CLAIMS

1. An *in vitro* method for the diagnosis of non-alcoholic steatohepatitis (NASH) and/or for determining the activity, the stage, or the severity of NASH in a subject, and/or for the classification of a subject as a receiver or non receiver of a treatment for NASH, and/or for the evaluation of the efficacy of a medical treatment, and/or for the determination of the progression or the regression of the pathology in NASH patients, and/or for the classification of a patient as a potential responder or non responder to a medical treatment, and/or for the prediction of disease outcome for a patient, and/or for the identification of surrogate markers of clinical relevant outcomes, comprising the measure of the level of blood, serum or plasma circulating hsa-miR-34 and YKL-40.

2. The method according to claim 1, further comprising the measure of the level of at least one other blood, serum, or plasma circulating marker of liver damage.

3. The method according to claim 1 or 2, for the diagnosis of NASH or for the classification of a subject as a receiver or non-receiver of a treatment for NASH.

4. The method according to claim 2 or 3, wherein said at least one other circulating marker of liver damage is selected in the group consisting of alpha 2 macroglobulin, glycated haemoglobin (HbA1c), fasting glucose level, fructosamine level, insulin, C-Peptide, Homeostasis Model Assessment (HOMA), N-terminal pro-peptide of collagen type III, hsa-miR-200, CK18-M30, CK18-M65, ALT, AST, Uninary Specific Gravity (Uri Spec Grav), uninary creatinine, basophils, High Sensitivity-Reactive Protein (HSCRP), Urinary β-NAG, leucocytes, neutrophils and fibrinogen;
wherein the method may further compris~~ing~~ measure of at least one other parameter such as the height of the subject.

5. The method according to claim 4, wherein the method comprises the measure of the level of hsa-miR-34a-5p, YKL-40, alpha 2 macroglobulin and HbA1c.

6. The method according any one of claims 1 to 4, comprising the steps of:
- measuring the level of hsa-miR-34, (in particular hsa-miR-34a and more particularly hsa-miR-34a-5p),
- measuring the level of YKL-40, and
- combining the results through a mathematical algorithm to obtain a NASH score.

7. The method according to claim 6, wherein a NASH score is calculated according to the following logistic function:

$$S = \frac{e^{Y}}{1 + e^{Y}}$$

wherein:

$$Y=k+a*A+b*B+c*C+d*D+f*F+g*G$$

wherein:

S is the NASH score;

A is the level of alpha 2 macroglobulin in g/L;

B is the level of HbA1c in percent;

C is the level of N-terminal pro-peptide of collagen type III in ng/mL;

D is the level of hsa-miR-34a (in particular hsa-miR-34a-5p) in Cq;

F is the level of hsa-miR-200 (in particular c hsa-miR-200a, more particularly hsa-miR-200a-3p) in Cq;

G is the level of YKL-40 in pg/ml.

k is the constant of the logistic function;

a is a coefficient associated to the level of alpha 2 macroglobulin;

b is a coefficient associated to the level of HbA1c;

c is a coefficient associated to the level of N-terminal pro-peptide of collagen type III;

d is a coefficient associated to the level of hsa-miR-34a (in particular hsa-miR-34a-5p);

f is a coefficient associated to the level of hsa-miR-200 (in particular hsa-miR-200a, more particularly hsa-miR-200a-3p); and

g is a coefficient associated to the level of YKL-40;

wherein either:

a) the method is derived from the median model, and:

k is a number comprised between 5.94 and 50.74, in particular 25.13;

a is a number comprised between 0 and 1.07, in particular 0.52;

b is a number comprised between 0 and 1.20, in particular 0.57;

c is a number comprised between 0 and 0.24, in particular 0.12;

d is a number comprised between -0.97 and 0, in particular -0.43;

f is a number comprised between -0.87 and 0, in particular -0.47;

g is a number comprised between 0 and 1.74E-05, in particular 9.54E-06;

wherein a NASH score higher than a threshold value comprised between 0.2017 and 0.4645, in particular equal to 0.2302, is indicative of a severe NASH, or of a moderate or high NASH activity; and thus is indicative of a patient having a steatosis score ≥ 1, a hepatocyte ballooning score ≥ 1, a lobular inflammation score ≥ 1, a NAS ≥ 4 and a fibrosis stage ≥ 2;

or wherein:

b) the method is derived from the bootstrap model, wherein:

k is a number comprised between 8.24 and 35.44, in particular 21.84;

a is a number comprised between 0.06 and 0.88, in particular 0.47;

b is a number comprised between 0.14 and 1.04, in particular 0.59;

c is a number comprised between 0.03 and 0.23, in particular 0.13;

d is a number comprised between -0.75 and -0.05, in particular -0.4;

f is a number comprised between -0.73 and -0.07, in particular -0.4; and

g is a number comprised between 3.59E-06 and 1.78E-05, in particular 1.07E-05;

wherein a NASH score higher than a threshold value comprised between 0.2718 and 0.6391, in particular equal to 0.3502, is indicative of a severe NASH, or of a moderate or high NASH activity; and thus is indicative of a patient having a steatosis score ≥ 1, a hepatocyte ballooning score ≥ 1, a lobular inflammation score ≥ 1, a NAS ≥ 4 and a fibrosis stage ≥ 2.

8. The method according to claim 6, wherein a NASH score is calculated according to the following logistic function:

$$S \sim \frac{e^{Y}}{1+e^{Y}}$$

wherein:

$$Y=k+a*A+b*B+c*C+d*D$$

wherein either:

a) the method is derived from the bootstrap model, and wherein

S is the NASH score;

A is the serum level of hsa-miR-34a (in particular hsa-miR-34a-5p) in Cq;

B is the serum level of alpha 2 macroglobulin in g/L;

C is the serum level of YKL-40 in pg/ml,

D is the level of HbA1c in percent (e.g. D is equal to 10 if measured HbA1c percentage is 10%);

k is the constant of the logistic function

a is a coefficient associated to the serum level of hsa-miR-34a (in particular hsa-miR-34a-5p);

b is a coefficient associated to the serum level of alpha 2 macroglobulin;

c is a coefficient associated to the serum level of YKL-40; and

d is a coefficient associated to the level of HbA1c;

wherein:

k is a number comprised between 9.51 and 34.37, in particular 21.94;

a is a number comprised between -1.17 and -0.47, in particular -0.82;

b is a number comprised between 0.02 and 0.84, in particular 0.43;

c is a number comprised between 6.10E-06 and 2.09E-05, in particular 1.35E-05;

d is a number comprised between 0.07 and 0.89, in particular 0.48;

wherein a NASH score higher than a threshold value comprised between 0.2013 and 0.5965, in particular equal to 0.4661, is indicative of a severe NASH, or of a moderate or high NASH activity; and thus is indicative of a patient having a steatosis score ≥ 1, a hepatocyte ballooning score ≥ 1, a lobular inflammation score ≥ 1, a NAS ≥ 4 and a fibrosis stage ≥ 2;

or wherein:

b) the method is derived from the median model, wherein:

k is a number comprised between 6.02 and 56.69, in particular 28.17;

a is a number comprised between -1.26 and 0.00, in particular -0.84;

b is a number comprised between 0.00 and 0.88, in particular 0.36,

c is a number comprised between 0.00 and 2.00E-05, in particular 1.23E-05;

d is a number comprised between 0.00 and 0.96in particular 0.41;

wherein a NASH score higher than a threshold value comprised between 0.9773 and 0.9955 is indicative of a severe NASH, or of a moderate or high NASH activity; and thus is indicative of a patient having a steatosis score ≥ 1, a hepatocyte ballooning score ≥ 1, a lobular inflammation score ≥ 1, a NAS ≥ 4 and a fibrosis stage ≥ 2.

9. The method according to claim 6, wherein a NASH score is calculated according to the following logistic function:

$$S \sim \frac{e^{Y}}{1+e^{Y}}$$

wherein:

$$Y=k+a*A+b*B+c*C+d*D$$

wherein:

S is the NASH score;

A is the serum level of hsa-miR-34a (in particular hsa-miR-34a-5p) in copy/µL (log10);

B is the serum level of alpha 2 macroglobulin in g/L;

C is the serum level of YKL-40 in pg/ml,

D is the level of HbA1c in percent (e.g. D is equal to 10 if measured HbA1c percentage is 10%);

k is the constant of the logistic function

a is a coefficient associated to the serum level of hsa-miR-34a (in particular hsa-miR-34a-5p);

b is a coefficient associated to the serum level of alpha 2 macroglobulin;

c is a coefficient associated to the serum level of YKL-40;

d is a coefficient associated to the level of HbA1c;

wherein either:

a) the method is derived from the bootstrap model, wherein:

k is a number comprised between -14.50 and -7.40, in particular -10.95;

a is a number comprised between 1.38 and 3.58, in particular 2.48;

b is a number comprised between 0.02 and 0.84, in particular 0.43;

c is a number comprised between 5.98E-06 and 2.08E-05, in particular 1.34E-05;

d is a number comprised between 0.07 and 0.89, in particular 0.48;

wherein a NASH score higher than a threshold value comprised between 0.1895 and 0.6089, in particular equal to 0.4255, is indicative of a severe NASH, or of a moderate or high NASH activity; and thus is indicative of a patient having a steatosis score ≥ 1, a hepatocyte ballooning score ≥ 1, a lobular inflammation score ≥ 1, a NAS ≥ 4 and a fibrosis stage ≥ 2;

or wherein:

b) the method is derived from the median model, wherein:

k is a number comprised between -27.16 and -0.78, in particular -11.03;

a is a number comprised between 0 and 3.97, in particular 2.59;

b is a number comprised between 0 and 0.89, in particular 0.38,

c is a number comprised between 0 and 1.98E-05, in particular 1.21E-05;

d is a number comprised between 0 and 0.97, in particular 0.40;

wherein a NASH score higher than a threshold value comprised between 0.1421 and 0.4556, in particular equal to 0.3201, is indicative of a severe NASH, or of a moderate or high NASH activity; and thus is indicative of a patient having a steatosis score ≥ 1, a hepatocyte ballooning score ≥ 1, a lobular inflammation score ≥ 1, a NAS ≥ 4 and a fibrosis stage ≥ 2.

10. The method according to claim 6, wherein a NASH score is calculated according to the following logistic function:

$$S \sim \frac{e^{Y}}{1+e^{Y}}$$

wherein:

$$Y=k+a*A+b*B$$

wherein:

S is the NASH score;

A is the level of hsa-miR-34a (in particular hsa-miR-34a-5p) in Cq;

B is the level of YKL-40 in pg/ml;

k is a number comprised between -116.08 and 40.97, in particular 12.87;

a is a number comprised between -0.82 and 0, in particular -0.42;

b is a number comprised between 0 and 1.88E-05, in particular 8.160E-06;

wherein a NASH score higher than a threshold value comprised between 0.1387 and 0.3481, in particular equal to 0.2187, is indicative of a severe NASH, or of a moderate or high NASH activity; and thus is indicative of a patient having a steatosis score ≥ 1, a hepatocyte ballooning score ≥ 1, a lobular inflammation score ≥ 1, a NAS ≥ 4 and a fibrosis stage ≥ 1.

11. The method according to claim 6, wherein a NASH score is calculated according to the following logistic function:

$$S \sim \frac{e^{Y}}{1+e^{Y}}$$

wherein:

$$Y=k+a*A+b*B+c*C+d*D+f*F$$

wherein:

S is the NASH score;

A is the level of hsa-miR-34a (in particular hsa-miR-34a-5p) in Cq;
B is the level of YKL-40 in pg/ml;
C is the level of urinary specific gravity (no units);
D is the level of basophils in 10e9/L;
F is the level of HSCRP in mg/dL
k is a number comprised between -124.81 and 1.13, in particular -61.84
a is a number comprised between -0.91 and -0.25, in particular -0.58;
b is a number comprised between 4.77e-06 and 2.39e-05, in particular 1.44E-05;
c is a number comprised between 17.21 and 141.51, in particular 79.36;
d is a number comprised between 2.80 and 60.74, in particular 31.77;
f is a number comprised between 0.01 and 0.23, in particular 0.12;

wherein a NASH score higher than a threshold value comprised between 0.5791 and 0.8269, in particular equal to 0.7758, is indicative of a severe NASH, or of a moderate or high NASH activity; and thus is indicative of a patient having a steatosis score ≥ 1, a hepatocyte ballooning score ≥ 1, a lobular inflammation score ≥ 1, a NAS ≥ 4 and a fibrosis stage ≥ 1.

12. A kit for the diagnosis of NASH, said kit comprising means for determining the level of:
(i) ~~hsa-miR-34, (in particular hsa-miR-34a and more particularly~~ hsa-miR-34a-5p~~)~~; and
(ii) YKL-40,
wherein the kit comprises as means antibodies specific for a protein to be quantified and/or primers useful for quantifying micro-RNA levels.

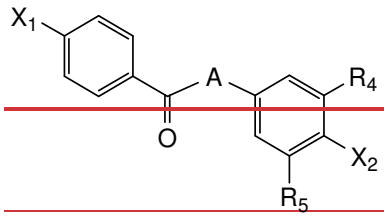
~~13. The kit according to claim 13, further comprising means for determining the level of at least one other blood, serum or plasma circulating marker of liver damage.~~

~~14~~13. The kit for the diagnosis of NASH according to claim 12~~ or 13~~, comprising means for determining the level of:
(i) ~~hsa-miR-34 (in particular hsa-miR-34a, more particularly~~ miR-34a-5p~~);~~,
(ii) YKL-40;
and at least one other blood, serum or plasma circulating marker selected in the group ~~selected from~~consisting of:
(iii) alpha 2 macroglobulin;
(iv) ~~at least one marker selected in the group consisting of~~ HbA1c~~, fasting glucose level, fructosamine level~~;
(v) N-terminal pro-peptide of collagen type III;
(vi) hsa-miR-200 ~~(in particular hsa-miR-200a, more particularly hsa-miR-200a-3p),~~

wherein the kit comprises as means antibodies specific for a protein to be quantified and/or primers useful for quantifying micro-RNA levels.

~~15. Use of an anti-NASH molecule in the manufacture of a medicament for the treatment of NASH, wherein said anti-NASH molecule is to be administered to a patient in need thereof diagnosed with NASH or classified according to the method of any one of claims 1 to 11, in particular classified as receiver of a treatment for NASH.~~

~~16. The use according to claim 15, wherein said molecule is of formula (I):~~



~~wherein:~~

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

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

~~X2 represents a G2-R2 group;~~

~~G1 and G2, identical or different, represent 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 halogen atoms, an alkoxy or an alkylthio group, cycloalkyl groups, cycloalkylthio groups or heterocyclic groups;~~

~~R2 represents an alkyl group substituted by at least a –COOR3 group, wherein R3 represents a hydrogen atom, or an alkyl group that is substituted or not by one or more halogen atoms, cycloalkyl groups, or heterocyclic groups.~~

~~R4 and R5, identical or different, representing an alkyl group that is substituted or not by one or more halogen atoms, cycloalkyl groups, heterocyclic groups;~~

~~or a pharmaceutically acceptable salt thereof.~~

~~17. The use according to claim 15 or 16, wherein the compound is selected in the group consisting of 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-carboxydimethylmethyloxyphenyl]prop-2-en-1-one, 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-isopropyloxycarbonyldimethylmethyloxyphenyl]prop-2-en-1-one, 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-tertbutyloxycarbonyldimethylmethyloxyphenyl] prop-2-en-1-one, 1-[4-trifluoromethylphenyl]-3-[3,5-dimethyl-4-tertbutyloxycarbonyl dimethylmethyloxyphenyl]prop-2-en-1-one, 1-[4-trifluoromethylphenyl]-3-[3,5-dimethyl-4-carboxydimethylmethyloxyphenyl]prop-2-en-1-one, 1-[4-trifluoromethyl oxyphenyl]-3-[3,5-dimethyl-4-tertbutyloxycarbonyldimethylmethyloxy~~

~~phenyl] prop-2-en-1-one, 1-[4-trifluoromethyloxyphenyl]-3-[3,5-dimethyl-4-carboxydimethylmethyl oxyphenyl]prop-2-en-1-one, 2-[2,6-dimethyl-4-[3-[4-(methylthio)phenyl]-3-oxo-propyl] phenoxy]-2-methylpropanoic acid, and 2-[2,6-dimethyl-4-[3-[4-(methylthio) phenyl]-3-oxo-propyl]phenoxy]-2-methyl-propanoic acid isopropyl ester; or a pharmaceutically acceptable salt thereof, in particular 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-carboxydimethylmethyloxy phenyl]prop-2-en-1-one or a pharmaceutically acceptable salt thereof.~~