Supporting Information

The dynamics of immune responses to *Mycobacterium tuberculosis* during different stages of natural infection: a longitudinal study among Greenlanders

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METHODS

Table A: Information on antigens and ratio cut-points used in definition of a positive immune response in the analyses for each *Mtb* antigen

Antigen							Ratio cut-points used in definition of a positive immune response in the analyses for each *Mtb* antigen		
Locus tag	Gene description[1]	Known as	Peptides	Length/ overlap	Purity	Origin	Specificity	Sensitivity	Ratio cut-point
-	-	PPD	-	-	-	SSI, Denmark	95%	76%	79.84
QFT antigens									
Rv3875	ESAT-6 protein EsxA	ESAT-6	1-7	25/10	>90%	Genecust, Luxemburg	95%	90%	35.87
Rv3874	EAST-6-like protein EsxB	CFP10	1-6	25/10	>90%	Genecust, Luxemburg	95%	98%	10.59
Constitutive									
Rv3614*	ESX-1 secretion-associated protein EspD	Rv3614c snm10	1-17	20/10	>80%	Genecust, Luxemburg	95%	56%	21.98
Rv3849*	ESX-1 transcriptional regulator EspR	-	1-12	20/10	>80%	Genecust, Luxemburg	95%	55%	8.94
Rv3865	ESX-1 secretion-associated protein EspF	-	1-9	20/10	>80%	Genecust, Luxemburg	95%	80%	4.22
Rv3872	PE family protein PE35	-	1-9	20/10	>80%	Genecust, Luxemburg	95%	78%	4.26
Early									
Rv0203	Hypothetical protein	-	1-13	20/10	>80%	Genecust, Luxemburg	95%	96%	24.29
Rv0642a*	Hydroxymycolate synthase MmaA4	Rv0642c	1-14	20/10	>80%	Genecust, Luxemburg	95%	58%	5.42
Rv0642b*	Hydroxymycolate synthase MmaA4	Rv0642c	15-29	20/10	>80%	Genecust, Luxemburg	95%	95%	3.13
Rv1196a	PPE family protein PPE18	mtb39a	1-19	20/10	>80%	Genecust, Luxemburg	95%	89%	9.39
Rv1196b	PPE family protein PPE18	mtb39a	20-33	20/10	>80%	Genecust, Luxemburg	95%	94%	14.73
LTBI									
Rv2031	Alpha-crystalin, HspX	Rv2031c acr	1-13	20/10	>80%	Genecust, Luxemburg	95%	95%	6.05
Rv2244*	Meromycolate extension acyl carrier protein acpM	-	1-11	20/10	>80%	Genecust, Luxemburg	95%	93%	3.13
Rv1284	Beta-carbonic anhydrase canA	-	1-15	20/10	>90%	Genecust, Luxemburg	95%	90%	16.95
Rv2659a	Prophage integrase	Rv2659c	1-13	20/10	>90%	Genecust, Luxemburg	95%	82%	15.64
Rv2659b	Prophage integrase	Rv2659c	14-25	20/10	>90%	Genecust, Luxemburg	95%	74%	78.26
Rv2659c	Prophage integrase	Rv2659c	26-37	20/10	>90%	Genecust, Luxemburg	95%	73%	57.40
Rv2660c	Hypothetical protein	Rv2660c	1-7	20/10	>90%	Genscript, USA	95%	87%	8.08

*No human or murine data. References are not exhaustive

[1]Pubmed, gene database

PPD, purified protein derivate, is in the tuberculin skin test (TST) and has a substantial antigenic overlap with BCG [1–3].

ESAT-6, Rv2660c and RV1196 are in novel TB vaccines currently in clinical testing [4].

ESAT-6 and CFP10 are two of the antigens used in QFT [1,5,6].

ESAT-6, CFP10, Rv3614, Rv3865, Rv3872 and Rv3849 are region of difference 1 (RD1) antigens and all but Rv3872 are part of the ESAT-6 antigen secretion system operon (ESX-1) [6–11].

Rv3872 and Rv1196 are part of the PE and PPE families, which constitute 10% of the *Mtb* genome [10–14].

Rv0203 is proposed to be a secreted heme transporter that mediate *Mtb* heme iron uptake [15,16].

Rv2660c, Rv1284, Rv2659, Rv2031, Rv2244 are all induced by either bacterial hypoxia or starvation, are a part of the enduring hypoxia response or dormancy regulon antigens (DosR) [17–27].

RESULTS

Table B: Further categorisation of year of QFT testing or TB/preventive treatment among participants at enrolment by *Mtb* infection stage among 65 young adults in East Greenland

Characteristics at enrolment	All		*Mtb* infection Undetectable by QFT		*Mtb*-infected All		Non-treated		Treated (Preventive treatment)		Treated (TB treatment)		All Subsequent TB	
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
All	65		11		54		22		12		20		5	
Year of:														
prior positive QFT or TST/ TB treatment / preventive treatment														
1996	1	(2)	-		1	(2)	0	(0)	0	(0)	1	(5)	0	(0)
2000	1	(2)	-		1	(2)	0	(0)	0	(0)	1	(5)	0	(0)
2005	2	(3)	-		2	(4)	1	(5)	0	(0)	1	(5)	0	(0)
2008	6	(9)	-		6	(11)	6	(27)	0	(0)	0	(0)	1	(20)
2009	5	(8)	-		5	(9)	5	(23)	0	(0)	0	(0)	0	(0)
2010	5	(8)	-		5	(9)	1	(5)	3	(25)	1	(5)	0	(0)
2011	26	(40)	-		26	(48)	6	(27)	9	(75)	11	(55)	1	(20)
2012	6	(9)	-		6	(15)	3	(14)	0	(0)	3	(15)	1	(20)
In TB treatment	3	(5)	-		-	-	-	-	-	-	3	(15)	-	-
None	11	(17)	11	(100)	0	(0)	0	(0)	0	(0)	0	(0)	2	(40)

Subsequent TB: tuberculosis disease notified through the National TB register at any point from study enrolment to end of follow-up.
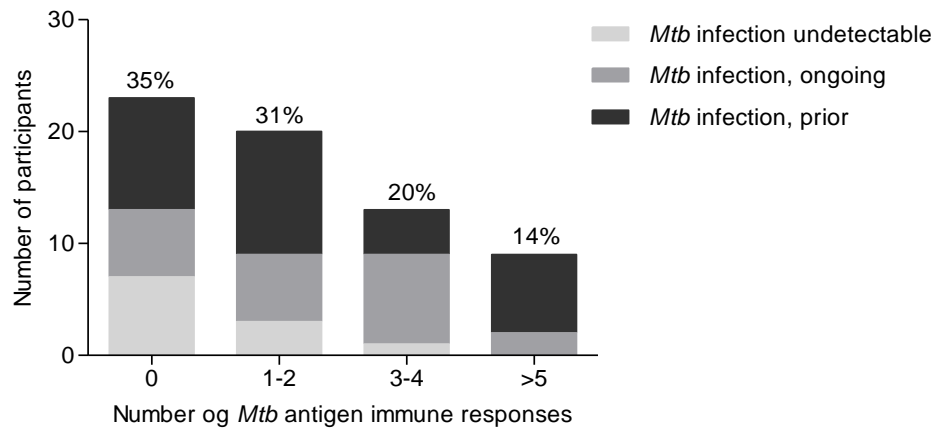QFT: QuantiFERON®-TB Gold test
TST: Tuberculin skin test

Table C: Characteristics for participants and non-participants and participation rate and OR for participation according to characteristics at enrolment

Characteristics at enrolment	Non-Participants N	Participants N	Participation rate %	OR (95% CI) for participants Unadjusted OR	P value
All	116	65			
Sex					
Women	53	36	40	1 (ref)	
Men	63	29	32	0.68 (0.37-1.25)	*0.21*
BCG-vaccination					
No	100	59	37	1 (ref)	
Yes	16	6	27	0.64 (0.24-1.71)	*0.37*

Of 181 eligible, 65 participants were enrolled and tested for *Mtb* antigen immune responses, after enrolment no participants were excluded. All participants had a PHA response >74.6 pg/ml IFNγ.

Fig A

Number of participants (y-axis, 0–30)

35%, 31%, 20%, 14% above bars for categories 0, 1-2, 3-4, >5

Number og *Mtb* antigen immune responses (x-axis)

Legend:
- *Mtb* infection undetectable
- *Mtb* infection, ongoing
- *Mtb* infection, prior

Fig A: Distribution of number of immune responses to non-QFT antigens per participant by *Mtb* infection stage at enrolment. Of 65 participants 42 (65%) participants had an immune response to at least one of the 12 non-QFT antigens. Among *Mtb*-infected participants, 70% had an immune response to at least one non-QFT antigen. 34% of participants had immune responses to three or more non-QFT antigens.

Table D: Prevalence of immune responses and median IFNy response among participants with prior *Mtb* infection at enrolment by treatment status, among 65 young adults in East Greenland

	Prior *Mtb* infection, Treated											
	All				**Treated with preventive monotherapy**				**Treated for notified TB**			
			Median	(range)			Median	(range)			Median	(range)
	%	(N)	pg/ml		%	(N)	pg/ml		%	(N)		
All		(32)				(12)				(20)		
QFT	97	(31)	157	(16-400)	92	(11)	104	(16-400)	100	(20)	203	(18-400)
7-day IGRAs												
PPD[a]	94	(30)	2177	(178-3741)	83	(10)	1397	(208-2560)	100	(20)	2463	(697-3735)
QFT Ags[b]												
(any[c]/max[d])	**97**	**(31)**	**764**	**(80-3647)**	**100**	**(12)**	**502**	**(80-3647)**	**95**	**(19)**	**764**	**(159-2807)**
ESAT-6	94	(30)	756	(80-3647)	92	(11)	485	(80-3647)	95	(19)	764	(159-2807)
CFP10	53	(17)	318	(50-784)	25	(3)	440	(50-784)	70	(14)	270	(56-765)
Constitutive Ags					17							
(any/max)	**17**	**(6)**	**171**	**(48-234)**		**(2)**	**174**	**(133-215)**	**20**	**(4)**	**151**	**(48-234)**
Rv3614	16	(5)	89	(42-215)	8	(1)	215	(-)	20	(4)	68	(42-209)
Rv3849[a]	16	(5)	92	(43-137)	8	(1)	137	(-)	20	(4)	73	(43-116)
Rv3865	6	(2)	90	(48-133)	8	(1)	133	(-)	5	(1)	47	(-)
Rv3872	3	(1)	234	(-)	0	(0)	-	(-)	5	(1)	234	(-)
Early Ags												
(any/max)	**44**	**(14)**	**106**	**(46-329)**	**33**	**(4)**	**94**	**(51-180)**	**50**	**(10)**	**106**	**(46-329)**
Rv0203[a]	19	(6)	85	(43-329)	8	(1)	131	(-)	25	(5)	74	(43-329)
Rv0642	3	(1)	130	(-)	0	(0)	-	(-)	5	(1)	130	(-)
Rv1196	44	(14)	87	(45-329)	33	(4)	57	(51-180)	50	(10)	106	(45-329)
LTBI Ags												
(any/max)	**60**	**(19)**	**177**	**(44-918)**	**50**	**(6)**	**58**	**(44-491)**	**65**	**(13)**	**184**	**(45-918)**
Rv2031[a]	22	(7)	184	(66-624)	17	(2)	163	(66-260)	25	(5)	184	(79-624)
Rv2244	9	(3)	57	(46-120)	0	(0)	-	(-)	15	(3)	56	(46-120)
Rv1284	22	(7)	71	(50-918)	8	(1)	50	(-)	30	(6)	92	(62-918)
Rv2659	47	(15)	62	(44-491)	33	(4)	47	(44-491)	55	(11)	71	(45-478)
Rv2660c	0	(0)	-	(-)	0	(0)	-	(-)	0	(0)	-	(-)
Combination of Ags												
Rv2659/Rv1196	63	(20)	90	(44-491)	58	(7)	51	(44-491)	65	(13)	124	(49-477)

[a] One donor missing one stimulation. [b] Ags: antigens. [c] Immune responses for groups of antigens (QFT, constitutive, early, LTBI, Rv2659/Rv1196) were defined as having an immune response to at least one of the antigens and [d] the IFNy response magnitude was defined by the maximum response.
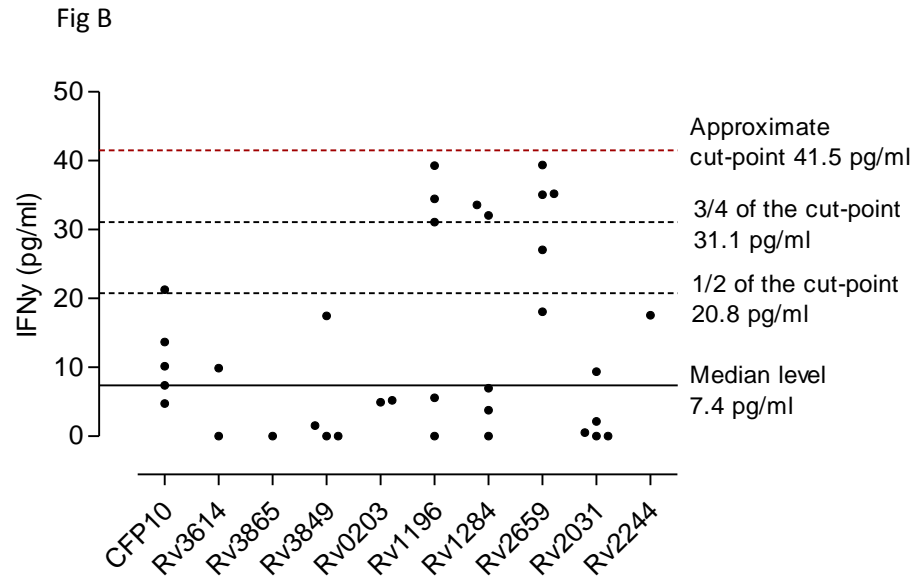
Fig B

Fig B: Level of IFNy among reverts from first to second assessment (35 immune responses to 10 antigens in 19 participants). Rv1196 and Rv2659 response levels were generated from the maximum response of the included pools, which partly explains higher values for these two antigens compared to other antigens. The median IFNy level was 7.4 pg/ml. 80% the IFNy levels were less than ¾ of the cut-point level. When not considering Rv1196 and Rv2659, 97% of the IFNy levels were less that ¾ of the cut-point level.

Table E: Estimated annual reversion risk during follow-up, among participants with *Mtb* antigen immune response at least once and with subsequent assessment

Antigens	All — Estimated annual reversion risk %	All — Revert during follow-up N	All — Total N	*Mtb* infection undetectable by QFT — Estimated annual reversion risk %	*Mtb* infection undetectable by QFT — Revert during follow-up N	*Mtb* infection undetectable by QFT — Total N	*Mtb* infection All — Estimated annual reversion risk %	*Mtb* infection All — Revert during follow-up N	*Mtb* infection All — Total N	Reversion risk: *Mtb*-infection QFT un-detectable vs detectable P value[a]	*Mtb* infection Ongoing, non-treated — Estimated annual reversion risk %	Ongoing, non-treated — Revert during follow-up N	Ongoing, non-treated — Total N	*Mtb* infection Prior, treated[d] — Estimated annual reversion risk %	Prior, treated — Revert during follow-up N	Prior, treated — Total N	Reversion risk: on-going vs. prior P value[b]
QFT	0	0	49	0	0	1	0	0	48	-	0	0	19	0	0	29	-
7-day IGRAs																	
PPD	5	2	52	0	0	4	5	2	48	-	6	1	20	4	1	28	0.79
QFT Antigens																	
Any[c]	5	2	53	0	0	3	5	2	50	-	6	1	20	4	1	30	0.78
ESAT-6	5	2	52	0	0	3	5	2	49	-	6	1	20	4	1	29	0.77
CFP10	34	10	35	100	1	1	32	9	34	1.00	8	1	15	48	8	19	0.06
Constitutive antigens																	
Any[c]	60	8	16	58	2	4	61	6	12	0.93	69	3	20	55	3	6	0.64
Rv3614	65	5	9	75	1	2	63	4	7	0.77	58	1	2	65	3	5	0.87
Rv3849	91	11	14	83	3	4	94	8	10	0.53	84	3	5	99	5	5	0.29
Rv3865	46	1	3	-	0	0	46	1	3	-	0	0	1	59	1	2	-
Rv3872	76	3	5	-	0	0	76	3	5	-	89	2	3	58	1	2	0.55
Early antigens																	
Any[c]	44	13	39	75	1	2	42	12	37	0.38	38	5	18	47	7	19	0.63
Rv0203	52	8	19	0	0	1	53	8	18	-	73	7	12	17	1	6	0.07
Rv0642	45	2	6	0	0	1	51	2	5	-	45	1	3	58	1	2	0.80
Rv1196	48	13	36	100	1	1	45	12	35	1.00	44	5	16	47	7	19	0.88
LTBI antigens																	
Any[c]	41	16	50	18	1	8	45	15	42	0.29	54	8	19	38	7	23	0.34
Rv2031	78	13	19	79	1	1	78	12	18	0.97	94	8	9	55	4	9	**<0.05**
Rv2244	95	9	9	-	0	0	95	9	9	-	91	2	2	96	7	7	0.72
Rv1284	93	12	14	75	1	2	95	11	12	0.48	100	4	4	90	7	8	-
Rv2659	49	17	47	18	1	8	55	16	39	0.18	63	8	18	49	8	21	0.43
Rv2660c	-	0	0	-	0	0	-	0	0	-	-	0	0	-	0	0	-
Combinations of antigens[c]																	
Rv2659/ Rv1196	28	11	54	18	1	8	29	10	46	0.60	29	4	20	30	6	26	0.92
CFP10 and Rv2659/ Rv1196	55	14	32	100	1	1	52	13	31	1.00	38	4	14	63	9	17	0.22

The table presents three columns per group (e.g. all, *Mtb* infection undetectable, *Mtb* infection). The right column is the total number of participants with *Mtb* antigen immune responses at either the first or the second assessment, thus have the possibility to revert (e.g. for Rv0203, 19 participants with *Mtb* antigen immune responses had the possibility to revert). The middle column shows the number of participants who revert during follow-up (e.g. for Rv0203, 8 of 19 participants revert during follow-up), and the left column shows the estimated annual reversion risk (e.g. for Rv0203 the annual conversion risk is 52%). [a] P values relate to homogeneity test for odds of *Mtb* antigen reversion among all participants by *Mtb* infection status (udetectable/detectable *Mtb* infection). [b] P values relate to homogeneity test for odds of *Mtb* antigen reversion among participants with *Mtb* infection by treatment status. [c] For groups of antigens (QFT antigens, constitutive, early, LTBI and Rv2659/1196) immune responses to at least one of the antigens defined having a positive immune response, while a negative immune response required all of the antigen immune responses within the group to be below the cut-point. [d] Treated participants were defined by both prior LTBI and prior TB treatment.

Table F. Adjusted Hazard ratios (HRs) for subsequent TB (N=5) by *Mtb* antigen immune response at enrolment, history of conversion and history of reversion during follow-up. Adjusted for *Mtb* infection status

| Antigens	Participants with *Mtb* antigen immune response at enrolment and risk of subsequent TB		History of conversion during follow-up and risk of subsequent TB		History of reversion during follow-up and risk of subsequent TB	
	HR (95%CI)	P value	HR (95%CI)	P value	HR (95%CI)	P value
QFT	-	-	-	-	-	-
7-day IGRAs						
PPD	-	-	3.77 (0.30-47.75)	0.31	-	-
QFT antigens						
Any	-	-	9.09 (0.64-129.28)	0.10	-	-
Esat-6	-	-	9.09 (0.64-129.28)	0.10	-	-
CFP10	0.38 (0.03-4.40)	0.44	3.86 (0.57-26.07)	0.17	3.28 (0.30-26.44)	0.33
Constitutive antigens						
Any	1.03 (0.11-9.69)	0.98	2.19 (0.33-14.42)	0.42	1.98 (0.18-21.23)	0.57
Rv3614	-	-	2.01 (0.29-13.83)	0.48	-	-
Rv3849	1.20 (0.13-11.53)	0.87	-	-	-	-
Rv3865	-	-	-	-	-	-
Rv3872	-	-	-	-	-	-
Early antigens						
Any	0.69 (0.06-7.88)	0.77	6.97 (0.73-66.39)	0.09	1.90 (0.19-19.52)	0.59
Rv0203	2.05 (0.17-24.92)	0.57	3.48 (0.46-26.09)	0.23	-	-
Rv0642	-	-	2.45 (0.19-30.86)	0.49	-	-
Rv1196	0.83 (0.07-9.26)	0.88	2.47 (0.41-15.05)	0.33	1.90 (0.19-19.52)	0.59
LTBI antigens						
Any	1.28 (0.21-7.81)	0.79	0.86 (0.14-5.43)	0.87	3.96 (0.48-32.73)	0.20
Rv2031	1.08 (0.12-10.20)	0.94	2.68 (0.37-19.34)	0.34	3.86 (0.55-26.99)	0.17
Rv2244	-	-	-	-	-	-
Rv1284	2.69 (0.24-30.49)	0.42	1.06 (0.10-10.92)	0.96	1.15 (0.11-12.05)	0.91
Rv2659	2.08 (0.34-12.59)	0.42	0.50 (0.07-3.62)	0.50	3.27 (0.37-29.01)	0.29
Combinations						
Rv2659/Rv1196	1.40 (0.23-8.46)	0.72	0.71 (0.11-4.56)	0.72	1.77 (0.17-18.19)	0.63
CFP10 and Rv2659/Rv1196	0.83 (0.07-10.32)	0.89	2.45 (0.37-16.13)	0.35	5.95 (0.72-49.06)	0.10

HRs relate to the risk of TB during follow-up.

Fig C
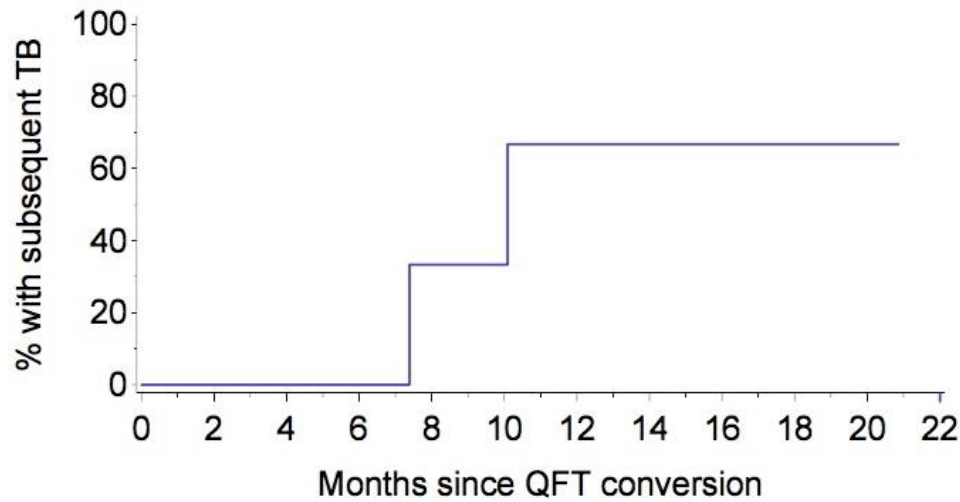
Fig C: Percentage with subsequent TB among individuals with QFT conversion during follow-up by months since QFT conversion (estimated by the Kaplan Meier estimator). Three participants had QFT conversion during follow-up, two progress to subsequent TB. The time from infection to TB was 7 and 10 months for two participants while no TB was observed in one participant during 21 months of follow-up.

Fig D
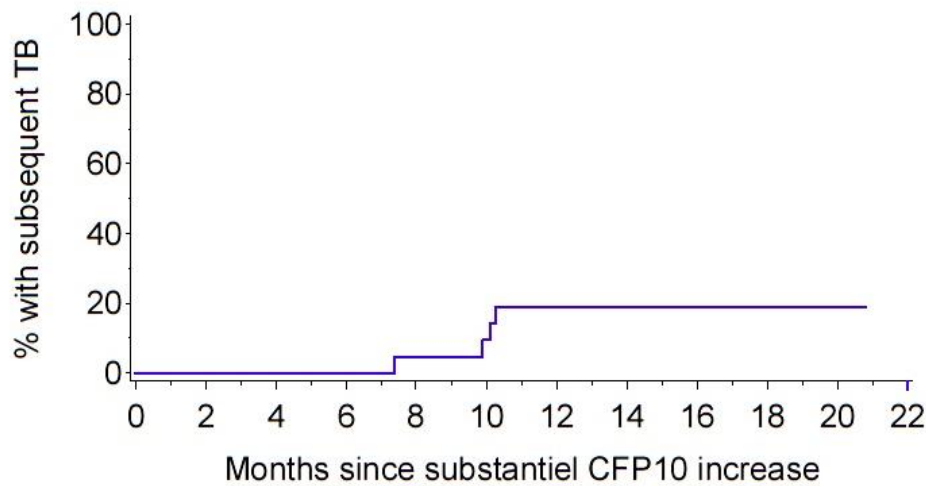
Fig D: Percentage with subsequent TB among individuals with a substantial CFP10 increase (≥103.39 pg/ml) during follow-up by months since CFP10 increase (estimated by the Kaplan Meier estimator). A substantial CFP10 increase was observed for 21 participants had during follow-up, four progress to subsequent TB. The time from CFP10 increase to TB was 7, 10, 10 and 10 months for four participants while no TB was observed in 17 participants until end of follow-up

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