Characterization of progressive HIV-associated tuberculosis using 2-deoxy-2-[\(^{18}\)F]fluoro-\(d\)-glucose positron emission and computed tomography

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Tuberculosis is classically divided into states of latent infection and active disease. Using combined positron emission and computed tomography in 35 asymptomatic, antiretroviral-therapy-naïve, HIV-1-infected adults with latent tuberculosis, we identified ten individuals with pulmonary abnormalities suggestive of subclinical, active disease who were substantially more likely to progress to clinical disease. Our findings challenge the conventional two-state paradigm and may aid future identification of biomarkers that are predictive of progression.

Tuberculosis (TB) is classically divided into an active-disease state (presence of symptoms characteristic of TB, in addition to microbiological confirmation of Mycobacterium tuberculosis (MTB) or evidence of typical pathology identified radiographically or histologically) and a preceding non-infectious, latent-infection state (evidence of immune sensitization by MTB without evidence of active TB). Currently available tests for latent tuberculosis poorly predict who will develop disease. We and others have suggested that this two-state paradigm is an oversimplification and that tuberculosis may be characterized as a spectrum of infection states, with transition from latent infection to active disease involving a subclinical phase of disease during which pathology evolves before symptomatic presentation. This view is supported by historical autopsy studies in which persons dying of causes other than TB frequently had evidence of minimally active disease, and also by mass screening and prevalence surveys in which asymptomatic persons are identified by either chest radiograph (CXR) abnormalities consistent with TB or sputum culture positive for MTB.

Chest radiography can be used to screen for evidence of subclinical TB, but it is insensitive and prone to reader variability. Sputum culture positivity in persons with a normal CXR is frequently described; in these cases it is probable that the CXR is insufficiently sensitive to detect the pulmonary pathology present. 2-deoxy-2-[\(^{18}\)F]fluoro-\(d\)-glucose ([\(^{18}\)F]FDG) positron emission tomography combined with computed tomography (PET–CT) is a more sensitive imaging modality that has the potential to detect early pathology in asymptomatic persons who are sputum-culture negative. [\(^{18}\)F]FDG uptake is increased in metabolically active cells; in TB this primarily relates to activated macrophages and neutrophils. [\(^{18}\)F]FDG PET–CT has been used in the clinical investigation of TB and to describe evolving pathology in animal models of active and latent TB. Here we used [\(^{18}\)F]FDG PET–CT to identify pathology consistent with subclinical pulmonary TB in asymptomatic HIV-1-infected persons living in Khayelitsha (a township in Cape Town, South Africa) who were diagnosed with latent TB by a positive QuantiFERON-TB Gold in-tube (QFN-GIT) test and were thus eligible for isoniazid preventive therapy (IPT).

We screened 265 HIV-1-infected, antiretroviral therapy (ART)-naïve, adult outpatients with no previous history of active TB (Supplementary Fig. 1). Notably, ten (4%) of these people screened were excluded because they had sputum cultures that were positive for MTB, five of whom had no TB symptoms or chest radiographic evidence of active TB (Supplementary Fig. 2). Thirty-five participants who were positive for the QFN-GIT test but had no history of IPT, a CXR without evidence of active TB, CD4 T cell counts ≥250/mm\(^3\) and no TB symptoms were recruited and underwent [\(^{18}\)F]FDG PET–CT following confirmation of negative sputum cultures. The median CD4 cell count was 517/mm\(^3\) (interquartile range (IQR) 393–658), and 91% of the participants were female. IPT was commenced following the initial PET–CT scan, with close clinical follow-up. The CD4 T cell threshold for ART initiation was below 350/mm\(^3\).

Participants were TB symptom free on the day of the initial PET–CT scan, with normal clinical parameters (Supplementary Table 1). Twenty-five participants (71.4%) had CT abnormalities within the lung parenchyma. These abnormalities were categorized into four groups: infiltrates, fibrotic scars, active nodules and discrete nodules (Figs. 1 and 2). The six participants with fibrotic scarring were significantly more likely to have infiltrates than those without scars (50% (3/6) versus 10% (3/29); \(P = 0.049\)). There were significant differences.

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in the anatomical distribution of abnormalities. Seventeen of the 18 (94%) infiltrates and scars were within the upper lobes (mainly located apico-posteriorly) (Figs. 1a,b), in contrast to 33% of discrete nodules and 42% of active nodules ($P < 0.0001$) (Fig. 2c). However, as compared to active nodules, discrete nodules were significantly more likely to be subpleurally located ($P = 0.001$) (Fig. 2c). We interpreted these abnormalities in relation to historical autopsy studies, particularly with regard to their spatial distribution and anatomical characteristics (Online Methods). We determined that the ten participants with either infiltrates and/or fibrotic scars (consistent with bronchogenic reactivation of TB; $n = 9$) or active nodules (consistent with hematogenous spread of TB; $n = 1$) showed evidence of subclinical TB, whereas the 25 participants with either normal lung parenchyma ($n = 10$) or discrete nodules only (anatomically consistent with Ghon foci of primary infection; $n = 15$) showed no evidence of subclinical pathology.

Those subjects with evidence of subclinical disease were significantly more likely to develop symptomatic active TB during the 6-month follow-up—four of ten participants required treatment with standard TB therapy (isoniazid and rifampicin for 6 months, supplemented with pyrazinamide and ethambutol for the first 2 months (2HRZE/4HR)), as compared to none of the 25 participants who had no evidence of subclinical pathology ($P = 0.0003$) (Fig. 1d). Of these four individuals, two were confirmed to have active TB by sputum culture (one with early evidence of cavitation on PET–CT), and two had progressive imaging changes consistent with active TB (Supplementary Fig. 3). All four individuals developed TB symptoms a median of 32 d after the PET–CT scan (range 7–90 d). The ten participants with subclinical pathology in lung parenchyma were also significantly more likely to have abnormal [18F]FDG uptake within the mediastinal lymph nodes as compared to the 25 participants without subclinical TB (80% versus 32%; $P = 0.022$), but not within the cervical or axillary lymph nodes ($P = 0.68$ and $P = 1.0$, respectively). Furthermore, in the 27 participants who underwent repeat [18F]FDG PET–CT 6 months after IPT or standard TB treatment, all of those with subclinical TB (six participants) had improvement in the baseline abnormalities within the lung parenchyma or mediastinal lymph nodes, as compared to only 1 of the 21 participants with no
using [18F]FDG PET–CT as a research tool, we defined a subgroup what is currently diagnosed and managed as latent tuberculosis. By Supplementary Table 1 not have evidence of subclinical TB (P = 0.005) (Fig. 1e,f). In all, 17 participants (48.6%) had evidence of mineralization within discrete nodules or lymph nodes, with no significant difference in mineralization found between those with or without evidence of subclinical pathology (50% versus 48%; P = 0.91). There were no significant differences in clinical characteristics between participants who did or did not have evidence of subclinical TB (Supplementary Table 1).

We have provided evidence of biological heterogeneity within what is currently diagnosed and managed as latent tuberculosis. By using [18F]FDG PET–CT as a research tool, we defined a subgroup of asymptomatic, HIV-1–infected persons who were eligible for IPT, had evidence of subclinical disease and were at higher risk of disease progression. We were not able to fully elucidate the natural history of subclinical disease, as all of the participants were treated with IPT or 2HRZE/4HR. This proof-of-concept study has implications for the optimal management of HIV-1–infected persons with evidence of immune sensitization by MTB. Our findings may also facilitate future development of more practical biomarkers that are predictive of disease progression.

Although [18F]FDG PET–CT is a nonspecific imaging tool, it is highly probable that the abnormalities we found related to subclinical TB and not to alternative pathology. All of the participants who were classified as having subclinical TB and who had a repeat scan showed evidence of improvement in baseline abnormalities in the regions of interest following specific therapy. Strict exclusion criteria and frequent clinical assessments minimized the possibility of alternative pathologies, and in our study setting, TB is considerably more common as a cause of asymptomatic, upper-lobe infiltration and scarring than alternative conditions (such as sarcoidosis or histoplasmosis).

Fibrotic scarring following spontaneous healing of TB infection is well established. Latently infected persons who show evidence of fibrotic scarring are up to 15 times more likely to develop disease12. We show here that subjects with fibrotic scarring, as assessed by [18F]FDG PET–CT, are significantly more likely to have infiltrates; however, the infiltrates were not directly associated with scars, as previously thought13, and could be located in distinct bronchopulmonary segments. These findings require confirmation but suggest that individuals with latent TB and fibrotic scarring may be undergoing repeated episodes of subclinical reactivation, which ultimately places them at greater risk of developing clinical disease. Participants with subclinical TB were significantly more likely to have [18F]FDG uptake within mediastinal lymph nodes, in keeping with observations in a recently published case series of [18F]FDG PET–CT performed in five HIV-uninfected individuals who were close contacts of persons with active TB14.

Our findings support autopsy results showing that TB pneumonia with bronchogenic spread is one of the earliest manifestations of pulmonary TB15. Progression of the disease may result in intermittent shedding of MTB into sputum, and pathology may eventually become visible on CXRs. Dowdy et al. have modeled data from community prevalence surveys for undiagnosed culture-positive TB that suggest that MTB may be shed into sputum for up to 13.5 months before clinical presentation16. As pathology progresses, the release of proinflammatory cytokines presumably contributes to the development of symptoms17. The identification of active TB cases by symptom screening or by CXRs typically shortens the duration of symptoms prior to diagnosis. However, the effect of these active case-finding strategies on reducing transmission has been disappointing, and identification of subclinical disease at an earlier stage may be required to have an effect18.

Zak and colleagues have recently described a predictive 16-transcript signature in whole blood that can identify people up to 12 months before they clinically present with TB with 54% sensitivity and 83% specificity. Our work suggests that such biomarkers may identify infected individuals during the subclinical phase of disease19.

Our findings raise issues around the optimal management of subclinical TB. Because we have shown that a proportion of HIV-1–infected adults with a negative screen for active TB (by sputum culture, CXR and symptom screening) who are eligible for IPT have evidence of subclinical disease, it is likely that isoniazid monotherapy is often inadvertently prescribed to persons with subclinical TB in clinical settings. Although isoniazid monotherapy may be adequate treatment for some individuals with subclinical TB, in a proportion of subjects this approach may not be successful in preventing clinical disease. Of note, during their investigation of HIV-1–infected persons who underwent isoniazid treatment for 6 months versus 36 months,
Samandari et al. showed that rates of active TB increased shortly after cessation of therapy in the 6-month group, only in those individuals who had a positive tuberculin skin test. It is possible that this reflects recrudescence of inadequately treated subclinical TB. Multidrug therapy may be preferable, but whether 6 months of standard four-drug therapy is necessary to adequately treat subclinical TB has yet to be established. Traditionally, studies to evaluate novel treatment regimens in individuals with asymptomatic TB infection have required large sample sizes and prolonged follow-up periods; our findings suggest that [18F]FDG PET–CT could provide a potential surrogate endpoint to evaluate novel regimens for subclinical TB.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

H.E., A.O., C.E.B. and R.J.W. designed the study with input from K.A.W., D.B.Y. and J.L.F.; H.E., G.W. and C.F.K. collected samples and data, and provided clinical care for participants during follow-up; J.M.W. led the radiologists and nuclear medicine physicians who reported the [18F]FDG PET–CT scans; K.A.W. and H.E. processed samples; H.E., M.L. and R.P.L. analyzed data with advice and input from R.J.W., K.A.W., C.E.B., J.M.W., A.K.C., C.M.G., A.O. and J.L.F.; R.J.W. supervised data analysis; H.E. and R.J.W. wrote the manuscript, with early input from A.O., C.E.B., D.B.Y., M.L. and J.L.F., and subsequently all authors provided advice and approved the final manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Online Methods

Recruitment of participants and controls. Ethical approval for this study was provided by the research ethics committees of the University of Cape Town (013/2011) and Stellenbosch University (N12/11/079). Recruitment and follow-up for the study took place between August 2011 and July 2013. All participants and control subjects were residents of and recruited in Khayelitsha, a peri-urban township of Cape Town, South Africa, where TB incidence is 917/100,000, and >95% of residents are of Xhosa ancestry. Informed consent was sought from all patients before screening and study entry.

The asymptomatic HIV-1-infected, ART-naïve persons with latent tuberculosis were recruited from local pre-ART wellness clinics. Participants were considered HIV-1 infected if they had a positive point-of-care (POC) test for HIV in their medical notes and either a positive HIV-1 viral load and/or a positive HIV-1 ELISA. Potential participants were screened for active TB by symptom screening (cough >1 week, hemoptysis, fever, night sweats and weight loss), digital posterior–anterior (PA) CXR, 2× smear and culture (induced if necessary) and latent TB by QFN-GIT. Potential participants also underwent regular clinical assessment for symptoms and signs of TB over a 6-week screening period. Potential participants were eligible for study participation if they met the following pre-established inclusion–exclusion criteria.

Inclusion criteria. HIV-1 infected; ART naïve; screening CD4 ≥ 350/mm³; QFN-GIT positive at initial screening visit; age ≥ 18 years.

Exclusion criteria. Screening sputum culture positive for TB; symptoms or signs of active TB; evidence of active TB on initial screening CXR; evidence of any acute or unexplained chronic illness; previously diagnosed or treated TB; previous IPT; known recent contact of multidrug-resistant (MDR) MTB; CXR abnormality known or suspected to relate to a condition other than TB; age > 50 years; smoker of > 30 pack years (1 pack year = 20 cigarettes per day per year); occupational history of mining or evidence of silicosis; previously diagnosed malignancy, chronic lung infection, chronic lung disease or chronic inflammatory condition; current corticosteroid use; uncontrolled diabetes mellitus; pregnant or planning pregnancy; breast feeding; inclusion in study to result in an inflammatory condition; current corticosteroid use; uncontrolled diabetes mellitus; or any factor felt to significantly increase the participant’s risk of suffering an adverse outcome.

Thirty-five eligible participants consented to study entry and underwent [18F]FDG PET–CT, blood sampling and sputum culture. They were commenced on IPT (or standard TB therapy if microbiological or clinical evidence of active TB developed during follow-up) and had a repeat [18F]FDG PET–CT after 6 months. No randomization took place.

Sample size considerations. For this study we took the previously uncharacterized approach of using [18F]FDG PET–CT as a research tool in human latent tuberculosis to define a subgroup of individuals with evidence of subclinical disease. There was no prior data to allow us to precisely establish the proportion of individuals that may have subclinical TB, and hence the study was exploratory in this respect.

We determined that if less than approximately 20% of our participants had evidence of subclinical TB, then our approach might need re-evaluation. We therefore prespecified a planned initial evaluation after the first 18 participants had undergone baseline PET–CT imaging. We determined that if no participants had abnormalities consistent with subclinical TB at this stage, then the true rate of abnormalities would unlikely be greater than 20% (the upper bound of one-sided 97.5% binomial exact confidence interval would be 0.19) and we would stop the study on grounds of futility. If at least one participant had abnormalities consistent with subclinical TB, then we planned to continue to enroll a total of 35 participants. We determined that if no more than 4 of the 35 participants had evidence of subclinical TB, then the true rate of abnormalities in this population was unlikely to be greater than 27% (95% binomial exact confidence intervals are 0.03 and 0.27).

Chest radiograph (CXR) reading. Chest radiographs were all performed using a digital X-ray machine (Delft Oldeca DR or Phillips Essenta DR) and captured posterior–anteriorly with the participant standing in full inspiration. The digital CXR images were viewed on 2-megapixel screens using the OsirIX version 3.8.1 32-bit software package (Pixmeo, Bernex, Switzerland) and reported independently by two medically qualified researchers blinded to clinical details. CXR were reported using a structured reporting form to ensure CXR were fully assessed for evidence of TB and then classified as consistent with active TB, inactive TB, abnormal but not consistent with TB or normal according to modified CDC criteria21. We modified these criteria by retaining calcified granuloma as evidence of inactive TB as in previous versions of the guidance. Any disagreement in reporting was resolved by consensus, which if not achieved, was resolved by a third reader.

Sputum processing for Mycobacterium tuberculosis. All sputum samples were processed in the accredited laboratories of the South African National Health Laboratory Services (NHLS) where auramine sputum smear and mycobacteria growth indicator tube (MGIT) liquid TB culture were performed. Cultures were kept for 42 d before being classified as negative. For quality control (QC) sterile mock sputa were sent weekly to the laboratory. None of 209 mock sputa sent to the NHLS laboratory for TB culture between August 2011 and June 2014 were found to be positive for MBT, demonstrating that cross contamination within this laboratory was very low. When the study began recruitment, sputum samples underwent auramine smear and MGIT culture, from 2012 MGIT culture was replaced by the GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA) nucleic acid amplification test.

Quantiferon-TB Gold-in-tube (QFN-GIT) assays. The QFN-GIT assay (Qiagen, Valencia, CA) was conducted and scored in accordance with the manufacturer’s instructions.

[18F]FDG positron emission and computed tomography (PET–CT). [18F]FDG PET–CT scans were performed at two different sites, both approximately 15 miles from the study site in Khayelitsha. Between August 2011 and May 2012 scans were performed at the Cape PET–CT center in Panorama Mediclinic using a Siemens Biograph PET–CT machine. From June 2012 to July 2013, [18F]FDG PET–CT scans were performed at the Western Cape Academic PET–CT center at Tygerberg provincial hospital using a Philips Gemini PET–CT machine. Imaging protocols were similar (PET: injected dose of [18F]FDG = 4 MBq per kg body weight (MBq/kg); CT: thorax, Kv = 120, mAs = 100, collimation = 16 × 1.5, field of view (FOV) = 660 mm, rotation time = 0.5 s, matrix = 512, slice thickness = 3 mm). All repeat imaging was performed on the same machine as the initial scan. The reporting of all images was carried out by the same group of radiologists and nuclear medicine physicians, using the same structured report irrespective of where the scan took place.

Participants undergoing [18F]FDG PET–CT fasted for 6 h before the scan and were escorted to the PET–CT center by a research worker. The PET–CT center a point-of-care assessment of blood glucose (BM) was performed to ensure that BM < 11.1 mmol/L. A POC pregnancy test was performed before scans for all female participants. Participants were prescribed 20 mg propranolol orally to minimize brown fat uptake of [18F]FDG. Thirty minutes later 4 MBq/kg of [18F]FDG was administered via a 22-G cannula. Sixty minutes after [18F]FDG administration, a PET–CT scan was performed. CT was limited to the thorax (neck to liver) to reduce radiation exposure. Total effective radiation dose per scan was approximately 10 mSv (varying with body weight and height). A second PET–CT was performed after approximately 6 months using a similar dose of [18F]FDG and an injection-to-scan time similar to that of the initial scan.

Images were reported, using a structured report focusing on the lung parenchyma and the mediastinal and hilar lymph nodes. Detailed reporting instructions were provided to all readers. The primary report of the PET–CT scan was provided by radiologists and Nuclear Medicine physicians at the Western Cape Academic PET–CT center, who blinded to clinical details. Parenchymal lesions were categorized as infiltrates, fibrotic scars, active nodules or discrete nodules according to the definitions below. ‘Infiltrate’ refers to the irregular, poorly defined airway consolidation, which may have further evidence of radiographic activity (tree-in-bud appearance, cavitation) and may display increased [18F]FDG–PET activity ([18F]FDG uptake greater than background lung). ‘Fibrotic scar’ indicates linear fibrotic or fibro-cystic abnormalities that slightly distort the surrounding lung tissue, which may have no radiographic signs of activity and may be calcified with only minimal [18F]FDG uptake.
'Active nodule' refers to the small, spherical, non-calcified opacities within the lung parenchyma, with poorly defined borders and that may have evidence of increased \([^{18}F]FDG\)-PET activity. 'Discrete nodule' refers to the small, spherical opacities present within the lung parenchyma, with clearly defined borders and no radiographic signs of activity, and which may be calcified with only minimal \([^{18}F]FDG\) uptake. The size and location of the lesions (both the lobar location and the relationship to pleura and secondary lobule) were described. The lesion were also evaluated for radiographic signs of disease activity (for example, cavitation, tree-in-bud appearance and poorly defined margins). \([^{18}F]FDG\) uptake within the parenchyma lesions was quantified by the maximal standardized uptake value (SUV\text{max}) and the visual score (VS) (VS = 0, no visible uptake of \([^{18}F]FDG\); VS = 1, \([^{18}F]FDG\) uptake within lesion greater than background lung parenchyma but less than that of mediastinal blood pool; VS = 2, \([^{18}F]FDG\) uptake within lesion greater than that of mediastinal blood pool but less than that of liver; VS = 3, \([^{18}F]FDG\) uptake within lesion greater than that of liver). Parenchymal lesions were considered to have abnormal \([^{18}F]FDG\) uptake for VS \(\geq 2\). SUV\text{max} and VS of mediastinal and hilar lymph nodes was assessed, and \([^{18}F]FDG\) uptake was considered abnormal for VS \(\geq 2\). Mediastinal and hilar lymph nodes were also assessed for size (considered abnormal for short axis widths >1 cm) and for evidence of mineralization. Abnormal lymph nodes were placed into one of the following lymph node basins following the convention of the International Association for Study of Lung Cancer (IASLC): right or left superior mediastinal (IASLC 2–4), aortic (IASLC 5 and 6), inferior mediastinal/subcarinal (IASLC 7–9), right or left hilar (IASLC 10–14). In addition to the mediastinal and hilar lymph nodes, any abnormal uptake within cervical lymph nodes, axillary lymph nodes, upper abdominal lymph nodes and the thymus were noted, as well as any other pathological abnormalities between the mandible and the base of the liver. All PET–CT scans were reviewed by a second reader, and any disagreement was resolved by a third reader.

Categorization of participants. As described above, the primary reporting and final classification of parenchymal lesions (infiltrates, scars and nodules) that were found on the \([^{18}F]FDG\) PET–CT image, and assessment of their radiographic and metabolic activity, were performed by nuclear medicine physicians and radiologists who were blinded to the clinical details of participants, unaware of clinical outcome, and not involved in laboratory components of the study or in data analysis. The summary maps of the different lesion types (infiltrates, scars, active nodules and discrete nodules) were created to identify patterns of spatial distribution for lesion types across the participants. They were created to be anatomically accurate as follows. First an outline of each lesion of interest on the CT image was digitally propagated in the coronal plane, and the location of the lesion was then captured at the level of the carina using OsiriX software. The location of each lesion was then digitally traced onto a representative CXR, anchored by anatomical reference points. A similar approach was used to create maps in the axial plane.

The radiographic abnormalities were then interpreted in relation to the pathology described in historical autopsy studies that were identified through literature searches, author collections and cross-referencing. We sought to identify studies published in English that were conducted in the pre-chemotherapeutic era as a systematic evaluation pathology relating to tuberculous infection and to early or minimal disease in persons who predominantly died of causes other than tuberculosis. Hence these were applicable to our imaging findings. Two studies were identified that provided descriptions of different lesion types and their detailed anatomical location, had a clear methodology and were primarily used to interpret our radiographic findings, along with those of a third study that focused solely on the anatomical distribution of primary lesions.

Infiltrates were determined to be consistent with reactivation and bronchogenic spread of TB, with scarring representing spontaneous healing. Active nodules were determined to be consistent with the hematogenous spread of TB. Participants with these three lesion types were considered to have evidence of subclinical disease. Discrete nodules were determined to be anatomically consistent with the Ghon foci of primary infection, and participants in whom these were the only parenchymal abnormality, in addition to those with normal lung parenchyma, were considered to have latent TB with no evidence of subclinical pathology.

Statistical analysis. Statistical analysis and data visualization were conducted in Stata ver. 12.1 (StataCorp) and Prism ver. 5.0a (GraphPad software). Gaussian distribution of data was determined by the Shapiro–Wilks test, and variance was compared by the F-test or Bartlett’s test. Nonparametric data was compared using the Mann–Whitney U test, and parametric data was compared by using the t-test. Proportions were compared by the \(\chi^2\) test or by Fisher’s exact test (if the contingency included a number \(\leq 5\)). Survival analysis was conducted by the log-rank test.

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