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Research article

Tuberculosis diagnosis: algorithm that May discriminate latent from active tuberculosis

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ABSTRACT

Diagnosis of tuberculosis still faces a lot of challenges and is one of the priorities in the field of tuberculosis management. Deciphering the complex tuberculosis pathogenicity network could provide biomarkers for diagnosis. We discussed the distribution of HLA-B17, -DQB and -DRB together with QuantiFERON test results in tuberculosis infection. A case control study was done during which a total of 337 subjects were enrolled comprising 227 active tuberculosis (ATB), 46 latent tuberculosis infection (LTBI) and 64 healthy controls (HC). Sequence-specific primer polymerase chain reaction and immune epitope database were used to genotype samples and determine the epitope binding ability of the over-represented alleles respectively. QuantiFERON test was done according to manufacturer's instructions. The peptides HLA-B*5801 and HLA-DRB1*12 and the peptides HLA-B*5802 and HLA-DQB1*03 were found to be associated with latent tuberculosis while the haplotypes DRB1*10-DQB1*02 and DRB1*13-DQB1*06 were found to be associated with active tuberculosis (All p-values$\leq$0.05). The association of HLA-B*5801 and HLA-B*5802 with latent tuberculosis was linked to their ability to bind or not mycobacterial antigens. DRB1*10-DQB1*02 haplotype was found to be over-represented in LTBI compared to ATB (p-value = 0.0015) while DRB1*13-DQB1*06 was found to be under-represented in LTBI compared to ATB (p-value = 0.0335). The DRB1*10-DQB1*02 haplotype was only found in the LTBI when compared with the ATB group. The present study suggests the following algorithm to discriminate LTBI from ATB: QuantiFERON+ and DRB1*10-DQB1*02 haplotype + may indicate LTBI; QuantiFERON+ and DRB1*10-DQB1*02 haplotype - may indicate ATB.

1. Introduction

As of late 2017, TB has been classified by the World Health Organization as the ninth leading cause of death worldwide and the leading cause of death from a single infectious agent, ranking above HIV/AIDS [1]. In Human, most TB diseases originate from an infection with *Mycobacterium tuberculosis* (*Mtb*) and occur in a series of stages ranging from asymptomatic latent stage (latent TB infection) to a more symptomatic clinically infectious stage (active TB). The aerobic bacillus preferentially infects the lungs (certainly due to its aerobic nature) to cause pulmonary TB but can also infect other organs such as the kidneys, bones, brain… (extra-pulmonary TB). Efforts to manage it are hampered by difficulties in diagnosis, prevention and treatment [2].

Pulmonary TB-infection begins when the mycobacteria reach the pulmonary alveoli, where they invade and replicate within endosomes of alveolar macrophages [3]. Macrophages identify the bacterium as

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"foreign" and attempt to eliminate it by phagocytosis. During this process, the entire bacterium is enveloped by the macrophage and stored temporarily in phagosomes. The phagosome fuses with a lysosome to form a phagolysosome. In the phagolysosome, the cell attempts to use reactive oxygen species and acid to kill the bacterium and generate antigenic epitopes like the *Mtb* 6 kDa early secretory antigenic target (ESAT-6), 10 kDa culture filtrate protein (CFP-10), and mycolyltransferase Ag85 A/B for presentation to T-lymphocytes. However, *Mtb* thick, waxy mycolic acid capsule protects it from these toxic substances. *Mtb* also survives in macrophages by inhibiting phagolysosome maturation. Some of these *Mtb* antigens (ESAT-6 and CFP-10) have been exploited in the development of quantiFERON blood TB test but unfortunately this test cannot discriminate between latent TB infection (LTBI) and active TB (ATB) [4].

Not every individual exposed to TB is infected (LTBI) and out of all those infected not everyone progresses to clinically express the active form (ATB) of the disease. The pathogenicity of TB is very complex being influenced by many factors ranging from immunogenetic, nutritional to environmental factors [5, 6]. In deciphering this complex network involved in TB infection and progression, it is possible to identify biomarkers that could be useful in TB diagnosis which is one of the priority fields in TB management. One of the genetic factors that has been described to regulate host immune responses are the major histocompatibility complexes (MHCs) which are peptides that are involved in presenting antigenic epitopes to immune cells known as human leukocyte antigens in humans (HLA). Chen *et al* in 2015 showed that two Class II HLA alleles; DRB1*03 and DRB1*07 could provide protective effects against TB susceptibility [7] while Kettaneh *et al* (2006) observed that susceptibility to TB was modulated by class I and II HLA antigens [8]. Oliveira-Cortez *et al* (2016) [9] reported the association of HLA-DRB1*08:03, HLA-DQB1*06:01, HLA-DQB1*06:09 and HLA-DQA1*01:01 with a higher susceptibility to pulmonary TB and the association of HLA-DRB1*07:01, HLA-DQB1*03:01, HLA-DQB1*04:02, HLA-DQA1*04:01, and HLA-DQA1*05:01 with protection against pulmonary TB. Wamala and colleagues in 2016 working on a Ugandan population found a negative correlation between HLA-DQB1*03:03 (a Class II allele) and susceptibility to TB [10]. While on the other hand, HLA-B type was previously described as the Class I HLA most strongly correlated with disease control in HIV patients [11]. The Class I HLA present antigens to CD8+ T cells whereas the Class II HLA present antigens to CD4+ T cells. CD8 T cells were initially thought to be less important than CD4 T cells in the immune response to *Mtb*, it has now been recognized that they play a critical but complex role. Like CD4+ T cells, CD8 T cells are able to produce IL2, IFN-γ, and TNF, cytokines that are known to have critical functions during *Mtb* infection. Importantly, CD8+ T cells have cytolytic functions to kill *Mtb*-infected cells via granule-mediated function (via perforin, granzymes, and granulysin) or Fas-Fas ligand interaction to induce apoptosis. In humans, CD8+ T cell can produce granulysin, which can kill *Mtb* directly [12].

In this work, we discuss the phenotypic distribution profile of one Class I HLA (B17 made up of two splits B*57 and B*58), two class II HLAs (DQB and DRB) and their haplotypes in TB infection, combined with quantiFERON test.

2. Materials and methods

2.1. Human subjects and ethical considerations

All subjects were enrolled at the Jamot hospital center Yaounde Cameroon between September 2013 and May 2015. For QuantiFERON test, blood was sampled according to the manufacturer's instructions. For HLA analysis, blood samples were collected and transported in ice coolers (temperature monitored) to the Chantal Biya International Reference Center (CBIRC). An ethical clearance N° 2013/05/242/CNERSH/SP was obtained from the Cameroonian National Ethical Committee for Research on human health and an authorization from the Ministry of Health N° D30-685AAR/MINSANTE/SG/DROS/CRC/CEA1/AB was also obtained. Only consenting participants were enrolled and a questionnaire was administered after which biological specimen were collected.

2.2. Active TB (ATB) patients

Active TB was confirmed by sputum smear microscopy and/or culture with complementary information from chest X-ray examined by a medical specialist. We enrolled a total of 227 active TB (ATB) patients.

2.3. Latent TB infection (LTBI) and healthy controls (HC)

Subjects in these two groups (LTBI and HC) were healthcare workers and interns working in the TB unit in contact with TB patients for at least three months. They showed no signs/symptoms of TB, apparently in good health and had no past history of TB infection. The quantiFERON-TB test was used to distinguish between LTBI and HC. Subjects with positive quantiFERON test were grouped as LTBI while those with negative quantiFERON test results, with negative chest radiography results for pulmonary lesions and no prior preventive therapy for LTBI were grouped as HC [13, 14]. We enrolled a total of 46 LTBI and 64 HC.

2.4. Blood collection

8 mL of blood were collected into Ethylene diamine tetra-acetate (EDTA) tubes through venous puncture. Blood samples were transported in ice coolers with temperature being monitored from the Jamot hospital center to the CBIRC. 1 ml of blood were collected into each of the three quantiFERON tubes (Nil, Antigen, and Mitogen tubes) and the remaining 5ml were centrifuged at 3000 rpm for 10 min. Buffy coat were aliquoted into 2 ml screw-cap tubes and stored at -30 °C till needed for DNA extraction.

2.5. Performing the quantiFERON test

The quantiFERON test was carried out following the manufacturer's instructions. We used the whole blood ELISA-based quantiFERON-TB Gold In-Tube (QFT-GIT) kit provided by Qiagen (Qiagen, Chadstone, Victoria, Australia, Cat. N° 0594–0201). The maximum time between blood sample collection and incubation was 3 h. Samples were incubated within a time range of 20–23 h with a modal incubation time of 22 h. ELISA was done using an automated washer (ELx50 Biokit bioelisa washer, Highland Park, Winooski, USA) and optical densities (ODs) were read using the Miray spectrophotometer (BA-88A Mindray, Eiffestraβe 80, Hamburg, Germany). These ODs were introduced into a software provided by Cellestis downloadable from their website.

2.6. DNA extraction

DNA was extracted from buffy coat samples using the QIAmp DNA mini kit (Qiagen, Strasse 1, Hilden Germany, Cat. N° 51306) following the manufacturer's instructions. Briefly, 200 μl of the buffy coat samples were lysed with proteinase K and DNA molecules adsorb onto silica-gel membranes in the presence of high chaotropic salt concentration. The resulting DNA were washed and eluted in 200 μl of buffer AE. The quality of DNA was assessed using a nanodrop (NanoDrop One, by Thermo-Scientific; Verona Rd, Madison, Assembled USA) prior to HLA genotyping by sequence-specific priming polymerase chain reaction (SSP-PCR).

2.7. HLA-B17 genotyping

The method used for HLA B17 genotyping was the AllSet + Gold SSP B17 high resolution Kit (One Lambda INC, Kittridge Street, Canoga Park, USA Catalogue No 541120D). The method was realized following the manufacturer's instructions with slight modifications (15 min were used for electrophoretic migration instead of 18–23 min recommended by

manufacturer). After the SSP-PCR, electrophoresis was conducted at 150 V, 250 mA for 15 min in a 2% agarose gel containing 0.01% ethidium bromide and DNA visualized by UV trans-illumination. Genotypes were attributed by analyzing the positive fragments (positivities) with the HLA Fusion v. 4.1 software (package installed by Lagitre-One-Lambda) and/or worksheets.

2.8. HLA-DQB and HLA-DRB genotyping

HLA-DQB and HLA DRB were genotyped using the AllSet + Gold SSP DRDQ low resolution Kit (One Lambda INC, Kittridge Street, Canoga Park, USA, Catalogue N^{o} 54380D) following the manufacturer's instructions with slight modifications (15 min were used for electrophoretic migration instead of 18–23 min recommended by manufacturer). This method permitted to simultaneously genotype the two locus on the same plate (multiplex). Electrophoresis was carried out at 150 V, 250 mA for 15 min in a 2% agarose gel containing 0.01% ethidium bromide and DNA visualized by UV trans-illumination (ChemiDoc XRS+, Bio-Rad laboratories, Inc, USA). Genotypes were attributed using HLA Fusion 4.1 software and/or worksheet.

2.9. In silico prediction of HLA molecules binding to Mtb antigenic epitopes

The prediction of the binding ability of *Mtb* antigenic epitopes with HLA class I and II molecules associated with TB were carried out by bioinformatics tools using analysis of algorithms ("silico mapping"). This was done with the Immune Epitope Database Analysis Resource (IEDB) version 2.19 (www.iedb.org/home_v3.php) with default settings.

2.10. Analysis of haplotypes

Haplotypes were analyzed by combining the following loci; DRB1-B17, DRB1-DQB1 and B17-DQB1. The distribution of these haplotypes was compared between the LTBI group and the ATB group.

2.11. Statistical analyses

GraphPad Prism version 6.0 was used as the statistical analysis tool. Chi square contingency test was used to compare the phenotypic frequencies between the groups (HC, LTBI, ATB) or the Fisher's exact test when an expected absolute cell frequency was less than 5. P-values less than or equal to 0.05 were considered significant in all analyses. Where appropriate the penalization method was used for analysis [15].

3. Results

3.1. Demographic and clinical characterization of study population

We enrolled a total of 337 subjects (non-HIV infected) among which were 227 ATB, 46 LTBI and 64 HC. The number of samples analyzed varied from one HLA to the other due to different in the number of kits available (Table 1). For HLA-B17, a total of 335 samples were analyzed among which 227 ATB, 44 LTBI and 64 HC. For HLA-DQB, a total of 288 samples were analyzed among which 179 ATB, 46 LTBI and 63 HC while for HLA-DRB, 277 samples were analyzed containing 170 ATB, 43 LTBI and 64 HC (Table 1).

Associations were found between, gender, mean ages and TB infection. We observed that more females presented with LTBI than males ($p < 0.0001$) but more males presented ATB than females ($p < 0.0001$). When the mean ages between the three groups (ATB, LTBI and HC) were compared, older people (mean age of 32 years) were more infected with LTBI and ATB than the young (mean age of 27 years) (p-value = 0.0015 and 0.0003 respectively).

Table 1
Demographic and clinical characteristics of study population.

Parameters	Active TB (ATB)	Latent TB infection (LTBI)	Healthy controls (HC)
Number of samples analyzed per HLA (HLA-B17/DQB/DRB)	(227/179/170)	(44/46/43)	(64/63/64)
Age (Mean ± SD) [Range]	(34 ± 15) [6, 76]	(32 ± 10) [19, 53]	(27 ± 6) [19, 56]
Gender (Female/Male)	(85/142)	(33/13)	(21/43)

3.2. Relative distribution of HLA B17 alleles in TB subjects

A total of 335 samples made up of 227 active TB (ATB) patients, 44 latent TB infection (LTBI) and 64 healthy controls (HC) were genotyped for the HLA B locus. One hundred and twenty-eight samples showed no positivity for the B17 locus and were excluded from the statistical analysis. Two hundred and seven samples comprising 146 ATB, 24 LTBI and 37 HC were compared statistically.

We identified a total of 15 HLA B alleles in our study population namely; B*1301, B*1424, B*15162, B*1501, B*1516, B*1520, B*1844, B*5201, B*5702, B*5703, B*5711, B*5801, B*5802, B*5820 and B*5828. Seven of these (B*5702, B*5703, B*5711, B*5801, B*5802, B*5820 and B*5828) were HLA B17 alleles (Table 2). The prevalence of B17 alleles in our study population was 40% (134/335) of which 29% (39/134) were of the B*57 split and 71% (95/134) of the B*58 split. The most prevalent B17 antigen was B*5802 with a frequency of 17% (57/335).

When we compared the distribution of the 7 HLA B17 alleles in the different groups (ATB, LTBI and HC) in our study population, the B*5802 antigen was found to be over-represented in LTBI compared to HC (p-value = 0.0108) (Table 2). HLA-B*5802 may therefore be associated with latency (LTBI). HLA-B*5801 was more frequent in HC compared to LTBI (p-value = 0.0534). This allele may be associated with protection against TB infection. No allele was found to be associated with ATB compared to LTBI.

3.3. Relative distribution of HLA DQB alleles in TB subjects

Two hundred and eighty-eight samples comprising 179 ATB, 46 LTBI

Table 2
Phenotypic distribution of the alleles of HLA B within different TB groups.

Alleles/ Antigens	Number of alleles N' (Phenotypic frequency %)			P-values		
	HC (N = 37)	LTBI (N = 24)	ATB (N = 146)	HC X LTBI	HC X ATB	LTBI X ATB
B*1301	0 (0)	0 (0)	1 (0.7)	1.0000	1.0000	1.0000
B*1424	0 (0)	0 (0)	1 (0.7)	1.0000	1.0000	1.0000
B*15162	2 (5.4)	1 (4.2)	2 (1.4)	0.4441	0.1825	0.3684
B*1501	1 (2.7)	1 (4.2)	6 (4.1)	0.4852	1.0000	1.0000
B*1516	1 (2.7)	1 (4.2)	10 (6.8)	0.4852	0.6973	1.0000
B*1520	28 (75.7)	21 (87.5)	109 (74.7)	0.1445	0.8985	0.1693
B*1844	3 (8.1)	0 (0)	3 (2.0)	0.2159	0.0981	1.0000
B*5201	0 (0)	0 (0)	6 (4.1)	1.0000	0.6025	0.5962
B*5702	2 (5.4)	0 (0)	3 (2.0)	0.3639	0.2661	1.0000
B*5703	6 (16.2)	3 (12.5)	24 (16.4)	0.2713	0.9740	0.7701
B*5711	0 (0)	0 (0)	1 (0.7)	1.0000	1.0000	1.0000
B*5801	8 (21.6)	1 (4.2)	27 (18.5)	0.0534[#]	0.6656	0.1328
B*5820	0 (0)	0 (0)	1 (0.7)	1.0000	1.0000	1.0000
B*5802	6 (16.2)	11 (45.8)	40 (27.4)	0.0108[#]	0.1614	0.0678
B*5828	1 (2.7)	0 (0)	0 (0)	0.6066	0.2022	1.0000

[#] Significantly different, p-value ≤ 0.05 at 95% CI. (N = total number of subjects, N' = number of alleles present within group, HC = Healthy controls, LTBI = Latent TB Infection, ATB = Active TB).

and 63 HC were genotyped for the HLA DQB locus. We identified a total of 5 HLA-DQB alleles in our study population namely; DQB1*02, DQB1*03, DQB1*04, DQB1*05, and DQB1*06 (Table 3). HLA-DQB1*06 was the most prevalent with a frequency of 62.5% (180/288).

When we compared the HLA-DQB allele distribution within the different groups (ATB, LTBI and HC), the DQB1*03 antigen was found to be over-represented in LTBI compared to the HC group (p-value = 0.0447) (Table 3). When we compared the distribution of the different HLA-DQB1*03 genotypes (homozygous or heterozygous for the allele) between those with LTBI and HC, we found an association (p = 0.0025). Majority of those with LTBI (60%) were homozygous for the HLA-DQB1*03 allele against 11% for the HC group. No allele was found to be associated with ATB compared to LTBI.

3.4. Relative distribution of HLA DRB alleles in TB subjects

Two hundred and seventy-seven samples comprising 170 ATB cases, 43 LTBI and 64 HC were genotyped for the HLA DRB locus. Sixteen HLA-DRB alleles were found to be present in our study population namely; DRB1*01, DRB1*02, DRB1*03, DRB1*04, DRB1*07, DRB1*08, DRB1*09, DRB1*10, DRB1*11, DRB1*12, DRB1*13, DRB1*14, DRB1*15, DRB1*16, DRB3*01, and DRB5*01. The DRB1*15 allele was the most prevalent with a frequency of 31.4% (87/277) (Table 4).

We analyzed the HLA-DRB allele distribution within different groups (ATB, LTBI and HC) and found out that the HLA-DRB1*12 allele was under-represented in the LTBI group compared to HC (p-value = 0.0371). No allele was found to be associated with ATB compared to LTBI.

The HC group and the LTBI group were merged as one group and compared to the ATB across the three loci (HLA-B17, HLA-DQB and HLA-DRB) but no associations were found (All p > 0.05).

3.5. In silico prediction of HLA molecules binding to Mtb antigenic epitopes

We estimated the ability of *Mtb* antigenic epitopes to bind with the HLA molecules (HLA-B*5801, HLA-B*5802, HLA-DQB1*03 and HLA-DRB1*12) that were found to be associated with TB and the results were recorded in Table 5. The specific HLA-B*5801 peptide was observed to bind the *Mtb* 10 kDa culture filtrate protein abbreviated CFP-10 (ESAT-6-like protein, EsxB) and the *Mtb* mycolyltransferase Ag85B while the specific HLA-B*5802 peptide could not bind any *Mtb* antigens.

3.6. Relative distribution of haplotypes in relation to the quantiFERON test results in TB subjects

The relative distribution of the haplotype associations DRB1-B17, DRB1-DQB1 and B17-DQB1 were evaluated between LTBI and ATB. All the LTBI and ATB subjects in our study population gave a positive quantiFERON-TB test result. The quantiFERON-TB test did not distinguish between the two groups, so we assessed the possibility of discriminating between these two groups by using haplotype associations.

Two haplotypes DRB1*10-DQB1*02 and DRB1*13-DQB1*06 were found to be distributed differently between the LTBI group and the ATB group (Table 6). DRB1*10-DQB1*02 was found to be over-represented in LTBI compared to ATB (p-value = 0.0015) while DRB1*13-DQB1*06 was found to be under-represented in LTBI compared to ATB (p-value = 0.0335). We then compared the phenotypic frequency of DRB1*10-DQB1*02 in those with LTBI who were positive responders to the quantiFERON-TB test to those with ATB who were positive responders to the quantiFERON TB test and found an association (p-value = 0.0015) (Table 7). The haplotype DRB1*10-DQB1*02 was only found in the LTBI-quantiFERON-TB + group when compared with the ATB-quantiFERON-TB + group. Using the penalization methods, the odds ratio (95% CI) of LTBI-quantiFERON + for DRB1*10-DQB1*02 Present compared to DRB1*10-DQB1*02 Absent was 6.82 (1.30–35.54).

Table 3
Phenotypic distribution of the DQB alleles or antigens within different TB groups.

Alleles/ Antigens	Number of alleles N' (Phenotypic frequency %)			P-values		
	HC (N = 63)	LTBI (N = 46)	ATB (N = 179)	HC X LTBI	HC X ATB	LTBI X ATB
DQB1*02	17 (27.0)	11 (23.9)	42 (23.5)	0.1654	0.5757	0.9489
DQB1*03	18 (28.6)	20 (43.5)	56 (31.3)	0.0447[#]	0.6877	0.1188
DQB1*04	6 (9.5)	5 (10.9)	11 (6.1)	0.2427	0.3668	0.2661
DQB1*05	16 (25.4)	8 (17.4)	47 (26.3)	0.1157	0.8936	0.2120
DQB1*06	41 (65.1)	26 (56.5)	113 (63.1)	0.1049	0.7819	0.4108

[#] Significantly different, p-value ≤ 0.05 at 95% CI. (N = total number of subjects, N' = number of alleles present within group, HC = Healthy controls, LTBI = Latent TB Infection, ATB = Active TB).

Table 4
Phenotypic distribution of the DRB alleles within different TB groups.

Alleles/ Antigens	Number of alleles N' (Phenotypic frequency %)			P-values		
	HC (N = 64)	LTBI (N = 43)	ATB (N = 170)	HC X LTBI	HC X ATB	LTBI X ATB
DRB1*01	13 (20.3)	7 (16.3)	20 (17.6)	0.1770	0.0940	0.4267
DRB1*02	0 (0)	0 (0)	2 (1.2)	1.0000	1.0000	1.0000
DRB1*03	9 (14.1)	8 (18.6)	27 (15.9)	0.1721	0.7309	0.6669
DRB1*04	3 (4.7)	5 (11.6)	7 (4.1)	0.1230	1.0000	0.0564
DRB1*07	4 (6.3)	1 (2.3)	6 (3.5)	0.2570	0.4675	1.0000
DRB1*08	6 (9.4)	5 (11.6)	25 (14.7)	0.2329	0.2836	0.6042
DRB1*09	0 (0)	1 (2.3)	4 (2.4)	0.4019	0.5771	1.0000
DRB1*10	13 (20.3)	14 (32.6)	33 (19.4)	0.0654	0.8772	0.0633
DRB1*11	20 (31.3)	11 (25.6)	49 (28.8)	0.1427	0.7167	0.6729
DRB1*12	11 (17.2)	2 (4.7)	19 (11.2)	0.0371[#]	0.2202	0.2609
DRB1*13	19 (29.7)	13 (30.2)	48 (28.2)	0.1698	0.8266	0.7958
DRB1*14	1 (1.7)	1 (2.3)	2 (1.2)	1.0000	1.0000	0.4934
DRB1*15	18 (28.1)	10 (23.3)	59 (34.7)	0.1536	0.3396	0.1518
DRB1*16	4 (6.3)	2 (4.7)	12 (7.1)	0.3175	1.0000	0.7404
DRB3*01	4 (6.3)	2 (4.7)	3 (1.8)	0.3175	0.0911	0.2656
DRB5*01	0 (0)	2 (4.7)	5 (2.9)	0.1592	0.3266	0.6309

[#] Significantly different, p-value ≤ 0.05 at 95% CI. (N = total number of subjects, N' = number of alleles present within group, HC = Healthy controls, LTBI = Latent TB Infection, ATB = Active TB).

4. Discussion

Not every individual exposed to TB is infected (LTBI) and out of all those infected not everyone progresses to clinically express the active form (ATB) of the disease. The pathogenicity of TB is very complex being influenced by many factors. The factors that influence TB pathogenicity range from immunogenetic, nutritional to environmental factors [5, 6]. In this study, we observed that gender was associated with TB infection with men being more likely to develop active TB than women. This difference may be due to biological, socioeconomic or cultural differences [16]. Men's greater involvement in certain activities outside the home such as smoking and drinking alcohol which favor the development of TB could account partly for this gender difference [16]. Tobacco has been shown to be an important factor which promotes the development of TB and some studies have reported more men to be involved in tobacco intake than women [16]. Age was also found to be associated with TB

infection with older people being infected with LTBI than the young and also older people having more ATB than the young. The association between age and TB infection is not yet clear but we could suggest that association of age with TB infection may be due to decrease in immunity as we advance in age. The function of the immune system generally peaks at around puberty and gradually declines thereafter with advancement in age [17]. Another plausible explanation may be the repeated and cumulative exposure to *Mtb* over time.

One of the genetic factors that has been described to regulate host immune responses are the major histocompatibility complexes (MHCs) which are peptides that are involved in presenting antigenic epitopes to immune cells. Here we discuss the phenotypic distribution profile of one Class I HLA (B17 made up of two splits B*57 and B*58) and two class II HLAs (DQB and DRB) in three groups (ATB, LTBI and HC).

Leslie and collaborators in 2010 [11] described HLA-B type as the Class I HLA most strongly correlated with disease control (progression and non-progression) in HIV patients. We assessed the role of HLA-B17 in TB patients by comparing the phenotypic frequencies between ATB, LTBI and HCs. We found no association between ATB and HC or between ATB and LTBI and HLA-B17. This might mean that HLA-B17 is not associated with TB infection in TB single infected patients. But we found that HLA-B*5801 which differ by just three amino acids from HLA-B*5802 was associated with protection against TB infection (LTBI) while HLA-B*5802 was associated with susceptibility to TB infection-LTBI. These results suggest that, the HLA-B17 split subtypes HLA-B*5801 (protection) and HLA-B*5802 (susceptibility) may be associated with TB infectivity-LTBI but not with ATB. Leslie *et al* (2010) [11] observed that HLA-B*5801 was associated with slow HIV disease progression while HLA-B*5802 was associated with rapid HIV disease progression. Ngumbela *et al* (2008) [18] also reported that HLA-B*5802 was associated with rapid HIV progression due to poor HIV-1-specific CD8+ T cell responses as a result of poor epitope presentation while HLA-B*5801 was associated with slow HIV disease progression due to proper epitope recognition. This result suggest that *Mtb* antigens and HIV antigens may exhibit cross reactivation on the same HLA B*58 groove.

In order to elucidate the difference in the influence of HLA-B*5801 and HLA-B*5802 in TB infection, we assessed their ability to bind *Mtb* virulent antigens. HLA-B*5801 was found to bind the *Mtb* antigens epitopes CFP-10 (ESAT-6-like protein) and mycolyltransferase Ag85B while HLA-B*5802 did not bind any of the *Mtb* antigens. ESAT-6 and CFP-10 are essential for *Mtb* virulence. CFP-10 has been described as a chaperon protein (folding protein) for ESAT-6 but recent studies have shown that, other than just being a folding protein, it is also involved in neutrophil recruitment and activation during *Mtb* infection for the benefit of the mycobacterium [19]. Mycolyltransferase Ag85B (T and B-cell immunogen) belong to a family of proteins responsible for the synthesis of *Mtb* cell wall components which have been shown to be involved in host entry [20]. We therefore suggest that, the association of HLA-B*5801 and protection against TB infection may be attributed to its ability to properly bind and present the *Mtb* virulent antigens CFP-10 and mycolyltransferase Ag85B to immune cells which induce a protective immune response. While the association of HLA-B*5802 with susceptibility to TB infection is attributed to its inability to bind any of the *Mtb* antigens.

We could not perform the prediction analysis for HLA-DQB1*03 and HLA-DRB1*12 generic peptides because the software only allows the specific subtypes of these generic peptides. We used a low resolution kit (only reads the first two digit alleles) which could not permit us obtain these specific subtypes.

When we assessed the effect of HLA-DQB in TB patients by comparing its phenotypic frequencies between ATB, LTBI and HCs, we found no association between ATB and HC nor between ATB and LTBI. This was in agreement with the study of Duarte *et al* (2010) [6] who working with a Portuguese population found no association between the phenotypic distribution of HLA-DQB in the healthy exposed group (healthy exposed positive and healthy exposed negative) and ATB. But we observed that HLA-DQB1*03 was associated with susceptibility to LTBI. This susceptibility to TB infection-LTBI was higher in the homozygous genotype when compared to the heterozygous genotype (p-value = 0.0025). The difference between genotype susceptibility within the same allele may be attributed to the "heterozygote advantage" (or the "homozygote disadvantage") which states that those who are MHC heterozygous may benefit a relatively dominant resistance compared to the MHC homozygous individuals. MHC heterozygosity confers a selective advantage by enhancing protection of host against infectious diseases [21]. The increase protective advantage in heterozygous genotype is due to its ability to present a variety of antigenic peptides compared to the homozygote. Our results suggest that HLA-DQB1*03 may be associated with susceptibility to TB infection (LTBI) but not with ATB. Wamala *et al* (2016) [10] in their study in Uganda found a negative correlation between HLA-DQB1*03:03 and susceptibility to TB indicating that this allele may confer protection against TB. We could not confirm this report in our study because we used a low resolution kit which did not permit us to conclude if it was actually the same HLA-DRB1*03 subtype or a different subtype that was associated with susceptibility to TB infection.

Table 5
Binding ability of HLA-B*58 subtypes to *Mtb* virulent antigens.

Mtb Antigens (Binding ability and peptide core sequence)		HLA-B*58 subtypes	
		HLA-B*5801	HLA-B*5802
ESAT-6	Binding ability	Non-binder	Non-binder
	Antigen core sequence	/	/
CFP-10	Binding ability	Binder	Non-binder
	Antigen core sequence	TAGSLQGQW	/
Mycolyltransferase Ag85B	Binding ability	Binder	Non-binder
	Antigen core sequence	QTYKWETFL	/

Table 6
Distribution of haplotypes significantly different between LTBI and ATB.

Haplotypes	Number of haplotypes N' (Haplotype frequency %)		P-values LTBI X ATB	Association with progression to ATB
	LTBI (N = 43)	ATB (N = 109)		
DRB1*10-DQB1*02	5 (11.6)	0 (0)	0.0015[#]	Protection
DRB1*13-DQB1*06	3 (7.1)	24 (22.0)	0.0335[#]	Susceptibility

[#] Significantly different, p-value $\leq$ 0.05 at 95% CI. (N = total number of subjects, N' = number of alleles present within group, LTBI = Latent TB Infection, ATB = Active TB). The N for ATB haplotypes (109) is lesser than the initial N for either DRB or DQB ATB groups (170 and 179 respectively) due to the absence of some corresponding pair values for the haplotype pairs.

Table 7
Distribution of DRB1*10-DQB1*02 in positive responders to quantiFERON-TB test.

Availability of Haplotype DRB1*10-DQB1*02	Number of subjects (Haplotype frequency %)		P-value
	LTBI-quantiFERON+	ATB-quantiFERON+	
DRB1*10-DQB1*02 Present	5 (11.6%)	0 (0%)	0.0015[#]
DRB1*10-DQB1*02 Absent	38 (88.4%)	109 (100%)	

[#] Significantly different, p-value $\leq$ 0.05 at 95% CI. (LTBI = Latent TB Infection, ATB = Active TB).

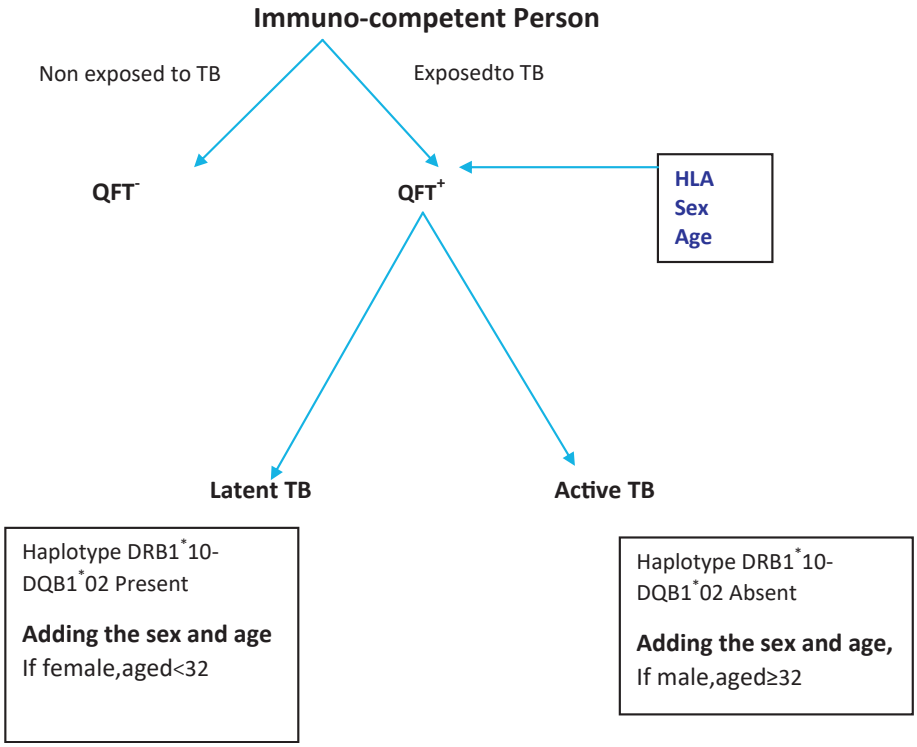

Fig. 1. Proposed algorithm to differentiate Latent TB from Active TB. Reading of the algorithm: For an immunocompetent person, who has been exposed to MTB, its quantiferon test is positive. He can be either infected latenly or actively infected. To now differentiate the two groups, if the person present the haplotype DRB1*10-DQB1*02, then the chance is high that Tb infection is latent and the probability of latency is even higher if the patient is female and young. If after genotyping, the haplotype DRB1*10-DQB1*02 is absent, the balance leaned towards active TB, and the risk is higher if the patient is male and aged more than 32.

Adekambi *et al*, in 2015 [14] had reported that HLA-DR + IFN-γ+ accurately classified ATB and LTBI at cutoff value of 60% with 100% specificity and greater than 96% sensitivity. Adekambi measured the HLA-DR on *Mtb*-specific CD4+ T cells where upregulation of DR on the cells reflected bacterial load. In this study, we evaluated the implication of the HLA-DRB locus in TB by comparing the phenotypic frequencies between ATB, LTBI and HC. We found no association between ATB and HC nor between ATB and LTBI. This was contrary to the study of Duarte *et al* (2010) [6] who working in a Portuguese population found that HLA-DRB1*14 was associated with susceptibility to active TB when compared with the healthy exposed positive group. This discrepancy could be attributed to difference in ethnicity or difference in the method used in subject enrolment. They used the tuberculin skin test in a population where BCG vaccine is systematically applied at birth [22, 23, 24]. We found an association between HLA-DRB1*12 and protection against TB infection-LTBI. Our results suggest that HLA-DRB1*12 may be associated with protection against TB infection (LTBI) but not with progression to ATB. A protective effect of HLA-DRB1*1202 was also found in a study carried out in Western Javanese Indonesia by Yuliwulandari *et al* (2010) [25].

The most prevalent (common) alleles in our study population were HLA-B*1520, HLA-DQB1*06 and HLA-DRB1*15 but none of them was associated with TB infection or progression. This can be attributed to the "rare allele advantage" (or the "common allele disadvantage") which argues that pathogens adapt to HLA alleles that are more prevalent (or common) in the population thus such alleles cannot really play a role in the pathogenicity of those pathogens [26].

When we assessed the role of haplotypes in influencing the progression from LTBI to ATB in those with positive quantiFERON-TB test results, two haplotypes DRB1*10-DQB1*02 (protection) and DRB1*13-DQB1*06 (susceptibility) were found to be associated. DRB1*10-DQB1*02 was only found in those with LTBI-quantiFERON-TB + while none was found in ATB-quantiFERON-TB + subjects. One of the main difficulties faced with the quantiFERON TB test is its inability to discriminate LTBI from ATB [4]. Generally, it is assumed that, those without signs and symptoms of ATB, without a history of ATB and with a positive quantiFERON-TB test have LTBI. Considering the fact that the haplotype DRB1*10-DQB1*02 was only found in LTBI and not in ATB and also considering the fact that all the LTBI and ATB subjects in our study population were quantiFERON-TB test positive, we developed an algorithm to discriminate LTBI from ATB. Those with a positive quantiFERON-TB Gold In-tube test, without a history of ATB and with the DRB1*10-DQB1*02 haplotype have a higher chance to have latent TB and not active TB. People carrying the DRB1*13-DQB1*06 may progress to ATB.

For the first time we report an HLA-quantiFERON-TB test based algorithm for discriminating LTBI from ATB (see Fig. 1). Our study also highlights the importance of the relationship between HLA and antigenic epitope recognition in elucidating an immune response. The limits of this study include a limited sample size, difference in gender and age between groups which could act as potential confounders to the results and the lack of validation study.

5. Conclusion

We found that the alleles HLA-B*5801 and HLA-DRB1*12 (protection) and the alleles HLA-B*5802 and HLA-DQB1*03 (susceptibility) are associated with latent TB infection while the haplotypes DRB1*10-DQB1*02 (protection) and DRB1*13-DQB1*06 (susceptibility) are associated with progression to active tuberculosis. The haplotype DRB1*10-DQB1*02 may be a useful biomarker that discriminates latent from active tuberculosis when associated with the quantiFERON-TB Gold In-tube test. Further studies are required to confirm this association and future studies may consider associating HLA in the management of tuberculosis especially in diagnosis.

Declarations

Author contribution statement

Elvis Ndzi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Celine Nkenfou: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed

reagents, materials, analysis tools or data; Wrote the paper.

Eric Walter Yone Pefura, Linda Chapdeleine Mekue, Elise Guiedem, Carine Nguefeu, Marie Nicole Ngoufack, Elise Elong, Laeticia Yatchou: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Alexis Ndjolo, Jules-Roger Kuiate: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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