

Supplementary information

Cytokine biosignature of active and latent *Mycobacterium tuberculosis* infection in children

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Supplementary Table S1. Median concentrations of Th17-related cytokines and inflammatory mediators in children's sera

Supplementary Table S2. The results of a linear model-based approach where three differences are tested: (1) between the HC and LTBI, (2) between HC and TB, and (3) between TB and LTBI.

Supplementary Table S3. The results of elastic-net multinomial regression model. The coefficients represent relative differences of serum expression of respective proteins between studied groups (under the optimal lambda parameter for the penalty function). The most informative set of markers was chosen based on the 5-fold cross-validation approach. The dashes correspond to non-informative predictors.

Supplementary Table S4. Levels of Th-17-related cytokines and inflammatory mediators in the sera from TB, LTBI and HC children with positive (TST-positive) or negative (TST-negative) skin reaction to tuberculin.

Supplementary Table S1. Median concentrations of Th17-related cytokines and inflammatory mediators in children's sera

Protein	TB	LTBI	HC
	Me; IQR (pg/ml)	Me; IQR (pg/ml)	Me; IQR (pg/ml)
Th-17-related cytokines			
IL-4	0; 0-57.7	0; 0-27.4	0; 0-50.6
IL-6	15.1; 3.8-35.7	2.14; 0-12.7	5.29; 0-24.8
IL-10	0; 0-25.0	0; 0-17.0	6.88; 0-34.5
IL-17F	17.5; 0-90.5	0; 0-41.0	5.34; 0-59.6
IL-21	46.4; 0-95.9	38.9; 0-56.2	4.26; 0-67.5
IL-22	7.22; 0-78.0	0; 0-39.6	0; 0-68.8
IL-25	0; 0-20.1	0; 0-11.1	4.8; 0-26.6
IL-31	725.0; 540.0-1133.0	680.0; 379.0-1226.0	822.0; 389.0-1228.0
IL-33	32.5; 12.9-215.0	30.2; 3.56-156.0	31.1; 0-272.0
IFN- γ	2.65; 0-42.2	4.88; 0-53.5	5.43; 0.66-121.0
sCD40L	2979.0; 1251.0-3562.0	2565.0; 1076.0-4688.0	1139.0; 538.0-3584.0
TNF- α	36.4; 19.9-108.0	30.3; 16.1-101.0	40.3; 18.6-101.0
IL-1 β	4.97; 0-39.7	0 ^a ; 0-16.7	6.68 ^a ; 0-44.0
IL-17A	0; 0-7.92	0 ^b ; 0-15.9	4.01 ^b ; 0-24.3
IL-23	24.5; 0-125.0	10.4 ^c ; 0-178.0	67.2 ^c ; 6.81-205.0
Inflammatory mediators			
APRIL/TNFSF13	864686 ^{d,e} ; 493031-1006100	439681 ^d ; 301873-816166	379919 ^e ; 287429-525611
BAFF/TNFSF13B	2412; 330-4834	1201; 631-3593	1195; 929-1634
sCD30/TNFRSF8	201; 53.1-513	320; 174-463	372; 270-555
sCD163	0.43; 0.19-2.88	0.19; 0.06-2.52	0.29; 0.07-0.98
Chitinase 3-like 1	3020; 1145-3723	2991; 1722-3666	2343; 1478-3494
gp130/sIL-6R	31926; 25440-33901	33448; 28523-40262	32038; 30185-35883
IFN- α 2	3.03; 1.39-3.92	3.14; 2.52-4.67	3.03; 2.52-3.77
IFN- β	2237; 1898-2372	2245; 2130-2336	2211; 2141-2294
IL-2	2682; 2379-2718	2659; 2609-2743	2665; 2567-2735
sIL-6R α	1376; 677-2428	1888; 1215-3703	2223; 1305-2967
IL-8	30.6; 6.92-398	8.29; 4.47-46.6	9.04; 5.13-43.4
IL-10	0.45; 0.01-0.6	0.54; 0.34-0.84	0.50; 0.27-0.70
IL-11	173; 80.6-192	165; 151-196	167; 144-188
IL-12 (p40)	7.82; 3.5-9.95	8.99; 7.21-11.0	8.25; 6.91-10.7
IL-12 (p70)	0.03; 0.01-0.04	0.04; 0.02-0.07	0.03; 0.02-0.04
IL-19	6.4; 3.4-9.7	7.69; 6.4-9.1	7.1; 5.9-8.2
IL-20	>5810	>5810	>5810
IL-22	5.98; 2.74-8.62	6.29; 4.24-9.33	5.67; 4.56-7.30
IL-26	3.72; 2.16-4.29	4.21; 3.49-4.97	4.13; 3.51-4.46
IL-27	5.64; 0-14.2	9.83; 3.63-20.8	7.71; 2.9-13.6
IL-28A/IFN- λ 2	0; 0-1813	1065; 0-2508	1065; 0-2070
IL-29/IFN- λ 1	14; 6.33-26.7	19.5; 12.3-25.6	14.4; 11.5-21.7
IL-32	0	0	0
IL-34	0; 0-1	0.45; 0-1.98	0; 0-0.18
IL-35	0; 0-5.95	0; 0-5.95	0; 0-1.99

LIGHT/TNFSF14	3.62; 0-19.2	1.17; 0-4.24	0.15; 0-2.61
MMP-1	28498; 13959-38039	25248; 20055-32649	24872; 20342-28479
MMP-2	1061 ^{f,g} ; 350-3589	3230 ^f ; 1651-5057	4541 ^g ; 3334-6087
MMP-3	369; 161-720	286; 187-418	310; 206-494
osteocalcin	>208184	>208184	>208184
osteopontin (OPN)	1867 ^{h,i} ; 634-3287	4370 ^h ; 1936-5738	3623 ⁱ ; 2377-5125
pentraxin-3 (PTX-3)	5235 ^j ; 4452-6435	6033; 5291-6639	6329 ^j ; 5531-7155
sTNF-R1	400; 111-970	546; 277-802	573; 373-763
sTNF-R2	254; 0-622	326; 149-567	312; 157-736
TSLP	25.4; 6.13-34.6	26.8; 19.8-42.9	24.6; 17.8-33.8
TWEAK/TNFSF12	56.7; 13.8-102	95.4; 61.1-140	78.2; 49.2-116

Abbreviations: TB – tuberculosis children; LTBI – children with latent *M.tb* infection, HC – healthy controls; Me – median, IQR – interquartile range.

^ap=0.035, ^bp=0.023, ^cp=0.031, ^dp=0.03; ^ep=0.0005; ^fp=0.02; ^gp=0.0001; ^hp=0.004; ⁱp=0.02; ^jp=0.03

Supplementary Table S2. The results of a linear model-based approach where three differences are tested: (1) between the HC and LTBI, (2) between HC and TB, and (3) between TB and LTBI.

Proteins	Coefficients			p value	adjective p value
	HC versus LTBI	HC versus TB	TB versus LTBI		
Th-17-related cytokines					
IL-21	-1.46E+02	-3.70E+01	-1.09E+02	p<0.001	p<0.001
sCD40L	-1.32E+03	-2.01E+03	6.92E+02	0.003	0.025
IL-10	1.51E+01	2.32E+01	-8.04E+00	0.034	0.174
IL-6	-1.87E+02	-8.01E+01	-1.07E+02	0.052	0.196
IL-31	8.22E+02	2.64E+02	5.58E+02	0.148	0.445
IL-17A	2.82E+01	3.24E+01	-4.14E+00	0.178	0.445
IL-23	1.96E+02	9.57E+02	-7.61E+02	0.254	0.543
TNF- α	1.58E+02	2.01E+02	-4.25E+01	0.349	0.641
IL-33	1.69E+02	1.99E+02	-3.00E+01	0.427	0.641
Inflammatory mediators					
MMP-2	1.55E+03	2.87E+03	-1.31E+03	p<0,001	0.003
IL-8	-1.71E+02	-1.13E+03	9.64E+02	0.003	0.057
sCD163	-1.04E+03	-4.48E+10	4.48E+10	0.012	0.151
LIGHT/TNFSF14	-2.46E+00	-3.13E+01	2.88E+01	0.028	0.261
IL-34	-1.93E+00	-1.42E+00	-5.13E-01	0.044	0.326
chitinase 3-like 1	-5.05E+02	-8.61E+02	3.57E+02	0.056	0.350
sTNF-R1	-1.89E+02	-2.95E+02	1.06E+02	0.075	0.401
IL-12 (p70)	-2.79E-02	-1.90E+00	1.87E+00	0.111	0.455
IFN- γ	4.72E+00	9.77E+00	-5.05E+00	0.117	0.455
osteopontin	-1.29E+03	1.20E+03	-2.49E+03	0.123	0.455

Supplementary Table S3. The results of elastic-net multinomial regression model. The coefficients represent relative differences of serum expression of respective proteins between studied groups (under the optimal lambda parameter for the penalty function). The most informative set of markers was chosen based on the 5-fold cross-validation approach. The dashes correspond to non-informative predictors.

	TB	LTBI	HC
Th17-related cytokines			
IL-1 β	-	-	-
IL-4	-	-	-
IL-6	-	-	-
IL-10	-	-	-
IL-17A	-	-	-
IL-17F	-	-	-
IL-21	-	-	-
IL-22	-	-	-
IL-23	-	-	-
IL-25	-	-	-
IL-31	-	-	-
IL-33	-	-	-
IFN- γ	-	-	-
sCD40L	-	-	-
TNF- α	-	-	-
Inflammatory mediators-			
Apil/TNFSF13	1.80E-02	-	-1.32E-03
BAFF/TNFSF13B	-	-	-
sCD30/TNFRSF8	-8.51E-03	-	9.92E-03
sCD163	-	-	-
Gp130/sIL-6Rbeta	-	-	-5.72E-03
IFN-alfa2	-	3.13E-01	-3.82E-03
IFN-gamma	-	-	-
IL-2	-	-	-
sIL-6Ralfa	-2.58E-03	4.26E-02	3.03E-03
IL-8	2.23E-04	-	3.65E-02
IL-10	-	-	-
IL-11	-	-	-7.29E-05
IL-12 (p40)	-	-	-
IL-12 (p70)	-	-	-
IL-19	-	-	-
IL-27	-	-	-
IL-28A/IFN lambda2	-	-	2.65E-01
IL-29/IFNlambda1	-	4.27E-02	-
IL-34IL-35	-	-	-
IL-35	-	-	-
LIGHT/TNFSF14	-	-	-
MMP-1	4.96E-02	-	-2.68E-02
MMP-2	-2.79E-02	-	2.26E-02
MMP-3	2.76E-02	-2.10E-02	-
osteopontin (OPN)	-2.35E-03	1.13E-02	-
Pentraxin 3	-	-	2.94E-02
sTNFR1	-	-	-
sTNFR2	-	-4.60E-03	1.20E-03
TSLP	-	1.49E-03	-
TWEAK/TNFSF12	-3.55E-03	-	4.76E-03

Supplementary Table S4. Levels of Th-17-related cytokines and inflammatory mediators in the sera from TB, LTBI and HC children with positive (TST-positive) or negative (TST-negative) skin reaction to tuberculin.

Protein	Concentration					
	Me (IQR)					
	TB		LTBI		HC	
	TST-positive	TST-negative	TST-positive	TST-negative	TST-positive	TST-negative
Th-17-related cytokines						
IL-4	0	0	0	0	0	0
	0-59.30	0	0-31.10	0-28.7	0-48.20	0-51
IL-6	14.5	43.8	0.965	6.51	2.86	5.84
	3.79-30.6	16.9-70.6	0-12.4	0.72-40.20	0-22.5	0.24-29.40
IL-10	0	8.88	0	1.12	9.75	6.17
	0-25.5	8.23-9.53	0-21.0	0-9.94	0-44.10	0-32.40
IL-17F	1.05	33.5	0	18.1	2.67	5.34
	0-92.9	17.5-49.5	0-54.2	0-30.5	0-72.8	0-59.5
IL-21	46.4	75.7	40.4	16.9	29.8	4.26
	0-93.7	4.26-147.0	0-57	4.1-56.6	0-94.7	0-65.6
IL-22	0	14.9	0	0	0	1.11
	0-88.9	7.22-22.6	0-42.7	0-29.2	0-51.8	0-74.2
IL-25	0	17.8	0	0.795	4.79	5.36
	0-17.8	6.45-29.1	0-13.9	0-5.27	0-29.2	0-24.6
IL-31	679	1967	680	645	861	804
	519-914	1256-2677	136-1279	491-1037	34.6-976	399-1304
IL-33	32.5	82	25.3	46	78.4	31.1
	13.9-217	0-164	3.56-291	13.7-115	0-368	0-268
IFN- γ	3.72	1.84	1.91	31.3	16.9	2.49
	0-81.7	1.02-2.65	0-50	3.02-76.7	1.04-160	0.64-120
sCD40L	3070 ^a	794 ^a	2473	3620	963	1139
	1940-4368	720-868	916-4712	1592-4192	404-3782	538-3446
TNF- α	35.4 ^b	73.3 ^b	30.3 ^c	61.9 ^c	20.2 ^d	42.1 ^d
	15.6-93.9	38.4-108	13.5-82.1	23.2-133	0.47-69	21.9-156
IL-1 β	3.54	91.3	0	20.3	1.06	5.76
	0-31.4	33.5-149.0	0-12.8	1.47-96.3	0-22.6	0-47.4
IL-17A	0	27.5	0	0	2.69	4.3
	0-6.53	7.92-47.2	0-22.2	0-3.97	0-18.3	0-24.3
IL-23	15.5	226	10.4	7.73	41.2	67.2
	0-111	119-333	0-243	0-47.3	5.66-194	6.83-213
Inflammatory mediators						
APRIL/TNFSF 13	842327	1147159	479407	331262	286638	386665
	470010-956723	944618-1349700	372325-816472	120142-737397	223971-422787	297992-567490
BAFF/TNFSF1 3B	1506	7689	1175	4601	1532	1188
	327-3938	3989-11389	617-2127	852-23118	737-1626	933-1771
sCD30/TNFRS F8	177	533	320	380	413	346
	46.6-334	513-553	170-458	231-629	381-557	252-558
Chitinase 3-like 1	3020	4346	2475	3080	3132	2042
	754-3565	1319-7373	1694-3712	2172-4559	1650-4391	1424-3493
gp130/sIL-6R	31631	110144	33329	33448	34131	31603
	22954-33298	32503-187786	28420-40658	29452-37300	33967-36255	30115-35557
IFN- α 2	3.03	3.33	3.08 ^e	5.45 ^e	3.32	2.97
	1.30-3.74	2.74-3.92	2.45-3.95	3.48-6.41	2.97-3.43	2.49-4.05
IFN- β	2237	2277	2219	2409	2245	2201
	1879-2346	2179-2376	2125-2317	2240-2429	2055-2283	2141-2302
IL-2	2682	2672	2645	2786	2698	2665
	2363-2714	2609-2735	2605-2732	2652-2825	2540-2707	2571-2742
sIL-6R α	1376	2576	1855	2193	2983	2015
	403-2282	1359-3793	1180-3719	1717-3179	1998-3701	1279-2916
IL-8	13.10	571.00	9.82	8.29	5.13	10.10
	6.92-254	289-853	4.31-38.4	4.81-3131	3.6-18.8	6.14-45.8
IL-10	0.45	0.47	0.52	1.1	0.49	0.52
	0.005-0.64	0.34-0.6	0.24-0.77	0.48-5.81	0.14-0.6	0.3-0.74
IL-11	173	166	163	179	167	168
	40.3-196	154-179	148-196	163-208	147-180	143-190
IL-12 (p40)	7.82	8.30	8.45 ^f	12.30 ^f	8.45	8.21
	3.24-10.1	7.37-9.23	6.84-10.2	10.3-25.6	6.61-8.6	6.91-11
IL-12 (p70)	0.03	0.06	0.03	0.06	0.04	0.03
	0.01-0.04	0.02-0.11	0.02-0.07	0.04-0.30	0.02-0.07	0.02-0.04
IL-19	6.40	7.43	7.53	11.1	7.04	7.04
	2.73-8.70	5.16-9.7	4.78-8.52	7.37-21.7	5.77-7.69	6.09-8.52
IL-20	>5810	>5810	>5810	>5810	>5810	>5810
	5.37	8.19	5.98 ^g	12.00 ^g	5.67	5.67
IL-22	1.86-7.86	7.59-8.79	3.78-7.34	7.95-250.00	3.97-5.98	4.59-7.51
	3.72	3.77	4.13	4.97	3.57	4.13
IL-26	1.78-4.46	3.49-4.05	3.14-4.91	4.85-20.60	3.18-4.13	3.57-4.46
	5.64	7.48	8.77	20.8	3.63	7.71
IL-27	0-13.10	0.79-14.2	0-14.40	8.77-96.4	1.7-12.00	3.14-14.2
	0	975	0 ^h	2508 ^h	1644	1065
IL-28A/IFN- λ 2	0-1813	0-1951	0-2219	1355-3042	0-2070	0-2289
	14	19.3	17.6	22.5	14.8	14
IL-29/IFN- λ 1	5.7-22.6	11.5-27.2	12.1-22.5	18.1-53.2	11.5-18.5	11.5-24.0

IL-32	0	0	0	0	0	0
IL-34	0 0-0.5	0.65 0-1.31	0.03 0-1.85	6 0.45-28	0.11 0-0.45	0 0-0.12
IL-35	0 0-7.46	0	0 0-5.95	0 0-37.4	0 0-2.61	0 0-1.36
LIGHT/TNFSF 14	3.62 0-34.4	5.75 0-11.5	0.86 0-3.93	3.2 1.89-57.2	0 0-1.86	0.3 0-2.81
MMP-1	28498 13517-36965	39611 25061-54161	24723 19056-31898	28498 25045-43179	22641 19797-28498	24872 20887-29072
MMP-2	1061 248-3757	1025 794-1256	3176 1505-5000	4463 2071-6358	3564 3396-3771	4720 3198-6682
MMP-3	313 95-694	566 411-720	271 121-397	341 274-675	286 181-608	313 215-490
osteocalcin	>208184	>208184	>208184	>208184	>208184	>208184
osteopontin (OPN)	1808 458-3181	2660 1872-3448	4187 1674-5633	4614 3011-23151	3157 1852-4036	3703 2463-5241
pentraxin-3 (PTX-3)	4832 4084-6492	5534 5235-5833	5776 ⁱ 4998-6485	7034 ⁱ 6237-8206	6635 6204-7213	6156 5342-7145
sTNF-R1	398 87-870	1348 787-1909	543 276-804	608 334-1148	704 381-744	562 351-804
sTNF-R2	254 0-595	634 206-1062	321 130-537	437 228-688	441 389-603	263 120-786
TSLP	25.4 5.8-36.9	26.4 20.3-32.4	24.9 ^j 19.3-40.7	48.9 ^j 33.6-65.8	24.1 12.6-33.3	25.2 18.4-37.4
TWEAK/TNFS F12	56.7 9.56-102	122 45.3-198	94.8 56.4-130	142 62.4-167	105 74.1-113	76.3 48-120

Abbreviations: TB – tuberculosis children; LTBI – children with latent *M.tb* infection, HC – healthy controls; Me – median, IQR – interquartile range.

^ap=0.02; ^bp=0.04; ^cp=0.0001; ^dp=0.0001; ^ep=0.04; ^fp=0.01; ^gp=0.04; ^hp=0.04; ⁱp=0.01; ^jp=0.02.