text{platelet} (\times 10^9/\text{L}) / \sqrt{\text{ALAT (U/L)}}$.

In a further particular embodiment, co1 is a value comprised between 2 and 3. In a more particular embodiment, co1 is comprised between 2.4 and 2.8. More particularly, co1 may be equal to 2.48 or 2.67. In a particular embodiment, co1 is equal to 2.48.

A S1 score of a subject higher than a cut-off value 1 (co1) is indicative of that said subject is cirrhotic and is not eligible to liver biopsy.

A S1 score of a subject lower than a cut-off value 1 (co1) is indicative of that said subject has a non-cirrhotic MASH.

In another more particular embodiment, the S2 is a score obtained by combining the levels of hsa-miR-34a and YKL-40 measured in a biological fluid sample of a subject in a mathematical function. In another more particular embodiment, the S2 is a score obtained by combining the levels of hsa-miR-34a and YKL-40 measured in a biological fluid sample of a subject and the gender of said subject in a mathematical function, for example that described in PCT/EP2023/059290.

In a particular preferable embodiment, the S2 is defined as a logistic function.:

$$S2 = \frac{1}{1 + \exp(-y)}$$

wherein

$y = \beta_0 + \beta_1 \cdot \log_{10}(\text{miR-34a-5p (Fold)}) + \beta_2 \cdot \log_{10}(\text{YKL-40 (ng/ml)}) + \beta_3 \cdot \text{Gender} + \beta_4 \cdot \log_{10}(\text{miR-34a-5p (Fold)}) \cdot \text{Gender}$

wherein

Gender is 0 if the subject is a female, or 1 if the subject is a male.

B0 is comprised between -3 and 3, in particular between -2 and 2.

B1 is comprised between 1 and 5, in particular between 2 and 4.

B2 is comprised between 0 and 4.5, in particular between 0.5 and 3.

B3 is comprised between -2 and 2, in particular between -1 and 1.

5 B4 is comprised between -1 and 2, in particular between 0 and 2.

In a more particular embodiment, the logistic function is derived from a generalized linear model.

In a further particular embodiment, co2 is a value comprised between 0.4 and 0.8. In a particular embodiment, co2 is comprised between 0.5 and 0.7. More particularly co2 may be 0.53 or 0.68.

10 In a particular embodiment, co2 is equal to 0.53.

A S2 score of a subject higher than a cut-off value 2 (co2) is indicative of that said subject has at-risk MASH.

A S2 score of a subject lower than a cut-off value 2 (co2) is indicative of that said subject a non-at-risk MASH patient, who is not eligible to liver biopsy.

15 In a particular embodiment, the method comprises the step of comparing :

- (i) the S1 score of a subject with a cut-off value co1 comprised between 2 and 3, in particular between 2.4 and 2.8, more particularly equal to 2.48 or 2.67, advantageously equal to 2.48; and
- (ii) the S2 score of the subject with a cut-off value co2 comprised between 0.4 and 0.8,
20 particularly between 0.5 and 0.7, more particularly equal to 0.53 or 0.68, advantageously equal to 0.53,

wherein a subject having the S1 score lower than the co1 and the S2score higher than the co2 is identified as a subject with non-cirrhotic at-risk MASH, and/or a subject as being eligible to liver biopsy, and/or a subject as a receiver of a treatment for MASH; and/or a subject as a
25 responder of a treatment for MASH.

It should be understood that the comparison (i) may be performed before, after or simultaneously with the comparison (ii).

The invention also relates to a kit comprising means for determining the levels of ALAT, ASAT, Platelets, hsa-miR-34a-5p and YKL-40. In a particular embodiment, said kit comprises

enzymatic reagents for measuring ALAT and ASAT, cell counters for measuring Platelets, antibodies directed against YKL-40 and primers and/or probes specific to hsa-miR-34a-5p.

The kit may also comprise reactional buffers and any other reagents necessary for measuring the levels of ALAT, ASAT, Platelets, hsa-miR-34a-5p and YKL-40.

- 5 Said kit may be used for diagnosing non cirrhotic at-risk MASH, and/or determining a subject as being eligible to liver biopsy, and/or a subject as a receiver of a treatment for MASH; and/or a subject as a responder of a treatment for MASH.

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
10 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 MASH therapy and/or liver fibrosis therapy to the patient, to treat at-risk MASH or advanced liver fibrosis.

According to another aspect, the invention relates to a computer program comprising
15 instructions that, when executed by a processor/processing means, cause the processor/processing means to:

- a) receive levels of ALAT, ASAT, Platelets, hsa-miR-34a, YKL-40, the age and the gender of the subject,
- b) calculate (i) a first score from the levels of ALAT, ASAT, Platelets and the age, and
20 (ii) a second score from the levels of hsa-miR-34a and YKL-40 and the gender,
- c) compare (i) the calculated first score to a first cutoff value and (ii) the calculated second score to a second cutoff value,
- d) if the first score is lower than the co1 and the second score is higher than the co2, assign the subject into a group of non-cirrhotic at-risk MASH subjects, and/or a
25 patient eligible to liver biopsy, and/or a subject classified as a receiver of a treatment for MASH, and/or a subject classified as a responder of a treatment for MASH; if the first score is higher than the co1 and/or the second score is lower than the co2, the subject is not assigned into a group of non-cirrhotic at risk MASH subjects, and/or a patient eligible to liver biopsy, and/or a subject classified as a receiver of a
30 treatment for MASH, and/or a subject classified as a responder of a treatment for MASH.

It should be understood that in step b) of the above computer program, the calculation (i) may be performed before, after or simultaneously with the calculation (ii) and that in step c) of said computer program, the comparison (i) may be performed before, after or simultaneously with the comparison (ii).

In a particular embodiment, the first score and the second score are calculated according to the mathematical functions described above, respectively.

In another particular embodiment, the cut-off value $co1$ is comprised between 2 and 3, in particular between 2.4 and 2.8, more particularly equal to 2.48 or 2.67, advantageously equal to 2.48.

In another particular embodiment, the cut-off value $co2$ is comprised between 0.4 and 0.8, particularly between 0.5 and 0.7, more particularly equal to 0.53 or 0.68, advantageously equal to 0.53.

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. Suitable media are RAM, CD-ROM, or disk drives.

The present invention also relates to a computer readable recording medium storing instructions for executing the method of the present invention.

In a particular embodiment, the computer readable recording medium comprises instructions for executing the steps a) to d) of the above-described computer program.

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

EXAMPLES

Example 1: NITs as a screening method for identification of patients for referral to liver biopsy in MASH clinical trials.

Prior to liver biopsy to screen at-risk MASH patients in a clinical trial, Resolve-IT® study, the levels of ALAT, ASAT, Platelets, hsa-miR-34a, YKL-40, the age and the gender of the subjects were recorded to obtain a S1 score and a S2 score.

- 5 The S1 score was calculated according to the following formula: $S1 = \text{age (years)} \times \text{ASAT (U/L)} / \text{platelet (x } 10^9/\text{L)} / \sqrt{\text{ALAT (U/L)}}$.

The S2 score was calculated according to the following formula.:

$$S2 = \frac{1}{1 + \exp(-y)}$$

wherein

- 10 $y = \beta_0 + \beta_1 * \log_{10}(\text{miR-34a-5p (Fold)}) + \beta_2 * \log_{10}(\text{YKL-40 (ng/ml)}) + \beta_3 * \text{Gender} + \beta_4 * \log_{10}(\text{miR-34a-5p (Fold)}) * \text{Gender}$

wherein

Gender is 0 if the subject is a female, or 1 if the subject is a male.

B0 is comprised between -3 and 3, in particular between -2 and 2.

- 15 B1 is comprised between 1 and 5, in particular between 2 and 4.

B2 is comprised between 0 and 4.5, in particular between 0.5 and 3.

B3 is comprised between -2 and 2, in particular between -1 and 1.

B4 is comprised between -1 and 2, in particular between 0 and 2.

- 20 Resolve-IT® is a randomized double-blind, placebo-controlled multicentre international phase 3 clinical trial (NCT02704403). During the screening window, clinical data, blood samples and liver biopsy results were collected for all patients. All liver biopsies were scored based on the MASH CRN (Clinical Research Network) classification scoring. Demographic and anthropometric characteristics (including bodyweight, age, sex and BMI), and medical history
25 (Type-2 diabetes, dyslipidemia and hypertension) were collected at clinical sites from patients' medical records. Haematology and biochemical analyses were done by the central laboratory of the Resolve-IT® trial (BARC-Europe, Ghent, Belgium). These data allowed the calculation of S2 score and S1 score.

Patients with available S1 and S2 scores, demographic and anthropometric data, and liver biopsies were analysed. Table 1 shows distribution of demography, comorbidity, blood dosage factors, histology and NITs values among the three groups of interest: not At-Risk MASH (patients who should not be included), At-Risk MASH (patients who should be included) and cirrhotic (patients who should not undergo a Liver Biopsy). The prevalence of at-risk MASH (without cirrhosis) was 40%. Descriptive results are shown through means $\pm$ SD for numeric variables and proportion (sample size) for binary variables.

The comparison of not at-risk MASH group with at-risk MASH and cirrhotic patients in Table 1 demonstrates that both S1 and S2 values increase in the At-Risk MASH group: S2 score increases from 0.4 $\pm$ 0.24 in not at-risk MASH up to 0.7 $\pm$ 0.21 in at-risk MASH and 0.71 $\pm$ 0.21 in cirrhotic patients; S1 score increases from 1.11 $\pm$ 0.66 in not at-risk MASH up to 1.58 $\pm$ 0.95 in at-risk MASH and 2.29 $\pm$ 1.1 in cirrhotic patients.

	Not-At-Risk MASH	At-Risk MASH ^a	Cirrhotic
	(n=1066)	(n=765)	(n=98)
Demographics			
Sex, male	64 (682)	56 (425)	44 (43)
Age (years)	53 $\pm$ 12	54 $\pm$ 12	58 $\pm$ 9
Clinical characteristics			
T2DM	34 (360)	48 (365)	64 (63)
Dyslipidemia	46 (487)	50 (385)	42 (41)
Arterial hypertension	52 (550)	60 (458)	69 (68)
BMI (kg/m ²)	33.39 $\pm$ 6.18	34.10 $\pm$ 6.09	33.61 $\pm$ 5.99
Biochemistry			
ASAT (IU/L)	31.06 $\pm$ 19.19	54.4 $\pm$ 36.22	56.41 $\pm$ 38.73
ALAT (IU/L)	44.72 $\pm$ 32.36	75.17 $\pm$ 53.89	61.20 $\pm$ 44.75
GGT (IU/L)	57.89 $\pm$ 71.21	85.11 $\pm$ 102.53	140.95 $\pm$ 144.82
FPG (mmol/L)	5.64 $\pm$ 1.50	6.27 $\pm$ 1.96	7.14 $\pm$ 2.78
HbA1c (%)	6.03 $\pm$ 0.94	6.39 $\pm$ 1.09	6.73 $\pm$ 1.21
Triglycerides (mmol/L)	1.90 $\pm$ 1.07	2.04 $\pm$ 1.08	1.74 $\pm$ 0.79
Platelets (10e9/L)	249.04 $\pm$ 69.04	237.78 $\pm$ 65.53	201.66 $\pm$ 68.58
Histology			
F (0/1/2/3/4), %	28.0/54.0/6.6/11.4/0	0/0/47.8/52.2/0	0/0/0/0/100

MAS (0-1/2-3/4-5/6-8), %	25.2/36.3/30.9/7.6	0/0/44.7/55.3	12.2/14.3/37.8/35.7
NITs			
S2 score	0.40 ± 0.24	0.70 ± 0.21	0.71 ± 0.21
S1 score	1.11 ± 0.66	1.58 ± 0.95	2.29 ± 1.10

Table 1 Descriptive results : patients characteristics of analysis cohort.

Values are expressed as mean ± SD or % (n), unless otherwise noted.

a:Without cirrhosis (F4). BMI, body mass index; F, fibrosis stage; FPG, fasting plasma glucose; GGT, gamma-glutamyl transferase; HbA1c, hemoglobin A1c; MAS, MASLD activity score; MASH, metabolic-associated steatohepatitis; NITs, non-invasive tests; T2DM, type 2 diabetes mellitus

The comparison of S2 and S1 scores was also analysed to compare patients between the three categories: not at-risk MASH, non cirrhotic at-risk MASH and cirrhotic. The aim was to assess the discrimination between categories by the scores.

On the one hand, it was observed that when using a high cut-off score (0.7), S2 score discriminates efficiently between at-risk MASH patents and not at-risk MASH patients. S2 score is therefore suitable to rule-in patients with At-Risk MASH in clinical study.

On the other hand, it was observed that S1 score is significantly higher in cirrhotic patients than in at-risk MASH patients (means of 2.29 vs 1.58, p<0.0001), suggesting S1 score is suitable for ruling-out F4 patients.

Example 2: Screening of patients for inclusion in MASH clinical trial with S2 score.

Patients included in MASH clinical trial are non cirrhotic at-risk MASH patients.

The screening method based on the S2 score of the patients was therefore compared to the usual screening pathway (USP) in Resolve-iT® study. In this usual screening pathway of patients for MASH clinical trials, patients are selected by investigators in each center, based on risk factors (e.g. type-2 diabetes, obesity, Metabolic Syndrome). If these criteria are positive for inclusion, blood dosages of the patient are performed and aliquots are kept for potential NIT measurement. S2 score of patients is calculated according to the mathematical formula described in Example

1. In the screening method based on S2 score, patients having a S2 score below a pecific cut-

off are excluded from liver biopsy. Patients above this specific cut-off are targeted for Liver Biopsy before inclusion in the Clinical Trial.

Performance criteria linked to Liver Biopsy Failure of Usual Screening pathway vs the screening method based on S2 score with cut-offs of interest are reported in Table 2. Usual Screening pathway suffers from the high LBFR (60.34 %) resulting in more than half of patients carrying out a Liver Biopsy being not At-Risk MASH or cirrhotic (1522 out of 2522). It is also observed that in order to have 1000 confirmed at-risk MASH patients to be included in the clinical trial, usual screening pathway need to screen 2522 patients, while using the screening method based on S2 score with a cut-off value at 0.6815, only 1455 patients are screened to have 1000 confirmed at-risk MASH patients. Said screening method with cut-offs of interest (0.4564 , 0.5328 , 0.6815) allows to avoid respectively 777 (-51%), 890 (-58%), 1067 (-70%) Liver Biopsy Failures for 1000 patients to include.

		screening method based on S2 score		
	Usual Screening Pathway (USP)	Cut-off = 0.4564 (low)	Cut-off = 0. 5328	Cut-off = 0.6815 (high)
Total liver biopsies	2522	1745	1632	1455
Liver Biopsy Failure	1522	745	632	455
Liver biopsy failure rate	0.6034	0.427	0.3872	0.3129
Liver Biopsy Failure avoided vs USP	0	777 (-51%)	890 (-58%)	1067 (-70%)

Table 2: Liver Biopsy Failure reduction with S2 score. Positive Liver Biopsies were set at 1000.

Table 2 shows that screening method based on S2 score improves liver biopsy failure rate (LBFR) in MASH clinical trials. Said screening method provides an improvement to rule-in patients in MASH clinical trials versus usual screening pathway.

Example 3 Screening of patients to liver biopsy in MASH clinical trials by the screening method with S1 and S2 scores.

The selection of patients to be included in MASH clinical trial requires the accurate identification of patients to be enrolled. The aim to reduce LFBR also comprises reducing the number of cirrhotic patients to avoid associated risk with these specific patients and the associated cost.

In order to compare the usual screening pathway (USP) with the screening method of the invention based on S1 and D2 scores, estimations were performed with respect to proportions of patients who would have ended up in each exclusion/inclusion group at the end of the screening. 1929 patients with available S1 and S2 scores, demographic and anthropometric data, and liver biopsies were analysed.

The potential of the screening method of the invention before sending patients to liver biopsy was analyzed and compared to the USP.

For cost estimation, In Resolve-IT®, 4 groups of costs generated invoices: Screening Visit procedures, S1 and S2 scores measurement, Liver Biopsy and Liver Biopsy preparation.

The results are shown in Table 3.

	Resolve-IT Screening Pathway (RSP)	S1 and S2 screening method			
S1 cutoff	-	<2.67	<2.48	<2.67	<2.48
S2 cutoff	-	≥0.53	≥0.53	≥0.68	≥0.68
Performances					
Sensitivity	-	0.78	0.77	0.60	0.59
Specificity	-	0.69	0.69	0.83	0.84
LR+	-	2.5	2.5	3.6	3.7
LR-	-	0.3	0.3	0.5	0.5
NNS	3220	4630	4792	6023	6252
LBs performed, n	2522	1648	1646	1450	1442
LBFR (n)	60% (1522)	39 % (648)	39 % (646)	31% (450)	31% (442)
Failures avoided vs RSP, n (% reduction)	-	874 (- 57%)	876 (- 58%)	1072 (- 70%)	1080 (- 71%)
Number of cirrhotic patients referred for LB	128	98 (- 23%)	89 (- 30%)	93 (- 27%)	81 (-37%)

Total cost of screening (\$US M)	15.0	13.6 (-9%)	13.9 (-7%)	14.9 (-1%)	15.2 (+1%)
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Table 3: Performances with selected cutoffs versus the Resolve Screening Procedure.

Sensitivity: the proportion of positive patients (non-cirrhotic at-risk MASH) detected by the tested method during patients referral before Liver Biopsy; Specificity: the proportion of negative patients (either not-at-risk MASH or cirrhotic) detected by the tested method during patients referral before Liver Biopsy; LBFR: Liver Biopsy Failure Rate; NNS: Number of patients Needed to Screen; LB: Liver Biopsy; RSP, RESOLVE-IT screening pathway, LR+: positive likelihood ratio, which is defined as the probability of a person who has the disease testing positive (non-cirrhotic at-risk MASH) divided by the probability of a person who does not have the disease testing positive (either not-at-risk MASH or cirrhotic); LR-: negative likelihood ratio, which is defined as the probability of a person who has the disease testing negative (non-cirrhotic at-risk MASH) divided by the probability of a person who does not have the disease testing negative (either not-at-risk MASH or cirrhotic); Positive liver biopsies were set at 1000.

The LBFR decreases from 60% with the RSP pathway to 39% with the method of the invention with S2 cut-off set at 0.53 and S1 cut-off set at 2.48 or 2.67. The Liver Biopsy Failure Rate could be further reduced by using higher S2 cut-off value (0.68), but at the cost of an increased Number of patients Needed to Screen (NNS) from 4630 patients (cut-off S1 < 2.67, cut-off S2 ≥ 0.53) and 4792 patients (cut-off S1 < 2.48, cut-off S2 ≥ 0.53) up to 6023 patients (cut-off S1 < 2.67, cut-off S2 ≥ 0.68) and 6252 patients (cut-off S1 < 2.48, cut-off S2 ≥ 0.68).

The screening method with the combination of S1 score and S2 score, with a S1 cut-off < 2.48 and a S2 cut-off ≥ 0.53 achieved a greater reduction in the number of cirrhotic patients referred for Liver Biopsy (-30%) and a 7% decreased cost compared to the usual screening pathway (USP) in Resolve-iT® study.

To demonstrate that this screening pathway method does not introduce bias in the selection of patients included in MASH clinical trial, patient characteristics were compared between the screening pathways: RSP and the screening methods with S1 and S2 scores (Table 4).

	RSP	S1 and S2screening method
S1 cutoff	-	<2.48
S2 cutoff	-	≥0.53
Age (years)	54 ± 12	53 ± 12
T2DM	47.7	47.9
Sex, male	55.6	54.7
F ≥3	52.2	54.1
MAS ≥6	55.3	58.6

Table 4 Characteristics of patients included with NITS screening methods/

Values are expressed as mean ± SD, or %. F, fibrosis stage; MAS, metabolic dysfunction-associated steatotic liver disease (MASLD) activity score; RSP, RESOLVE-IT screening pathway; T2DM, type 2 diabetes mellitus

In conclusion, in clinical trials the method of the invention has the potential to significantly improve the recruitment process by reducing significantly the Liver Biopsy Failure Rate, increasing the number of positive liver biopsy, decreasing the number of F4 patients referred to Liver Biopsy and reducing the screening cost with a manageable Number of patients Needed to be Screened. No selection bias was observed in patients included with the sequential pathway vs the Resolve-it® Standard Procedure regarding Type 2 Diabetes Mellitus prevalence, percentage of males, or histology which represent randomization parameters at inclusion.

The method of the invention results in a decrease in the number of cirrhotic patients referred for LB, with a better homogeneity in the profile of patients included and a manageable Number of patients Needed to be Screened.

CLAIMS

1. A method for the diagnosis of a subject with non-cirrhotic at-risk MASH, and/or for the screening of a subject as being eligible to liver biopsy, and/or for the classification of a subject as a receiver or non-receiver of a treatment for MASH, and/or for the classification of a subject as a responder or non-responder of a treatment for MASH, wherein said method comprises:
 - providing the levels of alanine aminotransferase (ALAT), aspartate transaminase (ASAT), platelets, hsa-miR-34a and YKL-40 in a biological fluid sample of said subject,
 - comparing the levels of ALAT, ASAT and platelets with reference levels obtained in samples from cirrhotic at risk MASH subjects and the levels of hsa-miR-34a and YKL-40 with reference levels obtained in samples from subjects without at-risk MASH.
2. The method according to claim 1, wherein the method is for the screening of a subject as being eligible to liver biopsy in MASH clinical trials.
3. The method according to claim 1 or 2 further comprising the determination of the age and the gender of the subject.
4. The method according to claim 3, wherein the provided levels of ALAT, ASAT, platelets and the age are combined in a first mathematical function to calculate a first score (S1) and, wherein the provided levels of hsa-miR-34a and YKL-40, and the gender are combined in a second mathematical function to calculate a second score (S2).
5. The method according to claim 4, wherein the first score is compared with a first cut-off value (co1), and the second score is compared with a second cut-off value (co2), wherein the co1 is calculated from the reference levels of ALAT, ASAT and platelets obtained in sample from cirrhotic at risk MASH subjects and the ages of said subjects, and the co2 is calculated from the reference levels of hsa-miR-34a and YKL-40 obtained in samples from subjects without at-risk MASH and the genders of said subjects,

wherein a subject having the first score lower than the $co1$ and the second score higher than the $co2$ is identified as a subject with non-cirrhotic at risk MASH, and/or a subject as being eligible to liver biopsy, and/or a subject as a receiver of a treatment for MASH; and/or a subject as a responder of a treatment for MASH.

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6. The method according to claim 4 or 5, wherein the first score ($S1$) is obtained from a mathematical function defined as $\text{age (years)} \times \text{ASAT (U/L)} / \text{platelet (x } 10^9/\text{L)} / \sqrt{\text{ALAT(U/L)}}$.

7. The method according to claim 6, wherein the first cut-off is comprised between 2 and 3, particularly comprised between 2.4 and 2.8, more particularly equal to 2.48 or 2.67, still more particularly equal to 2.48.

8. The method according to claim 4 or 5, wherein the second score ($S2$) is obtained from a logistic function:

$$S2 = \frac{1}{1 + \exp(-y)}$$

wherein

$$y = \beta_0 + \beta_1 \cdot \log_{10}(\text{miR-34a-5p (Fold)}) + \beta_2 \cdot \log_{10}(\text{YKL-40 (ng/ml)}) + \beta_3 \cdot \text{Gender} + \beta_4 \cdot \log_{10}(\text{miR-34a-5p (Fold)}) \cdot \text{Gender}$$

20 wherein

Gender is 0 if the subject is a female, or 1 if the subject is a male.

β_0 is comprised between -3 and 3, in particular between -2 and 2.

β_1 is comprised between 1 and 5, in particular between 2 and 4.

β_2 is comprised between 0 and 4.5, in particular between 0.5 and 3.

25 β_3 is comprised between -2 and 2, in particular between -1 and 1.

β_4 is comprised between -1 and 2, in particular between 0 and 2.

9. The method according to claim 8, wherein the second cut-off is comprised between 0.4 and 0.8, particularly between 0.5 and 0.7, more particularly equal to 0.53 or 0.68, still more particularly equal to 0.53.

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10. The method according to any one of claims 1 to 9, wherein the biological fluid sample is a sample of blood or of a blood-derived sample such as serum or plasma.
11. The method according to any one of claims 1-10, wherein hsa-miR-34a is hsa-miR-34a-5p.
12. A kit comprising means for determining the levels of ALAT, ASAT, Platelets, hsa-miR-34a-5p and YKL-40.
13. The kit according to claim 12, comprising enzymatic reagents for measuring ALAT and ASAT, cell counters for measuring Platelets, antibodies directed against YKL-40 and primers and/or probes specific to hsa-miR-34a-5p.
14. A computer program comprising instructions that, when executed by a processor/processing means, cause the processor/processing means to:
- a) receive levels of ALAT, ASAT, Platelets, hsa-miR-34a, YKL-40, the age and the gender of the subject,
 - b) calculate (i) a first score from the levels of ALAT, ASAT, Platelets and the age, and (ii) a second score from the levels of hsa-miR-34a and YKL-40 and the gender,
 - c) compare (i) the calculated first score to a first cutoff value and (ii) the calculated second score to a second cutoff value,
 - d) if the first score is lower than the co1 and the second score is higher than the co2, assign the subject into a group of non-cirrhotic at-risk MASH subjects, and/or a patient eligible to liver biopsy, and/or a subject classified as a receiver of a treatment for MASH, and/or a subject classified as a responder of a treatment for MASH; if the first score is higher than the co1 and/or the second score is lower than the co2, the subject is not assigned into a group of non-cirrhotic at-risk MASH subjects, and/or is not a patient eligible to liver biopsy, and/or a subject classified as a receiver of a treatment for MASH, and/or a subject classified as a responder of a treatment for MASH.
15. A computer readable recording medium storing instructions for executing the method of any one of claims 1 to 11.