Use of 18F-FDG PET/CT for Imaging Extrapulmonary Tuberculosis Patients

SUMMARY

Radionuclide imaging of infection has for long time relied almost exclusively on single-photon-emitting agents, evolving from early applications of $^{67}$Ga-citrate scintigraphy to scintigraphy with autologous leukocytes, labelled either directly (by in vitro incubation with agents such as $^{111}$In-Oxine or $^{99m}$Tc-HMPAO before reinfusion) or indirectly (e.g., administering radiolabeled antibodies binding to surface antigens expressed by granulocytes) (1). The latest entry for radionuclide imaging of infection in the clinical setting is represented by PET with $^{18}$F-FDG, based on nonspecific enhanced glucose consumption of inflammatory cells and/or growing bacteria at the site(s) of infection (1). Nearly 11% of all deaths from infectious diseases are caused by tuberculosis (TB) (2). Over 95% of TB deaths occur in low- and middle-income Member States, where TB is among the top three causes of death for women in reproductive age. This disease has become or is becoming a medical emergency not only in developing Member States but also in some high-income countries, because of migration of people from low-income to higher-income areas, because of frequent co-infection with HIV/AIDS, and because of the development of drug-resistant strains of TB (3).
Aim of this study is to engage Member States in a coordinated research project to develop a comprehensive TB imaging strategy through the use of 18F-FDG PET/CT for imaging TB patients with initially special focus on (a) extrapulmonary TB and its treatment initially, and subsequently on (b) drug resistant TB and its treatment in order to reduce rates and deaths from TB especially drug resistant TB. This will contribute to the overall improvement of health care by accurate and early diagnosis of intractable TB infections in high risk patients through a multicentre imaging trial using 18F-FDG PET/CT by increasing the detection rate of TB.

SCIENTIFIC BACKGROUND

The worldwide impact of infectious diseases on healthcare is impressive: every year 13 million people die because of infectious disease, and most of these deaths occur in developing countries. If considering only lower respiratory infections (including pneumonia), HIV/AIDS, and malaria, the overall healthcare costs amount to US $343.9 billion worldwide, ranking third immediately after the healthcare costs for cancer and heart diseases.

Overall, infectious disease constitutes a big burden to healthcare systems not only because of the direct costs related to treatments, but also in terms of parameters describing the overall economic and social burden deriving from associated disabilities and chronic debilitating illnesses, such as the disability-adjusted life years and the health-adjusted life expectancy. The top infectious diseases causing deaths worldwide are lower respiratory infections (including pneumonia and influenza), chronic obstructive pulmonary disease, diarrheal diseases, HIV/AIDS, and tuberculosis (TB). While the impact of radionuclide imaging in the first four such infectious conditions is rather limited, special attention should be paid to the potential role of nuclear medicine imaging in certain stages of TB infection. Such role is not to be seen in the diagnostic approach to TB, but rather in subsequent stages, for characterization of the disease and for assessing its response to therapy.

Nearly 11% of all deaths from infectious diseases are caused by tuberculosis (TB). Over 95% of TB deaths occur in low- and middle-income Member States, where TB is among the top three causes of death for women in reproductive age. This disease has become or is becoming a medical emergency not only in developing Member States but also in some high-income countries, because of migration of people from low-income to higher-income areas, because of frequent co-infection with HIV/AIDS, and because of the development of drug-resistant strains of TB (3). For instance, in the UK drug-resistant TB has increased by 26% in the last year alone, and London is now considered the “TB capital of Western Europe” (4).

While recent efforts have resulted in a global decline in TB incidence and mortality, the number of individuals infected with drug-resistant isolates continues to increase, presenting a serious global health threat. Both MDR-TB, defined by resistance to both first-line drugs isoniazid and rifampin, and XDR-TB, which in addition to the first-line drug resistance seen in MDR, is resistant to any fluoroquinolone and at least 1 second line injectable drug, are already widespread around the world. Globally, 3.7% of new TB cases—there were an estimated 8.7 million new cases of TB in 2011—and 20% of previously treated TB cases are MDR-TB, according to the World Health
Organization (WHO) (2).

Based on the above considerations and in particular low- to middle-income countries, the experts identified complicated TB infection (i.e., multidrug-resistant, extreme multidrug-resistant, and extra-pulmonary TB) as one of the target areas with potential considerable impact from radionuclide imaging.

TB-Related investigations

Although early diagnosis is crucial for optimized treatments, radionuclide imaging has limited role in the diagnostic approach to TB; in fact, diagnosis of TB is typically based on a combination of clinical data, in vitro diagnostics and radiological imaging.

Whereas, there are still some open issues from the clinical point of view concerning at least three important aspects of TB infections:

1) Distinguishing active from inactive disease,
2) Assessing the efficacy of therapy, and
3) Identifying a prognostic parameter as to the development of drug-resistant TB.

These issues have not been properly addressed even with the most advanced biomarker-based approaches (5). Nevertheless, the panel recognizes that additional comments by clinical experts in the field of TB infection should be sought to confirm these considerations and to advise on optimal design of clinical protocols.

Identification of TB as a target disease for radionuclide imaging of infection implies some important considerations concerning the potential candidate agents for implementing such modality. In fact, TB is typically a pauci-bacillary, slow-evolving infection; moreover, these pathogens are located intracellularly, i.e., in a space poorly accessible to most imaging agents. Therefore, some of the radiolabeled agents that image infection through their binding to actively growing bacteria in the extracellular space (such as, e.g., antimicrobial agents) are not expected to yield reliable results in patients with TB infection. Furthermore, TB lesions are typically constituted by inflammatory cells of the chronic phase, such as T-cells/monocytes/macrophages rather than by cells of the acute/sub-acute phase of infection, such as neutrophils. As a consequence, scintigraphy with autologous radiolabeled leukocytes (a mixed granulocyte population) is sub-optimal for identification and characterization of TB lesions.

There is scant data published concerning the assessment of response of TB infection to therapy based on radionuclide imaging. In this regard, although scintigraphy with the nonspecific cell-activation agent Tc-99m Sestamibi has been reported to be useful to distinguish active from inactive pulmonary TB, its use has not been explored to assess response to therapy (6-7).

Preliminary data obtained at the University of Pretoria (South Africa) suggest that assessment of response to anti-TB therapy would be possible through serial 18F-FDG PET/CT scans, although the optimal schedule for such sequence has yet to be defined (8). Currently, the above two clinical questions remain to be clarified. Therefore, the most promising option for evaluation of response to anti-TB therapy is to use two
commercially available radiopharmaceuticals, one for single-photon and the other for PET imaging, respectively.

In addition, wide discussion among the experts was devoted to consider the possibility of developing new agents tailored to this specific purpose, based on current knowledge and advances in the fields of radiochemistry and radiopharmacy, considering the cellular pathophysiology of TB infection. However the widely used radiopharmaceutical $^{18}$F-FDG could be an ideal tracer for active detection, therapy response, identification of complicated TB infection (i.e., multidrug-resistant, extreme multidrug-resistant, and extra-pulmonary TB).

Alternative approaches to target bacterial load directly were also considered through the utilisation of novel metabolic pathways specific to bacteria. For example, several sugar uptake systems present within the cell wall of bacterial cells are absent from the cell surface of mammalian cells, such as for the uptake of phosphorylated sugars. Labelling these sugars for SPECT or PET imaging could alleviate some concerns associated with non-specific identification of inflammatory cells, and remove the need for ex vivo patient WBC manipulations.

Besides the single-photon imaging agent, $^{99m}$Tc-Sestamibi, the natural antimicrobial agent Ubiquicidin labeled with $^{99m}$Tc has attracted attention as an overall infection imaging agent (9). Nevertheless, no reports have described the use of this agent in patients with TB infection, an option that deserves further investigation despite the fact that $^{99m}$Tc-Ubiquicidin belongs to a class of agents that are assumed to target bacteria and might therefore not be suitable for imaging TB lesions. On the other hand, preliminary investigations should demonstrate the ability of $^{99m}$Tc-Ubiquicidin scintigraphy to depict active TB infection, this would translate also in the possibility of labeling Ubiquicidin with $^{68}$Ga, provided that a suitable chelator is attached to this peptide. The promising results from University of Pretoria have shown that the biodistribution of $^{68}$Ga- NOTA-UBI30-41 in rabbits infected with S. aureus demonstrates increased tracer uptake in infected muscles when compared with healthy and inflamed muscles. This increased accumulation in infected muscles therefore supports an argument for a mechanism that, at least partly, involves bacteria-specific binding. This also correlated well with the in-vitro results, which demonstrated binding to bacteria (UBI). Clinical investigators would thus be placed in the unique position whereby the same imaging vector (Ubiquicidin) would be available in two different forms ($^{99m}$Tc-Chelator-Ubiquicidin and $^{68}$Ga-Chelator-Ubiquicidin) according to local availability of single-photon or PET imaging facilities, respectively.

$^{18}$F-FDG

$^{18}$F-FDG is known to accumulate at sites of infection, inflammation and in autoimmune and granulomatous diseases. The inflammatory cells produce an excess of glycolytic enzymes and also over express glucose transporter (GLUT) isotypes (mainly GLUT-1 and GLUT-3) (10).
PET can diagnose a variety of infections with a fairly high degree of certainty. Just a few examples include large-vessel vasculitis, abdominal infections such as inflammatory bowel disease, thoracic and soft-tissue infection. It is also useful in tumor induced fever, secondary to Hodgkin’s disease, aggressive non-Hodgkin’s lymphoma, colorectal cancer and sarcoma. In patients with fever of unknown origin (FUO), in vitro or in vivo labelled WBC methods are of limited value because of the rather low prevalence of granulocytic processes in this clinical setting (11).

Various in vitro studies of $^{18}$F-labelling of human leukocytes using mixed/pure preparations of neutrophils and mononuclear cells have been reported. Granulocyte uptake accounted for 78%–87% of the activity in mixed preparations. Labelling of WBCs with $^{18}$F-FDG was not stable and the labelling yield ranged from 40% to 80% when pure preparations of WBC were used. Neutrophils when stimulated for 60 min by N-formyl-methionyl-leucyl-phenylalanine, a chemotactic peptide, revealed a significant increase in $^{18}$ F-FDG uptake (12-15).

A comprehensive TB imaging strategy, led by IAEA, should be developed to seize the opportunity this collaboration presents to reduce rates and deaths from TB.
STUDY AIMS

Use of $^{18}$F-FDG PET/CT for imaging extrapulmonary TB in a tertiary cohort of patients

(A) To detect the extent of disease
(B) To assess its utility as a biomarker to monitor response to therapy

OUTCOME MEASURES

1- Map abnormal sites of FDG uptake: Incidence of organ involvement, sites and pattern (of extrapulmonary lesions only and Pulmonary & extrapulmonary)
2- Its impact on clinical management decisions (Change/No Change)
3- To monitor change in SUV$_{max}$, number and size of lesions using baseline, 2 months and end-of-therapy scans to predict and assess response to therapy.

METHODS

A. GENERAL CONSIDERATIONS

Recruit and qualify centers for study (IAEA will delegate the centers based on facilities: Name of all centres to add

1. The above centers selected will enroll the patients
2. Each center should enroll a minimum of 20 patients/year
3. A baseline scan (within 1 week of referral), an interval scan at 2 months and an end of treatment scan (done within 2 weeks of treatment)

PET/CT must represent local practice prior to the study initiation.
4. Prospective core lab analysis is based on retrospectively collected data:
5. Informed consent will be determined by the local institutional ethics committee.

6. Core lab for central review of images and collection of data forms will be established according to IAEA regulations.

B. CORE LAB REGULATIONS & REQUIREMENTS

1. Standardized approach to obtaining isolates for culture.

2. Regulations
   a. Core lab central review member site should not recruit patients
   b. All anonymous data will reside digitally and will be kept according to IAEA regulations

3. Requirements for enrolled institutions
   a. All submitted data will be anonymous
   b. Institutions will be required to pre-submit 3 representative paired studies (pre-therapy and early response study) for evaluation of image quality prior to being accepted.
   c. DICOM images for PET/CT must be submitted and must at a minimum include the following data
      i. net administered activity
      ii. blood glucose
      iii. time of injection and start of imaging
      iv. patient weight and height

C. PATIENT INCLUSION CRITERIA

1. Patient age must be greater than 18 years of age at the time of first PET/CT.

2. Patients fulfilling WHO criteria (see attached appendix) for extrapulmonary TB
EXCLUSION CRITERIA

1. Pregnant and lactating patients
2. Recent history of cancer and/or chemo/radiation therapy
3. Subjects with pan resistant isolates
4. Terminal illness and impending mortality.
5. Blood glucose levels greater than guideline (SNM/EANM/IAEA)
6. Drugs: Systemic investigational drugs
7. Retro-viral +ve pts
8. Any condition that the investigator believes would warrant exclusion.
9. 

D. DATA INTERPRETATION AND ANALYSIS

a) Demographic
b) % of +ve and –ve baseline scans
c) Impact of baseline PET/CT scan on clinical management
d) Predictive power of PET/CT to determine therapy response
e) Comparison of PET/CT interpretation between institutions and core lab for each patient

F. SPECIMENS TYPES

a. Minimum Set:
   Sputum/smear/aspirate/histology for AFB status (WHO based grading),
   Culture status on solid and liquid media

G. Timeline, Clinical Assessment: Baseline, 2 months and end of treatment
PATIENT DATA AND PET/CT TECHNICAL ASPECTS

1. A patient data sheet must be completed (electronic form) with demographic:
   - Age (yrs)
   - Sex
   - Ethnicity
   - Geographic location (Town, State, Country)
   - Date of study

3. Standardized patient therapy care according to WHO guidelines (see guidelines in Appendix)

4. Documenting extent of disease/outcome at:
   - baseline,
   - 2 months (time of 2nd PET scan)
   - End-of Treatment

TB treatment (centres to document which regimen patient was under) Otherwise we may have a mixture. E.g.

Group 1: First-line oral agents – isoniazid, rifampicin, ethambutol, and pyrazinamide
7. Standardized $^{18}$F-FDG PET/CT acquisition according to the following criteria:
   a. SNM guidelines
   b. EANM guidelines
   c. IAEA PET/CT – Standardized Operating Procedure guidelines
8. Acquisition parameters of the PET component at baseline PET/CT and interim PET/CT must be equal (see attached SOP)
   a. $^{18}$F-FDG PET/CT interpretation criteria
   b. Quantitative assessment:
      i. SUV max
      ii. Lean body mass (LBM)
      iii. BSA (body surface area)
      iv. BW (body weight)
1. **Measurements**

PET/CT details of scanning  
Lab description in brief  
Histology description in brief

2. **Data Analysis**

Statistics to be finalized at the first meeting

Sample size calculation for PET TB study (by Dr. Vera Mann)

Sample size for diagnostic value of PET (i.e. to be able to detect extrapulmonary TB).

I used the formula to calculate number size for desired sensitivity of a diagnostic test (Buderer NM Acad Emerg Med. 1996 Sep; 3(9):895-900).

\[
N = \frac{Z_{1-\alpha/2}^2 \cdot S_N \cdot (1-S_N)}{L^2 \cdot \text{Prevalence}}
\]

where:  

- \( N \) is the required sample size,  
- \( S_N \) is the anticipated sensitivity,  
- \( Z_{1-\alpha/2} \) is the standard normal deviate corresponding to the specified size of the critical region, and  
- \( L \) is the absolute precision desired on either side (half-width of the confidence interval) of sensitivity.

In my calculation I used 0.05 for \( \alpha \), 0.05 for \( L \) and assumed a desired sensitivity of 95% or 90%.

As the prevalence of extrapulmonary TB is 21% we can use the 30% prevalence (nearest figure) to do the calculation.

Updated sample size calculation when only extra-pulmonary TB patients are included

Compare desired detection proportion to proportion 1 (all patients have extra-pulmonary TB) with taking into account the multi-centre design (DEFF)
<table>
<thead>
<tr>
<th>Prevalence of XPTB in study</th>
<th>Desired % detection with PET</th>
<th>Total N needed</th>
<th>Number per centre (9 centres)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>90</td>
<td>15</td>
<td>243</td>
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<tr>
<td>1</td>
<td>95</td>
<td>32</td>
<td>519</td>
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DEFF = 1+(m-1)ICC = 16.2

m = 20

ICC = \( \frac{SD_b^2}{SD_b^2 + SD_w^2} \) = \( \frac{0.1^2}{0.1^2 + 0.05^2} \)
3. **Ethics**

This Protocol will be submitted to the Faculty of Health Sciences Research Ethics Committee, University of XXXX for approval. The study has been structured in accordance with the Declaration of Helsinki which deals with the recommendations guiding doctors in biomedical research involving human subjects.
### BUDGET

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<thead>
<tr>
<th>Item Description</th>
<th>Cost</th>
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<tbody>
<tr>
<td>FDG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stationary, printing toner, Phone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lab (all results), XXX</td>
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<td></td>
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<tr>
<td>Total Project Cost</td>
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### TIME LINES

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<th>Number</th>
<th>Task</th>
<th>Start</th>
<th>End</th>
<th>Duration (days)</th>
</tr>
</thead>
</table>
REFERENCES


World Health Organization (WHO). (2012). Global Tuberculosis Control. ISBN:


APPENDIX:
1. WHO DOTS programme document
2. SOP (FDG-PET/CT imaging)
3. CRF
4. Patient information sheet/Consent form (locally arranged in patient friendly language)