Abstract S144 Table

	PDGFRα	PDGFRβ	VEGFR	EGFR
DAKO score				
0	0%	31.4% (11/35)	86% (30/35)	45.7% (16/35)
1	23% (8/35)	37.1% (13/35)	11% (4/35)	25.7% (9/35)
2	40% (14/35)	23% (8/35)	0%	20% (7/35)
3	37% (13/35)	8.5% (3/35)	3% (1/35)	8.6% (3/35)

EGFR, endothelial growth factor receptor; PDGFR, platelet derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor.

agree with the problem of the sample size being very heterogenous; nevertheless, we conclude that an immediate ''tumour profiling'' already at initial diagnosis and a following, primary and adjusted anti-angiogenic therapy should be considered as an emerging necessity in the light of an individualised tumour therapy.

The science of tuberculosis: current concepts

S145 THE IDENTIFICATION OF DISTINCT GENE EXPRESSION PROFILES IN LATENT AND ACTIVE TUBERCULOSIS

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Introduction: Tuberculosis is a major and increasing cause of morbidity and mortality worldwide caused by infection with *Mycobacterium tuberculosis* (MTb). However, the majority of individuals infected with MTb remain asymptomatic, apparently retaining the infection in a quiescent form. It is thought that this latent infection is maintained by an active immune response. The immune response to MTb is complex and remains incompletely characterised. We hypothesised that obtaining whole genome expression profiles from the blood of patients with either latent or active tuberculosis could lead to improved insights into the protective immune response, as well as the development of potential biomarkers.
Methods: We recruited patients with active pulmonary tuberculosis and latent tuberculosis. All active tuberculosis patients included in the analysis were culture positive and all latent patients included were positive by both tuberculin skin test and interferon-gamma release assay (IGRA). Potentially immunosupressed individuals were excluded. We also recruited healthy controls, who tested negative by both skin test and IGRA. Whole blood was collected from all participants before treatment. RNA was extracted from the whole blood and microarray analysis performed using Illumina Sentrix Human-6 V2 BeadChip arrays (>48 000 probes). Statistical analysis and hierarchical clustering of samples was performed using Genespring, v7.1.3.
Results: After filtering out undetected transcripts and those with less than twofold change, 6269 transcripts were used for unsupervised clustering analyses by Pearson correlation. This revealed distinct expression profiles, or ''disease signatures'', corresponding to the clinical diagnosis of latent or active tuberculosis. These disease signatures held true across the broad number of ethnicities represented in the study. To improve discrimination between clinical groups, statistical comparisons were performed yielding a list of 119 genes. Unsupervised hierarchical clustering using this list separated participants into three distinct groups of active tuberculosis, latent tuberculosis and healthy controls.
Conclusions: We identified distinct gene expression profiles in active and latent tuberculosis, which were also distinct from control individuals. Our findings will yield insights into the potential mechanisms of immune protection (latent tuberculosis) versus immune pathogenesis (active tuberculosis). They also have value for the development of biomarkers for use in diagnosis.

S146 FREQUENCY OF *MYCOBACTERIUM TUBERCULOSIS* ANTIGEN-SPECIFIC IFN-Γ-SECRETING T CELLS CORRELATES WITH PRESENCE OF PATHOLOGY IN TUBERCULOSIS

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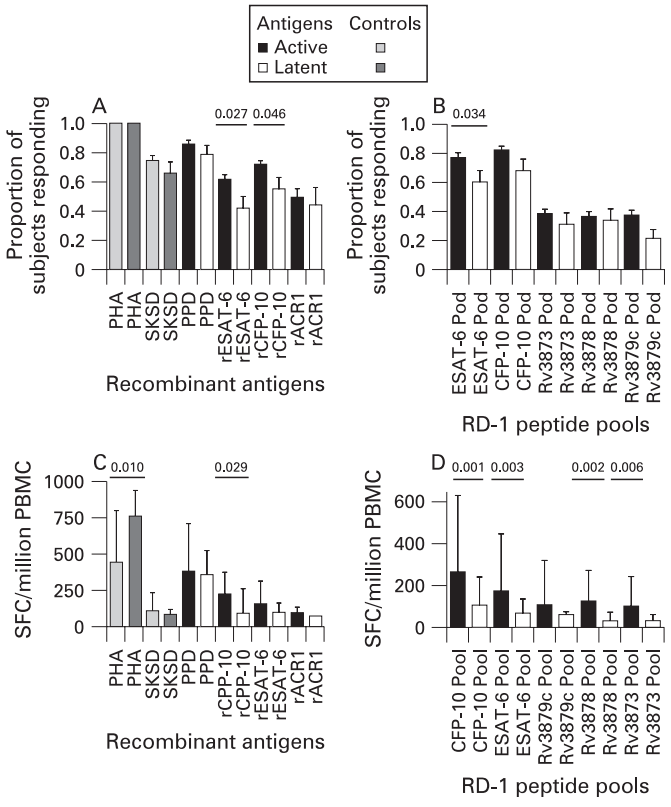
Introduction and Objectives: The majority of individuals infected with *Mycobacterium tuberculosis* achieve lifelong containment of the bacillus, but what constitutes this effective host immune response is poorly understood. Antigen-specific CD4 IFN-γ-secreting T-cell responses to mycobacterial antigens are believed to be pivotal, but the relationship between the strength of these responses and the development of protective immunity or of pathology is unclear.
Methods: Using the ex vivo enzyme-linked (ELISpot) assay, we compared frequencies of IFN-γ secreting T cells among 205 prospectively recruited individuals with either non-recent latent infection, who have successfully achieved immune control, or with active disease, whose immune responses have failed to contain the bacillus.
Results: Among subjects with an IFN-γ ELISpot response to one or more RD1-encoded antigens, T cells from subjects with active disease recognised a greater number of peptide pools from those antigens than did subjects with latent infection ($p = 0.002$). There was a trend for any given region of difference-1 (RD1) antigen or peptide pool to be recognised by a greater proportion of subjects with active disease than with latent disease and these differences were significant for early secretory antigenic target 6 (ESAT-6) peptide pools, recombinant ESAT-6 antigen and recombinant culture filtrate protein 10 (CFP-10) antigen (fig A,B). T-cell frequencies in responding wells were greater in active than latent infection for summed pools of RD1 peptides ($p \leqslant 0.006$), and for CFP-10 antigen ($p = 0.029$) (fig C,D). The hierarchy of immunodominance among antigens and peptides was highly correlated between active and latent disease. Responses to the DosR antigen α-crystallin were not associated with latency ($p = 0.373$). RD1-specific T-cell responses were greater among subgroups positive to tuberculin skin testing (TST). Proportions of individuals positive to TST were higher among active than latent cases (87.9% vs 44.1% $p<0.001$). Whereas RD1 antigen responses are higher in active infection, responses to PPD are strongly associated with TST ($p \leqslant 0.025$), but not with clinical status ($p>0.7$).
Conclusions: Our results suggest that RD1-specific IFN-γ-secreting T-cell frequencies correlate with the presence of pathology rather than with protective immunity.



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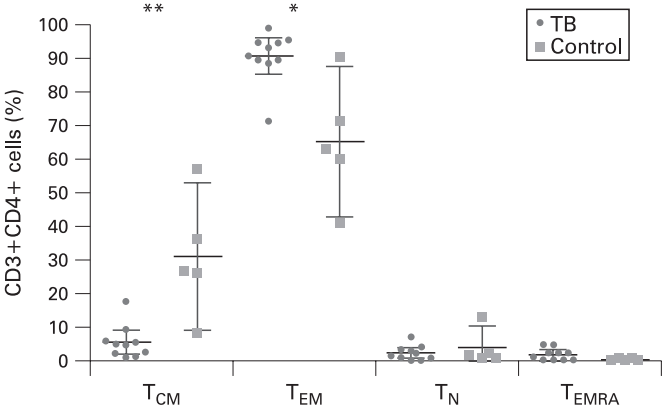

Abstract S147 Figure Distribution of CD3+CD4+ cells in induced sputurm based on CCR7 and CD45RA staining. *p = 0.01 **p = 0.003

S147 DISTINCT CD4 T-CELL DIFFERENTIATION MARKERS ARE PRESENT IN THE LUNGS OF SUBJECTS WITH ACTIVE TUBERCULOSIS

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Introduction: Protective host immunity against *Mycobacterium tuberculosis* requires tissue T-cell responses in sites such as the lung. Most reported work has used blood samples—and this may not reflect accurately the local immune response. The recent appreciation of the heterogeneous nature of memory T cells; together with the possibility of a compartmentalised tissue response, led us to investigate whether we could demonstrate distinct differences in blood and lung T-cell memory populations between tuberculosis patients and relevant controls.

Methods: T-cell differentiation markers were studied in paired blood and lung samples (the latter obtained by sputum induction). Seventeen subjects with active tuberculosis and six healthy controls were recruited. Tuberculosis was pulmonary in 11 cases, lymph node in three and abdominal, cerebral and renal in one each. Two of 17 subjects were HIV co-infected. Fresh samples were stained with CD3 and CD4 and with one of the following combinations: CD45RA and CCR7; CD27 and CD62L; CD27 and CD28; CD57 and CD62L. All panels were analysed using four-colour cytometry.

Results: There were significant differences between blood and lung T-cell populations. At the latter site, tuberculosis patients had a significantly smaller proportion of central memory (CD45 RA^-CCR7^+, T_{CM}) and a greater proportion of effector memory (CD45 RA^-CCR7^-,T_{EM}) T cells than healthy controls (fig). There was no significant difference in the same populations in blood ($p = 0.62$). In contrast, significant differences were demonstrated between CD27 CD62L, CD27 CD28 and CD57 CD62L CD4 T-cell populations ($p = 0.001$, $p = 0.001$ and $p = 0.03$, respectively) in blood but not sputum, when tuberculosis patients were compared with controls.

Conclusions: Memory T-cell populations differ markedly between blood and lung fluid and within specific populations at these sites. This highlights the limitations of blood-based assays when investigating tuberculosis pathogenesis. Furthermore, it suggests that simple phenotypic markers may lack the sensitivity required to discriminate those T-cell populations that are recruited and play an active role in immune regulation at tissue sites. The future use of antigen-specific assays may assist with this.

S148 COST EFFECTIVENESS OF QUANTIFERON-TB GOLD BLOOD TESTING FOR IMMIGRANT TUBERCULOSIS SCREENING IN UK

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Background: NICE guidelines for new entrant tuberculosis screening recommend chest *x* ray (CXR) for immigrants from countries with tuberculosis incidence greater than $40/10^5$, and tuberculin skin test (TST) for people with normal CXR from very high tuberculosis prevalence countries.[1] The Leeds tuberculosis screening service serves a population of 750 000. Based on previous audit, we piloted a revised screening policy using first-line QuantiFERON-TB Gold™ (QFT) in high-risk immigrants from 1 January 2007. We have evaluated the cost effectiveness of this approach.

Methods: Initially, TST was offered to immigrants from countries with tuberculosis incidence 200–$339/10^5$, and QFT to those from countries with incidence greater than $340/10^5$. When increased resources became available, all immigrants from countries with tuberculosis incidence greater than $200/10^5$ had QFT. Those with positive QFT were invited for CXR. Costs were estimated for our protocol and for NICE protocol based on NICE estimates for cost of CXR £23.24, TST £13.69 and QFT £25.67.[1] For the NICE protocol costings, the expected rate of positive TST for the group was estimated from the subgroup who had both tests, assuming that there would be no false negative TST.

Results: In 2007 we identified 2902 immigrants from countries with tuberculosis incidence greater than $200/10^5$, 1336 were invited for screening (TST or QFT as described above) with a 32% attendance rate. 280 patients had QFT, of which 38% were positive, with less than 2% being indeterminate. Using the NICE approach the cost of screening these 280 immigrants would be £13 334 (£47.62 per immigrant) and would identify 83 cases of latent tuberculosis infection (LTBI). Using first line QFT followed by CXR the cost was £9652 (£34.47 per immigrant) and identified 106 LTBI. The cost

to identify one case of LTBI following NICE guidelines would be £160.66 and using our protocol was £91.06.
Conclusions: Using QuantiFERON-TB Gold™ blood testing followed by CXR is more effective and more cost effective than NICE guideline for screening new entrants from high-risk countries.

S149 RESULTS OF SYSTEMATIC SINGLE-STEP TIGRA TESTING IN TUBERCULOSIS CONTACTS IN 2007

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Introduction: T-cell based interferon gamma release assays (TIGRA) are highly specific for the diagnosis of latent tuberculosis infection (LTBI) or tuberculosis disease. Given the relatively high incidence of tuberculosis in the city of Leicester, tuberculosis contacts were screened by Quantiferon (QFN) and/or chest *x* ray (CXR) in 2007.
Methods: All tuberculosis contacts below the age of 36 years and contacts of all ages of smear-positive pulmonary index cases were invited for TIGRA testing by QFN assay in 2007, 2–3 months after the notification date. Results are presented according to age and ethnicity of contacts as well as disease site, smear/culture positivity of the index case.
Results: 989 contacts of 269 index cases were identified and 464 contacts (47%) were QFN tested. 158/251 (63%) children (age <16 years), 220/383 (53%) of young adults (age 16–35 years), and 86/355 (24%) of older adults (age >35 years) were tested. QFN positivity was found in 11/158 (7%) children, 53/220 (24%) young adults and 37/86 (43%) adults above the age of 35 years. Indeterminate results were found in 11/58 (7%) children but in only 1/306 (0.3%) contacts above the age of 15 years. Positivity rates were similar for contacts of Indian subcontinent origin (80/399, 23.6%) and white caucasian contacts (13/58, 22.4%). Overall positivity rates were highly age dependent (table) but highly significant differences were seen between young contacts of respiratory and of non-respiratory index cases with the highest positivity rates in contacts of smear/culture-positive pulmonary index cases (age <10 years, 14.8%; age 10–19 years, 37%; age 20–29 years, 35.1%). Not a single positive QFN result was found in 34 contacts of non-respiratory index cases below the age of 20 years (age 0–19 years, 0%) but positivity rates increased above the age of 20 years even in contacts of non-respiratory index cases (age 20–35 years, 23.6%).
Conclusion: One-year results of a single step contact screening protocol involving TIGRA testing of all contacts below the age of 36 years are presented. Positivity is strongly correllated with smear/culture positivity and disease site of the index case. A probable cohort effect is seen in children and youngsters below the age of 20 years with no QFN positivity in those in recent contact with non-infectious index cases.

Abstract S149 Table Results summary of QFN testing in all contacts eligible for chemoprophylaxis by age groups (2007)

Age (years)	Total	Tested, n (%)	Positive, n (%)
0–4	101	49 (48.5)	1 (2.0)
5–9	77	57 (74.0)	4 (7.0)
10–14	66	42 (63.6)	5 (11.9)
15–19	91	47 (51.6)	8 (17.0)
20–24	99	37 (37.4)	7 (18.9)
25–29	77	40 (51.9)	10 (25.0)
30–34	43	27 (62.8)	5 (18.5)

Chronic obstructive pulmonary disease: exacerbations and clinical aspects

S150 GOLD VERSUS NICE DIAGNOSTIC CRITERIA FOR CHRONIC OBSTRUCTIVE PULMONARY DISEASE: IMPACT ON DISEASE PREVALENCE AND MORTALITY RISK WITHIN THE RENFREW/PAISLEY STUDY

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Objective: To compare chronic obstructive pulmonary disease (COPD) prevalence and mortality risk in a single population using alternative diagnostic criteria.
Methods: The global initiative for chronic obstructive lung disease (GOLD) is based on lung function alone, whereas the UK National Institute for Health and Clinical Excellence (NICE) criteria include lung function, respiratory symptoms and other risk factors. These criteria were applied to a Scottish prospective cohort (Renfrew/Paisley (Midspan) study) of 15 402 men and women aged 45–64 years at baseline and followed for over 30 years. All-cause and COPD mortality were modelled using Cox regression analysis.
Results: Overall COPD prevalence for men (women) was 31% (20%) applying GOLD criteria compared with 12% (5%) applying NICE criteria. Prevalence was strongly related to age (see table). Kaplan–Meier curves for COPD mortality by disease severity showed greater separation under NICE criteria compared with GOLD. Following Cox regression analysis (adjusting for age, smoking pack years, diastolic blood pressure, cholesterol, body mass index and social class), participants meeting NICE diagnostic criteria were found to have increased hazard ratios (HR) by disease severity for both all-cause and COPD mortality, compared with those meeting the GOLD criteria. The HR for COPD mortality for men in the most severe COPD group, compared with those without COPD, was 92 (95% CI 58 to 148) applying GOLD and 110 (95% CI 70 to 171) applying NICE. A separate analysis showed respiratory symptoms and pack years, independent of lung function, to be significant contributors to mortality risk for all-cause and COPD mortality.
Conclusion: COPD prevalence in this population is high. GOLD diagnostic guidelines are often considered the ''gold standard'' in COPD, yet they may overestimate the COPD burden: prevalence of 31% (20%) compared with 12% (5%) has important ramifications by way of planning and funding decisions at the micro and macro level. Mortality risk increased with disease severity and was higher using NICE compared with GOLD criteria: the inclusion of

Abstract S150 Table COPD prevalence by age, sex and diagnostic criteria (%) in the Renfrew/Paisley study

Age group (years)	n	GOLD	NICE
Men			
45–49	1787	24.9	8.8
50–54	1953	28.2	10.0
55–59	1655	33.5	13.1
60–64	1534	40.9	18.7
Overall prevalence (%)		31.4	12.4
Women			
45–49	2003	16.9	4.6
50–54	2263	19.2	4.6
55–59	2005	23.1	6.3
60–64	1945	20.9	3.1
Overall prevalence (%)		20.0	4.6

COPD, chronic obstructive pulmonary disease; GOLD, global initiative for chronic obstructive lung disease; NICE, National Institute for Health and Clinical Excellence.