

**Altered profiles of circulating cytokines in chronic liver diseases (NAFLD/HCC): Impact of the *PNPLA3*<sup>I148M</sup> risk allele**

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## **Supplemental information about the analytes and their quantification**

*Abbreviations and alternative names for the 22 analytes assessed for the full cohort (123 samples)*

$\beta$ -NGF (nerve growth factor), CTACK (cutaneous T cell-attracting chemokine, CCL27), GRO $\alpha$  (growth regulated  $\alpha$ , CXCL1), HGF (hepatocyte growth factor), IFN- $\alpha$ 2 (interferon-  $\alpha$ 2), IFN $\gamma$ , IL-16, IL-17A, IL-1Ra, IL-4, IL-6, IL-8, IL-9, IP-10 (IFN $\gamma$ -inducible protein 10 kDa, CXCL10), MCP-1 (monocyte chemoattractant protein-1, CCL2), M-CSF (macrophage-colony stimulating factor), MIF (macrophage migration inhibitory factor), MIG (monokine induced by gamma IFN), PDGF-BB (platelet-derived growth factor-BB), RANTES (regulated on activation, normal T cell expressed and secreted, CCL5), SCGF- $\beta$  (serum stem cell growth factor  $\beta$ ), and TRAIL (tumor-necrosis-factor related apoptosis inducing ligand). These 22 analytes were selected based on preliminary results suggesting potential changes in concentrations in chronic liver diseases and levels above the detection thresholds in a majority of samples.

*Other analytes tested in preliminary analyses*

A total of 59 analytes were assessed in preliminary analyses (with 16 or 30 samples, using the Bio-Rad Human Group I 27-plex panel, M50-00005L3, the Human Group II 21-plex panel, MF0-05KMII, and custom assays for 11 additional analytes, selected based on literature research (e.g., (1-4); leading to the inclusion of cytokines already known to be involved in hepatocarcinogenesis (-> “positive controls”) or of others for which little / no such information was available) as well as on our specific interest in IL-6-type cytokines. These included also: Eotaxin, FGF basic, G-CSF, GM-CSF\*, Gp130, IL-1 $\alpha$ \*, IL-1 $\beta$ , IL-2\*, IL-3\*, IL-2R $\alpha$ , IL-5, IL-7, IL-10, IL-11\*, IL-12 (p40)\*, IL-12 (p70), IL-13, IL-15\*, IL-17F\*, IL-18, IL-22\*, IL-25\*, IL-26\*, IL-27 (p28) \*, IL-32\*, IL-33\*, IL-35\*, LIF\*, MCP-3\*, MIP-1 $\alpha$ , MIP-1 $\beta$ , SCF, SDF-1 $\alpha$ , sIL-6R $\alpha$ , TNF $\alpha$ , TNF $\beta$  \*, and VEGF. Of note, those indicated by \* were below detection thresholds in a majority of samples.

*Analyte quantifications*

Sample concentrations were interpolated from the standard curves calculated by the program (Bio-Plex Data Pro software version 1.02, Bio-Rad). To avoid zero-values which would prevent log-transformation of the concentrations, values found below the standard curve were set to an arbitrary value of Lower Limit of Quantification (LLOQ) divided by 2. Values found below the blank were set to an arbitrary value of LLOQ divided by 3. Values found above the standard curve

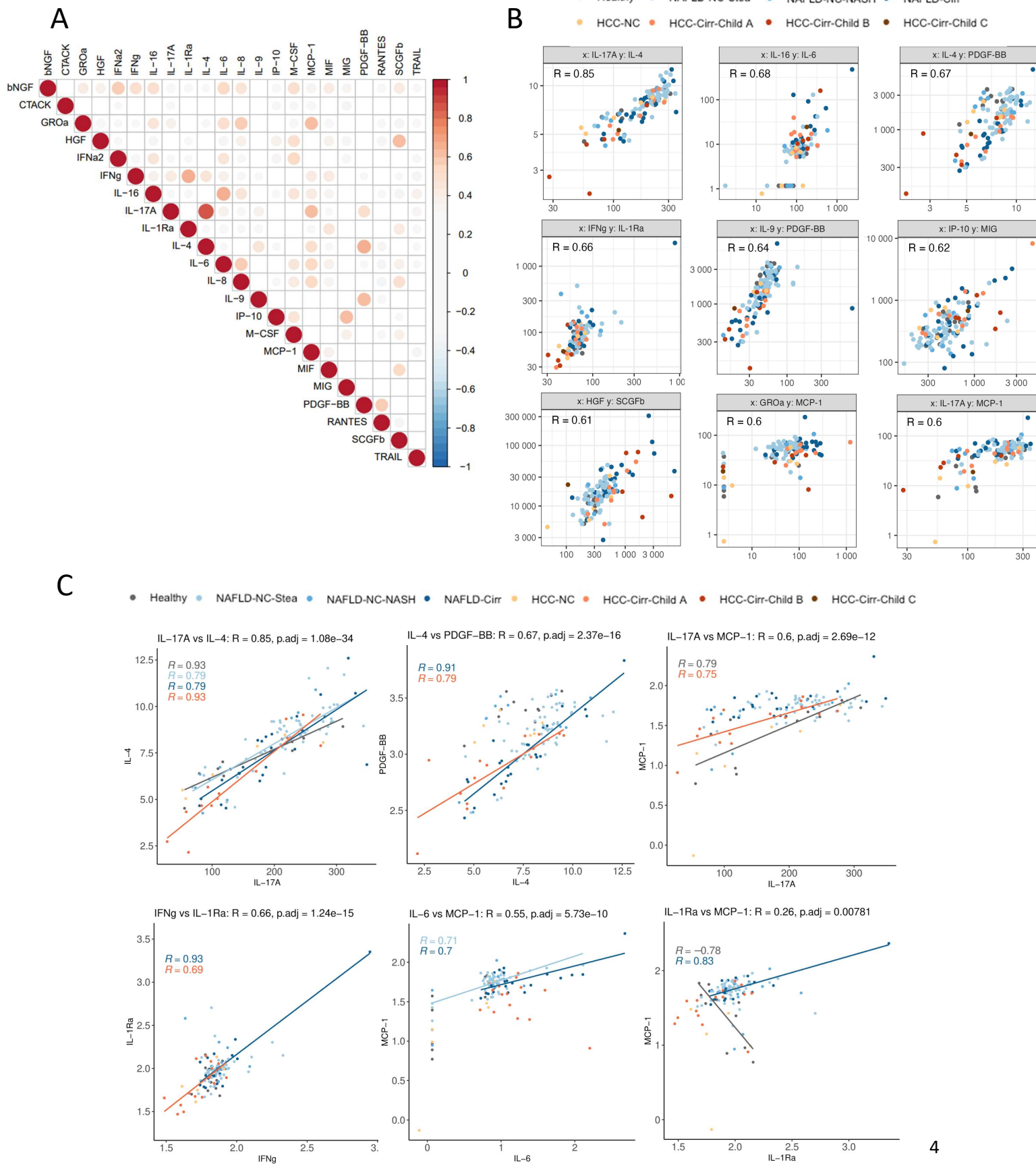
were set to an arbitrary value of Upper Limit of Quantification (ULOQ) multiplied by 2. The LLOQ/2, LLOQ/3, and ULOQx2 are represented in the graphs as empty circles. Only the non-recalculated values were considered “in the range”.

### Supplemental references

1. Capone F, Costantini S, Guerriero E, Calemma R, Napolitano M, Scala S, et al. Serum cytokine levels in patients with hepatocellular carcinoma. *Eur Cytokine Netw* 2010;21:99-104.
2. Budhu A, Wang XW. The role of cytokines in hepatocellular carcinoma. *J Leukoc Biol* 2006;80:1197-1213.
3. **Vansaun MN, Mendonsa AM**, Lee Gorden D. Hepatocellular proliferation correlates with inflammatory cell and cytokine changes in a murine model of nonalcoholic fatty liver disease. *PLoS One* 2013;8:e73054.
4. **Chen ZY, Wei W**, Guo ZX, Peng LX, Shi M, Li SH, et al. Using multiple cytokines to predict hepatocellular carcinoma recurrence in two patient cohorts. *Br J Cancer* 2014;110:733-740.

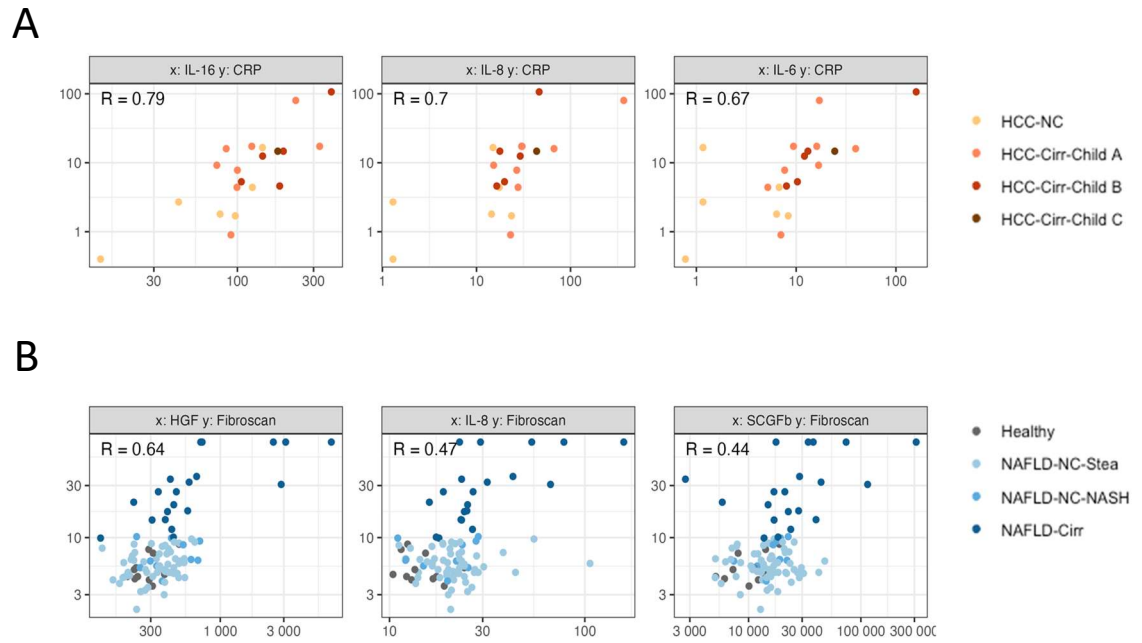
Author names in bold designate shared co-first authorship

# SDC, Figure S1



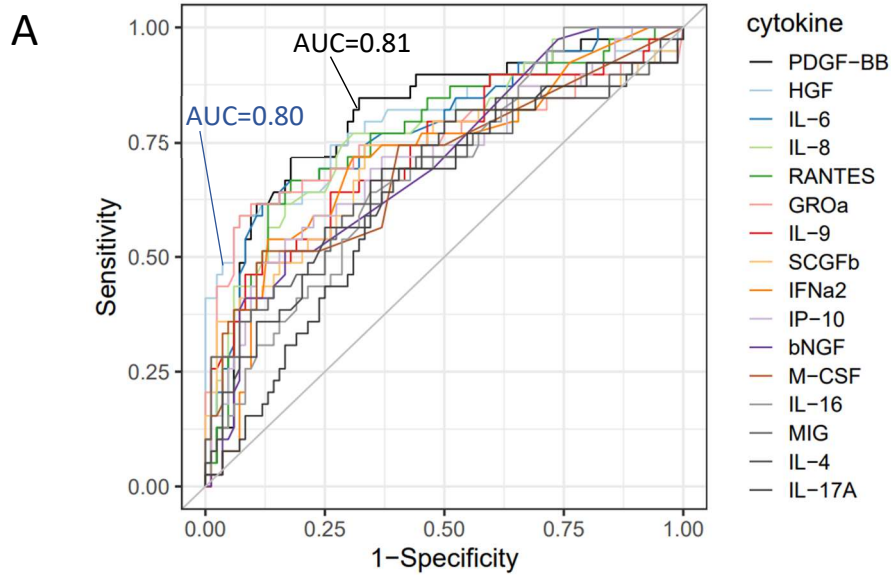
**Fig. S1: Pairwise correlations between cytokines.** (A): Graphical overview of Pearson's correlations between 2 analytes. The color code and the size of the circles reflect the R-values. (B): Only pairs of cytokines with high Pearson's correlations ( $R \geq 0.6$ ) are plotted; the overall R-value is indicated. (C): For the pairs of cytokines shown in the plots, significant correlations with R values  $\geq 0.7$  were observed in more than one subgroup of patients. Subgroup-specific R values are shown in color.

SDC, Figure S2



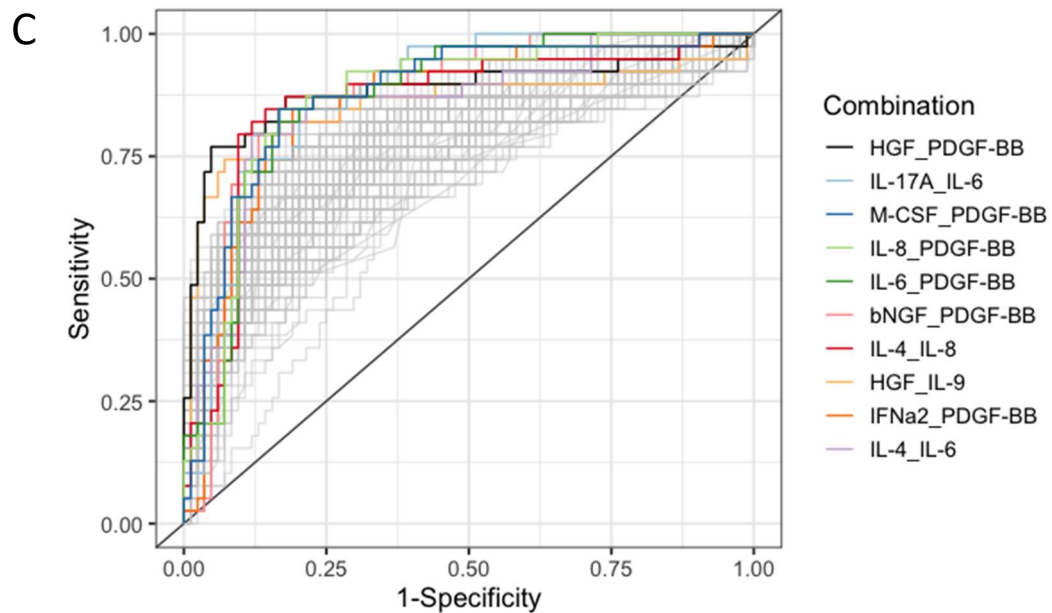
**Fig. S2: Pairwise correlations. (A):** Selected examples for the correlation of cytokines with CRP. **(B):** Selected examples for the correlation of cytokines with Fibroscan values.

SDC, Figure S3



**B**

Cytokine	AUC	PPV	NPV	SENS	SPEC	Cut-off	Equation
PDGF-BB	0.81	0.65	0.86	0.72	0.82	0.33	$y \sim 11.14 + -3.84 * x$
HGF	0.8	0.79	0.83	0.59	0.93	0.44	$y \sim -14.59 + 5.21 * x$
IL-6	0.78	0.71	0.83	0.62	0.88	0.35	$y \sim -4.01 + 3.34 * x$
IL-8	0.78	0.54	0.87	0.77	0.69	0.3	$y \sim -8.29 + 5.48 * x$
RANTES	0.77	0.63	0.84	0.67	0.82	0.38	$y \sim 2.29 + -0.00 * x$
GRO $\alpha$	0.75	0.79	0.83	0.59	0.93	0.4	$y \sim -3.79 + 1.70 * x$
IL-9	0.72	0.53	0.82	0.64	0.74	0.32	$y \sim 5.06 + -3.59 * x$
SCGF $\beta$	0.72	0.51	0.85	0.74	0.67	0.3	$y \sim -13.36 + 2.96 * x$
IFN $\alpha$ 2	0.71	0.52	0.84	0.72	0.69	0.36	$y \sim -2.21 + 1.18 * x$
IP-10	0.71	0.47	0.83	0.72	0.63	0.29	$y \sim -10.32 + 3.49 * x$
$\beta$ NGF	0.71	0.59	0.79	0.51	0.83	0.43	$y \sim -1.28 + 1.40 * x$
M-CSF	0.7	0.67	0.8	0.51	0.88	0.49	$y \sim -1.03 + 1.53 * x$
IL-16	0.68	0.38	1	1	0.25	0.2	$y \sim -6.63 + 2.82 * x$
MIG	0.68	0.46	0.8	0.67	0.63	0.29	$y \sim -7.03 + 2.38 * x$
IL-4	0.67	0.42	0.85	0.82	0.48	0.25	$y \sim 1.75 + -0.34 * x$
IL-17A	0.63	0.47	0.82	0.69	0.63	0.31	$y \sim 0.28 + -0.01 * x$



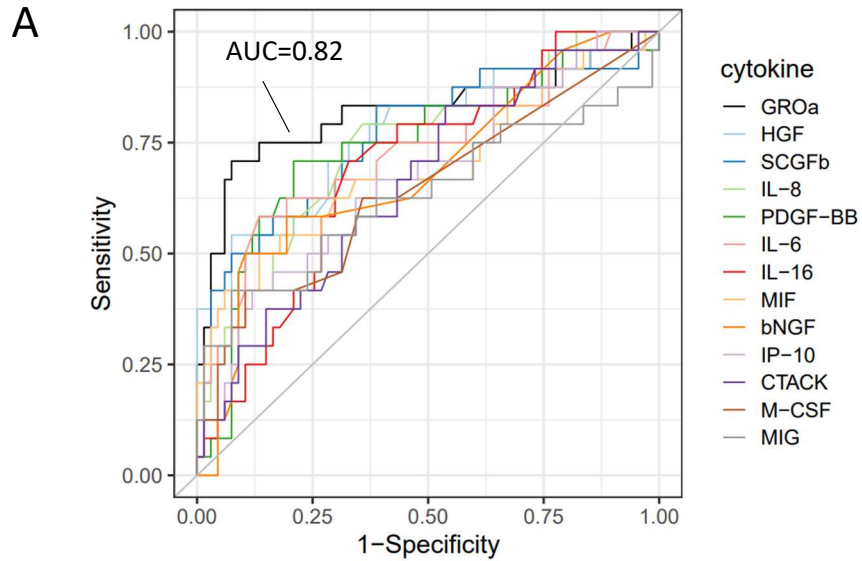
Cytokine Combination	AUC	PPV	NPV	SENS	SPEC	Cut-off	Equation
HGF_PDGF-BB	0.88	0.88	0.90	0.77	0.95	0.45	$y \sim -2.49 + 5.58 * x_1 + -4.21 * x_2$
IL-17A_IL-6	0.88	0.78	0.87	0.72	0.90	0.41	$y \sim -1.76 + -0.02 * x_1 + 4.10 * x_2$
M-CSF_PDGF-BB	0.88	0.70	0.92	0.85	0.83	0.27	$y \sim 11.31 + 1.69 * x_1 + -3.96 * x_2$
IL-8_PDGF-BB	0.87	0.70	0.92	0.85	0.83	0.31	$y \sim 2.76 + 5.45 * x_1 + -3.57 * x_2$
IL-6_PDGF-BB	0.87	0.70	0.91	0.82	0.83	0.32	$y \sim 7.06 + 3.23 * x_1 + -3.56 * x_2$
$\beta$ NGF_PDGF-BB	0.87	0.74	0.90	0.79	0.87	0.37	$y \sim 10.73 + 1.48 * x_1 + -3.88 * x_2$
IL-4_IL-8	0.86	0.79	0.90	0.79	0.90	0.39	$y \sim -6.31 + -0.51 * x_1 + 6.87 * x_2$
HGF_IL-9	0.86	0.83	0.89	0.74	0.93	0.38	$y \sim -8.99 + 5.73 * x_1 + -4.22 * x_2$
IFN $\alpha$ 2_PDGF-BB	0.86	0.67	0.92	0.85	0.81	0.32	$y \sim 10.72 + 1.50 * x_1 + -4.29 * x_2$
IL-4_IL-6	0.85	0.76	0.90	0.79	0.88	0.36	$y \sim -0.29 + -0.56 * x_1 + 3.89 * x_2$

**Fig. S3: ROC curve analysis for prediction of cirrhosis (all samples).**

(A): ROC curves for top 16 cytokines with adj. p-values < 0.05 allowing best to distinguish cirrhosis vs. non-cirrhosis are represented. (B): Scores for AUC, PPV, NPV, sensitivity, specificity, cut-off and equation for the ROC curves highlighted in (A). (C): ROC curves for the 10 cytokine pairs discriminating best between cirrhosis and non-cirrhosis are highlighted in colors. (D): Scores for AUC, PPV, NPV, sensitivity, specificity, cut-off and equation for the ROC curves highlighted in (C).

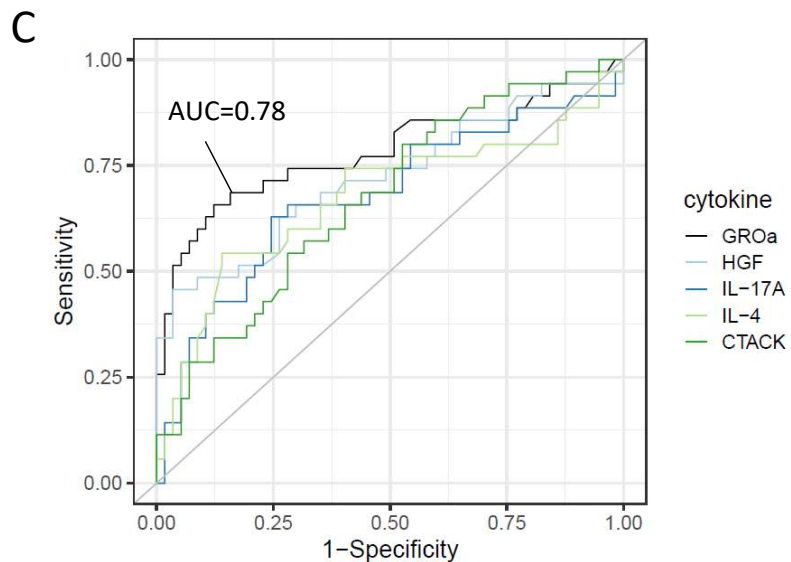


SDC, Figure S4



**B**

Cytokine	AUC	PPV	NPV	SENS	SPEC	Cut-off	Equation
GRO $\alpha$	0.82	0.77	0.9	0.71	0.93	0.37	$y \sim -10.56 + 5.07 * x$
HGF	0.77	0.72	0.85	0.54	0.93	0.39	$y \sim -15.02 + 5.28 * x$
SCGF $\beta$	0.76	0.71	0.84	0.5	0.93	0.39	$y \sim -17 + 3.73 * x$
IL-8	0.74	0.46	0.88	0.75	0.69	0.24	$y \sim -7.61 + 4.73 * x$
PDGF-BB	0.74	0.55	0.88	0.71	0.79	0.28	$y \sim 7.56 + -2.76 * x$
IL-6	0.73	0.61	0.85	0.58	0.87	0.28	$y \sim -3.59 + 2.55 * x$
IL-16	0.69	0.44	0.87	0.71	0.67	0.26	$y \sim -6.29 + 2.51 * x$
MIF	0.69	0.57	0.83	0.5	0.87	0.33	$y \sim -6.02 + 1.87 * x$
$\beta$ NGF	0.69	0.63	0.83	0.5	0.9	0.39	$y \sim -1.6 + 1.34 * x$
IP-10	0.67	0.38	0.84	0.67	0.61	0.25	$y \sim -8.68 + 2.81 * x$
CTACK	0.66	0.36	0.89	0.83	0.46	0.21	$y \sim -5.92 + 1.98 * x$
M-CSF	0.64	0.59	0.81	0.42	0.9	0.42	$y \sim -1.16 + 1.19 * x$
MIG	0.63	0.67	0.82	0.42	0.93	0.38	$y \sim -6.09 + 1.94 * x$



D

Cytokine	AUC	PPV	NPV	SENS	SPEC	Cut-off	Equation
GRO $\alpha$	0.78	0.77	0.81	0.66	0.88	0.47	$y \sim -6.24 + 3.14 * x$
HGF	0.72	0.58	0.77	0.66	0.7	0.39	$y \sim -9.27 + 3.36 * x$
IL-17A	0.68	0.59	0.77	0.66	0.72	0.39	$y \sim 1.36 + -0.01 * x$
IL-4	0.67	0.53	0.79	0.74	0.6	0.33	$y \sim 2.3 + -0.35 * x$
CTACK	0.67	0.47	0.82	0.86	0.4	0.29	$y \sim -5.76 + 2.15 * x$

**Fig. S4: ROC curve analysis for prediction of cirrhosis or “complication” within the NAFLD group.** (A): ROC curves for 13 cytokines allowing to distinguish between cirrhotic and non-cirrhotic cases within the NAFLD group (adj. p-values < 0.05). (B): Scores for PPV, NPV, sensitivity, specificity, cut-off and equation for the ROC curves highlighted in (A). (C): ROC curves for 5 cytokines allowing to distinguish between complicated and uncomplicated cases within the NAFLD group (adj. p-values < 0.05). (D): Scores for PPV, NPV, sensitivity, specificity, cut-off and equation for the ROC curves highlighted in (C).