In Vivo Prediction of Tuberculosis-Associated Cavity Formation in Rabbits

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The presence of cavitary lesions in patients with tuberculosis poses a significant clinical concern due to the risk of infectivity and the risk of antibiotic treatment failure. We describe 2 algorithms that use noninvasive positron emission tomography (PET) and computed tomography (CT) to predict the development of cavitary lesions in rabbits. Analysis of the PET region of interest predicted cavitary disease with 100% sensitivity and 76% specificity, and analysis of the CT region of interest predicted cavitary disease with 83.3% sensitivity and 76.9% specificity. Our results show that restricting our analysis to regions with high [18F]-fluorodeoxyglucose uptake provided the best combination of sensitivity and specificity.

Key words. CT; [18F]-FDG; PET; tuberculosis.

Mycobacterium tuberculosis is the primary etiological agent of tuberculosis in humans and was responsible for an estimated 1.4 million deaths in 2011 [1]. The initial infection is usually cleared or otherwise contained by the host immune system in a granuloma. One hypothesis is that cavities evolve when solid caseous necrotic granulomas liquefy [2]. Cavities are a risk factor for disease transmission [3]. The tissue destruction that results from cavitation contributes to the morbidity and mortality of tuberculosis [2, 4, 5]. Cavitary disease is also an indicator of treatment failure and disease relapse [6].

There are no clinical tests that are designed to assay the risk of cavitary lesion development [7]. Imaging markers provide an attractive option because of their benefit of providing a real-time, noninvasive tool. Additionally, noninvasive imaging tests allow for monitoring disease progression within a patient over time.

We used a rabbit cavitary model of tuberculosis to develop imaging markers predictive of cavitary lesion development. In this study, we demonstrate that, although inflammation, as measured by [18F]-fluorodeoxyglucose ([18F]-FDG) uptake, does not positively correlate with cavitary disease, changes in lung density, as measured by CT, are predictive of cavitary lesion development. We believe this novel method can be used as a noninvasive tool to analyze the progression of tuberculosis cavitary lesions. Such imaging biomarkers could shorten the time and cost of tuberculosis drug trials and are valuable in evaluating therapeutics that target the cavitation process.

MATERIALS AND METHODS

Ethics Statement
All animal experiments were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, and all procedures were approved by the Johns Hopkins University Animal Care and Use Committee.

Modified Rabbit Cavitary Model
Sensitization and infection of New Zealand White female rabbits were done as previously described [8]. Briefly, rabbits received 5 separate injections of 10⁸ colony-forming units (CFUs) of heat-killed Mycobacterium bovis Ravenel. Twenty-five days after the final injection, the animals were given a skin test (purified protein derivative) to measure hypersensitivity. Positivity was defined as any measurable induration. Animals for which the skin test revealed no conversion were still included in this study. Following receipt of the skin test results, rabbits were challenged with 10⁴ live M. tuberculosis H37Rv bacilli. The bacterial suspension was delivered to the right lower lung lobe by bronchoscopy. Inoculum dose was determined by plating the inoculum on 7H11 selective plates.

[18F]-FDG PET/CT Imaging
Rabbits were anesthetized with ketamine (20 mg/kg), xylazine (5 mg/kg), and acepromazine (10 mg). Animals were maintained under 3 L/min O₂ and 1% isoflurane for the duration...
of the imaging. Because the imaging facility is located in a biosafety level 2 facility, the infected animals were placed into a biosafety imaging chamber (Mediso). This chamber allowed for the filtration of gas exchange and for the safe transport of the animal. A total of 2 mCi of [18F]-FDG was administered into the marginal ear vein. PET images were acquired on a Philips Mosaic PET scanner. Forty-five minutes after injection, PET data were acquired by a 30-minute static scan. CT images were obtained with a clinical 8-slice Ceretom CT (Neurológica) scanner.

**Imaging Processing, Segmentation, Coregistration, and Regions of Interest (ROIs)**

An overview of the image processing is summarized in Supplementary Figure 1. Fiducial markers were used to coregister PET images to the CT images. A group of 5 thin-walled polymerase chain reaction (PCR) tubes containing 5 μCi of [18F]-FDG were used as the fiducial markers. Rigid image coregistration was done using AMIRA (Visualization Science Group). Image segmentation was done using both AMIRA and the ANNOTATION TOOL (National Institutes of Health) software packages. Methods for image segmentation accorded with those described by Bagci et al [9]. Briefly, lung segmentation was conducted by an adaptive region growing algorithm in which the user-defined voxels were labeled as “lung” or “nonlung” regions [10]. Explicitly labeled voxels are used to determine the status of the unlabeled voxels. Furthermore, manual interaction for refinement of dense pathological regions within the lung was also possible, using the brush tools or random walk region segmentation algorithm [9, 11]. The segmented lung regions were then converted into binary masks such that nonlung regions were removed from the raw images. PET ROIs were defined by thresholding the top fifth percentile of [18F]-FDG uptake. CT ROIs were defined as being between −200 and 200 Hounsfield units (HU).

**Statistical Analyses**

Statistical analysis was done using PRISM (GraphPad Software). For analysis that consisted of comparing multiple time points, statistical significance was determined by a repeated-measures 1-way analysis of variance with a Bonferroni multiple comparison test. For pairwise analysis, a 2-tailed, unpaired Student’s t test was done. P values of >.05 were considered nonsignificant.

**RESULTS**

**Rabbit Model of Cavitary Disease Training Set**

Twelve rabbits were presensitized and infected with 10^4 CFUs of *M. tuberculosis* H37Rv. Rabbits underwent PET and CT at the following time points: before infection and on days 14, 21, 28, 35, 70, and 140 after infection. In total 114 image sets, comprised of 57 PET and 57 CT data sets, were collected and analyzed. Cavitary progressive disease was defined by visual assessment of a cavity structure during necropsy or observing a region with density of less than −900 HU as measured by CT. Animals in which no cavitary disease was observed were labeled as having noncavitary progressive disease.

**PET and CT ROIs**

Raw PET and CT images were acquired, coregistered, and then segmented. The PET ROI was defined as a global threshold of the top fifth percentile of [18F]-FDG uptake, and the CT ROI was defined by the density range of −200 to 200 HU. The PET and CT ROIs were applied to the segmented PET and CT data sets, respectively (Supplementary Figure 1). The PET ROI can be summarized as defining a region with high inflammation, and the CT ROI can be summarized as defining a consolidated region. Both regions identify abnormalities that can be interpreted as signs of disease. A summary of the main types of pathology that are visualized by CT are summarized in Figure 1A. Because PET and CT images have been coregistered, application of the ROI is not restricted to the data set used to generate the ROI. For example the spatial volume defined by the PET ROI can be applied to the CT image set and also the PET data set.

**Changes in Lung Density During Disease Progression**

Progression of active tuberculosis in the rabbit model leads to structural changes, such as fibrosis, that can be measured as an increase in lung tissue density (Figure 1B). A significant difference (P = .0038) was found when comparing the lung density distribution in cavitary progressive animals at the week of cavitation versus the week prior to cavitation (Figure 1B). A significant difference was also observed when measuring the density distribution at the week of cavitation, compared with the week prior to cavitation, using the PET ROI (2-tailed unpaired t test; Figure 1C). The density distribution of cavitary progressing animals did show an increasing trend in the 0:100 HU domain in the CT ROI (Figure 1D).

**Defining an Imaging Marker of Cavitation**

We observed that the shifts in the density distribution from the PET ROI produced a peak in the −200 to 200 HU region (Figure 1B–D). It was also observed that a significant increase (P = .0003) in lung density in range of −200 to 200 HU occurs after infection (Supplementary Figure 2A). There was a significant difference (P = .0001) between cavitary progressing and noncavitary progressing animals when measuring the percentage of lung within the range of −200 to 200 HU (Supplementary Figure 2B).

**Assessment of the Predictive Power of the Imaging Markers, Using the Test Set**

We developed 2 methods for predicting cavitary lesion progression, using the data we collected from the training set series of
animals. The methods quantify the density distribution, as measured by CT, using differently defined ROIs. The area under curve (AUC) cutoffs of >90 and >115 for the PET and CT ROIs, respectively, were positive predictors for the development of cavitary disease. These criteria were designed to provide the best combination of both sensitivity and specificity. Images defined as positive were the week of cavitation and the week prior to cavitation. Therefore, the training set consisted of 10 positive imaging sets and 47 negative imaging sets (Table 1).

To validate the usefulness of these methods, these predictive criteria were applied to a new test set of rabbits. The test set was subjected to the same infection conditions as the training set group. Three of 4 animals developed cavitary disease from the test set. The test set was imaged before infection and 1, 2, 3, and 5 weeks after infection and consisted of 6 positive images and 13 negative images (the week 5 image from 1 rabbit was not used because it was obtained 2 weeks after cavitation occurred). Use of the PET ROI predicted cavitary disease with 100% sensitivity

Table 1. Sensitivity and Specificity of Cavitary Predictive Radiology Markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Training Set</th>
<th>Test Set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region of interest</td>
<td>PET  CT</td>
<td>PET  CT</td>
</tr>
<tr>
<td>Area under the curve</td>
<td>90  115</td>
<td>90  115</td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>90  80</td>
<td>100  83.333</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>78.182  72.727</td>
<td>76.923  76.923</td>
</tr>
</tbody>
</table>

Use of the PET ROI outperformed the CT ROI.

Abbreviations: CT, computed tomography; PET, positron emission tomography; ROI, regions of interest.

a There were 10 true positive and 55 true negatives.

b There were 6 true positives and 13 true negatives.
DISCUSSION

Tuberculosis biomarkers for monitoring disease outcome are urgently needed. The use of imaging biomarkers could have an immediate impact in a clinical trial setting in which resources are more abundant. At present, the cost of clinical trials for new tuberculosis vaccines and drugs is staggeringly high because of poor biomarkers. Identification of robust biomarkers to monitor treatment outcome could dramatically reduce both the financial cost and study duration needed to evaluate new therapeutics [7]. These technologies may also have value in clinical care and management as costs decline. This tool can also be used for reducing the number of animals used for studies and also for mitigating animal suffering. The number of animals used for studies can be reduced because the noninvasive imaging techniques described do not require animals to be euthanized at each time point.

The improved sensitivity of predicting cavitary disease for the PET ROI, compared with the CT ROI, is likely due to a positive correlation between $[^{18}\text{F}]-\text{FDG}$ uptake and inflammation. Inflammation results in the release of enzymes capable of remodeling the extracellular matrix. The role of proteases and collagenases and their necessity for producing the disease pathology typically observed in tuberculosis has previously been reported [2, 4, 5].

It is important to note that increasing uptake of $[^{18}\text{F}]-\text{FDG}$ was observed during disease progression, consistent with findings from recent studies [12–14]. These studies established that $[^{18}\text{F}]-\text{FDG}$ uptake is correlated with CFU burden. While it has been reported that cavities provide an environment for high bacterial burden, it is unknown whether a high bacterial burden is a necessary prerequisite for cavitation formation [2, 8, 15]. It is plausible that the CFU burden prior to cavitation was similar between cavitary and noncavitary groups, and therefore there was a similar uptake of $[^{18}\text{F}]-\text{FDG}$. The density region that we observed to be increased during the course of disease progression was also independently reported in a marmoset model recently published by Via et al [12]. This suggests that the density region that we identified in this study may be applicable in model animals other than rabbits.

Limitations of the proposed study were that the predictive power of the algorithm is only 1 week at present. Lin et al [14] reported a similar lung density increase in $M.~\text{tuberculosis}$-infected nonhuman primates. While we are optimistic that our algorithm can be extended to other species beyond rabbits, this has not been demonstrated. Noninvasive tests, such as sputum smear microscopy, interferon-γ release assays, and quantification of lung matrix and break down products, could be integrated into a multi-biomarker disease activity (MBDA) matrix for risk assessment. Similar approaches have been done for rheumatoid arthritis. This tool could be used to advance the understanding of cavity lesion development by identifying precavitary lesions.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes


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References