Supplemental information

Host biomarker-based quantitative

rapid tests for detection and treatment

monitoring of tuberculosis and COVID-19

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SUPPLEMENTAL INFORMATION

SUPPLEMENTARY TABLE S1 *Description of the cohorts, Related to Figures 1-4 and Tables 1-2*

Cohort	Country of sampling	Group	*N*	Age mean (range)	Sex (% female)
TB cohort 1	Italy	TB	30	34 (18 – 52)	43
		LTBI	29	45 (19 – 75)	69
TB cohort 2	The Netherlands	TB	20	44 (15 – 84)	20
		LTBI	18	35 (18 – 60)	61
COVID-19/healthy controls	The Netherlands	COVID-19	102	63 (18 – 91)	23
		healthy controls	39	42 (22 – 72)	59
		pre-COVID-19	*27*	*33 (22 – 60)*	*70*
		during COVID-19	*12*	*64 (60 – 72)*	*33*

Overview of the different cohorts including country of sampling, group, number of samples per group, age, and sex. COVID-19: coronavirus disease 2019; LTBI: latent tuberculosis infection; TB: tuberculosis.

SUPPLEMENTARY TABLE S2	*Country of origin of the participants, Related to Figures 1-4 and Tables 1-2*		
Cohort	**Group**	**Country of origin**	***N***
TB cohort 1	TB	*Albania*	2
		Colombia	1
		Georgia	1
		Italy	10
		Kosovo	1
		Moldova	1
		Romania	14
	LTBI	*Afghanistan*	1
		Colombia	1
		Italy	20
		Lithuania	1
		Peru	1
		Romania	5
TB cohort 2	TB	*Angola*	2
		Italy	1
		Jakarta	2
		Morocco	2
		the Netherlands	9
		Somalia	2
		South Africa	1
		Sri Lanka	1
	LTBI	*France*	1
		the Netherlands	14
		Netherlands Antilles	1
		Sri Lanka	2
COVID-19/healthy controls		*Dutch citizens**	141

Overview of the country of origin for the participants in the different cohorts. *Individuals from the COVID-19/healthy controls cohort were all Dutch citizens. COVID-19: coronavirus disease 2019; LTBI: latent tuberculosis infection; TB: tuberculosis.

| SUPPLEMENTARY TABLE S3		*Overview of different NUM scores per cohort comparison with corresponding AUC, sensitivity, and specificity, Related to Figures 1-3 and Table 2*			
Cohort comparison	**# of markers used in NUM score**	**Markers used in NUM score**	**AUC**	**Cut-off**	**Sn/Sp**
TB vs. LTBI	6	ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2	0·94	≥3	83%/97%
	5	CRP, ferritin, IL-6, IP-10, SAA1/A2	0·93	≥2	87%/86%
	4	CRP, IL-6, IP-10, SAA1/A2	0·93	≥2	87%/86%
	3	CRP, IL-6, SAA1/A2	0·94	≥2	80%/97%
	2	CRP, SAA1/A2	0·91	≥1	83%/97%
	1	CRP*	0·87	≥1	77%/97%
	1	SAA1/A2*	0·87	≥1	100%/77%
COVID-19 vs. healthy controls	7	ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, S100A12	0·99	≥4	93%/100%
	6	ApoA1, CRP, ferritin, IP-10, SAA1/A2, S100A12	1·00	≥3	98%/100%
	5	ApoA1, CRP, ferritin, SAA1/A2, S100A12	1·00	≥3	95%/100%
	4	CRP, ferritin, SAA1/A2, S100A12	0·99	≥2	96%/97%
	3	CRP, ferritin, SAA1/A2	0·99	≥2	95%/97%
	2	ferritin, SAA1/A2	0·98	≥1	99%/92%
	1	SAA1/A2	0·98	≥1	92%/97%
COVID-19 vs. TB	7	ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, S100A12	0·95	≥4	91%/87%
	6	ApoA1, CRP, ferritin, IL-6, SAA1/A2, S100A12	0·95	≥3	98%/83%
	5	ApoA1, CRP, ferritin, SAA1/A2, S100A12	0·94	≥3	93%/83%
	4	ApoA1, CRP, SAA1/A2, S100A12	0·95	≥3	88%/89%
	3	CRP, SAA1/A2, S100A12	0·94	≥2	90%/87%
	2	CRP, SAA1/A2	0·93	≥1	94%/80%
	1	CRP	0·93	≥1	78%/96%

Overview of the different NUM scores per cohort comparison with corresponding AUC, sensitivity, and specificity. A cut-off ratio for positivity for each biomarker was determined by calculating the maximal Youden's index. Using the cut-offs for positivity, a NUM score was calculated; the number of biomarkers that scored above the threshold of positivity. Three comparisons were made: TB vs. LTBI, COVID-19 vs. healthy controls, and COVID-19 vs. TB. Biomarkers were deleted from the NUM score based on their contribution (AUC); the biomarker with the lowest AUC was removed first, ending up with the most discriminatory marker for that comparison. *Since CRP and SAA1/A2 both showed an AUC of 0·87 in the comparison of TB vs. LTBI, both options as a final marker remaining are shown. AUC: area under the curve; COVID-19: coronavirus disease 2019; LTBI: latent tuberculosis infection; Sn/Sp: sensitivity/specificity; TB: tuberculosis.

A   TB patients

IL-6

SAA1/A2

IP-10

CRP

Ferritin

ApoA1

S100A12

B   COVID-19 patients

IL-6

SAA1/A2

IP-10

CRP
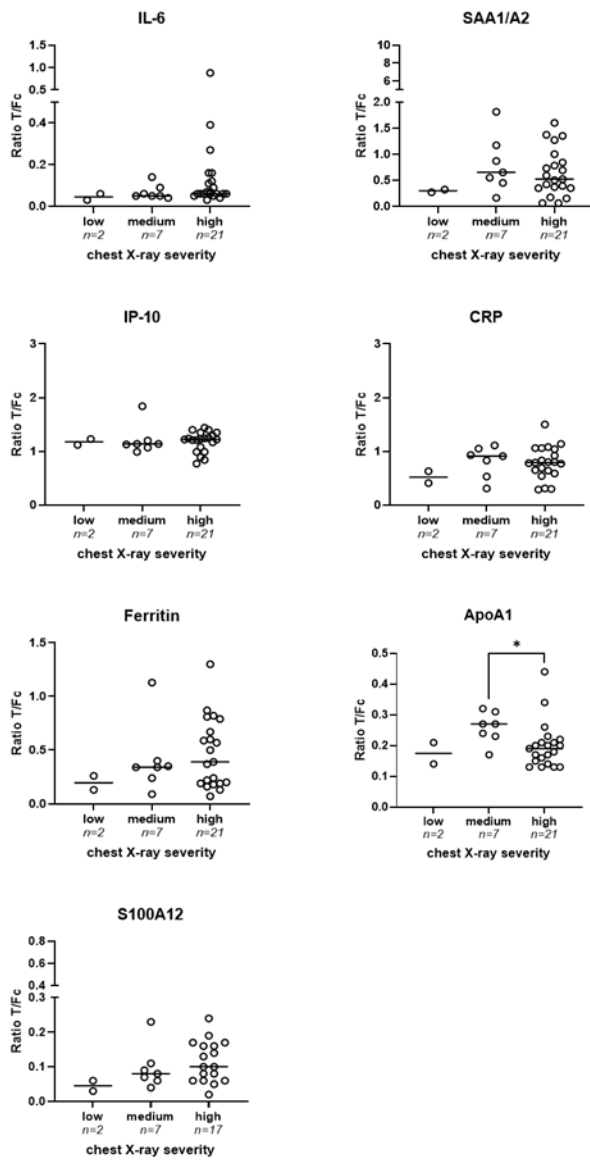
Ferritin
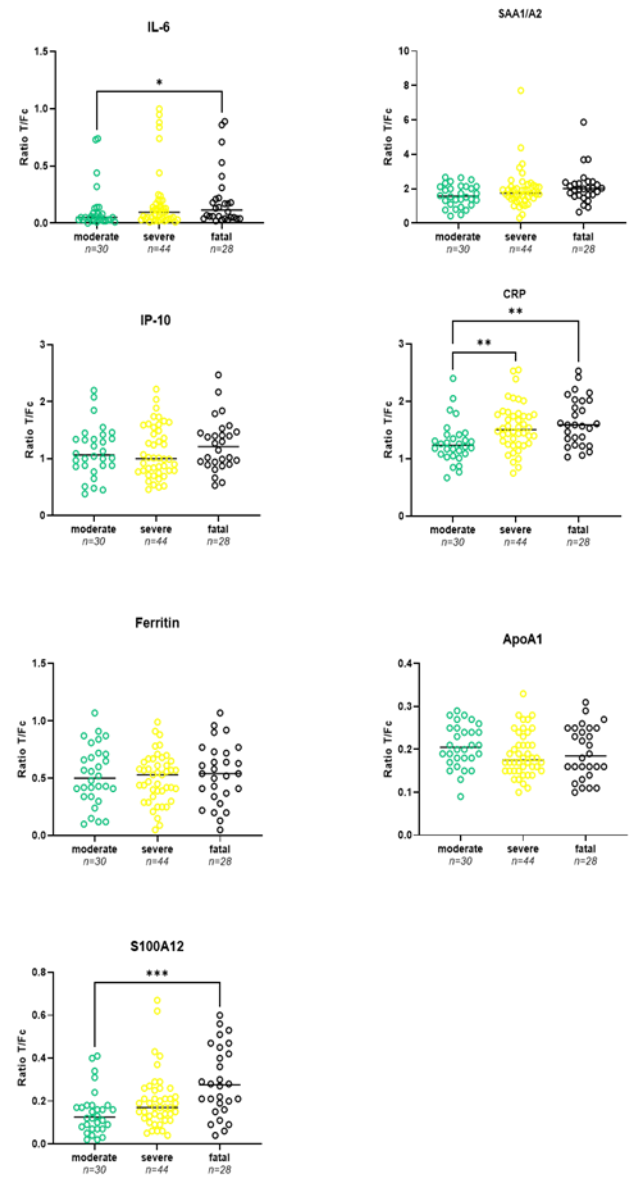
ApoA1

S100A12

SUPPLEMENTARY FIGURE S1: *Evaluation of seven biomarkers for disease severity of TB and COVID-19, Related to Figures 1-2 and Table 1*

ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12 were measured by singleplex strips in serum samples of TB patients with low (n=2), medium (n=7) and high (n=21) chest X-ray severity (A) and serum samples at hospital admission of COVID-19 patients with ultimate moderate disease (n=30), severe disease (n=44), and fatal outcome (n=28) from the Netherlands (B). Median values for each group are indicated by horizontal bars. A Kruskal-Wallis test was performed to determine the statistical significance between groups (p-values: *p≤0·05, **p≤0·01, ***p≤0·001, ****p≤0·0001). Green dots: COVID-19 patients with moderate disease, yellow dots: COVID-19 patients with severe disease; black dots: COVID-19 patients with a fatal outcome. COVID-19: coronavirus disease 2019; Fc: flow control line; T: test line; TB: tuberculosis.
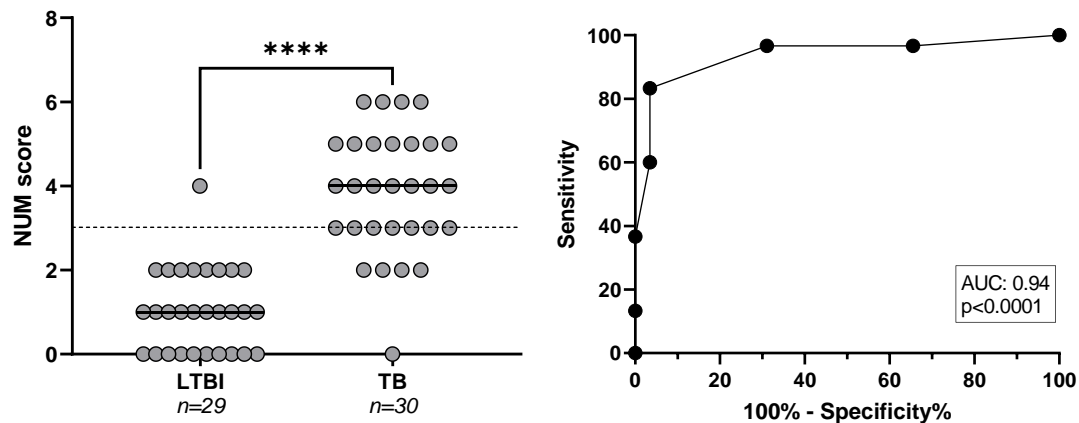
Cut-off	Sensitivity (%)	95% CI	Specificity (%)	95% CI
≥ 1	97	83·3% to 99·8%	34	19·9% to 52·7%
≥ 2	97	83·3% to 99·8%	69	50·8% to 82·7%
≥ 3	**83**	**66·4% to 92·7%**	**97**	**82·8% to 99·8%**
≥ 4	60	42·3% to 75·4%	97	82·8% to 99·8%
≥ 5	37	21·9% to 54·5%	100	88·3% to 100%
≥ 6	13	5·3% to 29·7%	100	88·3% to 100%

SUPPLEMENTARY FIGURE S2: *NUM score evaluation of six biomarkers for TB and LTBI in European cohort, Related to Figure 1*

ApoA1, CRP, ferritin, IL-6, IP-10, and SAA1/A2 were measured by singleplex strips in serum samples of TB patients (n=30) and LTBI (n=29) from Europe. The NUM score (the number of positive biomarkers) is indicated on the *y*-axis. Median values for each group are indicated by horizontal bars. A Mann-Whitney U test was performed to determine the statistical significance between groups (p-values: *p≤0·05, **p≤0·01, ***p≤0·001, ****p≤0·0001). The cut-off with optimal sensitivity and specificity is written in bold and depicted as a dotted horizontal line in the graph. AUC: area under the curve; CI: confidence interval; LTBI: latent tuberculosis infection; TB: tuberculosis.

A   6-marker NUM score
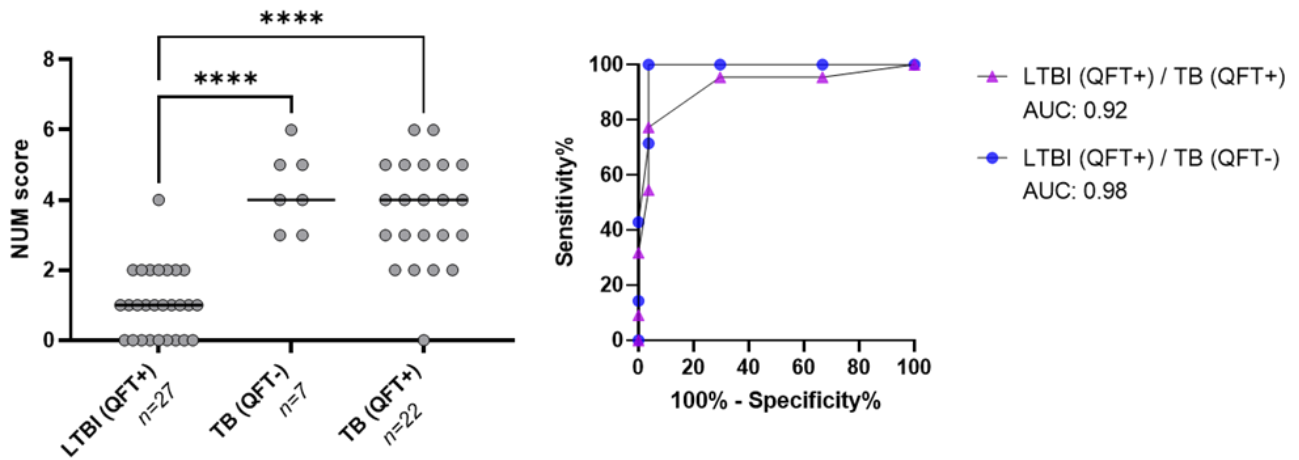

B   2-marker NUM score
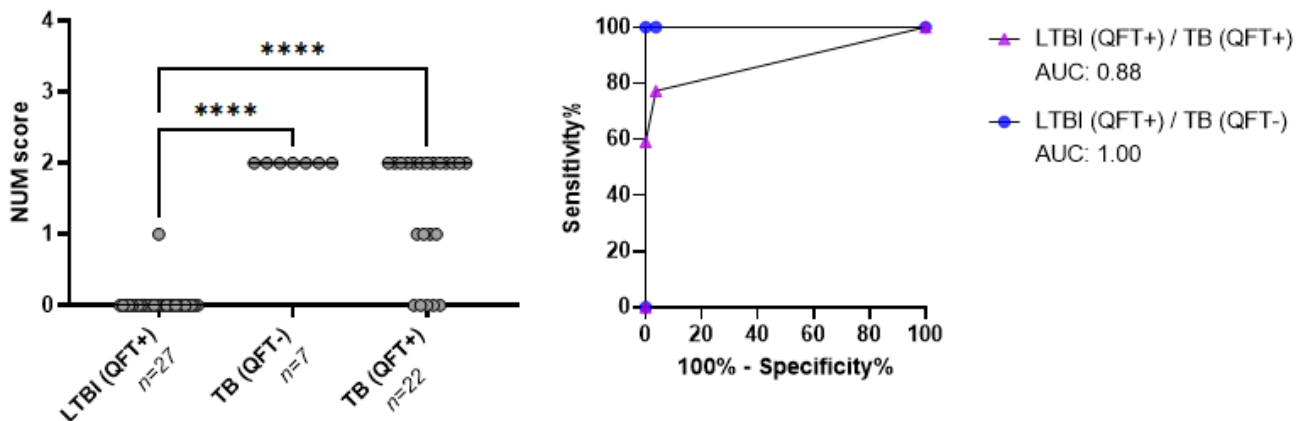
SUPPLEMENTARY FIGURE S3: *Comparison of QuantiFERON and NUM score results for TB and LTBI patients, Related to Figure 1*

ApoA1, CRP, ferritin, IL-6, IP-10, and SAA1/A2 were measured by singleplex strips in serum samples of: QuantiFERON-negative TB patients (n=7) and QuantiFERON-positive LTBI (n=27) and TB (n=22) patients from Europe. The NUM score (the number of positive biomarkers) is indicated on the *y*-axis; ApoA1, CRP, ferritin, IL-6, IP-10, and SAA1/A2 were combined into a 6-marker NUM score (A) and CRP and SAA1/A2 were combined into a 2-marker NUM score (B). Median values for each group are indicated by horizontal bars. A

Kruskal-Wallis test was performed to determine the statistical significance between groups (p-values: $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, $****p \leq 0.0001$). AUC: area under the curve; LTBI: latent tuberculosis infection; QFT: QuantiFERON; TB: tuberculosis.
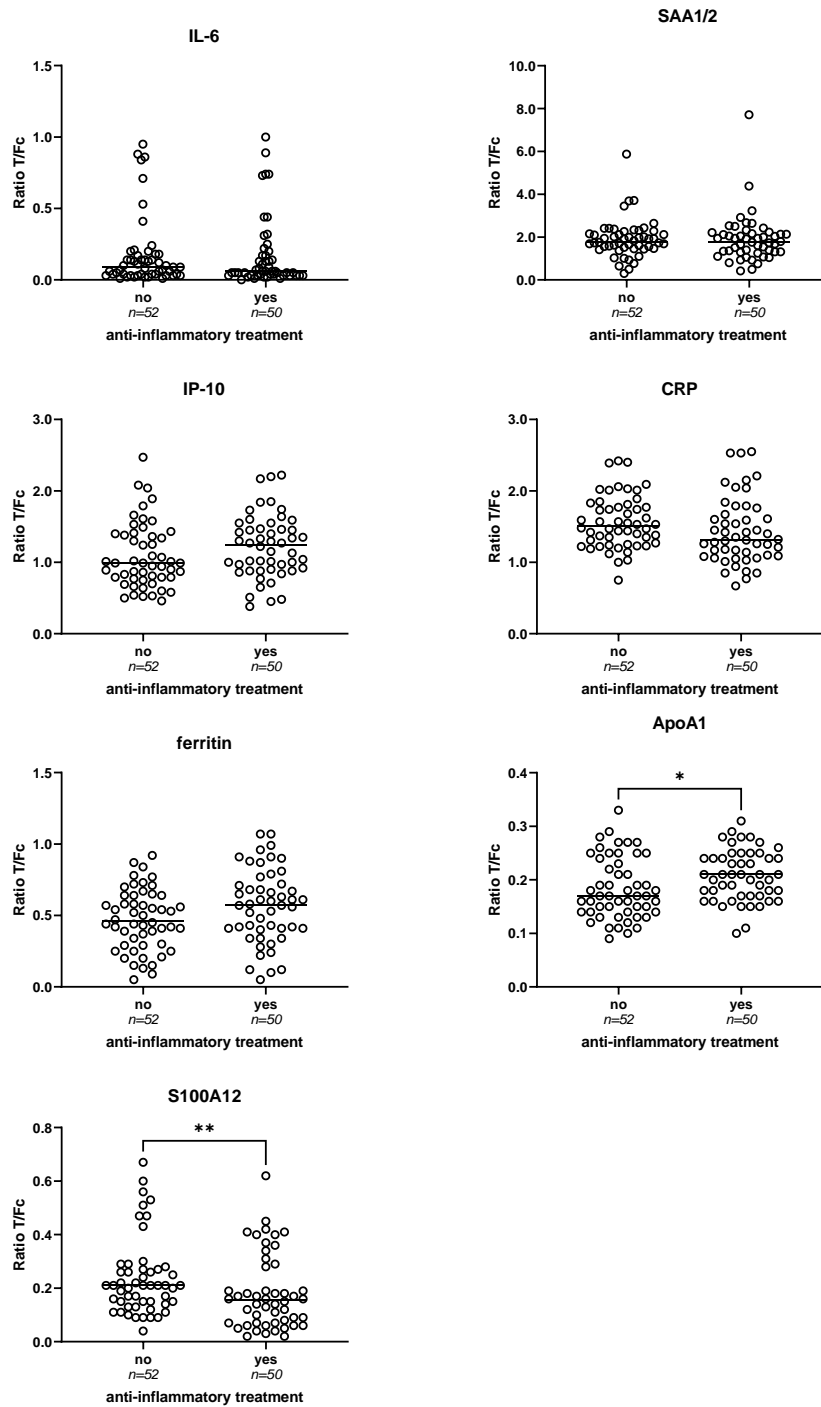
SUPPLEMENTARY FIGURE S4: *Effect of anti-inflammatory treatment on seven host proteins in COVID-19 patients, Related to Figure 2*

ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12 were measured by singleplex strips in serum samples at hospital admission of COVID-19 patients which had already received anti-inflammatory treatment before the first sample collection (n=50) and those who

had not (n=52). Anti-inflammatory treatment included betamethasone, dexamethasone, hydrocortisone, methylprednisolone or prednisolone. Median values for each group are indicated by horizontal bars. A Mann-Whitney U test was performed to determine the statistical significance between groups (p-values: *p≤0·05, **p≤0·01, ***p≤0·001, ****p≤0·0001). Fc; flow control line; T: test line.
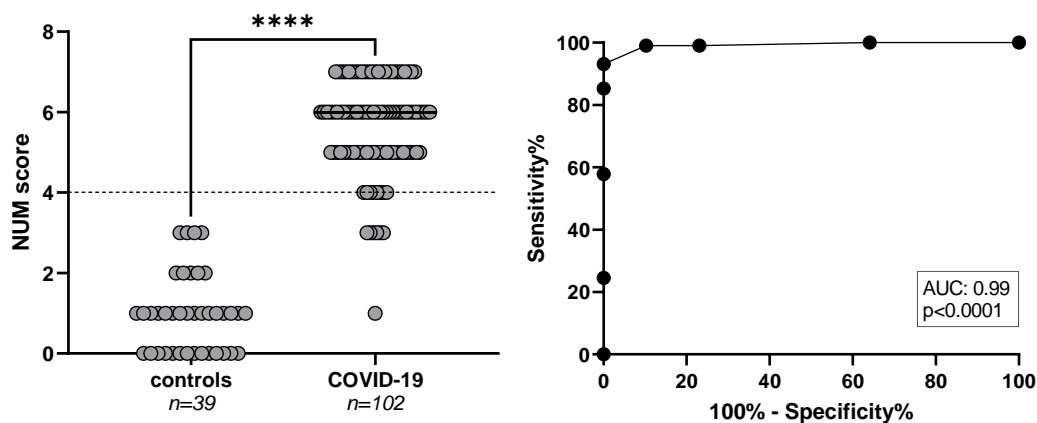
Cut-off	Sensitivity (%)	95% CI	Specificity (%)	95% CI
≥ 1	100	96·4% to 100%	36	22·7% to 51·6%
≥ 2	99	94·7% to 100%	77	61·7% to 87·4%
≥ 3	99	94·7% to 100%	90	76·4% to 95·9%
≥ 4	**93**	**86·5% to 96·6%**	**100**	**91·0% to 100%**
≥ 5	85	77·2% to 90·9%	100	91·0% to 100%
≥ 6	58	48·2% to 67·0%	100	91·0% to 100%
≥ 7	25	17·2% to 33·7%	100	91·0% to 100%

SUPPLEMENTARY FIGURE S5: *NUM score evaluation of seven biomarkers for healthy controls and COVID-19 patients from the Netherlands, Related to Figure 2*

ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12 were measured by singleplex strips in serum samples of controls (n=39) and COVID-19 patients (n=102) from the Netherlands. The NUM score (the number of positive biomarkers) is indicated on the *y*-axis. Median values for each group are indicated by horizontal bars. A Mann-Whitney U test was performed to determine the statistical significance between groups (p-values: *p≤0·05, **p≤0·01, ***p≤0·001, ****p≤0·0001). The cut-off with optimal sensitivity and specificity is written in bold and depicted as a dotted horizontal line in the graph. AUC: area under the curve; CI: confidence interval; COVID-19: coronavirus disease 2019.
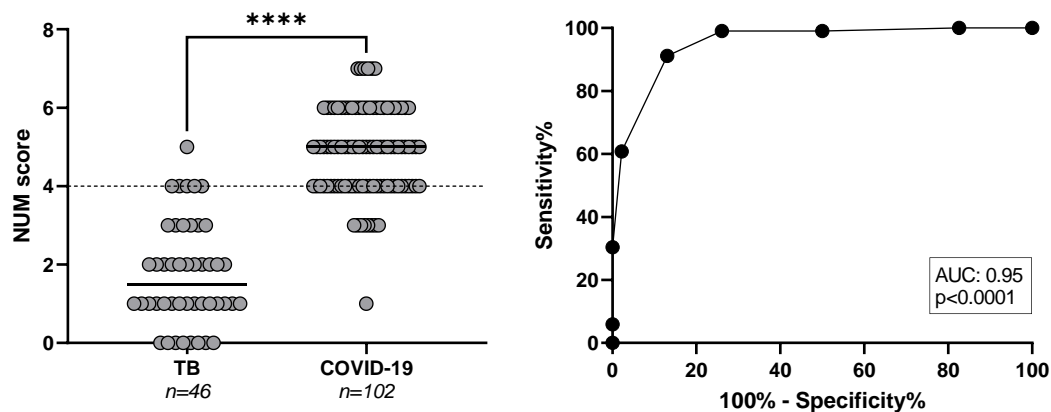
Cut-off	Sensitivity (%)	95% CI	Specificity (%)	95% CI
≥ 1	100	96·4% to 100%	17	9·1% to 30·7%
≥ 2	99	94·7% to 100%	50	36·1% to 63·9%
≥ 3	99	94·7% to 100%	74	59·7% to 84·4%
≥ 4	**91**	**84·1% to 95·3%**	**87**	**74·3% to 93·9%**
≥ 5	61	51·1% to 69·7%	98	88·7% to 99·9%
≥ 6	30	22·3% to 39·9%	100	92·3% to 100%
≥ 7	6	2·7% to 12·2%	100	92·3% to 100%

SUPPLEMENTARY FIGURE S6: *NUM score evaluation of seven biomarkers for TB and COVID-19 patients, Related to Figure 3*

ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12 were measured by singleplex strips in serum samples of TB patients (n=46) from Europe and COVID-19 patients (n=102) from the Netherlands. The NUM score (the number of positive biomarkers) is indicated on the *y*-axis. Median values for each group are indicated by horizontal bars. A Mann-Whitney U test was performed to determine the statistical significance between groups (p-values: *p≤0·05, **p≤0·01, ***p≤0·001, ****p≤0·0001). The cut-off with optimal sensitivity and specificity is written in bold and depicted as a dotted horizontal line in the graph. AUC: area under the curve; CI: confidence interval; COVID-19: coronavirus disease 2019; TB: tuberculosis.

TB patients

IL-6

SAA1/A2

IP-10

CRP

Ferritin
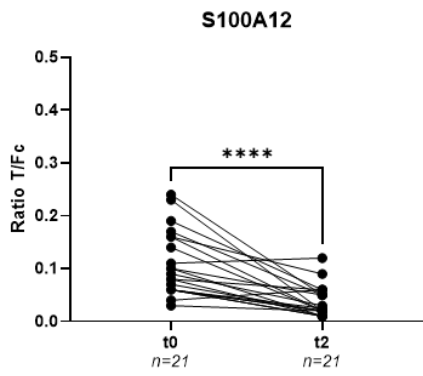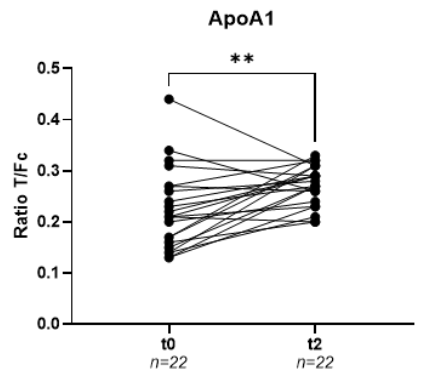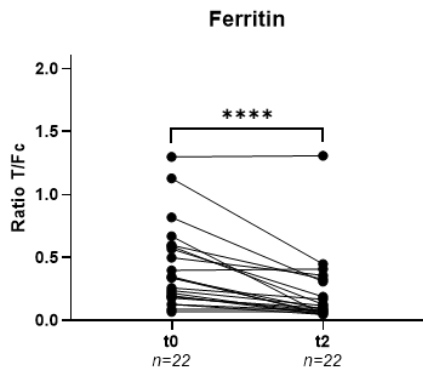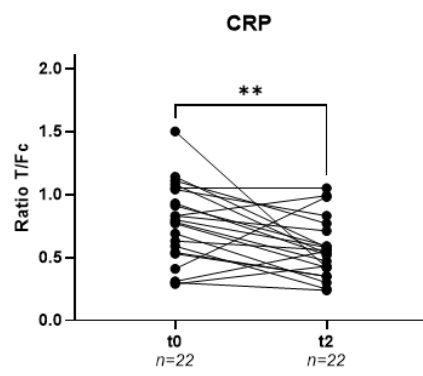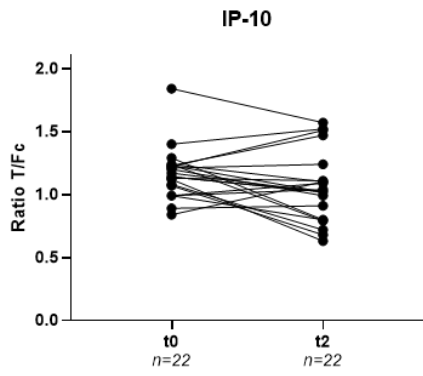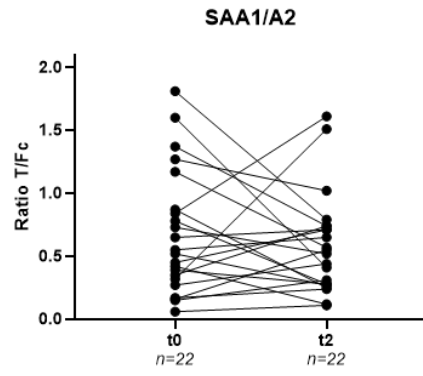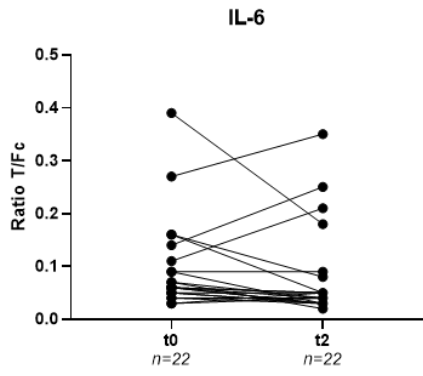
ApoA1

S100A12

SUPPLEMENTARY FIGURE S7: *Paired visualization of treatment monitoring for TB, Related to Figure 4*

Treatment monitoring of TB patients (n=22) over time. S100A12 data were missing for one patient. Levels of IL-6, IP-10, ferritin, SAA1/A2, CRP, ApoA1, and S100A12 were measured by UCP-LFA in serum samples of pulmonary TB patients before treatment (t_0), and months 5 – 9 (t_2). Wilcoxon matched pairs signed rank tests were performed to determine the statistical significance between timepoints (p-values: *$p \leq 0 \cdot 05$, **$p \leq 0 \cdot 01$, ***$p \leq 0 \cdot 001$, ****$p \leq 0 \cdot 0001$). Fc: flow control line; T: test line; TB: tuberculosis.