CLAIMS

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

a) measuring the circulating levels of Soluble Vascular Cell Adhesion Molecule-1 (sVCAM), Thrombospondin 2 (TSP-2) and alpha 2 Macroglobulin (A2M) in a biological fluid sample isolated from said subject;

b) comparing the levels of sVCAM, TSP-2 and A2M with reference levels of sVCAM, TSP-2 and A2M, wherein the comparison between measured levels and reference levels is indicative of the presence or absence of advanced liver fibrosis or liver cirrhosis.

2. The method according to claim 1, wherein the biological fluid sample is a saliva sample, an interstitial liquid sample, an urine sample, a blood sample, a plasma sample or a serum sample.

3. The method according to claim 1 or 2, wherein in step (b) a score A (SA) is compared to a cut-off value, said SA being obtained from the levels of sVCAM, TSP-2 and A2M measured in step (a), said cut-off value being obtained from reference levels of sVCAM, TSP-2 and A2M, said SA and cut-off value being calculated using an algorithm equation.

4. The method according to any of claims 1 to 3, wherein the SA is calculated through the following algorithm equation:

$SA = e^{y}/(1 + e^{y})$ with

$y = k + a \times A + b \times B + c \times C$

A is the level of A2M in log10 g/L;

B is the level of sVCAM in log10 ng/mL;

C is the level of TSP-2 in log10 ng/mL;

k is the constant of algorithm equation;

a is a coefficient associated to the level of A2M;

b is a coefficient associated to the level of sVCAM;

c is a coefficient associated to the level of TSP-2;

and wherein:

k is a number comprised between -34.3 and -24.53, in particular -30.0;

a is a number comprised between 2.002 and 4.359, in particular 3.100;

b is a number comprised between 4.08 and 7.379, in particular 6.111; and

c is a number comprised between 5.524 and 7.544, in particular 6.210.

5. The method according to claim 3 or 4, wherein SA higher than a cut-off value co1 is indicative of advanced liver fibrosis, particularly co1 being comprised between 0.220 and 0.511, more particularly co1 being equal to 0.3471.

6. The method according to claim 3 or 4, wherein SA higher than a cut-off value co2 is indicative of a liver cirrhosis, particularly co2 being comprised between 0.513 and 0.790, more particularly co2 being equal to 0.6315.

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

a) measuring the circulating levels sVCAM, TSP-2 and A2M in a biological fluid sample isolated from said subject, and

b) comparing said levels with levels of sVCAM, TSP-2 and A2M previously measured in the same subject.

8. The method according to claim 7, wherein in step (b) a score C (SC) is compared to a score B (SB), SC being a score obtained from the measured levels of sVCAM, TSP-2 and A2M of step (a) and SB being a score obtained from levels of sVCAM, TSP-2 and A2M previously measured in the same subject, SB and SC being calculated using an algorithm equation; and 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.

9. The method according to claim 8, 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.

10. The method according to any one of claims 7 to 9, wherein SC or SB are calculated through the following algorithm equation:

SC or SB = $e^y/(1 + e^y)$ with

$y = k + a \times A + b \times B + c \times C$

A is the level of A2M in log10 g/L;

B is the level of sVCAM in log10 ng/mL;

C is the level of TSP-2 in log10 ng/mL;

k is the constant of the algorithm equation;

a is a coefficient associated to the level of A2M;

b is a coefficient associated to the level of sVCAM;

c is a coefficient associated to the level of TSP-2;

and wherein:

k is a number comprised between -34.3 and -24.53, in particular -30.0;

a is a number comprised between 2.002 and 4.359, in particular 3.100;

b is a number comprised between 4.08 and 7.379, in particular 6.111; and

c is a number comprised between 5.524 and 7.544, in particular 6.210.

11. Use of an anti-fibrotic agent in the manufacture of medicament for treating 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 6, 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.

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

a) measuring the circulating levels of sVCAM, TSP-2 and A2M 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

b) comparing said levels of sVCAM, TSP-2 and A2M with the levels of sVCAM, TSP-2 and A2M, previously measured before administration of the anti-fibrotic agent to the same subject to assess the efficacy of said anti-fibrotic agent.

13. The method according to claim 12, wherein in step (b) a score E (SE) is compared to a score D (SD),

- SD being a score obtained from the levels of sVCAM, TSP-2 and A2M measured before administration of an anti-fibrotic agent to the subject, and
- SE being a score obtained from the levels of sVCAM, TSP-2 and A2M measured after administration of an anti-fibrotic agent to the subject,
- SD and SE being calculated through an algorithm equation; and

wherein a decrease of SE compared to SD indicates the efficacy of the anti-fibrotic agent.

14. The method according to claim 13, wherein SE and SD are calculated through the following algorithm equation:

SE or SD = $e^y/(1 + e^y)$ with

$y = k + a \times A + b \times B + c \times C$

A is the level of A2M in log10 g/L;

B is the level of sVCAM in log10 ng/mL;

C is the level of TSP-2 in log10 ng/mL;

k is the constant of the algorithm equation;

a is a coefficient associated to the level of A2M;

b is a coefficient associated to the level of sVCAM;

c is a coefficient associated to the level of TSP-2;

and wherein:

k is a number comprised between -34.3 and -24.53, in particular -30.0;

a is a number comprised between 2.002 and 4.359, in particular 3.100

b is a number comprised between 4.08 and 7.379, in particular 6.111; and

c is a number comprised between 5.524 and 7.544, in particular 6.210.

15. A kit for diagnosing advanced liver fibrosis or liver cirrhosis in a subject, said kit comprising means for determining the levels of sVCAM, TSP-2and A2M, wherein the kit comprises at least one specific positive control for TSP-2 and/or at least one specific positive control for sVCAM.

16. The kit according to claim 15, comprising an antibody or an aptamer or a peptide directed against sVCAM, an antibody or an aptamer or a peptide directed against TSP-2 and an antibody or an aptamer or a peptide directed against A2M.

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

- receive measured levels of sVCAM, TSP-2 and A2M;
- calculate a SA score from these measured levels, from the mathematical function

SA = $e^y/(1 + e^y)$ with

$y = k + a \times A + b \times B + c \times C$

A is the level of A2M in log10 g/L;

B is the level of sVCAM in log10 ng/mL;

C is the level of TSP-2 in log10 ng/mL;

k is the constant of algorithm equation;

a is a coefficient associated to the level of A2M;

b is a coefficient associated to the level of sVCAM;

c is a coefficient associated to the level of TSP-2;

and wherein:

k is a number comprised between -34.3 and -24.53, in particular -30.0;

a is a number comprised between 2.002 and 4.359, in particular 3.100;

b is a number comprised between 4.08 and 7.379, in particular 6.111; and

c is a number comprised between 5.524 and 7.544, in particular 6.210; 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.

18. A data-processing device comprising means for carrying the method of any one of claims 1 to 14.

19. 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 14.

20. 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 14.