Expression and production of the *SERPING1*-encoded endogenous complement regulator C1-inhibitor in multiple cohorts of tuberculosis patients

Rosalie Lubbers[a], Jayne S. Sutherland[b], Delia Goletti[c], Roelof A. de Paus[d], Douwe J. Dijkstra[e], Coline H.M. van Moorsel[f], Marcel Veltkamp[f], Stefan M.T. Vestjens[g], Willem J.W. Bos[g,h], Linda Petrone[c], Stephanus T. Malherbe[i], Gerhard Walzl[i], Kyra A. Gelderman[j], Geert H. Groeneveld[d], Annemieke Geluk[d], Tom H.M. Ottenhoff[d], Simone A. Joosten[d,1], Leendert A. Trouw[e,*,1]

[a] *Department of Rheumatology, Leiden University Medical Center, Leiden, the Netherlands*
[b] *Vaccines and Immunity Theme, Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine, Banjul, The Gambia*
[c] *Translational Research Unit, Department of Epidemiology and Preclinical Research, National Institute for Infectious Diseases L. Spallanzani-IRCCS, Rome, Italy*
[d] *Department of Infectious Diseases, Leiden University Medical Center, Leiden, the Netherlands*
[e] *Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, the Netherlands*
[f] *Department of Pulmonology, St. Antonius Hospital, Nieuwegein, the Netherlands*
[g] *Department of Internal Medicine, St. Antonius Hospital, Nieuwegein, the Netherlands*
[h] *Department of Nephrology, Leiden University Medical Center, Leiden, the Netherlands*
[i] *DST/NRF Centre of Excellence for Biomedical TB Research and SAMRC Centre for TB Research, Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa*
[j] *Sanquin Diagnostic Services, Amsterdam, the Netherlands*

ARTICLE INFO

ABSTRACT

Background: To facilitate better discrimination between patients with active tuberculosis (TB) and latent TB infection (LTBI), whole blood transcriptomic studies have been performed to identify novel candidate host biomarkers. *SERPING1*, which encodes C1-inhibitor (C1-INH), the natural inhibitor of the C1-complex has emerged as candidate biomarker. Here we collated and analysed *SERPING1* expression data and subsequently determined C1-INH protein levels in four cohorts of patients with TB.

Methods: *SERPING1* expression data were extracted from online deposited datasets. C1-INH protein levels were determined by ELISA in sera from individuals with active TB, LTBI as well as other disease controls in geographically diverse cohorts.

Findings: *SERPING1* expression was increased in patients with active TB compared to healthy controls (8/11 cohorts), LTBI (13/14 cohorts) and patients with other (non-TB) lung-diseases (7/7 cohorts). Serum levels of C1-INH were significantly increased in The Gambia and Italy in patients with active TB relative to the endemic controls but not in South Africa or Korea. In the largest cohort (n = 50), with samples collected longitudinally, normalization of C1-INH levels following successful TB treatment was observed. This cohort, also showed the most abundant increase in C1-INH, and a positive correlation between C1q and C1-INH levels. Combined presence of increased levels of both C1q and C1-INH had high specificity for active TB (96 %) but only very modest sensitivity 38 % compared to the endemic controls.

Interpretation: *SERPING1* transcript expression is increased in TB patients, while serum protein levels of C1-INH were increased in half of the cohorts analysed.

* Corresponding author at: Department of Immunohematology and Blood Transfusion, E3-P, Leiden University Medical Center, PO Box 9600, 2300 RC, Leiden, the Netherlands.
  E-mail address: L.A.Trouw@lumc.nl (L.A. Trouw).
  [1] These authors contributed equally.

1. Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*). Most people infected with *Mtb* remain latently infected (LTBI) and only a minority (5–10 %) of those infected progress to active TB disease (WHO, 2018). Most recent estimates from the World Health Organization (WHO), showed that, 10 million people became infected with *Mtb* in 2017 and 1.6 million died from TB that year (0.3 million co-infected with HIV). Although diagnostic testing and successful treatment has increased survival globally, there is a clear need for better, user friendly diagnostic tools, particularly to provide easy access in remote areas (WHO, 2018).

Current clinical diagnostic tests, other than mycobacterial culture, are based on detection of immunological memory (i.e. IFNγ production by T-cells) towards *Mtb* antigens and therefore fail to discriminate between individuals that are latently infected and those that have active disease (Goletti et al., 2018a) or cured TB (Goletti et al., 2018b). In addition, treatment efficacy monitoring of TB, could benefit from more accurate biomarkers of TB disease activity (Goletti et al., 2018b). Many recent studies have assessed general transcriptional biomarkers, such as *GBP5* and *FCGR1A* (Anderson et al., 2014; Berry et al., 2010; Joosten et al., 2013; Kaforou et al., 2013; Ottenhoff et al., 2012; Penn-Nicholson et al., 2019; Petruccioli et al., 2016; Zak et al., 2016). Upregulation of the expression of genes encoding proteins of the complement system have been identified as components of TB biomarker signatures (Cai et al., 2014; Cliff et al., 2013; Gupta et al., 2020; Jiang et al., 2017; Scriba et al., 2017; Wang et al., 2015). Recently, we reported that increased levels of C1q can be used a host serum biomarker that can discriminate active TB disease from LTBI and non-TB pulmonary infections or sarcoidosis (Lubbers et al., 2018).

Next to activators, the complement system also includes several inhibitors, such as C4b-binding protein (C4BP), Factor H (FH) and the degrading enzyme Factor I (Sjoberg et al., 2009). In addition, membrane-bound inhibitors exist such as CD46, CD55 and CD59. In serum, C1q forms a complex with C1r and C1s to generate the full C1 molecule which can activate the classical pathway of the complement system. Importantly, the C1 complex is regulated by a natural inhibitor, namely C1-inhibitor (C1-INH). Transcriptional biomarker signatures have indicated that expression of *SERPING1*, which encodes the inhibitor C1-INH, discriminates between active and latent TB disease. The expression of *SERPING1* was increased in active TB patients compared to LTBI subjects (Berry et al., 2010; Blankley et al., 2016; Cliff et al., 2013; Esmail et al., 2018; Jacobsen et al., 2007; Joosten et al., 2013; Maertzdorf et al., 2011b) and also in LTBI individuals that subsequently progressed to active TB (Esmail et al., 2018). Furthermore, *SERPING1* expression levels declined upon treatment initiation (Thompson et al., 2017). Most recently, *SERPING1* expression was also shown to associated with TB disease progression in mice and macaques (Ahmed et al., 2020). However, information on the circulating levels of C1-INH are lacking.

C1-INH is a member of the serine protease inhibitor family and regulates both the classical and the lectin pathway of the complement system (Nuijens et al., 1989). Additionally, C1-INH is a regulator of the contact system, which is a system of proteins related to coagulation, as an inhibitor for Factor XIa, Factor XIIa and kallikrein (Beinrohr et al., 2008). C1-INH protein functions as a suicide inhibitor, it binds to its target, and upon enzymatic cleavage C1-INH undergoes a permanent conformational change and therefore C1-INH can only work once. C1-INH is synthesized as a single-chain protein which is heavily glycosylated. C1-INH, C4BP and FH are mainly produced by hepatocytes but can also be produced by monocytes and macrophages (Breda et al., 2018; Lubbers et al., 2017; Stavenhagen et al., 2018). C1q is also produced by cells from the myeloid lineage: monocytes, macrophages and dendritic cells (Castellano et al., 2004), but not by hepatocytes (Lubbers et al., 2017). C1-INH is an acute phase protein which can be increased upon inflammation, deficiency of C1-INH is clinically associated with

Table 1
Description of the cohorts.

Country	Classification	N	Age mean (range)	Sex (% male)
Italy	Control	15	38 (25–57)	40
	Latent TB	19	37 (21–77)	33
	Active TB	19	38 (23–67)	89
	Treated TB	20	39 (18–70)	35
The Gambia	Control	50	31 (15–60)	30
	Active TB	50	34 (17–62)	62
South Korea	Control	10	23 (21–25)	90
	Active TB	10	51 (24–77)	40
South Africa	Control	31	32 (18–56)	26
	Active TB	20	32 (19–57)	65
Multiple* the Netherlands	Leprosy reactions	53	(18–69)	68
	Leprosy†	33	34 (18–57)	62
	Sarcoidosis	50	43 (26–57)	60
	Control	92	37 (21–67)	36
	Pneumonia (Leiden)	40	66 (23–93)	60
	Pneumonia (Nieuwegein)	28	73 (34–91)	57

* Nepal, Brazil, Ethiopia † Diagnosis made in the Netherlands.

recurrent episodes of angio-oedema (Hofman et al., 2016; Zeerleder and Levi, 2016).

Here we have determined serum levels of *SERPING1* encoded C1-INH in four geographically diverse cohorts of patients with active TB, LTBI, disease controls and patients that have been successfully treated for TB, to investigate possible application as host biomarker for active TB.

2. Materials and methods

2.1. Patients and controls

The demographic data and classification of the cohorts are presented in Table 1 (Lubbers et al., 2018). Serum samples were collected from all individuals locally following written informed consent and shipped to the laboratory at LUMC for assessment. Maximal transport time was 48 h, the shipments were dry ice monitored and samples frozen upon arrival. Below, the inclusion criteria per cohort are specified.

2.1.1. Patients with pulmonary TB, latent TB and controls

Smear, PCR or culture positive pulmonary TB patients, LTBI patients and treated TB patients as well as endemic controls were included from various geographic locations: Italy (Joosten et al., 2016; Lubbers et al., 2018), the Gambia, South Korea and South Africa (Table 1) (Lubbers et al., 2018). Patients with active pulmonary TB disease, referred to as 'TB' below, were diagnosed based on local guidelines and routine methodology. Active pulmonary TB was sputum-culture confirmed (BACTEC™ MGIT™, Becton-Dickinson, USA), or based on positive Xpert Mtb/RIF assay (Cephaid Inc., Sunnyvale, CA, USA), patients were included within 7 days of TB treatment initiation. LTBI was defined as Quantiferon TB Gold-in-tube positivity (Qiagen, Germany) in absence of signs of active disease.

Stored samples from participants recruited in South Africa from six public health care clinics around Tygerberg Academic Hospital in Northern Cape Town. These included 20 newly diagnosed pulmonary TB cases (4 HIV+) and 31 healthy controls (no HIV+) from the same area. TB cases were confirmed by a positive sputum MGIT culture for *Mtb* or GeneXpert in addition to a chest X-ray suggestive of active pulmonary TB (Vrieling et al., 2018). The non-TB controls were asymptomatic individuals from the same communities, with a negative sputum culture for *Mtb* and a chest X-ray not suggestive of TB.

Additionally, from Italy both LTBI (QuantiFERON TB Gold-In-tube-positive) individuals and successfully treated TB patients (2–72 months

after end of therapy) were included. TB patients from the Gambia were followed over time (one, two and six months after diagnosis), until completion of successful treatment.

2.1.2. Other mycobacterial diseases

Patients infected with *Mycobacterium leprae* (mostly immigrants with mixed ethnic backgrounds) were included in the Netherlands after having been diagnosed with leprosy without reactions. In addition, patients with type-1 leprosy reactions were enrolled in Brazil, Nepal and Ethiopia (Khadge et al., 2015; van Hooij et al., 2018).

2.1.3. Other pulmonary diseases

Hospital admitted patients with community-acquired pneumonia in the Netherlands were included. One cohort comprised patients admitted to a tertiary care hospital in Leiden (one patient was HIV-infected with a normal CD4 count and one suffered from sarcoidosis) and the other cohort comprised patients admitted to a hospital ward of a non-academic teaching hospital in Nieuwegein. From both groups of pneumonia patients paired serum samples from the time of diagnosis and after recovery (10–124 days later) were available. Lastly, serum samples of patients with sarcoidosis from the Netherlands that had pulmonary involvement were included, obtained prior to initiation of treatment.

2.1.4. Additional control group

As a reference group we included a panel of Dutch healthy controls (n = 92), not suffering from major infections or autoimmune diseases.

2.2. Ethics statement

Blood was obtained from individuals upon signing informed consent. All studies comply with the Helsinki declaration. The use of the samples in this study was approved by local ethical committees. For Italy, Ethical Committee of the Lazzaro Spallanzani National Institute of Infectious diseases (02/2007 and 72/2015); The MRC/Gambian government joint ethics committee (SCC1333); South Korea, Institutional Review Board for the Protection of Human Subjects at YUHS; South Africa, Health Research Ethics Committee of the Faculty of Medicine and Health Sciences at Stellenbosch University (N13/05/064); Brazil, National Council of Ethics in Research and UFU Research Ethics Committee (#499/2008); Nepal, Health Research Council (NHR#751); Ethiopia, Health Research Ethical Review committee Ethiopia (NERC#RDHE/127-83/08); The Netherlands leprosy patients, (MEC-2012-589); sarcoidosis patients, Medical research Ethics Committees United of the St Antonius (#R05.08A); control group, (P237/94); pneumonia Leiden (P12.147); pneumonia Nieuwegein (C-04.03 and R07.12).

2.3. Gene expression analysis

Microarray data from comparative studies (Anderson et al., 2014; Berry et al., 2010; Blankley et al., 2016; Bloom et al., 2013; Esterhuyse et al., 2015; Kaforou et al., 2013; Lee et al., 2016; Maertzdorf et al., 2016, 2011a; Maertzdorf et al., 2011b, 2012; Ottenhoff et al., 2012; Walter et al., 2016), publicly available in Gene Expression Omnibus (GEO) (GSE37250, GSE19491, GSE34608, GSE39941, GSE42834, GSE70478, GSE25534, GSE28623, GSE56153, GSE74092, GSE73408, GSE62525, GSE83456), were retrieved from GEO and re-analysed. Several of the studies described multiple independent populations, which we analysed separately. All data were extracted from GEO and compared in the same way using GEO2R, thus not relying on the analysis performed in the original manuscript. GEO2R compared two or more groups of samples in order to identify genes that are differentially expressed across experimental or clinical conditions. Here, lists of differentially expressed genes between TB and LTBI or TB and other diseases (with a significance of $p < 0.05$ and a factorial change of > 2

or < 0.5) were generated. A list of complement protein inhibitors (*SERPING1*, *CFH*, *C4BPA*, *CD46*, *CD55* and *CD59*) and two reference genes (*FCGR1A* and *GBP5*) was used to assess possible differential expression for each individual gene for each study population. In addition, information on the differential expression of the three different chains for C1q (*C1QA*, *C1QB* and *C1QC*) was retrieved from the same datasets for comparative purposes. All studies/ populations with significantly differential expression for a particular gene between patients with TB compared to healthy controls/ LTBI/ other diseases were enumerated and expressed as the percentage of the total number of comparisons investigated.

2.4. Detection of C1-INH by ELISA

C1-INH levels in sera were measured using an in-house developed ELISA. Maxisorp plates (nunc) were coated for two hours at room temperature on a shaker with mouse anti-human C1-INH (RII A280 2.8 RUCHIRA) in coating buffer (0.1 M NA_2CO_3, 0.1 M $NaHCO_3$, pH 9.6). Plates were washed with PBS/0.05 %Tween and the samples were diluted in PBS/0.2 %Gelatin/0.1 %Tween (PTG). Human sera were diluted 1:1000. A serial dilution of a pool of normal human serum (NHS) was applied as a standard with a known concentration and samples were added to the plate, all in duplicate, and incubated for one hour at room temperature on a shaker. After washing, plates were incubated for one hour on the shaker at room temperature with rabbit-anti-human C1-INH labelled with biotin (Sanquin) and diluted in PTG/1% normal mouse serum. Plates were washed and incubated with streptavidin-HRP (GE Healthcare cat#GERPN1231) for a maximum of 30 min on the shaker. Next, the plates were washed and TMB BD cat# 555214 was added as a substrate, the signal was stopped using 2 M H_2SO_4. Signal was measured as the absorbance at 450 nm minus the absorbance at 540 nm with the SpectraMax i3 × . The measured C1-INH is expressed as μg/ml as compared to the standard. the levels of C1-INH were compared to the levels of C1q that were previously determined in the same samples by ELISA as described (Lubbers et al., 2018).

2.5. Statistics

Statistical analyses were carried out using SPSS statistics version 23 (IBM) or Graphpad Prism version 8. To compare C1-INH levels the Mann-Whitney U test, Kruskall-Wallis and Dunn's multiple comparisons test were used. In all graphs median values are shown unless indicated otherwise. Receiver operating characteristic (ROC) analyses were performed to assess the sensitivity and specificity of C1-INH serum levels and was expressed as Area Under the Curve (AUC). Increased serum levels were based upon the 95th percentile of a reference group, Dutch healthy controls, of either C1q (Lubbers et al., 2018) or C1-INH.

3. Results

3.1. Patients with active TB have upregulated expression of SERPING1

Publicly available microarray data were retrieved from GEO from thirteen studies (Anderson et al., 2014; Berry et al., 2010; Blankley et al., 2016; Bloom et al., 2013; Esterhuyse et al., 2015; Kaforou et al., 2013; Lee et al., 2016; Maertzdorf et al., 2016, 2011a; Maertzdorf et al., 2011b, 2012; Ottenhoff et al., 2012; Walter et al., 2016), from different populations across the world, and all data were ranked according to differential expression between TB patient and Healthy Controls (HC), LTBI or other lung-diseases. The number of studies that reported on differential gene expression between patients with TB and HC (Fig. 1A), LTBI (Fig. 1B) or other lung-diseases (Fig. 1C) was plotted for complement system inhibitors: *SERPING1*, *CFH*, *C4BPA*, *CD46*, *CD55* and *CD59*. In addition, two genes, *FCGR1A* and *GBP5*, that are consistently top-differentially expressed genes in many of the TB cohorts, were included for comparative analysis. *SERPING1* expression was increased in
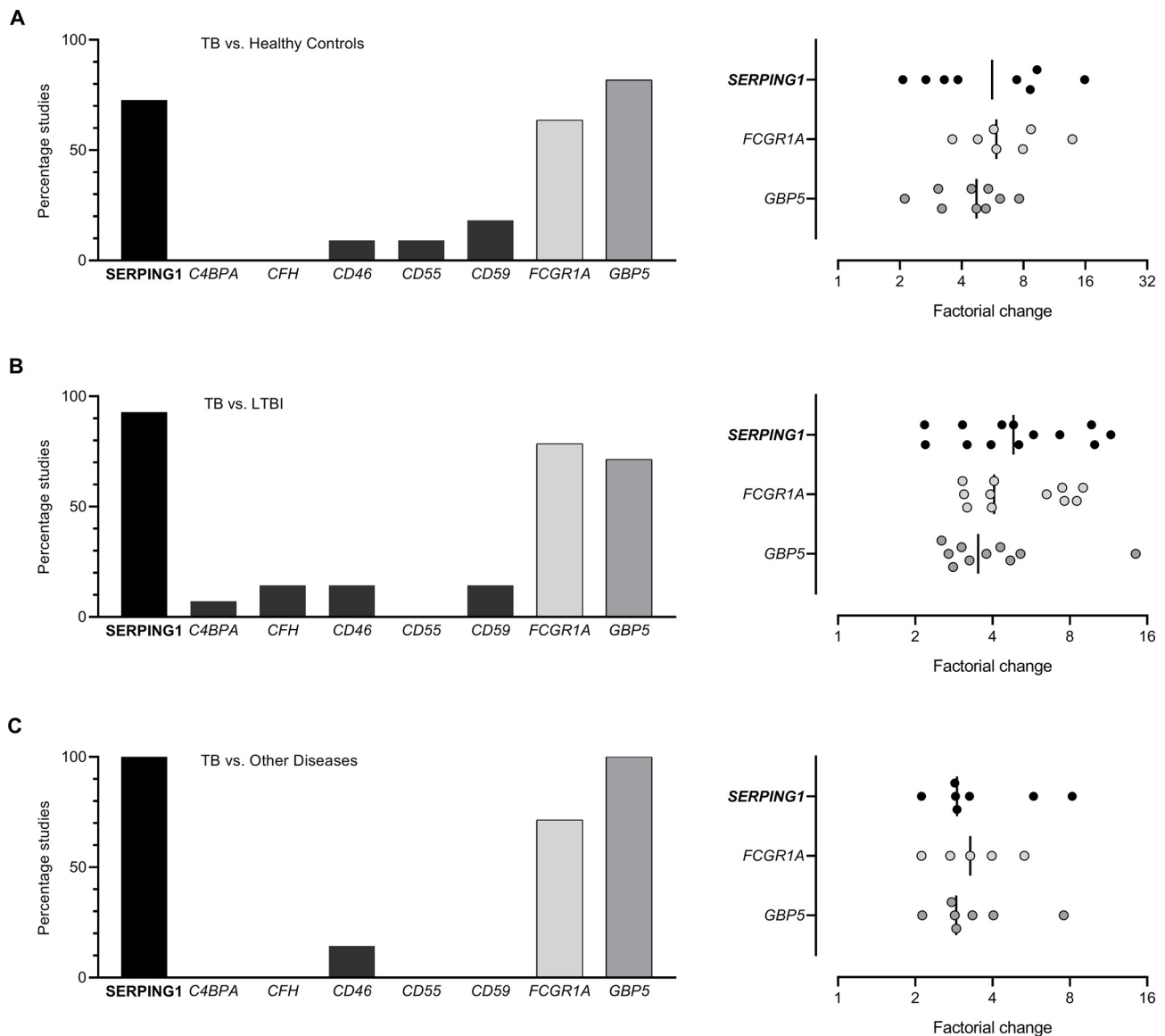
Fig. 1. Differentially expressed complement inhibitor genes in whole blood tuberculosis transcript signatures.
Tuberculosis (TB) specific blood transcript signatures, from various populations, were investigated for the presence of differentially expressed genes encoding inhibitors of the complement system and two well-known biomarkers, *FCGRIA* and *GBP5*. Publicly available transcriptome data was retrieved from Gene Expression Omnibus (Anderson et al., 2014; Berry et al., 2010; Blankley et al., 2016; Bloom et al., 2013; Esterhuyse et al., 2015; Kaforou et al., 2013; Lee et al., 2016; Maertzdorf et al., 2016, 2011a; Maertzdorf et al., 2011b, 2012; Ottenhoff et al., 2012; Walter et al., 2016) and analysed using GEO2R. Data were available for comparing patients with active TB to healthy controls for 11 cohorts (**A**); compared to Latent TB for 14 cohorts (**B**) and to other diseases for 7 cohorts (**C**). The left side of the figure shows the number of times the gene was significantly differentially expressed as percentage of the total number of studies analysed. The right side of the figure shows the factorial change measured in the positively reporting studies for *SERPING1* and *FCGR1A* and *GBP5*.

TB patients compared to HC (8/11 studies), LTBI (13/14 studies) and patients with other lung-diseases (7/7 studies). Other genes encoding complement inhibitory proteins were not consistently increased in active TB disease. Subsequently, in the studies that found differential gene expression the factorial changes were analysed for *SERPING1*, and control *FCGR1A* and *GBP5* (right panel Fig. 1). The factorial changes for these three genes were in the same order of magnitude. Since *SERPING1* expression results distinguished TB patients from both LTBI subjects and patients with other lung-diseases, we next measured the *SERPING1* protein product, C1-INH.

3.2. C1-INH protein levels in serum of patients with active TB and other diseases

Sera from cohorts of patients with pulmonary TB from distinct geographical locations were analysed for C1-INH protein levels by ELISA. Sera collected from TB patients in South Korea or South Africa showed no differences in C1-INH levels compared to controls from the same area (Fig. 2A, B). In samples from the Italian cohort, however, C1-INH levels were significantly increased (p = 0.026) in TB patients compared to controls (Fig. 2C). Similarly in samples from Gambian TB patients there was a significant increase in serum C1-INH levels compared to endemic controls (LTBI; p < 0.0001) (Fig. 2D). Next, C1-INH levels were measured in samples from patients with other lung diseases (sarcoidosis and community-acquired pneumonia) that present clinical symptoms comparable to those found in active TB, as well as patients with a non-TB related mycobacterial disease (leprosy). These were compared to a control group which comprised Dutch healthy controls (n = 92) (Fig. 2E). In none of these patient groups C1-INH was increased compared to healthy controls, however, the C1-INH levels in
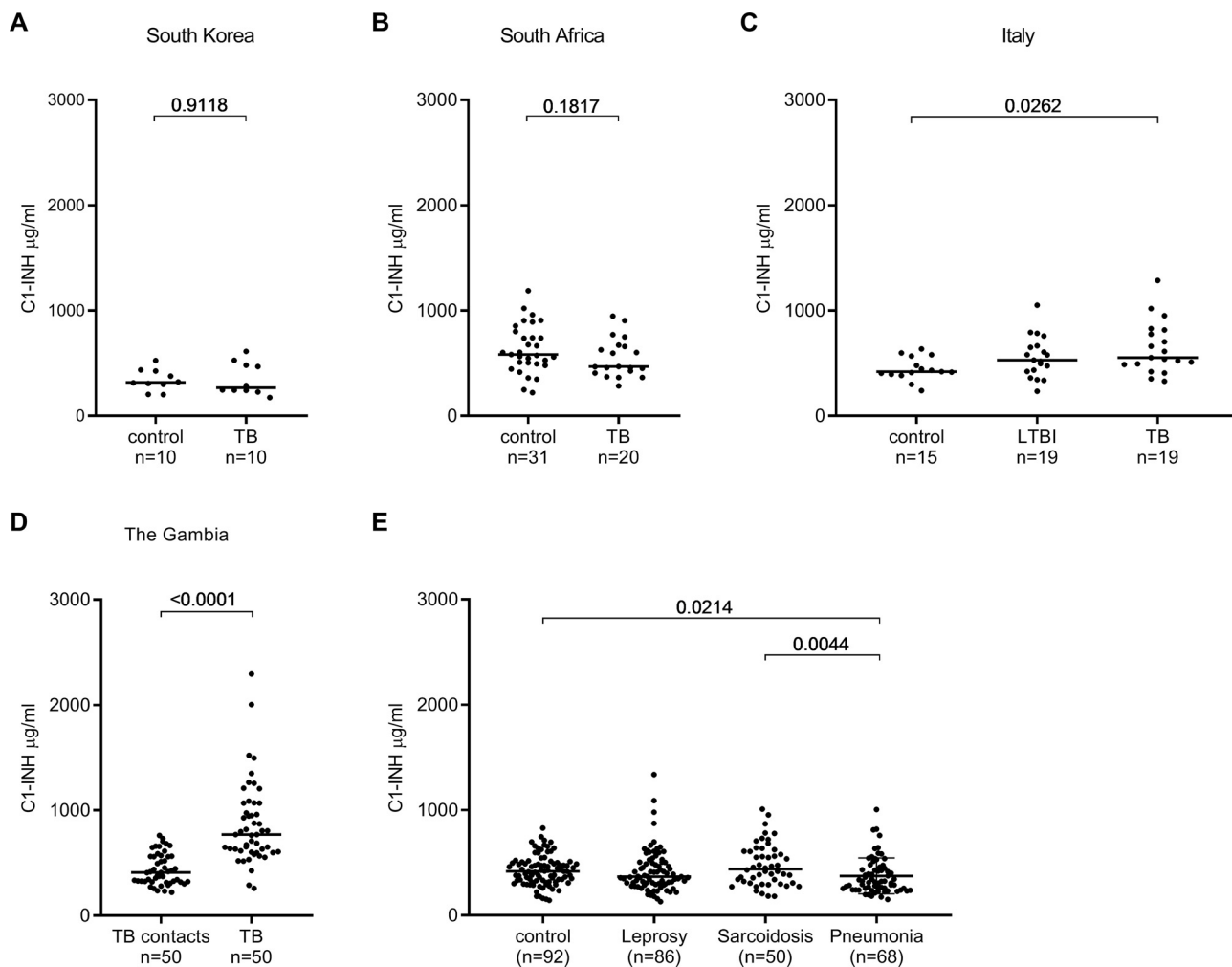
Fig. 2. C1-inhibitor serum levels in various patient cohorts worldwide.
C1-inhibitor (C1-INH) levels (μg/ml) were measured by ELISA in sera from patients with active tuberculosis (TB) and controls from different cohorts. The results from the independent and geographically different TB cohorts are depicted: South Korea (**A**), South Africa (**B**), Italy (**C**) and The Gambia (**D**). Dutch healthy controls (n = 92) were compared to other diseases: leprosy (n = 86), sarcoidosis (n = 50) and community acquired pneumonia (n = 68) (**E**). Differences between groups were analysed using using the Mann-Whitney *U* test, > 2 groups Kruskall-Wallis and Dunn's multiple comparisons test and a p-value of < 0.05 was considered statistically significant.

pneumonia patients were significantly decreased compared to the controls (p = 0.021) and sarcoidosis (p = 0.004). The elevation of serum levels of C1-INH in TB patients (found in two out of four cohorts) thus seems related to *Mtb* infection and not an indirect consequence of other infections or inflammation. However, it is unclear why a significant increase in serum C1-INH protein levels was only present in the Italian and Gambian the cohort analysed, whereas on transcriptomic level the *SERPING1* is more consistently increased.

3.3. C1-INH serum levels normalize over course of successful TB treatment

The Italian and The Gambian cohort showed significantly increased serum levels of C1-INH in TB. From these two cohorts additional samples were available from TB patients that were successfully treated for pulmonary TB or longitudinal samples of patients on treatment. From the Italian cohort, samples from patients with active TB and patients successfully treated for TB were collected cross-sectionally. C1-INH serum levels were significantly lower in the treated patients as compared to the untreated active TB samples (Fig. 3A). In The Gambia, samples were collected longitudinally from TB patients over the course of their treatment, after one, two and six months (completion of therapy). Already after one month of treatment the C1-INH levels

significantly dropped and even further decreased after two and six months of therapy, albeit not completely to the level of the control population (Fig. 3B).

Next to C1-INH levels, C1q serum levels of these Gambian patients were also available (Lubbers et al., 2018). First, the average serum levels of C1-INH or C1q were both set at 100 % for the control group (TB contacts from the Gambia), such that serum values could be expressed as relative levels compared to controls. The medians with the 25th-75th percentiles were plotted for the patients during treatment compared to the TB-contacts (Fig. 3C). The C1q levels were 135 % higher during active TB whereas C1-INH was nearly doubled compared to TB contacts (175 %). C1-INH levels had already decreased after one month of treatment, whereas C1q levels did not decrease up to two months of treatment. These data indicate that the patients do not have a genetic predisposition to high C1q or C1-INH levels as treatment resulted in complete normalisation of C1q serum level and a significant decrease of C1-INH serum level.

3.4. Combining C1q and C1-INH measurement has added value in identifying patients with TB in the Gambia

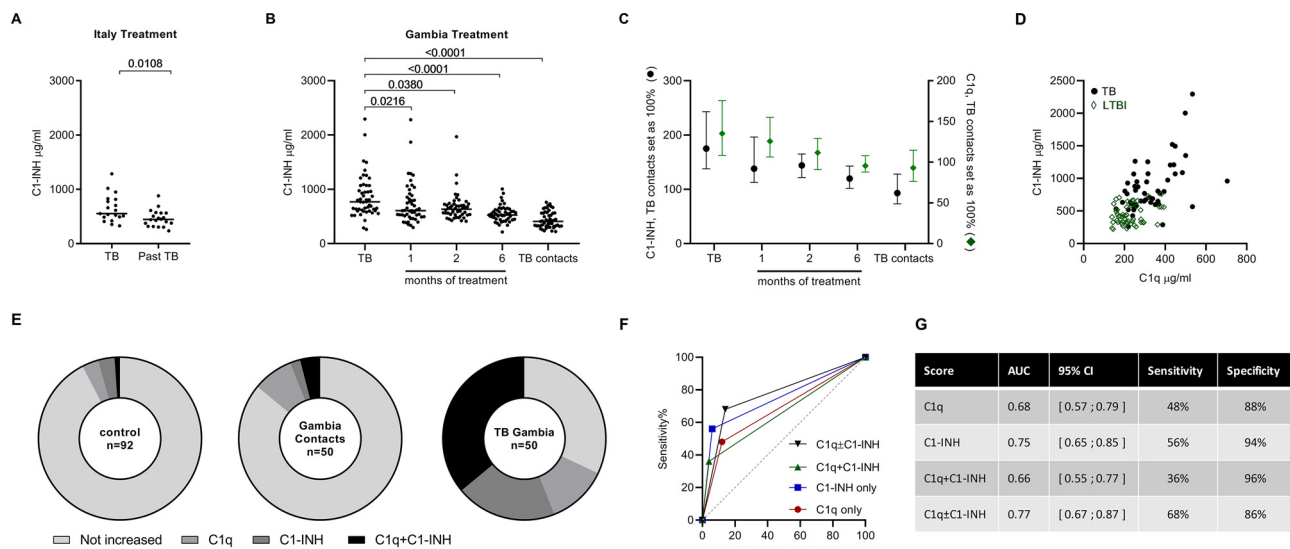In the samples from TB patients and controls, collected in The

Fig. 3. C1-inhibitor and C1q serum levels following treatment and combined scoring in The Gambian population.
C1-inhibitor (C1-INH) was measured by ELISA in TB patients and Past TB (patients that were successfully treated for TB, cross-sectionally enrolled) from Italy. Results were analysed using the Mann-Whitney *U* test (**A**). In patients from The Gambia, C1-INH was measured by ELISA and combined with C1q data that was available for these patients. These TB patients were followed over time during treatment, TB contacts are shown on the right (n = 50). The treatment months were compared to TB diagnosis with Friedman Test and Dunn's multiple comparisons test (**B**). The level of C1-INH or C1q was set at 100 % for the TB contacts from the Gambia and the median with the 25th -75th percentile was plotted for the Gambia active TB samples with treatment follow up (**C**). The C1-INH and C1q levels were analysed with Spearman (**D**). Subsequently, we further analysed this cohort and scored each sample for positivity for either C1q or C1-INH, or both. Positivity was based on the respective level of the Dutch healthy controls group (n = 92) at 95th percentile, when higher, the sample scored positive. Positivity was categorised in: not increased, single positive for C1q, single positive for C1-INH and finally double positive. These scoring results are visualized as a fraction of the total per group in doughnut charts (**E**). Additionally, these scores were analysed and the Area Under the Curve (AUC) was calculated comparing TB with the TB contacts. A separate ROC was generated based on a sample being positive for either one of the proteins (or both) and was annotated as C1q ± C1-INH (**F**). All AUC were calculated and summarized in the table, accompanied by the respective sensitivity and specificity (**G**).

Gambia, we combined C1q levels (previously published in (Lubbers et al., 2018)) with C1-INH levels determined in this study, in order to investigate if improved discriminatory value could be achieved or whether these molecules are possibly co-regulated. On the transcriptomic level the factorial changes were plotted (from the publicly available transcriptome data as also analysed in Fig. 1) for TB compared to LTBI for the different C1q chains and *SERPING1* (Supplementary Fig. 1). Spearman analyses showed significant correlations between *SERPING1* vs *C1QB* (p = 0.0098; r = 0.75) and *SERPING1* vs *C1QC* (p = 0.028; r = 0.79). C1-INH and C1q protein levels showed a significant (p < 0.0001) moderate correlation (r = 0.56) (Fig. 3D). Subsequently, we scored each sample for containing increased concentrations of either C1q or C1-INH, or both. A sample was scored as 'increased' if the value exceeded the 95th percentile of the Dutch healthy control group. These scoring results are visualized as a fraction of the total per group in Fig. 3E. Increased protein concentrations were categorized as: not increased, increased for C1q only, increased for C1-INH only, or increased for both C1q and C1-INH. Additionally, these results were analysed with ROC, comparing TB at diagnosis with TB contacts. Next to the categories in Fig. 3D, a separate ROC was generated based on a sample being increased for either one of the proteins (or both) and was annotated as C1q ± C1-INH (Fig. 3F, G). C1q alone resulted in an AUC of 0.68, whereas when C1q, C1-INH or both were increased an AUC of 0.77 was obtained. By using this scoring system, the presence of increased levels of both proteins is highly specific (96 %) but is accompanied by a relatively low sensitivity (36 %) in this cohort from the Gambia. Discrimination of TB patients from the control group based on C1q levels (Lubbers et al., 2018) resulted in a sensitivity of 48 % and a specificity of 88 %. In this comparison, incorporating the measurement of the C1-INH serum levels led to a remarkable increased sensitivity up to 68 % with a minor reduction of the specificity to 86 % in this specific cohort. Therefore, combination of C1q and C1-INH protein levels in serum, resulted in both acceptable specificity and sensitivity for active

TB detection in the cohort collected in The Gambia.

Overall, in contrast to the apparent abundant expression changes in *SERPING1*, measurement of C1-INH protein levels did not permit discrimination of TB patients from latently infected individuals in all cohorts. However, there may be added value in combined measurement of C1-INH and C1q in particular cohorts that warrant further investigation in the potential value of C1-INH as biomarker of TB disease.

4. Discussion

The major health threat posed by tuberculosis can likely only be tackled efficiently when a proper and fast identification of patients with active TB is available. Recently, complement gene expression and/or complement protein levels have been put forward as possible markers for such fast identification. In this study we collected available gene expression data and determined serum levels of C1-INH in four separate cohorts. We observed that *SERPING1* gene expression was higher in patients with active TB compared to healthy controls, LTBI and patients with other lung-diseases. This increase in *SERPING1* appears to be unique, as C4BP and FH, which are also soluble complement inhibitors with similar production sites as C1-INH, were not differently expressed in whole blood. For application in the field, it could be more feasible to measure protein levels using field friendly tests instead of gene expression, the latter being more expensive, labour intensive, time consuming and requiring more infrastructure. Therefore, we measured C1-INH protein serum levels, the product of *SERPING1*, in various world-wide cohorts of patients with active TB and other lung-diseases or mycobacterial exposure.

C1-INH serum levels were significantly increased in TB patients compared to endemic controls in two out of four cohorts analysed. In addition, in the Gambian cohort with TB patients followed over time the increased C1-INH decreased rapidly upon initiation of TB treatment. It is interesting that *SERPING1* expression is consistently increased in

patients with TB whereas its protein product C1-INH is found to be increased in only half of the cohorts analysed in this study. It is unclear why C1-INH upregulation was observed in only two out of four cohorts. Possible explanations may be sought in the production sites of C1-INH. C1-INH has multiple sources of production, like C1q, C1-INH is produced by circulating immune cells. But unlike C1q, C1-INH is also produced by hepatocytes and therefore may display a less clear relationship between mRNA levels in circulating cells versus protein levels in the circulation. The differences in expression in the microarray studies were measured in blood, which indicates that peripheral regulation may be affected by TB disease but this is not necessarily influencing circulating protein levels.

C1-INH levels were also measured in sera from patients that were diagnosed with diseases that resemble the symptoms of active TB, such as sarcoidosis and pneumonia, or caused by *Mycobacterium leprae*, leprosy. In contrast to active TB, in none of these patient cohorts, C1-INH was increased compared to the control group. Therefore, the increased C1-INH serum levels, observed in the two TB cohorts, seem to be specific for TB disease and not for inflammation in general.

TB patients from the Gambia were followed during treatment and samples were available over the full course of therapy. At recruitment, 28 of the 50 samples had C1-INH levels above the 95th percentile of the Dutch healthy control group. Of those 28 samples, 20 had decreased C1-INH levels after 1 month, 24 after 2 months, and 25 after 6 months of treatment. A significantly decreased C1-INH level was already observed after one month of therapy, whereas C1q levels at one month were indistinguishable from C1q levels at diagnosis (Lubbers et al., 2018). An explanation for this difference could be that different cellular sources have different response properties. Perhaps, hepatocytes decrease their production of C1-INH faster, resulting in a faster decrease of C1-INH in circulation, compared to the production of C1q. In the Gambia, where we found C1-INH to be significantly increased in TB compared to endemic controls, there was a positive correlation for C1q and C1-INH serum levels and the combination of these results had added value in discriminating active TB patients in this particular cohort. Moreover, it has been shown that the magnitude of the systemic inflammatory perturbation in pulmonary TB may be associated with different qualitative changes in inflammatory profiles (Oliveira-de-Souza et al., 2019) in samples from individuals coming from countries at different TB prevalence (Italy, Gambia, Korea, South Africa) and likely with different *Mtb* lineages, as previously shown (Romagnoli et al., 2018).

Overall, upon comparing C1q and C1-INH protein level in the different populations analysed, C1q was uniformly increased in TB cohorts whereas C1-INH in two out of four TB cohorts. We exclude that these differences between C1q and C1-INH in the measured TB cohorts were caused by freeze-thaw cycles or other technical issues with the samples, since the exact same samples were used for both measurements. For the differential diagnosis, leprosy, sarcoidosis and pneumonia, neither C1q nor C1-INH was increased. Both C1q and C1-INH decrease after successful TB treatment. How and why *Mtb* infection leads to increased levels of C1q and C1-INH in a part of the population is currently unknown, but it is interesting to speculate about the possible implications. C1q has been shown to be of importance in $CD8^+$ T-cell biology where C1q dampens the $CD8^+$ T-cell responses (Ling et al., 2018). Given that *Mtb* is an intracellular pathogen it could be of importance for *Mtb* to stimulate higher C1q production which will subsequently contribute to decreased $CD8^+$ T-cell anti-microbial activity. Simultaneous increases in C1q and C1-INH could limit activation of the classical pathway of the complement system. Recently, it was demonstrated in a $SERPING1^{-/-}$ mice that upon *Mtb* infection no differences were detected compared with the wildtype. However, more inflammation was observed in the $SERPING1^{-/-}$ mice, possibly due to the lack of complement inhibition (Ahmed et al., 2020).

In this study, the microarray data analysis demonstrated consistent increased expression of *SERPING1* in patients with TB compared to controls. However, the protein product of *SERPING1*, C1-INH, was increased in two out of four cohorts of patients with active TB analysed in this study. Thus, as yet, we conclude that C1-INH is not generally applicable as single protein host biomarker for TB. Cohorts with and without significant increases in C1-INH levels were from different geographical regions, however, at present it is unknown whether that explains the different outcomes. Additional, yet unidentified, factors may contribute as well and more (replication) studies are needed to provide insight into this finding.

The observation that increased C1-INH serum levels were only detected in cohorts of patients with active TB, but not in patients with similar clinical conditions and the notion that C1-INH decreased rapidly during TB treatment, suggests that *Mtb* is actively regulating these complement proteins. An increase in C1q and C1-INH could therefore represent an immune-escape mechanism of *Mtb* enabling immunosuppressive actions of C1q, without enhancing the classical pathway activity.

Author contributions statement

RL, AG, TO, SJ and LT designed the study. RL, RP and KG performed analyses. JS, DG, CM, MV, SV, WB, LP, STM, FN, GW, and GG oversaw recruitment and collection of specimens. RL, SJ and LT interpreted the data. All authors critically revised and approved the manuscript.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.molimm.2020.02.006.

References

Ahmed, M., Thirunavukkarasu, S., Rosa, B.A., Thomas, K.A., Das, S., Rangel-Moreno, J., Lu, L., Mehra, S., Mbandi, S.K., Thackray, L.B., Diamond, M.S., Murphy, K.M., Means, T., Martin, J., Kaushal, D., Scriba, T.J., Mitreva, M., Khader, S.A., 2020. Immune

correlates of tuberculosis disease and risk translate across species. Sci. Transl. Med. 12.

Anderson, S.T., Kaforou, M., Brent, A.J., Wright, V.J., Banwell, C.M., Chagaluka, G., Crampin, A.C., Dockrell, H.M., French, N., Hamilton, M.S., Hibberd, M.L., Kern, F., Langford, P.R., Ling, L., Mlotha, R., Ottenhoff, T.H.M., Pienaar, S., Pillay, V., Scott, J.A.G., Twahir, H., Wilkinson, R.J., Coin, L.J., Heyderman, R.S., Levin, M., Eley, B., 2014. Diagnosis of childhood tuberculosis and host RNA expression in Africa. N. Engl. J. Med. 370, 1712–1723.

Beinrohr, L., Dobo, J., Zavodszky, P., Gal, P., 2008. C1, MBL-MASPs and C1-inhibitor: novel approaches for targeting complement-mediated inflammation. Trends Mol. Med. 14, 511–521.

Berry, M.P., Graham, C.M., McNab, F.W., Xu, Z., Bloch, S.A., Oni, T., Wilkinson, K.A., Banchereau, R., Skinner, J., Wilkinson, R.J., Quinn, C., Blankenship, D., Dhawan, R., Cush, J.J., Mejias, A., Ramilo, O., Kon, O.M., Pascual, V., Banchereau, J., Chaussabel, D., O'Garra, A., 2010. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature 466, 973–977.

Blankley, S., Graham, C.M., Turner, J., Berry, M.P., Bloom, C.I., Xu, Z., Pascual, V., Banchereau, J., Chaussabel, D., Breen, R., Santis, G., Blankenship, D.M., Lipman, M., O'Garra, A., 2016. The transcriptional signature of active tuberculosis reflects symptom status in extra-pulmonary and pulmonary tuberculosis. PLoS One 11, e0162220.

Bloom, C.I., Graham, C.M., Berry, M.P., Rozakeas, F., Redford, P.S., Wang, Y., Xu, Z., Wilkinson, K.A., Wilkinson, R.J., Kendrick, Y., Devouassoux, G., Ferry, T., Miyara, M., Bouvry, D., Valeyre, D., Gorochov, G., Blankenship, D., Saadatian, M., Vanhems, P., Beynon, H., Vancheeswaran, R., Wickremasinghe, M., Chaussabel, D., Banchereau, J., Pascual, V., Ho, L.P., Lipman, M., O'Garra, A., 2013. Transcriptional blood signatures distinguish pulmonary tuberculosis, pulmonary sarcoidosis, pneumonias and lung cancers. PLoS One 8, e70630.

Breda, L.C.D., Vasconcellos, S.A., de Moraes Vasconcelos, D., Isaac, L., 2018. Binding of human complement C1 sterase inhibitor to Leptospira spp. Immunobiology 223, 183–190.

Cai, Y., Yang, Q., Tang, Y., Zhang, M., Liu, H., Zhang, G., Deng, Q., Huang, J., Gao, Z., Zhou, B., Feng, C.G., Chen, X., 2014. Increased complement C1q level marks active disease in human tuberculosis. PLoS One 9, e92340.

Castellano, G., Woltman, A.M., Nauta, A.J., Roos, A., Trouw, L.A., Seelen, M.A., Schena, F.P., Daha, M.R., van Kooten, C., 2004. Maturation of dendritic cells abrogates C1q production in vivo and in vitro. Blood 103, 3813–3820.

Cliff, J.M., Lee, J.S., Constantinou, N., Cho, J.E., Clark, T.G., Ronacher, K., King, E.C., Lukey, P.T., Duncan, K., Van Helden, P.D., Walzl, G., Dockrell, H.M., 2013. Distinct phases of blood gene expression pattern through tuberculosis treatment reflect modulation of the humoral immune response. J. Infect. Dis. 207, 18–29.

Esmail, H., Lai, R.P., Lesosky, M., Wilkinson, K.A., Graham, C.M., Horswell, S., Coussens, A.K., Barry 3rd, C.E., O'Garra, A., Wilkinson, R.J., 2018. Complement pathway gene activation and rising circulating immune complexes characterize early disease in HIV-associated tuberculosis. Proc. Natl. Acad. Sci. U. S. A. 115, E964–E973.

Esterhuyse, M.M., Weiner 3rd, J., Caron, E., Loxton, A.G., Iannaccone, M., Wagman, C., Saikali, P., Stanley, K., Wolski, W.E., Mollenkopf, H.J., Schick, M., Aebersold, R., Linhart, H., Walzl, G., Kaufmann, S.H., 2015. Epigenetics and Proteomics Join Transcriptomics in the Quest for Tuberculosis Biomarkers. mBio 6, e01187–1115.

Goletti, D., Lee, M.R., Wang, J.Y., Walter, N., Ottenhoff, T.H.M., 2018a. Update on tuberculosis biomarkers: from correlates of risk, to correlates of active disease and of cure from disease. Respirology (Carlton, Vic.).

Goletti, D., Lindestam Arlehamn, C.S., Scriba, T.J., Anthony, R., Cirillo, D.M., Alonzi, T., Denkinger, C.M., Cobelens, F., 2018b. Can we predict tuberculosis cure? What tools are available? Eur. Respir. J. 52.

Gupta, R.K., Turner, C.T., Venturini, C., Esmail, H., Rangaka, M.X., Copas, A., Lipman, M., Abubakar, I., Noursadeghi, M., 2020. Concise whole blood transcriptional signatures for incipient tuberculosis: a systematic review and patient-level pooled meta-analysis. Lancet Respir. Med.

Hofman, Z.L., Relan, A., Zeerleder, S., Drouet, C., Zuraw, B., Hack, C.E., 2016. Angioedema attacks in patients with hereditary angioedema: local manifestations of a systemic activation process. J. Allergy Clin. Immunol. 138, 359–366.

Jacobsen, M., Repsilber, D., Gutschmidt, A., Neher, A., Feldmann, K., Mollenkopf, H.J., Ziegler, A., Kaufmann, S.H., 2007. Candidate biomarkers for discrimination between infection and disease caused by Mycobacterium tuberculosis. J. Mol. Med. 85, 613–621.

Jiang, T.T., Shi, L.Y., Wei, L.L., Li, X., Yang, S., Wang, C., Liu, C.M., Chen, Z.L., Tu, H.H., Li, Z.J., Li, J.C., 2017. Serum amyloid A, protein Z, and C4b-binding protein beta chain as new potential biomarkers for pulmonary tuberculosis. PLoS One 12, e0173304.

Joosten, S.A., Fletcher, H.A., Ottenhoff, T.H., 2013. A helicopter perspective on TB biomarkers: pathway and process based analysis of gene expression data provides new insight into TB pathogenesis. PLoS One 8, e73230.

Joosten, S.A., van Meijgaarden, K.E., Del Nonno, F., Baiocchini, A., Petrone, L., Vanini, V., Smits, H.H., Palmieri, F., Goletti, D., Ottenhoff, T.H., 2016. Patients with tuberculosis have a dysfunctional circulating B-Cell compartment, which normalizes following successful treatment. PLoS Pathog. 12, e1005687.

Kaforou, M., Wright, V.J., Oni, T., French, N., Anderson, S.T., Bangani, N., Banwell, C.M., Brent, A.J., Crampin, A.C., Dockrell, H.M., Eley, B., Heyderman, R.S., Hibberd, M.L., Kern, F., Langford, P.R., Ling, L., Mendelson, M., Ottenhoff, T.H., Zgambo, F., Wilkinson, R.J., Coin, L.J., Levin, M., 2013. Detection of tuberculosis in HIV-infected and -uninfected African adults using whole blood RNA expression signatures: a case-control study. PLoS Med. 10, e1001538.

Khadge, S., Banu, S., Bobosha, K., van der Ploeg-van Schip, J.J., Goulart, I.M., Thapa, P., Kunwar, C.B., van Meijgaarden, K.E., van den Eeden, S.J., Wilson, L., Kabir, S., Dey, H., Goulart, L.R., Lobato, J., Carvalho, W., Bekele, Y., Franken, K.L., Aseffa, A.,

Spencer, J.S., Oskam, L., Ottenhoff, T.H., Hagge, D.A., Geluk, A., 2015. Longitudinal immune profiles in type 1 leprosy reactions in Bangladesh, Brazil, Ethiopia and Nepal. BMC Infect. Dis. 15, 477.

Lee, S.W., Wu, L.S., Huang, G.M., Huang, K.Y., Lee, T.Y., Weng, J.T., 2016. Gene expression profiling identifies candidate biomarkers for active and latent tuberculosis. BMC Bioinformatics 17 (Suppl 1), 3.

Ling, G.S., Crawford, G., Buang, N., Bartok, I., Tian, K., Thielens, N.M., Bally, I., Harker, J.A., Ashton-Rickardt, P.G., Rutschmann, S., Strid, J., Botto, M., 2018. C1q restrains autoimmunity and viral infection by regulating CD8(+) T cell metabolism. Science 360, 558–563.

Lubbers, R., van Essen, M.F., van Kooten, C., Trouw, L.A., 2017. Production of complement components by cells of the immune system. Clin. Exp. Immunol. 188, 183–194.

Lubbers, R., Sutherland, J.S., Goletti, D., de Paus, R.A., van Moorsel, C.H.M., Veltkamp, M., Vestjens, S.M.T., Bos, W.J.W., Petrone, L., Del Nonno, F., Bajema, I.M., Dijkman, K., Verreck, F.A.W., Walzl, G., Gelderman, K.A., Groeneveld, G.H., Geluk, A., Ottenhoff, T.H.M., Joosten, S.A., Trouw, L.A., 2018. Complement component C1q as serum biomarker to detect active tuberculosis. Front. Immunol. 9, 2427.

Maertzdorf, J., Ota, M., Repsilber, D., Mollenkopf, H.J., Weiner, J., Hill, P.C., Kaufmann, S.H., 2011a. Functional correlations of pathogenesis-driven gene expression signatures in tuberculosis. PLoS One 6, e26938.

Maertzdorf, J., Repsilber, D., Parida, S.K., Stanley, K., Roberts, T., Black, G., Walzl, G., Kaufmann, S.H., 2011b. Human gene expression profiles of susceptibility and resistance in tuberculosis. Genes Immun. 12, 15–22.

Maertzdorf, J., Weiner 3rd, J., Mollenkopf, H.J., Bauer, T., Prasse, A., Muller-Quernheim, J., Kaufmann, S.H., 2012. Common patterns and disease-related signatures in tuberculosis and sarcoidosis. Proc. Natl. Acad. Sci. U.S.A. 109, 7853–7858.

Maertzdorf, J., McEwen, G., Weiner 3rd, J., Tian, S., Lader, E., Schriek, U., Mayanja-Kizza, H., Ota, M., Kenneth, J., Kaufmann, S.H., 2016. Concise gene signature for point-of-care classification of tuberculosis. EMBO Mol. Med. 8, 86–95.

Nuijens, J.H., Eerenberg-Belmer, A.J., Huijbregts, C.C., Schreuder, W.O., Felt-Bersma, R.J., Abbink, J.J., Thijs, L.G., Hack, C.E., 1989. Proteolytic inactivation of plasma C1-inhibitor in sepsis. J. Clin. Invest. 84, 443–450.

Oliveira-de-Souza, D., Vinhaes, C.L., Arriaga, M.B., Kumar, N.P., Cubillos-Angulo, J.M., Shi, R., Wei, W., Yuan, X., Zhang, G., Cai, Y., Barry 3rd, C.E., Via, L.E., Sher, A., Babu, S., Mayer-Barber, K.D., Nakaya, H.I., Fukutani, K.F., Andrade, B.B., 2019. Molecular degree of perturbation of plasma inflammatory markers associated with tuberculosis reveals distinct disease profiles between Indian and Chinese populations. Sci. Rep. 9, 8002.

Ottenhoff, T.H., Dass, R.H., Yang, N., Zhang, M.M., Wong, H.E., Sahiratmadja, E., Khor, C.C., Alisjahbana, B., van Crevel, R., Marzuki, S., Seielstad, M., van de Vosse, E., Hibberd, M.L., 2012. Genome-wide expression profiling identifies type 1 interferon response pathways in active tuberculosis. PLoS One 7, e45839.

Penn-Nicholson, A., Hraha, T., Thompson, E.G., Sterling, D., Mbandi, S.K., Wall, K.M., Fisher, M., Suliman, S., Shankar, S., Hanekom, W.A., Janjic, N., Hatherill, M., Kaufmann, S.H.E., Sutherland, J., Walzl, G., De Groote, M.A., Ochsner, U., Zak, D.E., Scriba, T.J., 2019. Discovery and validation of a prognostic proteomic signature for tuberculosis progression: a prospective cohort study. PLoS Med. 16, e1002781.

Petruccioli, E., Scriba, T.J., Petrone, L., Hatherill, M., Cirillo, D.M., Joosten, S.A., Ottenhoff, T.H., Denkinger, C.M., Goletti, D., 2016. Correlates of tuberculosis risk: predictive biomarkers for progression to active tuberculosis. Eur. Respir. J. 48, 1751–1763.

Romagnoli, A., Petruccioli, E., Palucci, I., Camassa, S., Carata, E., Petrone, L., Mariano, S., Sali, M., Dini, L., Girardi, E., Delogu, G., Goletti, D., Fimia, G.M., 2018. Clinical isolates of the modern Mycobacterium tuberculosis lineage 4 evade host defense in human macrophages through eluding IL-1beta-induced autophagy. Cell Death Dis. 9, 624.

Scriba, T.J., Penn-Nicholson, A., Shankar, S., Hraha, T., Thompson, E.G., Sterling, D., Nemes, E., Darboe, F., Suliman, S., Amon, L.M., Mahomed, H., Erasmus, M., Whatney, W., Johnson, J.L., Boom, W.H., Hatherill, M., Valvo, J., De Groote, M.A., Ochsner, U.A., Aderem, A., Hanekom, W.A., Zak, D.E., 2017. Sequential inflammatory processes define human progression from M. Tuberculosis infection to tuberculosis disease. PLoS Pathog. 13, e1006687.

Sjoberg, A.P., Trouw, L.A., Blom, A.M., 2009. Complement activation and inhibition: a delicate balance. Trends Immunol. 30, 83–90.

Stavenhagen, K., Kayili, H.M., Holst, S., Koeleman, C.A.M., Engel, R., Wouters, D., Zeerleder, S., Salih, B., Wuhrer, M., 2018. N- and O-glycosylation Analysis of Human C1-inhibitor Reveals Extensive Mucin-type O-Glycosylation. Mol. Cell Proteomics 17, 1225–1238.

Thompson, E.G., Du, Y., Malherbe, S.T., Shankar, S., Braun, J., Valvo, J., Ronacher, K., Tromp, G., Tabb, D.L., Alland, D., Shenai, S., Via, L.E., Warwick, J., Aderem, A., Scriba, T.J., Winter, J., Walzl, G., Zak, D.E., 2017. Host blood RNA signatures predict the outcome of tuberculosis treatment. Tuberculosis Edinb. (Edinb) 107, 48–58.

van Hooij, A., Tjon Kon Fat, E.M., Batista da Silva, M., Carvalho Bouth, R., Cunha Messias, A.C., Gobbo, A.R., Lema, T., Bobosha, K., Li, J., Weng, X., Salgado, C.G., Spencer, J.S., Corstjens, P., Geluk, A., 2018. Evaluation of immunodiagnostic tests for leprosy in Brazil, China and Ethiopia. Sci. Rep. 8, 17920.

Vrieling, F., Ronacher, K., Kleynhans, L., van den Akker, E., Walzl, G., Ottenhoff, T.H.M., Joosten, S.A., 2018. Patients with Concurrent Tuberculosis and Diabetes Have a Pro-Atherogenic Plasma Lipid Profile. EBioMedicine 32, 192–200.

Walter, N.D., Miller, M.A., Vasquez, J., Weiner, M., Chapman, A., Engle, M., Higgins, M., Quinones, A.M., Rosselli, V., Canono, E., Yoon, C., Cattamanchi, A., Davis, J.L., Phang, T., Stearman, R.S., Datta, G., Garcia, B.J., Daley, C.L., Strong, M., Kechris, K., Fingerlin, T.E., Reves, R., Geraci, M.W., 2016. Blood transcriptional biomarkers for active tuberculosis among patients in the United States: a case-control study with systematic cross-classifier evaluation. J. Clin. Microbiol. 54, 274–282.

Wang, C., Wei, L.L., Shi, L.Y., Pan, Z.F., Yu, X.M., Li, T.Y., Liu, C.M., Ping, Z.P., Jiang,

T.T., Chen, Z.L., Mao, L.G., Li, Z.J., Li, J.C., 2015. Screening and identification of five serum proteins as novel potential biomarkers for cured pulmonary tuberculosis. Sci. Rep. 5, 15615.

World Health Organization (WHO), 2018. Global Tuberculosis Report 2018. available from: https://www.who.int/tb/publications/global_report/en/. .

Zak, D.E., Penn-Nicholson, A., Scriba, T.J., Thompson, E., Suliman, S., Amon, L.M., Mahomed, H., Erasmus, M., Whatney, W., Hussey, G.D., Abrahams, D., Kafaar, F., Hawkridge, T., Verver, S., Hughes, E.J., Ota, M., Sutherland, J., Howe, R., Dockrell, H.M., Boom, W.H., Thiel, B., Ottenhoff, T.H.M., Mayanja-Kizza, H., Crampin, A.C., Downing, K., Hatherill, M., Valvo, J., Shankar, S., Parida, S.K., Kaufmann, S.H.E., Walzl, G., Aderem, A., Hanekom, W.A., 2016. A blood RNA signature for tuberculosis disease risk: a prospective cohort study. Lancet (London, England) 387, 2312–2322.

Zeerleder, S., Levi, M., 2016. Hereditary and acquired C1-inhibitor-dependent angioedema: from pathophysiology to treatment. Ann. Med. 48, 256–267.