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Original article

CXCL10/IP-10 release is induced by incubation of whole blood from tuberculosis patients with ESAT-6, CFP10 and TB7.7☆,☆☆

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Abstract

IFN-γ responses to *Mycobacterium tuberculosis* specific antigens are used as in vitro diagnostic tests for tuberculosis infection. The tests are sensitive and specific for latent and active tuberculosis disease, but sensitivity may be reduced during immunosuppression. The objective of the study was to explore new ways to improve the diagnosis of tuberculosis infection using CXCL10 and IL-2 as alternative markers to IFN-γ. CXCL10, IL-2, and IFN-γ responses to stimulation with ESAT-6/CFP10/TB7.7 were assessed in 12 Quantiferon positive, 8 Quantiferon negative tuberculosis patients and 11 Quantiferon negative controls. CXCL10 and IL-2 were determined by multiplex and IFN-γ by the Quantiferon ELISA. The median antigen specific CXCL10, IFN-γ, and IL-2 responses in patients with tuberculosis were 870 pg/ml (range 261–1576 pg/ml), 217 pg/ml (81–1273 pg/ml), 59 pg/ml (14–276 pg/ml) respectively, and the CXCL10 responses were significantly higher than any of the other cytokines measured ($p = 0.001$). In 4/7 individuals with a negative ($n = 6$) or indeterminate ($n = 1$) Quantiferon test, antigen specific CXCL10 responses were detectable at high levels ranging from 196–532 pg/ml. In conclusion CXCL10 was strongly induced after *M. tuberculosis* specific stimulation and sensitivity appeared superior to the Quantiferon test. Our findings suggest that CXCL10 may serve as an alternative or additional marker for the immunodiagnosis of tuberculosis.

Keywords: Tuberculosis; CXCL10; ESAT-6; CFP10; TB7.7; Interferon-γ; IL-2; Prevention and control; Quantiferon

1. Introduction

Recently, two immunodiagnostic tests for tuberculosis (TB) infection have been introduced (rev in refs. [1,2]). The tests, Quantiferon® TB Gold, Quantiferon® TB-Gold In Tube (Cellestis Ltd., Carnegie, Australia) and T-SPOT TB ELISPOT® (Immunotech, Oxford, UK) all measure Interferon-γ (IFN-γ) release by sensitized T cells after stimulation with peptides of *M. tuberculosis* specific antigens ESAT-6, CFP-10, and the Quantiferon In Tube test also uses TB7.7. These IFN-γ release assays (IGRAs) have proven both sensitive (75–97%) and specific (>95%) for the diagnosis of TB infection, and appear to be more accurate than the Tuberculin skin test (TST)

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☆☆ Conflicts of interests: Copenhagen University Hospital has applied for a patent disclosing CXCL10 as marker for *M. tuberculosis* infection. Morten Ruhwald, Pernille Ravn and Jesper Eugen-Olsen are registered as co-inventors. If the research leads to development of an improved *in vitro* tuberculosis test, the inventors will make every effort to assure access to the test in the Bandim Health Project, Guinea-Bissau.

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(rev in refs. [2,3]). There is an ongoing debate about the sensitivity of the IGRAs [4–8] and there is a great concern that the sensitivity may be reduced in certain risk groups. Although this has not been formally shown for the IGRAs, the concern is very relevant and the purpose of the present study was to explore new ways to improve the diagnosis of infection with *M. tuberculosis* using alternative markers than IFN-γ.

A preliminary screening of 25 cytokines, chemokines and soluble cytokine receptors using the xMAP multiplex technology (Luminex) indicated that a number of markers were released after antigen stimulation. CXCL10 and IL-2 were selected on the basis of a low spontaneous release and a high expression after stimulation with *M. tuberculosis* specific antigens.

CXCL10, also named IFN-γ-inducible protein 10 (IP-10), is a member of the CXC-chemokine family. It is expressed in lymphocytes and monocytes [9] and is involved in trafficking monocytes and activated Th1 cells to inflamed foci through interaction with the CXCR3 chemokine receptor. High levels of CXCL10 has been found in delayed type hypersensitivity reaction to Tuberculin Purified Protein Derivative (PPD) [9], in lymph node and lung tuberculous granulomas [10], in pleural effusions of TB patients [11], in the plasma of TB patients [12,13], and TB-HIV co-infected experiencing immune reconstitution syndrome [14].

IL-2 a well known pro-inflammatory cytokine involved in a wide range of T cell functions e.g. clonal expansion of antigen specific T cells. High levels of *in vitro* IL-2 production have been described following stimulation of whole blood from TB patients with PPD [15] and *M. tuberculosis* peptides [16], and IL-2 concentration was found to be elevated in plasma of patients with TB and normalize with treatment [17].

In this small first series of TB patients and healthy controls tested, we report that expression of CXCL10, IL-2, and IFN-γ is induced by ESAT-6, CFP-10 and TB7.7 challenge. We demonstrate that CXCL10 is expressed in a highly *M. tuberculosis* specific manner and suggest that CXCL10 holds the potential as a novel and sensitive marker for the immunodiagnosis of infection with *M. tuberculosis*.

2. Materials and methods

2.1. Study population

Patients with active TB were included from the study area of the Bandim Health Project, Guinea-Bissau, West Africa and from the Department for Infectious Diseases at the Copenhagen University Hospital, Hvidovre, Denmark.

Initially 12 patients were tested, 8 were from Guinea-Bissau, and 4 from Denmark all had sputum microscopy and/or culture positive TB and a known positive Quantiferon In Tube (QFT-IT) test. Eleven had pulmonary TB and 1 had extrapulmonary TB. Eleven were HIV tested and found HIV seronegative. The mean age was 38 years (range 16–70 years), and 6 were male. Eleven were included within the first week of TB chemotherapy and one had blood drawn at week 10 after initiation of TB chemotherapy.

Seven patients with known microscopy and/or culture positive TB were selected because they had a negative ($n = 6$) or indeterminate QFT-IT test ($n = 1$). Two had extrapulmonary TB and 5 had pulmonary TB. Details are shown in Table 2. Six were HIV tested and 5 were HIV positive of these 4 had a CD4 cell count measurement showing a median of 317 cells/μl (range 32–767 cells/μl).

Eleven healthy, Danish volunteers with no history of exposure to a case of active TB and with a known negative QFT-IT test were selected as controls. The mean age was 33 years (range 26–43 years), and 4 were male. None were HIV tested.

The study was approved by the Ethical Committee of Copenhagen and Frederiksberg Commune (KF 01278477), and the Ethical Committee of Guinea-Bissau.

2.2. Whole blood stimulation

Briefly, 1 ml of blood was drawn directly into vaccutainer tubes from Cellestis. The tubes were precoated with saline (negative control), peptides of ESAT-6, CFP10 and TB 7.7 (*M. tuberculosis* specific antigens), or PHA (positive mitogen control). The tubes were incubated for 20–24 h at 37 °C, and plasma was harvested and frozen until further analysis.

2.3. IFN-γ determination by ELISA

The amount of IFN-γ produced was determined by ELISA using the commercially available assay following the manufacturer's instructions as previously described [18]. In order

Table 1
Plasma IFN-γ, CXCL10 and IL-2 release in antigen stimulated whole blood culture

	IFN-γ CXCL10 and IL-2 production (pg/ml) in plasma of saline, antigen or mitogen stimulated whole blood from:	
	Controls ($n = 11$)	TB patients ($n = 12$)
IFN-γ (ELISA)		
Saline	8 (7–12)[a]	10 (7–135)
Antigens	9 (5–14)	224 (99–1283)[b,c]
Mitogen	1613 (46–1799)	273 (32–1751)
IFN-γ (multiplex)		
Saline	5 (<5–12)	5 (<5–70)
Antigens	5 (<5–12)	91 (37–464)[c]
Mitogen	292 (<5–2981)	161 (<5–2913)
CXCL10		
Saline	27 (18–141)	151 (61–992)[d]
Antigens	40 (20–159)	1025 (497–2080)[c]
Mitogen	994 (164–>2800)	843 (384–2588)
IL-2		
Saline	6 (<6–58)	6 (<6–16)
Antigens	6 (<6–46)	65 (20–282)[c]
Mitogen	287 (<6–1395)	13 (<6–127)[e]

[a] Median (range).
[b] Differences between controls and patients were determined using Kruskal–Wallis test.
[c] $p < 0.0001$.
[d] $p < 0.0005$.
[e] $p < 0.008$.

Table 2
Patients with active TB and negative or indeterminate QFT-IT test result

Patient no.	Country[a]	Age (years)	Diagnosis[b]	Microscopy/ culture	HIV	CD4 (cells/μl)	IFN-γ (pg/ml)[c]			QFT-IT result[d]	CXCL10 (pg/ml)[c]			CXCL10 result[e]
							Saline	Antigens	Mitogen		Saline	Antigens	Mitogen	
1	GB	50	pTB	+/n.d.[f]	pos	300	8	7	32	neg	72	383	520	pos
2	GB	45	pTB	+/n.d.	pos	333	13	13	596	neg	97	106	757	neg
3	GB	50	pTB	+/n.d.	pos	n.d.	7	10	1446	neg	98	107	2800	neg
4	DK	39	epTB	+/+	pos	767	3	2	1051	neg	29	39	2800	neg
5	DK	36	pTB	+/+	neg	n.d.	38	44	249	neg	716	1040	2800	pos
6	DK	41	pTB	+/+	n.d.	n.d.	7	20	291	neg	31	227	556	pos
7	DK	26	epTB	+/+	pos	32	20	28	30	ind.[g]	301	834	394	pos

[a] GB, Guinea-Bissau; DK, Denmark.
[b] pTB, pulmonary TB, epTB, extrapulmonary TB.
[c] Whole blood was stimulated 20–24 h with either saline, *M. tuberculosis* specific antigens (ESAT-6, CFP10, and TB 7.7) or mitogen (PHA). Cytokine production was measured by ELISA (IFN-γ) and multiplex (CXCL10).
[d] A positive QFT-IT response was defined as an antigen specific response of 17.5 pg/ml above nil according to the manufacturer guidelines.
[e] A positive CXCL10 response was defined as an antigen specific CXCL10 response of 36 pg/ml above nil arbitrarily based on the mean results of healthy controls +3 standard deviations.
[f] n.d., not done.
[g] Indeterminate.

to compare cytokine and chemokine levels all results are shown as pg/ml. One International Unit (IU) of IFN-γ corresponds to 50 pg according to the National Institute for Biological Standards and Control, (Hertfordshire, United Kingdom). The QTF-IT result was considered ''positive'' if the antigen specific response (i.e. cytokine concentration in plasma of antigen stimulated whole blood after subtracting the cytokine concentration of unstimulated whole blood) was ≥17.5 pg/ml (0.35 IU/ml) and the stimulation index (i.e. the cytokine concentration in plasma of antigen stimulated whole blood divided to the cytokine concentration of unstimulated whole blood) was ≥1.25, regardless of the mitogen stimulated IFN-γ response; ''negative'' if the antigen specific response was <17.5 pg/ml (0.35 IU/ml) and the mitogen stimulated IFN-γ response was ≥25 pg/ml (0.5 IU/ml). A test was considered ''indeterminate'' if either both the antigen specific response was <17.5 pg/ml and the mitogen stimulated IFN-γ response was ≤25 pg/ml (0.5 IU/ml) or if the IFN-γ response in the unstimulated sample was ≥400 pg/ml (8 IU/ml) regardless of antigen specific or mitogen stimulated response.

2.4. Cytokine and chemokine determination by multiplex

Cyto- and chemokines (CXCL10, IL-2 and IFN-γ) were measured in unstimulated, Ag stimulated and mitogen stimulated plasma by xMAP multiplex technology on the Luminex platform (Luminex, Austin TX, USA), using Biosource reagents (Biosource, Camarillo, USA) acquired and analyzed with the STarStation v2.0 software (Applied Cytometry Systems, United Kingdom). Washes were performed using 96-well multiscreen filter plates (Biosource), a multiscreen vacuum manifold, and a Chemical Duty Pump (Milipore). xMAP allows multiplexing of analytes in solution with flow cytometry. Using a propriety technique, Luminex internally color codes xMAP microspheres by combining different ratios of two fluorescent dyes. Each bead set is conjugated with a different capture antibody. The use of R-phycoerythrin-labeled detection antibodies allows quantification of antigen-antibody reactions occurring on the microsphere surface, by measurement of the relative fluorescence intensity. The system is capable of measuring up to 100 different analytes in a single 50 μl sample. The assay was performed according to the Biosource protocol as described in ref. [19]. Initially 25 inflammatory and immunomodulatory cytokines/chemokines and soluble cytokine receptors were screened: IL-1β, IL-2, IL-4, IL-5 IL-6, IL-7, IL-10, IL-12p40, IL-13, IL-15, IL-17, IFN-α, IFN-γ, TNF-α, IL-1RA, IL-2R, CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CCL11/Eotaxin, CXCL8/IL-8, CXCL9/MIG, CXCL10/IP-10, GM-CSF. CXCL10 and IL2 were selected for further analysis. Bead suspensions from individual CXCL10, IL-2 and IFN-γ kits were combined in pre-wetted filter 96 plate wells. The beads were washed twice with wash solution and incubation buffer was added. Patient samples (50 μl) were diluted 1:1 in assay dilution and added to the plate. The plate was incubated 2 h at room temperature at 600 rpm on a titer plate shaker. After two washes, 100 μl detection antibody cocktail was added per well, and the plate was incubated at room temperature for one hour on a titer plate shaker. After two washes, 100 μl of strepavidin-RPE solution was added per well. Finally, after 30 min incubation and three washes, 100 μL of wash solution was added to each well and the plate was placed in the XY platform of the Luminex. From each well, a minimum of 100 analyte specific beads were analyzed for both bead- and RPE fluorescence.

Cytokine measurements in plasma from unstimulated and antigen stimulated whole blood were performed in duplicates, measurements in mitogen stimulated whole blood was performed once. All measurements were performed blinded from clinical data. A curve fit was applied to each standard curve according to the manufacturer's manual and sample concentrations were interpolated from the standard curves.

The limits of quantification of cytokines using multiplex were 5–2800 pg/ml for CXCL10, 6–12,264 pg/ml for IL-2

and 5–27,200 pg/ml for IFN-γ. Cytokine concentrations outside the limits of quantification were assigned the limits of quantification values.

2.5. Statistics

Concentration of CXCL10, IL-2 and IFN-γ were compared using Kruskal–Wallis test and Wilcoxon signed rank test. Correlation between CXCL10, IL-2, and IFN-γ were compared using Spearman correlation analysis. Data was analysed using SAS 9.1 (SAS institute, Cary, USA).

3. Results

3.1. Cytokine measurements

An initial screening of 25 cytokines, chemokines, and soluble receptors suggested that CXCL10 and IL-2 had potential as biomarkers as they appeared to be both sensitive and specific for *M. tuberculosis* exposure (data not shown). In order to confirm these preliminary findings, whole blood from 12 patients with confirmed TB and a positive QFT-IT test and 11 healthy controls with a negative QFT-IT test was incubated with ESAT-6, CFP-10, and TB7.7 using the pre-coated QFT-IT tubes.

We found that significant expression of CXCL10 was induced by stimulation with *M. tuberculosis* specific peptides in all the TB patients whereas no CXCL10 was induced in similarly stimulated whole blood from healthy unexposed controls (Table 1). The difference in median levels of CXCL10 between TB patients and healthy controls was highly significant ($p < 0.0001$). Expression of IL-2 and IFN-γ determined by the multiplex technology was evaluated in parallel, and we found significant differences between patients and controls ($p < 0.0001$), but the magnitude of responses were lower (Table 1).

Spontaneous release of IFN-γ and IL-2 was low, in all patients and healthy, whereas the median spontaneous CXCL10 release by TB patients was 5.6 fold higher compared to that of the healthy controls (Table 1) ($p = 0.0005$).

After mitogen stimulation all donors mounted increased CXCL10 and IFN-γ production determined by ELISA and multiplex, and there were no significant differences between patients and controls. The level of IL-2 release after mitogen stimulation was lower among TB patients than healthy controls (Table 1, $p = 0.008$) and only 6/11 of the TB patients expressed detectable IL-2 after mitogen stimulation (data not shown).

3.2. Agreement between IFN-γ measurements by either multiplex or ELISA

We compared the levels of IFN-γ determined by QFT-IT ELISA versus the multiplex technology in plasma of mitogen stimulated whole blood from both patients and controls and in plasma of antigen stimulated whole blood from the QFT-IT positive TB patients. There was a strong correlation between the two methods ($r = 0.75$, Spearman, $p < 0.0001$), but the QFT-IT ELISA measurements were consistently higher than the multiplex ($p < 0.0001$).

3.3. The diagnostic potential of CXCL10 and IL-2

In order to compare the diagnostic potential of CXCL10, IL-2, and IFN-γ determined by multiplex technology to that of the QFT-IT ELISA the following calculations are based on antigen specific cytokine responses; i.e. the cytokine or chemokine concentration after subtracting the value of the spontaneous cytokine or chemokine release. Concentrations of antigen specific CXCL10, IL-2, and IFN-γ from QFT-IT positive TB patients and controls are shown in Fig. 1 The difference in median antigen specific CXCL10 responses between patients (870 pg/ml, range 261–1576 pg/ml) and controls (9 pg/ml, range 1–28 pg/ml) was highly significant ($p < 0.0001$). The corresponding median IFN-γ (ELISA) response was 217 pg/ml (range 81–1273 pg/ml) for the patients compared to 1 pg/ml (range 0–3.5 pg/ml) for the controls ($p < 0.0001$) and the median IL-2 response was 59 pg/ml

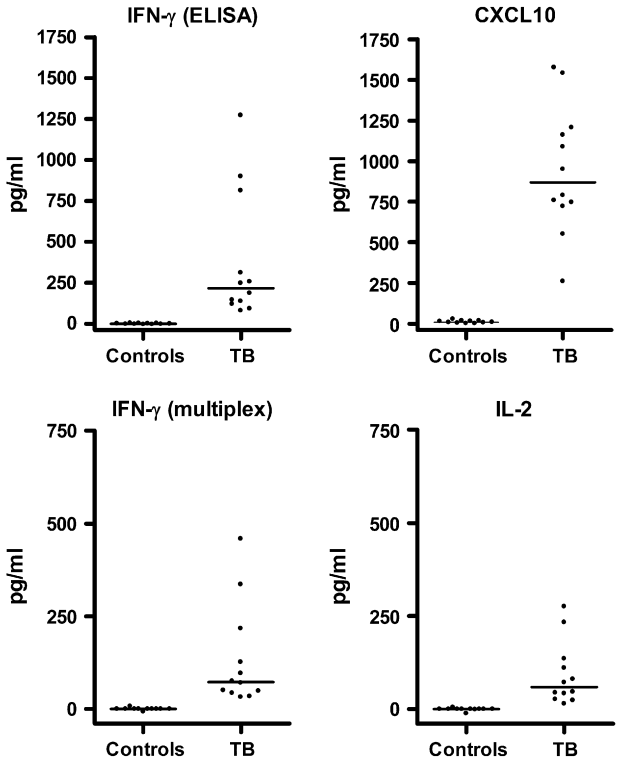

Fig. 1. Antigen specific IFN-γ, CXCL10 and IL-2 production. Whole blood of 12 Quantiferon in tube positive TB patients and 11 healthy unexposed controls was stimulated 20–24 h with either saline or *M. tuberculosis* specific antigens. Cytokine production was measured in plasma supernatant by ELISA (IFN-γ) and multiplex technology (CXCL10, IFN-γ and IL-2). Antigen specific production represent the antigen stimulated sample subtracted the unstimulated sample. Straight lines represent median values. Differences between healthy controls and patients were all significant $p < 0.0001$ (Kruskal–Wallis). The difference between the level of CXCL10 and IFN-γ (ELISA) among the patients was significant 870 pg/ml versus 217 pg/ml, $p = 0.001$ (Wilcoxon signed rank test).

(range 14–276 pg/ml) and 0 pg/ml (0–4 pg/ml) for TB patients and controls respectively ($p < 0.0001$). The amount of CXCL10 release in TB patients was significantly higher than the amount of IFN-γ release determined by QFT-IT ELISA ($p = 0.001$).

Although the study was not designed to evaluate sensitivity and specificity of CXCL10 and IL-2 as diagnostic markers for TB infection, we did select arbitrary cut off points for positive tests at mean antigen specific production in the control group +3 standard deviations. The resulting cut off points of 36 pg/ml and 13 pg/ml for CXCL10 and IL-2, respectively, differentiated completely between TB patients and controls.

3.4. The potential of improving diagnosis using CXCL10 in patients with active TB and negative QFT-IT test

To explore if CXCL10 had the potential to improve sensitivity of the current QFT-IT test, we tested the CXCL10 responses in patients with negative or indeterminate QFT-IT test results (for patient characteristics and individual measurements see Table 2). Fig. 2 shows the antigen specific CXCL10 and IFN-γ responses in 7 patients with TB and a negative or indeterminate QFT-IT test. The antigen specific IFN-γ responses, determined by QFT-IT ELISA, ranged from 0–12.8 pg/ml whereas, the antigen specific CXCL10 responses ranged from 0–532 pg/ml. Of the 7 patients, 3 were also CXCL10 unresponsive with an antigen specific response below 10 pg/ml, whereas the other 4 patients responded with a median CXCL10 level of 318 pg/ml (range 196–532 pg/ml). Of the 4 patients with a negative QFT-IT test and positive CXCL10 response, 2 were HIV co-infected with a CD4 cell count of 32 cells/μl and 300 cells/μl respectively. The mitogen specific CXCL10 release was high in all donors ranging from 394 to 2800 pg/ml.

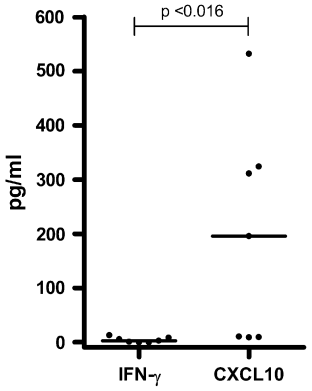

Fig. 2. Antigen specific IFN-γ and CXCL10 production in patients with a negative Quantiferon test. Whole blood of 6 Quantiferon in tube (QFT-IT) negative and one QFT-IT indeterminate TB patient was stimulated 20–24 h with either saline or *M. tuberculosis* specific antigens. Cytokine production was measured in plasma supernatant by ELISA (IFN-γ) and multiplex technology (CXCL10). Antigen specific production represent the antigen stimulated sample subtracted the unstimulated sample. Straight lines represent median values, (Wilcoxon signed rank test).

4. Discussion

The present study is the first to describe CXCL10 expression following stimulation with *M. tuberculosis* antigen (ESAT-6/CFP-10/TB7.7) and the main findings are: (i) that CXCL10 is expressed *in vitro* in response to antigen stimulation in significantly higher amounts in patients with active TB than in un-exposed controls; (ii) that CXCL10 is expressed in higher amounts than IFN-γ determined by either ELISA or multiplex and IL-2; (iii) compared to the commercially available QFT-IT test, CXCL10 showed at least similar power to discriminate between infected and uninfected individuals, and CXCL10 appeared to be even more sensitive than QFT-IT in TB patients with negative QFT-IT response; and (iv) finally, we found that the concentration of CXCL10 in plasma from un-stimulated whole blood was elevated in patients with active TB.

There is a concern that antigen specific sensitivity is reduced [4–8] due to the findings of indeterminate test results among risk groups such as HIV positive individuals [18], immunosuppressed patients[5,20–23] and infants [24]. None of these studies were designed to show an effect of immunosuppression on the antigen specific sensitivity. It is, however, well known that *in vivo* and *in vitro* antigen specific responses i.e. to Tuberculin PPD are impaired in severely immuocompromised patients [25]. We have shown that the magnitude of IFN-γ release is correlated with the level of CD4 cells [18]. Another recent study showed that IFN-γ levels after mitogen stimulation were lower in a subgroup of HIV positive compared to HIV negative and that the median antigen specific release of IFN-γ was lower in HIV positive than in HIV negative TB patients [21]. Using the current TST, the problems with low sensitivity has been overcome by differentiating the cut points according to the immune status of the patient and the risk of progressing to active TB, at the potential risk of loss of specificity. The sensitivity of the IGRAs could be adjusted in a similar manner or as suggested here by adding or using an alternative marker.

Despite an intensive search for new biomarkers for the diagnosis and prognosis of TB [26–28], none have yet identified a biomarker with superior sensitivity or specificity to the *M. tuberculosis* specific IGRAs. Other groups have described that monokine Induced by IFN-γ (CXCL9/MIG) was expressed *in vitro* after stimulation with *M. tuberculosis* specific antigens (ESAT-6/CFP10) and PPD. The amount of CXCL9 released was low and sensitivity of CXCL9, was only 40% compared to IFN-γ [28]. Another study based on intracellular cytokine cytometry in CD4 + T cells following ESAT-6 stimulation, showed that IFN-γ, IL-2, IL-4, IL-10, and the activation marker CD40L could distinguish TB infected from non-TB infected. CD40L was the only examined marker comparable to IFN-γ [16].

IL-2 which theoretically and from our initially screening showed potential as a diagnostic marker was inferior to IFN-γ and CXCL10 due to the low detectable levels. Interestingly, we found very low levels of IL-2 after mitogen stimulation, and in patients the mitogen induced IL-2 production was

virtually absent in over half the studied patients. This phenomenon of IL-2 hypo-responsiveness among tuberculosis patients has been described by others [15,29] and renders IL-2 an inadequate stand alone markers for T cell anergy in an *M. tuberculosis* diagnostic test. Whether low plasma IL-2 concentration in mitogen stimulated whole blood could be utilized alone or in combination with measurements of CXCL10 plasma concentration of unstimulated whole blood as a compound marker for active TB remains to be explored.

Neither of the commercially available IGRAs can discriminate between latent and active TB infection. In the present study we found that the median CXCL10 levels in plasma of unstimulated whole blood was 5.6 fold higher in patients with active TB compared to controls suggesting that with assay optimization CXCL10 in plasma of unstimulated whole blood might serve as a marker which can discriminate active from latent TB infection. This hypothesis is supported by the findings by Azurri [12] and others [13] who found increased concentrations of CXCL10 in unstimulated plasma from TB patients, but a recent paper was unable to reproduce the findings [30]. Whether CXCL10 will be able to serve as a marker that can discriminate between active and latent infection needs to be evaluated in properly designed studies.

In infants and young children, the amounts of blood drawn is critical and even the minimum of 3 ml of blood drawn for the QTF-IT test may limit its use in this specific group. Our preliminary findings suggest that the plasma samples can be diluted at least 8 times without loosing antigen specific sensitivity (unpublished). Thus, if antigen specific CXCL10 sensitivity is maintained with dilution, it should be possible to develop a ''micro assay'' using capillary blood and it should be possible to develop a simplified ELISA test format applicable for field use like a filter paper test or dipstick test where readout is a colour change directly visible by the naked eye. In addition, because CXCL10 is produced in such high concentrations and seems able to detect antigen specific reactivity in QFT-IT negative patients, it may be a more robust marker for use in immunocompromised patients. Finally, CXCL10 could be utilized as a sensitive marker in the evaluation of vaccine induced T-cell responses.

The present study demonstrates that *M. tuberculosis* specific CXCL10 release may have potential as a new tool to differentiate between TB infected and uninfected. The limitations of the present study are the relatively low number of patients and the fact that the controls were selected from a group of healthy individuals from a low incidence country in order to demonstrate the specificity under optimal conditions. A bias may have been introduced because patients were selected on the basis of a positive or negative QFT-IT response. Prospective studies comparing the two markers in patients and healthy individuals from high incidence populations are needed to confirm our findings.

In conclusion, our findings suggest high sensitivity and specificity of CXCL10 as a novel biomarker for infection with *M. tuberculosis*. We propose a novel generation of potentially more sensitive *in vitro M. tuberculosis* diagnostic tests based on CXCL10 measurements. Further studies are needed to develop a standardised assay format, to define CXCL10 cut off points for a positive test result, and to explore the potential of CXCL10 as a replacement of or in addition to IFN-γ for the diagnosis of active and latent TB infection in healthy, children, and immunocompromised patients.

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