PET-CT-guided characterisation of progressive, preclinical tuberculosis infection and its association with low-level circulating *Mycobacterium tuberculosis* DNA in household contacts in Leicester, UK: a prospective cohort study

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Summary

Background Incipient tuberculosis, a progressive state of *Mycobacterium tuberculosis* infection with an increased risk of developing into tuberculosis disease, remains poorly characterised. Animal models suggest an association of progressive infection with bacteraemia. Circulating *M tuberculosis* DNA has previously been detected in pulmonary tuberculosis by use of Actiphage, a bacteriophage-based real-time PCR assay. We aimed to investigate whether serial [^{18}F]fluorodeoxyglucose ([^{18}F]FDG)-PET-CT could be used to characterise the state and progressive trajectory of incipient tuberculosis, and examine whether these PET-CT findings are associated with Actiphage-based detection of circulating *M tuberculosis* DNA.

Methods We did a prospective 12-month cohort study in healthy, asymptomatic adults (aged ≥16 years) who were household contacts of patients with pulmonary tuberculosis, and who had a clinical phenotype of latent tuberculosis infection, in Leicester, UK. Actiphage testing of participants' blood samples was done at baseline, and [^{18}F]FDG PET-CT at baseline and after 3 months. Baseline PET-CT features were classified as positive, indeterminate, or negative, on the basis of the quantitation (maximum standardised uptake value [SUV_{max}]) and distribution of [^{18}F]FDG uptake. Microbiological sampling was done at amenable sites of [^{18}F]FDG uptake. Changes in [^{18}F]FDG uptake after 3 months were quantitatively categorised as progressive, stable, or resolving. Participants received treatment if features of incipient tuberculosis, defined as microbiological detection of *M tuberculosis* or progressive PET-CT change, were identified.

Findings 20 contacts were recruited between Aug 5 and Nov 5, 2020; 16 of these participants had a positive result on IFNγ release assay (QuantiFERON-TB Gold Plus [QFT]) indicating tuberculosis infection. Baseline PET-CT scans were positive in ten contacts (all QFT positive), indeterminate in six contacts (three QFT positive), and negative in four contacts (three QFT positive). Four of eight PET-CT-positive contacts sampled had *M tuberculosis* identified (three through culture, one through Xpert MTB/RIF Ultra test) from intrathoracic lymph nodes or bronchial wash and received full antituberculosis treatment. Two further unsampled PET-CT-positive contacts were also treated: one with [^{18}F]FDG uptake in the lung (SUV_{max} 9·4) received empirical antituberculosis treatment and one who showed progressive [^{18}F]FDG uptake received preventive treatment. The ten untreated contacts with [^{18}F]FDG uptake at baseline (seven QFT positive) had stable or resolving changes at follow-up and remained free of tuberculosis disease after 12 months. A positive baseline Actiphage test was associated with the presence of features of incipient tuberculosis requiring treatment (p=0·018).

Interpretation Microbiological and inflammatory features of incipient tuberculosis can be visualised on PET-CT and are associated with *M tuberculosis* detection in the blood, supporting the development of pathogen-directed blood biomarkers of tuberculosis risk.

Funding MRC Confidence in Concept.

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Introduction

Mycobacterium tuberculosis, the causative pathogen for tuberculosis disease, is estimated to infect almost a quarter of the global population.[1] The vast majority of *M tuberculosis* infections are asymptomatic (referred to as tuberculosis infection) and are a reservoir for potential future tuberculosis disease. Tuberculosis infection is defined by antigen-specific T-cell immune responses on IFN-γ release assays (IGRAs) or a positive tuberculin skin test, in the absence of clinical or radiological features of tuberculosis disease.[2] This definition is imprecise as it encompasses a spectrum of infection states that carry variable risks of

Research in context

Evidence before this study

We did two searches of the MEDLINE database from database inception to Sept 4, 2022, for studies investigating the use of PET-CT (search terms "positron emission tomography", "PET CT", and "tuberculosis") and those investigating bacteraemia (search terms "bacteraemia", "bacteremia", "mycobacteremia", "mycobacteraemia" and "tuberculosis") in tuberculosis infection. Publications written in English were reviewed. There were 560 publications for the first search and 488 for the second. Of the animal studies reviewed, non-human primate studies most closely resemble human tuberculosis infection and have used PET-CT to characterise the state and longitudinal trajectory of *Mycobacterium tuberculosis* infection, suggesting an association between culturable bacilli and high metabolic activity of lesions on PET-CT. Two prospective studies reporting PET-CT features in human tuberculosis infection were identified, of which one reported PET-CT features associated with progression to tuberculosis disease despite preventive treatment in an HIV-positive cohort. No prospective studies were found that followed up untreated tuberculosis infection longitudinally with PET-CT. Two recent human studies have reported detectable circulating *M tuberculosis* DNA in HIV-negative people with tuberculosis infection. In one study, *M tuberculosis* DNA was also detected in participants with a negative T-cell IFNγ response to *M tuberculosis* antigens. In the other study, a possible association was suggested between *M tuberculosis* DNA in the blood and prospective progression to tuberculosis disease.

Added value of this study

In this study, we use serial [^{18}F]fluorodeoxyglucose ([^{18}F]FDG)-PET-CT, complemented by invasive bronchoscopic sampling, to systematically characterise the state and trajectory of *M tuberculosis* infection over 12 months of prospective follow-up in a cohort of healthy, HIV-negative contacts of patients with pulmonary tuberculosis. Using this approach, we isolated *M tuberculosis* in culture and with Xpert MTB/RIF at sites of [^{18}F]FDG uptake from a subset of contacts, providing evidence of actively replicating bacilli in participants with a clinical phenotype of latent tuberculosis infection. We showed heterogeneity in the PET-CT trajectory of untreated contacts after 3 months and suggest that progressive metabolic changes on PET-CT and microbiological detection of *M tuberculosis* by targeted invasive sampling, before sputum production or evidence of disease on chest radiography, are features consistent with the definition of incipient tuberculosis. Finally, we showed a statistically significant association of this state with low-level *M tuberculosis* DNA in the blood using a novel bacteriophage-based assay.

Implications of all the available evidence

PET-CT provides a highly sensitive imaging modality that can support both mechanistic and translational studies of human tuberculosis infection. We describe features of metabolically active tuberculosis infection in a higher proportion than the 5–10% of infected people who develop tuberculosis disease, suggesting transience of this state in most people. Our observations provide a framework to enable accelerated development of tuberculosis risk biomarkers. While biomarkers hitherto have focused solely on the host immune response, our data support a role for developing pathogen-directed biomarkers of incipient tuberculosis.

progressing to disease.[3] Risk stratification of tuberculosis infection focuses on the characterisation of incipient tuberculosis, a conceptually defined state of preclinical infection that shows no radiographical or microbiological evidence of active tuberculosis but is at high risk of progressing to disease.[4] Implicit within this definition is the presence of metabolically active and replicating *M tuberculosis* that can overcome protective immune responses to drive progressive infection. In contrast to subclinical tuberculosis, which also describes a predominantly asymptomatic phenotype of tuberculosis infection, evidence for replicating *M tuberculosis* and associated pathology in incipient tuberculosis is not demonstrable with programmatic tools for screening—namely chest radiography and sputum microbiology. As such, most studies to date have sought to provide retrospective characterisation of incipient tuberculosis by focusing on the minority of individuals who develop disease during prospective observation of tuberculosis infection cohorts.

[^{18}F]fluorodeoxyglucose ([^{18}F]FDG)-PET-CT is a highly sensitive imaging tool that provides both structural and metabolic information that has been effectively used to inform non-human primate models of tuberculosis infection.[5,6] These studies reported elevated [^{18}F]FDG uptake in intrathoracic lymph nodes and emergent granulomas 4 weeks after *M tuberculosis* exposure, with increasing avidity and number of lesions described as imaging characteristics of progressive infection.[5,6] There are currently few high-quality data on the use of PET-CT in human tuberculosis infection.[7] One small prospective study in five close contacts of patients with pulmonary tuberculosis reported [^{18}F]FDG uptake in intrathoracic lymph nodes similar to that in non-human primates that resolved with treatment,[7] supporting interspecies commonality of *M tuberculosis* infection pathogenesis. In another study of a cohort of 35 people with HIV and latent tuberculosis infection, baseline PET-CT lesions were described that were associated with tuberculosis disease developing within the following 6 months, suggesting that PET-CT imaging features might reveal characteristics of incipient tuberculosis before the development of pathological changes that are visible with chest radiography.[8]

Although pathogen-directed biomarkers for incipient tuberculosis are yet undeveloped, a rationale for studying biomarkers of *M tuberculosis* or its products in the blood is supported by evidence that bacterial escape from

granulomas at the site of primary infection occurs during progressive infection,[9] and that escape might occur into the bloodstream.[10] Studies investigating bloodborne *M tuberculosis* in human tuberculosis infection have hitherto been limited by the methodological challenges of detecting low bacterial numbers circulating within peripheral blood mononuclear cells (PBMCs),[11] with evidence for circulating *M tuberculosis* reported predominantly in HIV-positive populations and those with severe multisystem disease.[12] With use of more sensitive molecular approaches, there is recent evidence of circulating *M tuberculosis* DNA in healthy cohorts of people with and without IGRA-defined tuberculosis infection in a setting with a high tuberculosis burden.[11] Actiphage (PBD Biotech, Birmingham, UK) is a novel bacteriophage-based assay that uses mycobacteriophage D29 to infect and lyse viable *M tuberculosis* within the PBMC fraction of fresh blood samples, releasing bacterial DNA for improved PCR-based detection.[13] Using this assay, we previously reported detection of circulating *M tuberculosis* DNA in immunocompetent adults with pulmonary tuberculosis[14] and preliminary evidence of an association with progressive tuberculosis infection in their household contacts.

In this exploratory study of *M tuberculosis*-exposed, HIV-negative, immunocompetent adults, we aimed to test the hypothesis that phenotypic heterogeneity of the state and trajectory of asymptomatic, preclinical tuberculosis infection can be characterised with serial [^{18}F]FDG-PET-CT scans and targeted microbiological sampling at sites of increased [^{18}F]FDG uptake. We hypothesised that this approach could support the prospective characterisation of incipient tuberculosis by identifying metabolically active and replicating *M tuberculosis* in individuals with no radiographical or sputum-based evidence of disease. As a secondary objective, we explored whether circulating *M tuberculosis* DNA detected with the Actiphage assay is associated with PET-CT-characterised incipient tuberculosis.

Methods

Study design and participants

We did a 12-month, prospective, observational cohort study of healthy, immunocompetent individuals (aged ≥16 years) who had household contact with a patient with pulmonary tuberculosis, identified by the contact tracing programme at University Hospitals of Leicester National Health Service (NHS) Trust (Leicester, UK). Full eligibility criteria are provided in appendix 1 (p 6). Participants were recruited at the National Institute for Health and Care Research (NIHR) Leicester Respiratory Biomedical Research Centre, as previously described.[15] Potential participants were approached by the study team after being identified by specialist tuberculosis nurses conducting contact tracing immediately after index notification, or after being recently identified with *M tuberculosis* infection but refusing preventive treatment. All participants provided written informed consent at enrolment and were prospectively followed up for 12 months at 3-month intervals with clinical review and symptom questionnaires. The study was approved by the Research Ethics Committee for East Midlands—Nottingham 1 (Nottingham, UK; number REC 15/EM/0109).

At the baseline visit, all recruited participants completed a self-reported demographics questionnaire, and symptom questionnaire, and underwent chest radiography and IGRA testing with QuantiFERON-TB Gold Plus (QFT; Qiagen, Hilden, Germany) in accordance with the manufacturer's guidelines. Blood was also sent to the Royal Veterinary College (London, UK) for Actiphage testing. Investigators at the Royal Veterinary College were masked to all participant details and outcomes. The QFT assay was repeated at 3 months if the results were negative at baseline to identify those who had converted. PET-CT scans were done as soon as baseline QFT results were known. Scans were preferentially offered to all QFT-positive participants and to a subset of QFT-negative contacts. Based on funding constraints, the first four QFT-negative participants who agreed to undergo PET-CT were recruited. Participants with positive PET-CT findings were investigated for tuberculosis disease with appropriate clinical tests and, if negative, had a further scan 3 months later. Treated participants were offered a post-treatment scan within 3 months of completing the course of treatment.

As this study was done during the COVID-19 pandemic, all participants were screened for current or recent symptoms of SARS-CoV-2 infection before each study visit, and any participants reporting symptoms had a PCR test of nasopharyngeal swab samples to exclude SARS-CoV-2 infection before assessment.

Invasive clinical sampling and analysis

Participants with positive PET-CT scans had further diagnostic investigation with bronchoscopy or endobronchial ultrasound-guided transbronchial needle aspiration, or both, as indicated by the imaging findings. Bronchoscopy was done in accordance with the British Thoracic Society guideline for diagnostic flexible bronchoscopy (appendix 1 p 11).[16] All participants were required to have had a negative SARS-CoV-2 PCR result from a swab sample obtained within 72 h of their procedure.

Microscopy, culture, and Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, USA) testing was done on all samples, in accordance with approved clinical laboratory protocols at University Hospitals of Leicester NHS Trust (appendix 1 p 11). Mycobacterial culture-positive samples were routinely sent for whole-genome sequencing at the National Mycobacterial Reference Laboratory (Birmingham, UK) as part of England's tuberculosis surveillance programme.[17]

See Online for appendix 1

Diagnosis and treatment of tuberculosis infection and disease

A diagnosis of tuberculosis disease was made according to standard clinical criteria, on the basis of compatible clinical signs and symptoms, coupled with radiological changes on routine clinical imaging modalities and supporting histological or microbiological evidence of mycobacterial infection. On the basis of the consensus view of our local clinical

tuberculosis network, contacts with isolated *M tuberculosis* were treated with a full 6-month course of antituberculosis treatment. Participants with progressive changes on PET-CT in the absence of any clinical or microbiological evidence of disease received a 3-month course of tuberculosis-preventive treatment with rifampicin–isoniazid. All other participants with tuberculosis infection who did not meet these criteria were observed without treatment for the duration of the study and offered treatment at study completion. Actiphage results, when available, were explicitly excluded from clinical assessment or decision making.

Actiphage assay

15 mL of blood was collected into sodium heparin tubes (Sarstedt, Nümbrecht, Germany) and processed within 24–48 h of collection. Blood was processed according to the lysis stages of the MolYsis kit (Molzym, Bremen, Germany) with some adaptations for use with Actiphage (appendix 1 p 10). The Actiphage assay was then done according to the instructions of the manufacturer (PBD Biotech). Briefly, the sample was resuspended in 110 µL of Actiphage reagent and incubated for 3·5 h at 37°C. After incubation, the samples were transferred into the top of the Actiphage tubes and centrifuged (13 000×*g*; 3 min). The flow-through containing released DNA was cleaned and concentrated (Monarch PCR & DNA Cleanup Kit [5 µg]; New England Biolabs, Hitchin, UK). The purified DNA was tested with the MTBC PCR DNA amplification kit (PBD Biotech). A positive and valid test was defined by amplification of the target PCR signal with no evidence of inhibition from the internal amplification control; corresponding positive and absent signals were used for positive and negative controls, respectively (appendix 2 p 1).

See Online for appendix 2

[^{18}F]FDG-PET-CT scans

[^{18}F]FDG-PET-CT scans were done with the GE Discovery PET-CT 710 according to local procedure guidelines (appendix 1 pp 10–11). Patients received an intravenous injection of 3·5 MBq/kg ± 10% (to a maximum of 400 MBq) of [^{18}F]FDG, which was followed by image acquisition from the skull vertex to the proximal third of the femur 1 h after administration. PET images were reconstructed with use of the ordered subset expectation maximisation method.

All PET-CT scans were independently reviewed and reported by a consultant nuclear medicine radiologist masked to the outcome of other investigations, according to the criteria described below. Baseline PET-CT findings were classified as positive (one or more [^{18}F]FDG-avid intrathoracic lesions with a maximum standardised uptake value [SUV_{max}] >5, a threshold selected on the basis of its reported association with *M tuberculosis* infected granulomas in non-human primate models),[18] indeterminate (intrathoracic nodal lesions with absolute [^{18}F]FDG avidity of SUV_{max} <5 but greater than that of the liver,[19] or uptake at extrathoracic sites clinically compatible with *M tuberculosis* infection, or both), or negative (no foci of [^{18}F]FDG uptake exceeding physiological levels).

Target lesions were defined as sites showing [^{18}F]FDG uptake on PET-CT. Up to three representative target lesions were selected on baseline PET-CT scans in descending order of [^{18}F]FDG avidity (SUV_{max}) for comparison at follow-up PET-CT to quantitatively evaluate the trajectory of infection. For scans showing more than three [^{18}F]FDG-avid lesions, lung parenchymal and extrathoracic lesions were considered separately. On the basis of previous studies, we considered a change of at least 20% in the [^{18}F]FDG avidity of target lesions from baseline to be significant to inform the trajectory.[20] Therefore, changes were categorised as progression if there was at least 20% increase in SUV_{max} in one or more target lesions or appearance of new lesions with SUV_{max} greater than 5; resolving if at least 20% reduction was observed in SUV_{max} of one or more lesions; stable if there was less than 20% variation in the SUV_{max} of all lesions; and mixed if there were concurrent resolving and progressing lesions. Resolving, stable, and mixed changes were collectively considered to represent non-progressive, controlled *M tuberculosis* infection.

Outcomes

The study primary outcome was progressive tuberculosis infection requiring antituberculosis treatment, defined as a progressive trajectory on serial PET-CT, or microbiological detection of *M tuberculosis*, or both. A secondary outcome was the association between baseline Actiphage test positivity and treatment of tuberculosis infection.

Statistical analysis

As this was an exploratory study examining two previously uncharacterised endpoints (longitudinal PET-CT changes after exposure and Actiphage results) a formal power calculation could not be done. χ^2 tests were done to assess the relationship between PET-CT results and categorical variables in demographic data. The distribution of continuous variables was examined by visual inspection of their frequency histograms to determine normality. To assess associations with baseline PET-CT categories, *t* tests for independent samples were used for continuous variables with parametric distributions and Mann Whitney *U* test for those with non-parametric distributions. A two-tailed p value of less than 0·05 was considered statistically significant. Fisher's exact test was used to evaluate the association between Actiphage results and incipient tuberculosis. Data were analysed with use of SPSS (version 26).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

35 asymptomatic adult household contacts of 14 index patients with pulmonary tuberculosis (*M tuberculosis* culture positive) completed a screening baseline visit between Aug 5 and Nov 5, 2020 (figure 1). All potential participants were

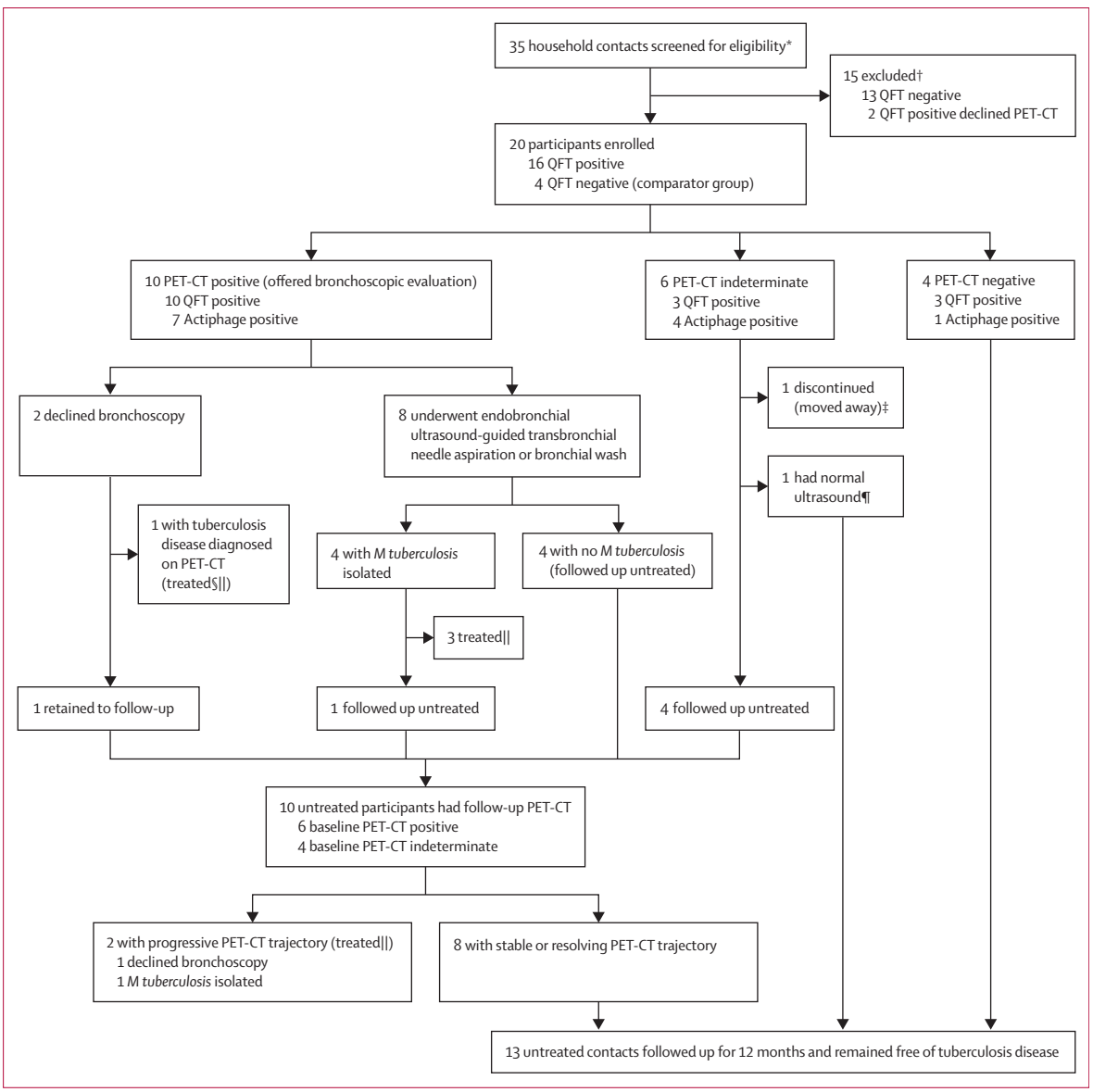


Figure 1: **Study profile**

M tuberculosis=Mycobacterium tuberculosis. QFT=QuantiFERON TB Gold Plus. *Clinical assessments, chest radiograph, QFT, and Actiphage tests were conducted for household contacts of patients with confirmed pulmonary tuberculosis. †All QFT-positive participants and a proportion of QFT-negative participants were offered a PET-CT scan; participants who declined or were not offered a PET-CT scan were excluded from the study. ‡One participant who left the area after the first PET-CT scan was excluded from outcome analyses. §One contact with a positive PET-CT scan was treated due to having potentially infectious tuberculosis. ¶One participant with an indeterminate PET-CT scan had non-specific subcentimetre bilateral [^{18}F]fluorodeoxyglucose uptake in cervical nodes alone that was further evaluated by ultrasound. ||All six treated contacts had post-treatment PET-CT scans showing resolving or resolved changes.

HIV negative, had no clinical features of immunodeficiency, and had no history of tuberculosis or treatment for tuberculosis infection. 16 of 18 QFT-positive contacts, including four who had QFT conversion between baseline and 3 months, and four persistently QFT-negative contacts (comparator group) were recruited to the prospective study (figure 1). The 20 participants were contacts of nine index patients with pulmonary tuberculosis. Eight cases were smear positive and the median time to positive sputum culture was 8 days (IQR 6–9). In keeping with local tuberculosis epidemiology, the recruited contact cohort comprised an even sex distribution (nine [45%] males and 11 [55%] females), and primarily consisted of non-smokers (16 [80%] participants) and people who had migrated to the UK (18 [90%] participants), predominantly from the Indian subcontinent. Three participants had a history of well controlled, non-insulin-dependent, type 2 diabetes, and one participant had hypothyroidism (table 1). A consultant thoracic radiologist reported baseline chest radiographs to be normal or exhibiting features unrelated to tuberculosis in

	Overall (n=20)	Baseline PET-CT (n=20)			Study outcome at 12 months* (n=19)		
		PET-CT negative or indeterminate (n=10)	PET-CT positive (n=10)	p value†	No tuberculosis disease (n=13)	Incipient tuberculosis (n=6)	p value†
Sex	..	..	..	0·178	..	..	0·095
Male	9 (45%)	6 (60%)	3 (30%)	..	8 (62%)	1 (17%)	..
Female	11 (55%)	4 (40%)	7 (70%)	..	5 (38%)	5 (83%)	..
Mean age	37·4 (15·5)	38·0 (15·8)	36·7 (16·0)	0·857	37·8 (14·9)	38·2 (19·0)	0·882
Country of birth	..	..	..	0·136	..	..	0·329
UK	2 (10%)	2 (20%)	0	..	2 (15%)	0	..
Other‡	18 (90%)	8 (80%)	10 (100%)	..	11 (85%)	6 (100%)	..
Comorbidities§							
Diabetes	3 (15%)	2 (20%)	1 (10%)	0·392	2 (15%)	1 (17%)	0·943
Other	1 (5%)	0	1 (10%)	NA	0	1 (17%)	NA
Smoking status	..	..	..	0·356	..	..	0·209
Current smoker	4 (20%)	3 (30%)	1 (10%)	..	4 (31%)	0	..
Ex-smoker	1 (5%)	0	1 (10%)	..	1 (8%)	0	..
Never smoker	15 (75%)	7 (70%)	8 (80%)	..	8 (62%)	6 (100%)	..
QFT positive	16 (80%)	6 (60%)	10 (100%)	0·025	10 (77%)	6 (100%)	0·143
Baseline blood tests							
Mean haemoglobin concentration, g/L	135·7 (22·2)	147·4 (21·3)	126·4 (19·0)	0·042¶	142·8 (23·9)	121·7 (7·6)	0·054
Mean monocyte count, $\times 10^9$	0·40 (0·12)	0·39 (0·11)	0·42 (0·14)	0·580	0·39 (0·10)	0·44 (0·16)	0·470
Mean lymphocyte count, $\times 10^9$	2·16 (0·65)	1·97 (0·63)	2·32 (0·64)	0·259	2·12 (0·58)	2·26 (0·81)	0·680
Median monocyte–lymphocyte ratio	0·16 (0·14–0·22)	0·17 (0·14–0·28)	0·16 (0·14–0·20)	0·657	0·17 (0·14–0·25)	0·15 (0·13–0·31)	0·925
Median C-reactive protein concentration, mg/L	<5	<5	<5	NA	<5	<5	NA

Categorical variables are presented as n (%) and were compared by χ^2 test. Parametric variables are presented as mean (SD) and were compared by *t* test for independent samples. Non-parametric variables are presented as median (IQR) and were compared by Mann Whitney *U* test. NA=not applicable. QFT=QuantiFERON TB Gold Plus. *One contact who moved away was excluded from the analysis. †All p values are two-sided. ‡18 participants were born in other countries: India (n=13), Bangladesh (n=2), Pakistan (n=1), the Philippines (n=1), and Italy (n=1). §Three participants had well controlled type 2 diabetes, and none were receiving immunosuppressive or immunomodulatory medication. ¶Difference in mean haemoglobin was attributable to the high proportion of female participants of child-bearing age in the PET-CT-positive group; one female participant had anaemia and the remaining participants' haemoglobin concentrations were within normal limits.

Table 1: **Demographic and clinical characteristics of study participants**

all but one of the contacts (who had possible subtle hilar enlargement).

The median time from index notification to PET-CT scan was 54·0 days (IQR 39·0–164·3). We categorised baseline PET-CT imaging characteristics as positive, indeterminate, or negative on the basis of specified imaging criteria (figure 2). Ten (50%) contacts had positive PET-CT scans, showing [^{18}F]FDG uptake in the mediastinal and hilar lymph nodes, with an SUV_{max} greater than 5. Concomitant [^{18}F]FDG uptake (SUV_{max} >2·5) in the adjacent lung parenchyma was also observed in five of these contacts, none of whom had evidence of abnormalities in these regions on chest radiographs. All contacts in this group were QFT positive, including three with QFT conversion. Notably, the contact with possible hilar enlargement at screening had a positive PET-CT scan, with [^{18}F]FDG uptake at additional intrathoracic nodal sites that were not visualised on the chest radiograph.

Six (30%) contacts had indeterminate PET-CT scans ([^{18}F]FDG uptake below the threshold for a positive PET-CT). In all these cases, [^{18}F]FDG uptake (SUV_{max} >2) was detected in the intrathoracic lymph nodes and some extrathoracic sites that can manifest tuberculosis, including cervical lymph nodes and terminal ileum (figure 2). Three of these contacts were QFT positive, including one contact with QFT conversion (appendix 2 p 4).

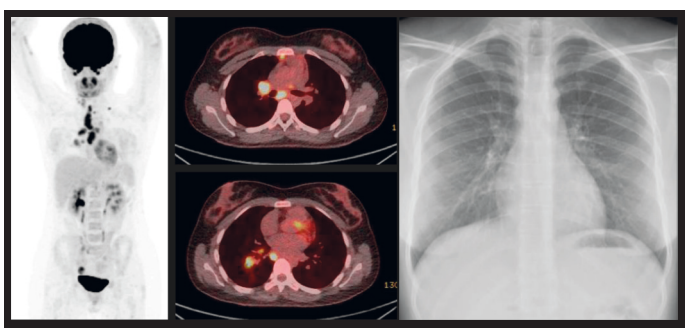
Four (20%) participants (three QFT positive) had a negative baseline PET-CT scan with no structural or metabolic evidence of tuberculosis infection.

Eight contacts with positive PET-CT scans consented to invasive sampling with bronchial wash (at [^{18}F]FDG-avid lung parenchymal sites) and endobronchial ultrasound-guided transbronchial needle aspiration ([^{18}F]FDG-avid mediastinal lymph node stations). Of the two contacts who declined invasive testing, one was treated empirically for probable infectious tuberculosis on public health grounds, based on highly [^{18}F]FDG-avid lung parenchyma (SUV_{max} 9·4; figure 3E). The other elected to have a review after follow-up PET-CT (figure 1).

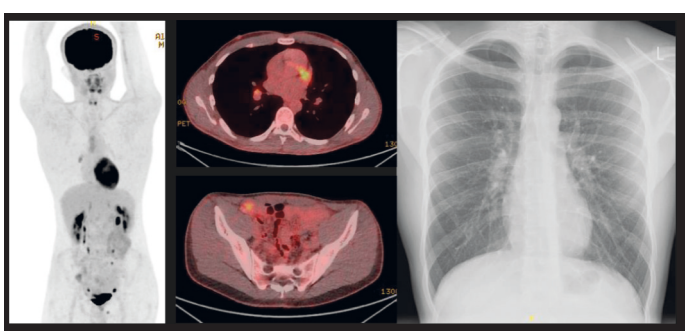
All nodal samples yielding sufficient tissue showed histological features of granulomatous inflammation with variable necrosis (appendix 2 p 4). *M tuberculosis* was cultured from bronchial wash in one contact and detected from mediastinal lymph node aspirates in three further contacts (two culture positive and one Xpert MTB/RIF Ultra positive alone; figure 3A–D). Time to positive culture ranged from 25 to 39 days indicating low bacterial burden. A genotypic match to the corresponding index isolates was identified in

Baseline characterisation

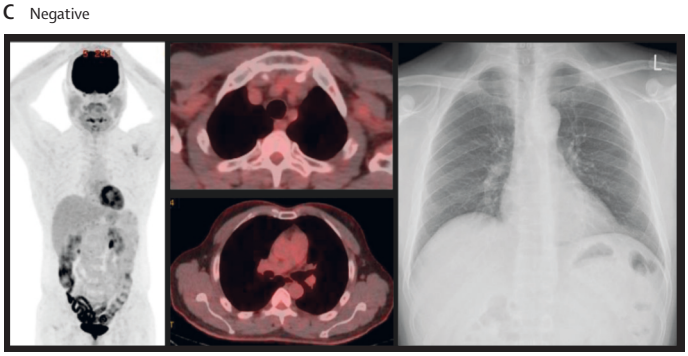
A Positive



B Indeterminate



C Negative

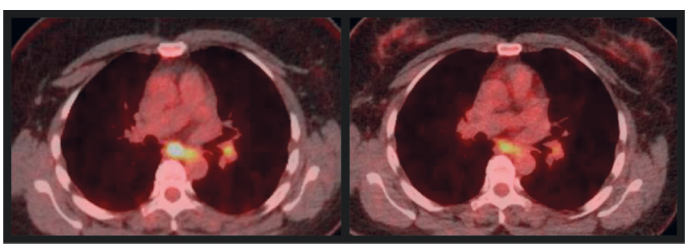


D

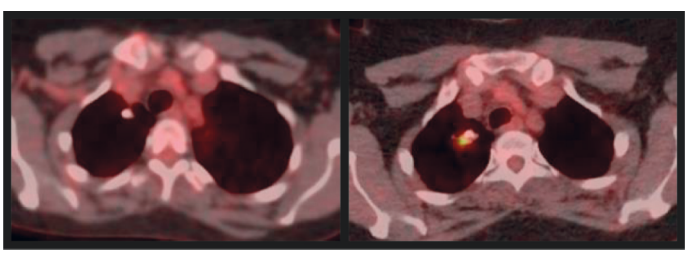
Classification of baseline PET-CT findings	
Positive	[^{18}F]FDG-avid mediastinal or hilar lymph nodes (SUV_{max} >5), with or without uptake in lung parenchyma
Indeterminate	Low absolute [^{18}F] FDG uptake (SUV_{max} <5); extrathoracic uptake at sites associated with *M tuberculosis* infection
Negative	No [^{18}F]FDG uptake exceeding physiological uptake

Longitudinal characterisation

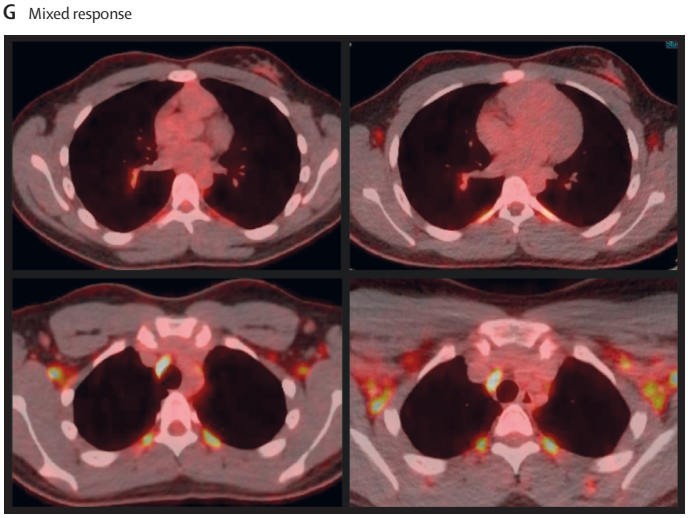
E Partial response



F Progression



G Mixed response



H

Classification of longitudinal PET-CT changes	
Resolved	Complete resolution of lesions seen on initial PET-CT scan
Partial response	≥20% reduction in SUV_{max} in all lesions
Stable	<20% variation in SUV_{max}
Mixed response	Sites with ≥20% increase and sites with ≥20% reduction in SUV_{max}
Progression	≥20% increase in SUV_{max}; new area of [^{18}F]FDG uptake with SUV_{max} >5

Figure 2: **Baseline and longitudinal PET-CT features observed following *M tuberculosis* exposure**

(A–C) Representative examples of positive, indeterminate, and negative scans (maximum intensity projection PET images [left] and fused axial PET-CT images [middle]) with corresponding chest radiographs (right). All chest radiographs shown were reported as normal by a consultant thoracic radiologist. (A) [^{18}F]FDG uptake (SUV_{max} 11·3) in mediastinal or hilar nodes (top) and [^{18}F]FDG-avid (SUV_{max} 7·4) lung lesion (bottom). (B) Low-grade [^{18}F]FDG uptake in hilar nodes (SUV_{max} 3·8, top) and terminal ileum (SUV_{max} 4·6, bottom). (C) No [^{18}F]FDG uptake above basal physiological level observed. (D) Classification model defining [^{18}F]FDG-PET-CT features at baseline. (E–G) Representative examples of longitudinal changes from baseline (left) in target lesions of untreated contacts after 3 months (right). (E) Resolving infection with decreasing [^{18}F]FDG uptake (38% reduction in SUV_{max}) in mediastinal or hilar nodes (left to right). (F) Increasing [^{18}F]FDG uptake (321% increase in SUV_{max} from 1·4 to 5·9) in lung lesion (left to right). (G) Decreasing in [^{18}F]FDG uptake (26% reduction in SUV_{max}) in right hilar nodes (top left and right), and increasing [^{18}F]FDG uptake (20% increase in SUV_{max}) in mediastinal nodes (bottom left and right). (H) Classification model defining longitudinal changes in [^{18}F]FDG PET-CT features. [^{18}F]FDG=[^{18}F]fluorodeoxyglucose. *M tuberculosis*=*Mycobacterium tuberculosis*. SUV_{max}=maximum standardised uptake value.

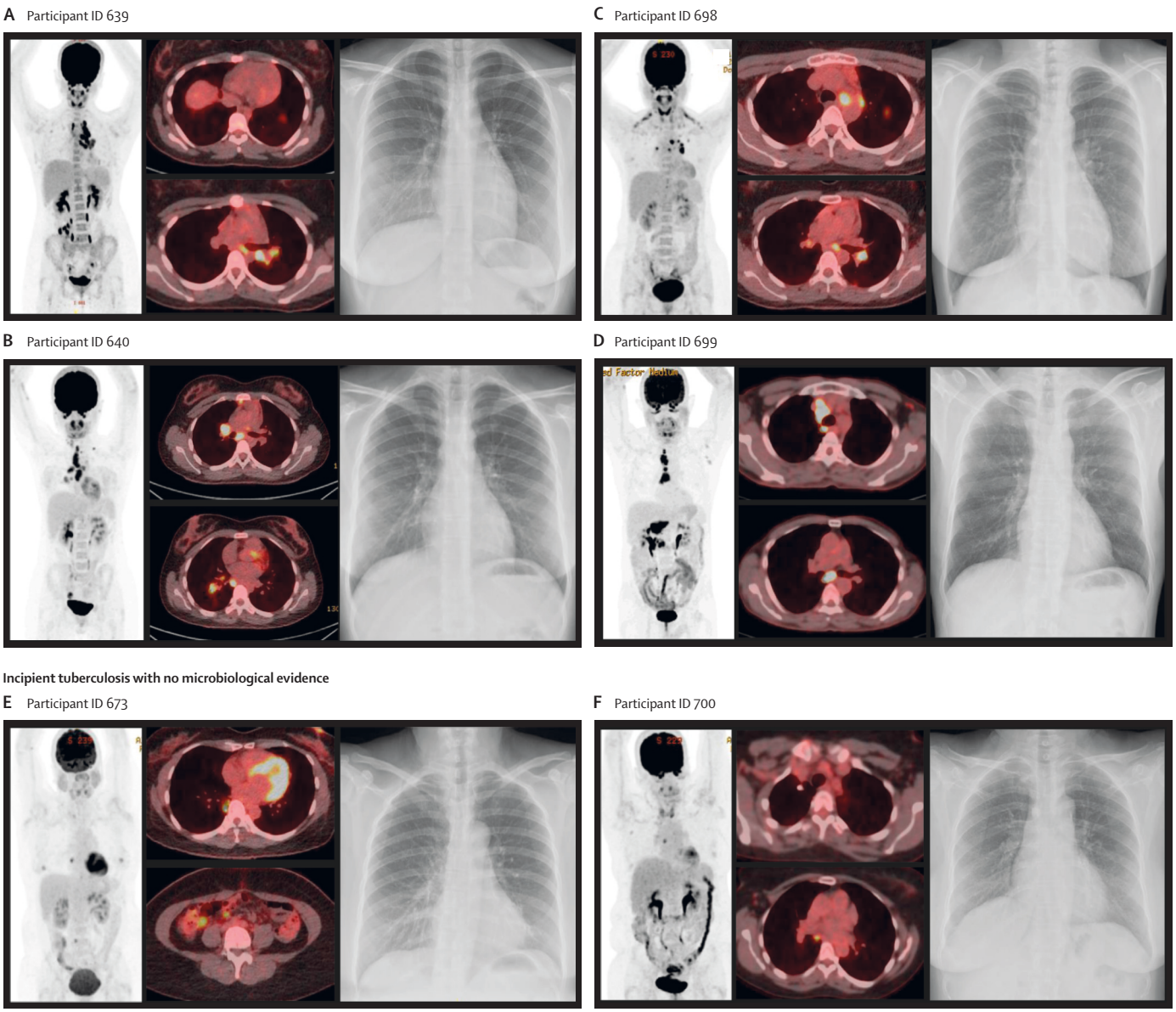


Figure 3: **Baseline [^{18}F]FDG-PET-CT and chest radiograph features of incipient tuberculosis**
Scans are maximum intensity projection PET images (left), fused axial PET-CT images (middle), and chest radiographs (right). (A) PET-CT shows a low grade [^{18}F]FDG uptake in the lung parenchyma (left and middle top) and intense uptake in the intrathoracic lymph nodes (left and middle bottom). Chest radiography shows normal anatomy (right). (B) PET-CT shows an [^{18}F]FDG uptake in the intrathoracic lymph nodes (left and middle top) and lung parenchyma (left and middle bottom). Chest radiography shows normal anatomy (right). (C) PET-CT shows a low-grade [^{18}F]FDG uptake in the lung parenchyma (left and middle top) and intense uptake in the intrathoracic lymph nodes (left and middle bottom). Chest radiography shows subtle hilar enlargement (right). (D) PET-CT shows intense uptake in the intrathoracic lymph nodes (left and middle). Chest radiography shows normal anatomy (right). (E) PET-CT shows [^{18}F]FDG uptake in the lung parenchyma (left and middle top) and abdomen (left and middle bottom). Chest radiography shows normal anatomy (right). (F) PET-CT shows a non-avid calcified granuloma (left and middle top) and low-grade [^{18}F]FDG uptake in the mediastinum (left and middle bottom). Chest radiography shows normal anatomy (right). See appendix 2 (p 2) for further participant information. [^{18}F]FDG=[^{18}F]fluorodeoxyglucose.

two of the contacts on whole-genome sequencing, confirming transmitted infection. In the third culture-positive contact, the strain was not linked to any existing local isolates. Two contacts with positive cultures and the contact with a positive Xpert MTB/RIF Ultra test commenced full anti-tuberculosis treatment at this time; the third contact with a positive culture chose to have a follow-up PET-CT scan before commencing treatment (figure 1; appendix 2 p 4).

We evaluated the natural trajectory of infection by repeating PET-CT scans after 3 months in the ten untreated contacts with baseline [^{18}F]FDG uptake who were retained to follow-up (figure 1). Quantitative categories of within-participant

	Incipient tuberculosis at 12 months	No tuberculosis disease at 12 months	p value*
All participants	n=6	n=13	0·018
Actiphage positive	6	5	..
Actiphage negative	0	8	..
QFT-positive participants	n=6	n=10	0·034
Actiphage positive	6	4	..
Actiphage negative	0	6	..

One QFT-negative contact who moved away was excluded from the analysis. QFT=QuantiFERON TB Gold Plus. *Two-sided p values from Fisher's exact tests assessing the associations between the Actiphage results and participant outcomes.

Table 2: **Association between Actiphage test results and participant outcomes**

change from baseline were defined as progressive, mixed or stable, and partial response or resolved (figure 2H). Two baseline PET-CT positive contacts (figure 3D, F) had progressive PET-CT features on their second scan and received antituberculosis treatment without further investigation (figure 1). This included the culture-positive participant who received antituberculosis treatment and the contact that declined bronchoscopy who received preventive tuberculosis treatment in the absence of any positive microbiological samples being obtained. The remaining eight contacts, including all contacts with indeterminate baseline features, had persistent or resolving changes and remained free of tuberculosis disease after 12 months.

Overall, six asymptomatic contacts (30% of the whole study cohort and 38% of the 16 QFT-positive contacts) received treatment, five with full antituberculosis treatment and one with a preventive regimen, based on a composite of PET-CT and microbiological features of progressive metabolically active tuberculosis infection (figures 1, 3). Using existing clinical tools and criteria, routine contact screening would have identified only the contact with a subtly abnormal chest radiograph to have—or be at higher risk of developing—tuberculosis. Comparing the baseline PET-CT features of contacts who did and did not receive treatment, we found that [^{18}F]FDG uptake in the lung parenchyma with an SUV_{max} greater than 2·5 was a specific discriminating feature of the prospectively treated group of asymptomatic contacts. A post-treatment PET-CT scan in all six contacts shows either partially or fully resolved changes (appendix 2 p 4).

Actiphage results were positive in 12 (60%) contacts at baseline. The test was positive in all six of the treated PET-CT-positive contacts, but negative in three of the four remaining PET-CT-positive contacts who had stable or resolving changes at follow-up (appendix 2 p 2). Actiphage was also negative in three of the four contacts with a negative baseline PET-CT scan. The four remaining positive Actiphage tests occurred in the indeterminate PET-CT group, and included two contacts who were persistently QFT negative. The cycle threshold (Ct) values for *M tuberculosis* DNA detection among Actiphage-positive contacts with negative or indeterminate PET-CT findings were similar to those obtained in Actiphage-positive contacts with positive PET-CT scans (appendix 2 p 2). There was also no correlation between Actiphage Ct values and quantitative QFT results, using either the TB1 or TB2 antigens provided with the QFT kit (appendix 2 p 2). There was a statistically significant association between a positive baseline Actiphage test and prospectively receiving treatment (Fisher's exact test p=0·018). A subgroup analysis restricted to the 16 QFT-positive contacts remained statistically significant (p=0·034; table 2).

There was no correlation between the QFT titre of contacts and baseline PET-CT features (appendix 2 p 3). Within the QFT-positive subgroup, the median response to TB1 and TB2 antigens was higher in treated contacts than in untreated contacts, but this difference did not reach statistical significance (median TB1 response 4·7 IU/mL [IQR 3·5–6·3] *vs* 2·9 IU/mL [0·7–5·8]; p=0·181), and there was considerable overlap in QFT values between the two groups (appendix 2 p 3). Other potential blood biomarkers of tuberculosis infection measured at baseline included the monocyte–lymphocyte ratio[21] and C-reactive protein concentration,[22] but neither showed any difference between contacts who did and did not receive treatment (table 1).

Discussion

In this study, we used [^{18}F]FDG-PET-CT to characterise early stages of human tuberculosis infection according to the amount and distribution of [^{18}F]FDG uptake near the time of developing adaptive immunity; the microbiological and histological features of infection at sites of targeted sampling; and the 3-month trajectory of inflammatory changes after tuberculosis exposure, before any treatment intervention. On this basis, we made three novel observations. First, from culture and whole-genome sequencing, we found direct microbiological evidence of actively replicating *M tuberculosis* during early infection that disseminates to local draining lymph nodes. Second, we observed heterogeneity in the natural trajectory of infection after 3 months, with a subset of contacts showing progressive changes over this period. We contend that evidence of replicating bacteria and progressive PET-CT changes observed before any abnormalities at clinical screening align with definitions proposed for incipient tuberculosis.[23] Third, based on the Actiphage assay, we report preliminary evidence of early haematogenous dissemination of *M tuberculosis* in contacts with a progressive phenotype of tuberculosis infection.

PET-CT provides a non-invasive modality for visualising metabolic activity and structural changes in early tuberculosis infection before changes become visible using conventional chest radiographs. Non-human primate studies have identified discriminatory features of active infection using PET-CT.[6,18] However, this high sensitivity is accompanied by poor specificity. A key strength of our study was inclusion of a cohort without significant comorbidities and with well characterised recent exposure in a setting with a low incidence of tuberculosis, reducing the likelihood of

multiple *M tuberculosis* exposure events. Heterogeneity in the volume and distribution of [^{18}F]FDG uptake at baseline was categorised into two groups on the basis of confidence of ascertainment of tuberculosis infection. While features observed in the positive PET-CT group were consistent with those reported in non-human primates[6] and a previous study in tuberculosis contacts,[7] the category of indeterminate PET-CT features has not previously been reported. As we describe, [^{18}F]FDG uptake in this category was less avid and more heterogeneously distributed at extrathoracic sites. Obtaining evidence to support tuberculosis infection through invasive sampling was generally not feasible in this subgroup because of the risk of sampling associated with the small size and occasional inaccessibility of lesions. In all cases, the SUV_{max} at sites of uptake was greater than 2, which is a threshold that has been associated with PET-CT features of tuberculosis infection in previous human studies.[7,24] Furthermore, QFT conversion was observed in one contact with indeterminate PET-CT.

In the PET-CT-positive group, sampling provided direct evidence of replicating *M tuberculosis* at the site of primary infection and in local draining lymph nodes during the early phase of infection. These findings are consistent with transmitted pathogens being in a metabolically active state, with early replication occurring before establishment of effective immune control. Furthermore, our observations suggest that *M tuberculosis* bacteria transported to local draining lymph nodes for antigen presentation are also actively replicating, supporting the Trojan horse theory of immune cells facilitating the transport of viable *M tuberculosis* out of the lung.[10] We were unable to systematically determine whether microbiological *M tuberculosis* detection was associated with a progressive PET-CT trajectory, as bacillary detection prompted treatment. However, in the one culture-positive contact who opted to have a follow-up PET-CT scan before starting treatment, there was objective evidence of progressive inflammatory change that was not seen in the microbiologically negative subgroup. As culture-based detection of *M tuberculosis* and progressive metabolic changes on PET-CT were observed in contacts who had neither clinical nor radiological features of disease, we suggest that these features are consistent with the definition of incipient tuberculosis.[23] In our cohort, features of incipient tuberculosis were identified in 38% of QFT-positive contacts, supporting the concept that progressive infection occurs in a substantial proportion of individuals after infection acquisition. However, this infection is subsequently controlled in most people, as epidemiological studies have consistently shown a 5–10% risk of developing disease. Further studies extending prospective observation to identify the subset with persisting incipient features are needed.

As methods for characterising pre-disease states evolve, the inference that all microbiologically positive tuberculosis infections constitute disease will need re-evaluation. Furthermore, there is growing recognition that shorter courses of antituberculosis treatment might be sufficient in paucibacillary disease and disease of limited extent.[25] Of the six contacts treated in this study, only one had chest radiographic evidence of subtle lymphadenopathy that would have been diagnosed as subclinical disease at routine contact screening. The other five contacts would have been diagnosed with tuberculosis infection and received preventive treatment. In a previous study of our local cohorts, we found no cases of tuberculosis arising among contacts with clinically defined tuberculosis infection who adhered to a 3-month regimen of rifampicin–isoniazid,[26] supporting the concept that a shorter course of treatment would have been sufficient in this cohort.

Consistent with recent studies,[11,14] we detected *M tuberculosis* DNA in the blood of a substantial proportion of our cohort. Using Actiphage, we found a significant association between *M tuberculosis* DNA in the blood and progressive infection, supporting the hypothesis developed in animal models of bacteraemia arising from inadequate immunological containment of metabolically active infection. This finding has important implications for biomarker development, as pathogen-directed blood biomarkers could complement host-immune biomarkers to improve the characterisation of tuberculosis infection and tuberculosis risk. Specifically, while IGRAs have high sensitivity for identifying tuberculosis infection,[27] pathogen-directed blood biomarkers could additionally inform the trajectory of infection to improve the specificity of risk evaluation.

Actiphage positivity was not confined to the PET-CT-positive group. Actiphage detected circulating *M tuberculosis* in four of six contacts with indeterminate baseline PET-CT scans. Given the benign course of early infection observed on follow-up PET-CT in this group, we postulate that circulating *M tuberculosis* in the context of indeterminate PET-CT scans might be pathologically distinct from the bacterial dissemination detected with progressive infection, and might more closely resemble the cohorts described by Belay and colleagues.[11] Specifically, detectable *M tuberculosis* associated with low-level [^{18}F]FDG uptake at extrapulmonary sites might be evidence of low-grade bacterial dissemination[28] that naturally occurs to seed infection at sites associated with extrapulmonary disease. These observations are speculative, and further studies in larger cohorts are needed.

This study was limited by the small and selective nature of our cohort. Specifically, we focused our recruitment on QFT-positive contacts and did not conduct prospective imaging in contacts with negative baseline PET-CT scans. As such, our findings are not generalisable and further validation studies in larger cohorts are needed. Our preliminary observations in QFT-negative contacts are limited by small numbers, but support heterogeneity in this group and the potential for PET-CT to reveal IFNγ-independent phenotypes of tuberculosis infection. Although we advocate a role for PET-CT in the study of tuberculosis infection, there are important ethical considerations, most notably increased radiation dose and handling of incidental findings. Approaches to limit radiation exposure, including limiting the body area for scan

and using alternative imaging modalities such as MRI, should be investigated. Our prospective follow-up period was limited to 12 months. Although a substantial proportion of incident disease arising from recent infection occurs during this period,[29] it is possible that some events of progression to disease might have been identified with more extended follow-up. Although not the focus of this study, there are notable mechanistic limitations. We did not conduct mycobacterial blood culture in study participants and therefore cannot provide conclusive evidence of bacteraemia. However, historical evidence is consistent with the concept that existing culture-based approaches are likely to be inadequate for this purpose. In this respect, a strength of the Actiphage method is incorporation of bacteriophage infection that is specific to metabolically active *M tuberculosis*. The cellular location of circulating *M tuberculosis* DNA was not investigated. Previous studies have reported *M tuberculosis* DNA to be either confined to[30] or more commonly present[11] in lineage-negative PBMCs isolated from human donors. Despite these limitations, we make several novel observations of potential mechanistic importance in human tuberculosis infection that need independent replication in larger and less selective cohorts.

In conclusion, heterogeneity of tuberculosis infection following recent *M tuberculosis* exposure can be seen with high-sensitivity [^{18}F]FDG PET-CT. We postulate that incipient tuberculosis is a transitional infection state objectively characterised by microbiological detection of *M tuberculosis* and a worsening inflammatory trajectory in the absence of symptoms or abnormal chest radiographic features. We provide early evidence that escape of *M tuberculosis* into circulation occurs during progressive infection and propose that pathogen-directed blood biomarkers might have an important synergistic role with the current pipeline of host immune biomarkers to improve risk stratification in tuberculosis infection.

Contributors

PH and JWK conceived the study and designed the study protocol with input from RV and GW. CR and BS developed the protocol for the Actiphage method. JL, PH, JN, and JWK contributed to enrolment of participants and clinical data collection. KB, BS, JN, JWK, and CS did the Actiphage and PCR assays. AK developed the protocol for analysis of PET-CT scans, with input from PH and JWK. PET-CT scans were reviewed and reported by AK and MS. JWK and PH wrote the manuscript, and all authors contributed to the final manuscript. JWK, KB, JN, and PH accessed and verified the data underlying the study. All authors had full access to all the data in the study and take final responsibility for the decision to submit for publication.

Declaration of interests

PH has received consultancy fees for two advisory board meetings with PBD Biotech since study inception. CR and BS hold stocks in PBD Biotech, the company commercialising the Actiphage technology. CR and BS have a pending patent on Actiphage bacteriophage-mediated mycobacterial DNA release assay testing (PCT/GB2020/050524). PBD Biotech supplied PCR reagents and components of the Actiphage kit for use in the study but had no role in design, execution, analysis, or reporting. All other authors declare no competing interests.

Data sharing

De-identified patient-level data are provided in the figures and supplementary appendices. Requests for additional data not included in the paper will be considered, but might be subject to additional ethical review.

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