TB: epidemiology and diagnosis

S1 HIGH LEVELS OF LATENT TB INFECTION, BLOOD BORNE VIRUSES AND UNMET NEED AMONG HARD TO REACH GROUPS IN LONDON: THE TB REACH STUDY

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Background Urban homeless people have high levels of disease and often present late for healthcare. Despite high rates of active TB in London's large homeless population, limited data are available regarding the prevalence of latent TB infection (LTBI) and blood borne viruses (BBV) - HIV, Hepatitis B & C. We have undertaken a TB/BBV screening programme to assess the prevalence of LTBI, infection with BBV and co-infection within hard to reach groups (homeless people and substance misusers) in homeless hostels and residential drug services in London.

Method Residents screened for TB on a mobile chest x-ray unit were approached and with consent, blood was drawn for TB IGRA (Quantiferon In-Tube) and BBV. Results were fed back to participants with onward referral as necessary.

Results Of 413 eligible participants, 390 (94%) reported a history of homelessness. Of these 390 participants, 89% were male, 68% were 16–49 years of age and 66% UK born. 17% were IGRA positive, 1% HIV positive (all previously known), and 10% had current and 4% past Hepatitis C. 1% of those screened had current Hepatitis B infection, 10% past infection, 18% had vaccine induced protective levels of immunity and 71% had insufficient or no Hepatitis B immunity. 29% of subjects with Hepatitis C were LTBI co-infected. Multivariate analysis identified increasing age e.g. 30–49 age group (odd ratio [OR], 2.15; 95% confidence interval [CI₉₅], 0.84–5.49) compared to the under 30, foreign birth (OR, 6.59; CI₉₅, 3.50–12.39), smoking hard drugs (OR, 2.19; CI₉₅, 1.02–4.64), and injecting hard drug (OR, 2.36; CI₉₅, 1.08–5.16) such as heroin, crack or cocaine (although 95% of injectors also smoked hard drugs) as risk factors for LTBI. Injecting drug use was the only factor associated with increased risk for Hepatitis C infection (OR, 19.62; CI₉₅, 8.23–46).

Conclusion Extremely high rates of LTBI, Hepatitis C and co-infection are present in our urban study population. Despite targeted Hepatitis B vaccination programmes, a high proportion of participants appear unvaccinated. These levels of unmet need have major implications for public and personal healthcare planning and should be recognised through appropriate targeted health and social policy.

S2 ETHNIC VARIATION IN INFLAMMATORY PROFILE IN TUBERCULOSIS

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Introduction and objectives *Mycobacterium tuberculosis* (MTB) emerged as a pathogen in Africa and has co-evolved with humans following the migration to Europe and Asia some 70,000 years ago. Distinct phylogenetic lineages of MTB associate with hosts of particular genetic ancestry, both in their regions of origin and in distant

cosmopolitan urban settings where human populations of different ancestry intermingle. These different strains induce distinct patterns of cytokine and chemokine secretion ('inflammatory profiles') in human macrophages. Circulating and antigen-stimulated inflammatory profiles might therefore be expected to vary significantly between tuberculosis patients of different ethnic origin. We therefore conducted a study to determine whether such variation exists.

Methods We measured circulating and antigen-stimulated concentrations of 43 soluble inflammatory mediators and 14 haematological parameters in 45 patients of African ancestry and 83 patients of Eurasian ancestry receiving intensive-phase antimicrobial therapy for smear-positive pulmonary tuberculosis in London, UK. Host and bacillary genotypes were also determined. Statistical analyses were performed to compare inflammatory profiles in patients of African vs Eurasian ancestry; to investigate the influence of host and bacillary genotype on inflammatory profile; and to determine immunological correlates of speed of elimination of MTB from sputum.

Results Tuberculosis patients of African vs Eurasian ancestry had similar clinical characteristics, but exhibited distinct inflammatory profiles. Patients of African ancestry had lower neutrophil counts, lower serum concentrations of CCL2, CCL11 and vitamin D binding protein (DBP), and lower antigen-stimulated CCL11 secretion than those of Eurasian ancestry, but higher serum CCL5 concentrations and higher antigen-stimulated interleukin 1 receptor antagonist and IL-12 secretion. These differences did not relate to MTB strain variation between groups, but they did associate with ethnic variation in host DBP genotype. Ethnic differences in inflammatory profile became more marked following initiation of antimicrobial therapy, and immunological correlates of speed of elimination of MTB from the sputum were distinct for patients of African vs. Eurasian ancestry.

Conclusions Our study demonstrates a hitherto unappreciated degree of ethnic heterogeneity in inflammatory profile in tuberculosis patients. Candidate immunodiagnoses and immunological biomarkers of response to antimicrobial therapy should therefore be derived and validated in tuberculosis patients of different ethnic origin.

S3 T CELL RESPONSES TO VITAMIN D ARE BLUNTED IN LATENT TUBERCULOSIS

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Introduction Low vitamin D status is associated with an increased risk of *Mycobacterium Tuberculosis*. Consistent with this, the active form of vitamin D, 1,25(OH)₂D₃ exerts potent effects on immune cells.

T cells are thought to contribute to the control of latent tuberculosis in which patients are infected with *Mycobacterium tuberculosis* but show no signs or symptoms of the disease. How vitamin D influences T cell responses in latent patients is therefore of interest.

It has been shown that the co-suppressive protein, CTLA4, which is expressed on regulatory T cells and induced in T cells upon activation, is strongly up regulated by vitamin D and that it increases the frequency of CTLA-4+FoxP3+ cells [1]. Furthermore, vitamin D conditioned T cells are functionally suppressive. In this study we have therefore compared the effect of vitamin D upon CTLA-4 expression and the frequency of CD25+ FoxP3+ CTLA4+ cells in healthy and latent TB patients.

Methods Peripheral blood cells from healthy control donors (n=21) and patients with latent tuberculosis (n=12) were cultured with SEB or PPD with or without 1,25(OH)₂D₃ or inactive 25(OH)D₃. CD25+ FoxP3+ CTLA4+ frequency and the median CTLA-4

expression (MFI) of CD25+ cells was then monitored after two days by flow cytometry.

Results 1,25(OH)₂D₃ significantly increased CTLA4 MFI in both healthy and latent populations following stimulation with SEB ($p \leq 0.01$) or PPD ($P = 0.026, 0008$). 25(OH)D₃ also enhanced CTLA-4 expression in SEB cultures ($p \leq 0.01$). Induction of CTLA-4 was however reduced in PPD cultures (median 121) compared to SEB (median 360). Interestingly, the magnitude of CTLA-4 induction by 1,25(OH)₂D₃ or 25(OH)D₃ also differed for healthy and latent populations in response to SEB (1,25(OH)₂D₃ ($p = 0.01$) and 25(OH)D₃ ($p = 0.006$), with a similar trend in PPD stimulated cells ($p = 0.092$).

Conclusion The shift towards a T reg population as a result of vitamin D is blunted in latent TB compared to health. Differential response of memory cells in latent disease could account for this.

1. Jeffery, LE, et al., *1,25-Dihydroxyvitamin D₃ and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3*. Journal of immunology, 2009. **183**(9): p. 5458–67.

S4 ANTI-INFLAMMATORY EFFECTS OF VITAMIN D ARE INFLUENCED MORE BY GENETIC BACKGROUND THAN MYCOBACTERIAL INFECTION

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Introduction Although vitamin D has antimicrobial effects that may be beneficial in mycobacterial infection, supplementation trials have been disappointing [1]. Response to vitamin D is heterogeneous, perhaps due to genetic variants in the vitamin D axis (vitamin D binding protein; GC or vitamin D receptor; VDR). We aimed to assess stability and magnitude of anti-inflammatory effect of vitamin D in vitro in mycobacterial infection and health, and the influence of GC and VDR variants.

Methods Peripheral blood monocytes from healthy controls ($n = 20$) and patients with mycobacterial infection ($n = 8$) were cultured and incubated with LPS (100 ng/ml), 25(OH)D₃ (50 nmol) and 1,25(OH)₂D₃ (50 nmol). IL6 in cell supernatants was measured by ELISA. Taqman was used to type 4 SNPs: rs7041 & rs4588 (GC), rs2228570 & rs1544410 (VDR). The effect of the protein product of GC in culture was assessed by addition of plasma and subsequent Luminex array.

Results Change in IL6 between pairs of repeated cultures did not vary ($p = 0.21$), indicating stability of response to LPS. There was no difference between HCs and patients in response to vitamin D (both $p > 0.32$), however in both groups there were responders ($n = 7$ controls, $n = 5$ TB), in whom IL6 fell with addition of vitamin D (1,25D, $p = 0.001$; 25D, $p = 0.02$) and non-responders ($n = 11$ controls, $n = 3$ TB) in whom it did not.

Response to 1,25D was influenced by rs7041 (T allele; $p = 0.04$), and tended to associate with GC2 ($p = 0.06$). Response to 25D was not affected by genotype until plasma was added when GC haplotype appeared to have an effect. There was no influence of VDR SNPs.

Conclusion Anti-inflammatory effects of vitamin D are influenced by GC genotype in vitro, consistent with previous mouse work [2], however it is unclear whether this occurs in humans in vivo. Such effects are equally relevant in health and mycobacterial infection. Further work to characterise changes in LPS responsiveness following high dose vitamin D replacement is ongoing.

1. Martineau AR, et al. Lancet. (2011) 377:242–50.
2. Zella LA, et al. Endocrinology. (2008) 149:3656–67.

S5 MOLECULAR IMMUNODIAGNOSIS OF TB INFECTION: A PILOT STUDY

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Introduction Current interferon-gamma (IFN- γ) release assays (IGRAs) are not sufficiently sensitive to be used as a “rule-out” test for tuberculosis (TB). Antigen-specific gene expression of chemokines downstream of, and amplified by, IFN- γ might demonstrate such sensitivity, and can be performed with very small volumes of blood.¹

Here we assess the performance and sensitivity of two IFN- γ -dependent gene expression platforms in the peripheral blood mononuclear cells of individuals with and without TB.

Methods 23 individuals with active TB, 12 individuals with latent TB infection (LTBI), and 18 controls had simultaneous IFN- γ ELISpot assays and qRT-PCR of CXCL-9 and CXCL-10, following stimulation with the immunodominant antigens ESAT-6, CFP-10 and EspC (6 gene expression assays in total). Test performances of the 6 gene expression assays were compared to the ELISpot.

Results Gene expression of CXCL-9 and CXCL-10 was antigen specific, correlating well with each other and with the IFN- γ ELISpot (Spearman Rank Correlations ranging from $r = 0.648$ to 0.74). Gene expression of either was not significantly different between those with active TB and LTBI.

Receiver-operating characteristic curves indicated good test performances for all the gene expression assays (AUC ranging from 0.918 to 0.959) and agreements between the ELISpot and gene expression platforms was good (κ statistic ranging from 0.403–0.496).

After constructing cut-offs for sensitivity for individual antigens, with cut-offs for specificity matching or exceeding that of the IFN- γ ELISpot, the sensitivity of TB diagnosis with gene expression was superior to ELISpot in 5 out of the 6 assays, although these differences were not statistically significant. Sensitivity was equivalent when antigens were combined, as in the commercially available T-Spot®.TB.

Conclusions These pilot data indicate that gene expression of IFN- γ -dependent cytokines is a robust, sensitive and specific method of TB diagnosis, and carries potential to be a more sensitive platform than the current gold standard – IFN- γ ELISpot. Larger studies with appropriate power are now required in similar populations to investigate this approach definitively.

References

1. Kaspruwicz VO, Mitchell JE, Chetty S, Govender P et al. A molecular assay for sensitive detection of pathogen-specific T-cells. PLoS One. 2011; 6(8):e20606. Epub 2011 Aug 11.

Targets for asthma therapy

S6 THE PHENOTYPE TRIAL: INHALED CORTICOSTEROID DOSE-RESPONSE USING DOMICILIARY EXHALED NITRIC OXIDE IN PERSISTENT ASTHMA

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Background International guidelines advocate a standard approach to asthma management for all despite its heterogeneity. ‘Personalised’ treatment for inflammatory asthma phenotypes confers superior benefits. We wished to evaluate dose-response to inhaled corticosteroids (ICS) in asthmatics with an elevated exhaled nitric oxide (FeNO) phenotype using domiciliary measurements.