METABOLIC IMAGING OF INFECTION

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Abstract

Metabolic imaging of infection has come to occupy a prominent place in the diagnosis and management of microbial infection. Molecular probes available for infection imaging have undergone a rapid evolution starting with the use of non-specific agents that accumulate similarly in infection, sterile inflammation and neoplastic tissue to more targeted probes that seek to identify specific microbial species. This focus review describes the metabolic and molecular imaging techniques currently available for clinical use in infection imaging and those that have demonstrated promising results in preclinical studies with the potential for clinical applications.

Introduction

The diagnosis and treatment of infection remains challenging in clinical practice. The gold standard for diagnosis is by culture of a specimen obtained from the infection site. Obtaining a sample is often difficult because the site of infection may be unknown or biopsy may be too invasive. Biopsy for microbiological evaluation may also be fraught with sampling error leading to false negative results. Imaging is therefore often necessary in view of these challenges. Anatomic imaging with radiological techniques evaluates structural changes in tissue architecture in the assessment of infection. These anatomic changes only become apparent when the infectious process is advanced and tissue damage has occurred. Similarly, restoration of tissue architecture lags behind successful treatment of infection making anatomic imaging less ideal for early diagnosis and therapy response assessment.

Molecular imaging of infection has been the focus of many human studies resulting in the introduction of a wide variety of molecular probes in the clinical or preclinical setting for imaging infection.

Infection Imaging: The Inflammatory Cascade

The presence of an infectious organism in the tissue elicits a series of vascular and cellular responses termed inflammation. In response to the presence of microbes, the regional microcirculation undergoes a series of changes aimed at delivering elements of the immune system to the infection site. The vascular events include vasodilation and increased vascular permeability to allow leucocytes and acute phase proteins access the infection site. In the cellular events, leucocytes are attracted to site of infection in large numbers in response to

endogenous and exogenous (bacterial products) chemoattractants. Leucocytes are activated at the site of infection and they increase the oxygen and energy substrate utilization.

The different steps of this inflammatory cascade have been explored in the development of molecular probes for metabolic imaging of infection. The objectives of molecular imaging of infection include non-invasive and early diagnosis of infection, differentiation of infection from sterile inflammation, monitoring response to antimicrobial therapy, prognostication and ability to identify the offending agent without the need for the often difficult, time-consuming and sometimes unsuccessful process required to obtain samples and culture the microbe *(1)*.

Imaging Leucocyte Migration: Radiolabeled White Cells

Single photon emission computed tomography (SPECT) imaging of radiolabeled leucocyte with Indium- 111 oxime (In-111) or Tc-99m Hexamethylpropyleneamine oxime is a useful molecular imaging modality in clinical practice.

Radiolabeled leucocyte imaging is the modality of choice in imaging skeletal infection. Triple phase bone scintigraphy provides sufficient sensitivity and specificity for diagnosing infection in unviolated bones. The specificity of bone scan drops significantly in violated bones such as following trauma or surgery. Radiolabeled leucocyte imaging either alone or in combination with marrow imaging provides high sensitivity and specificity above 90% for diagnosing skeletal infection in this setting *(2,3)*.

Infection of cardiovascular implantable devices is rare. Its occurrence is however associated with high morbidity and mortality *(4)*. Radiolabeled leucocyte SPECT/CT imaging

provides a highly specific molecular imaging modality for diagnosing infection in this critical condition *(5)*. The success with molecular imaging techniques of cardiac devices has led to the inclusion of positive finding on these modalities as a minor criteria in the modified Duke Criteria for the diagnosis of endocarditis by the European Cardiac Society *(6)*.

The relatively limited resolution of SPECT imaging compared to the positron emission tomography (PET) imaging has led to the attempt at labelling white cells with F-18 FDG for PET imaging. This endeavor has been faced with challenges. The labeling efficiency is variable and is lower than what has been described for radiolabeled leukocyte with Tc-99m hexamethylpropyleneamine oxime or In-111 oxime *(7)*. Furthermore, F-18 FDG elutes from the leucocyte and the limited half-life of the radionuclide (approximately 110 minutes) makes delayed imaging impossible. Concerns also exist for radiation exposure to the cells being labelled especially the long-lived lymphocyte *(8)*.

Limitations of radiolabeled leucocyte include a variable uptake in spondylodiscites hence it is not used in the evaluation of spinal infection. There is also early diffuse accumulation of the radiolabeled leucocyte in the lungs after re-injection which limits its application in lung infection imaging. Normal level of circulating leucocytes is necessary for optimum cell labelling. Therefore, radiolabeled leucocyte imaging has limited role in evaluation of patients with febrile neutropenia. Moreover, in-vitro labelling of leucocyte is laborious, time consuming and exposes staff to blood-borne infections. These challenges have stimulated interest in in-vivo labeling of leucocyte. Sulesomab is a murine monoclonal IgG antibody fragment that binds to non-cross reacting antigen 90 on polymorphs and their precursors. Sulesomab labeled with Tc-99m

(Leucoscan®), being an antibody fragment, is able to promptly penetrate into the infectious foci for in-vivo labeling of polymorphs.

Imaging Cellular Metabolism: F-18 Fluoro Deoxy-D-Glucose (F-18 FDG)

The better resolution of PET makes it a more sensitive technique than SPECT in infection imaging. F-18 FDG accumulates at sites of infection, sterile inflammation and neoplastic tissue resulting in a relatively reduced specificity of F-18 FDG PET as an infection imaging technique. While radiolabeled leukocyte scintigraphy offers higher specificity, F-18 FDG PET imaging provides better sensitivity in the setting of infection imaging *(5)*. The addition of morphologic imaging to either technique in the form of SPECT/CT or PET/CT or more recently magnetic resonance imaging (MRI) as PET/MRI improves the diagnostic accuracy.

F-18 FDG PET/CT imaging of infection is completed within a relatively shorter time, easier to perform, requires no handling of blood and allows for easy quantification of uptake which is useful for treatment response assessment. Studies have shown clinical utility of F-18 FDG PET/CT in the evaluation of osteomyelitis. In a head-to-head comparison of F-18 FDG PET/CT and MRI in patients with suspected spondylodiscitis, FDG PET/CT outperformed MRI with a sensitivity and specificity of 96% and 95% respectively versus 67% and 84% for MRI *(9)*. In a setting of pedal osteomyelitis complicating diabetic foot syndrome, FDG PET/CT has a sensitivity, specificity and accuracy of 88.3%, 96.8% and 93.8% respectively *(10)*. In this study, elevated blood sugar level did not affect the diagnostic performance of the test.

The number of cardiac devices implanted across the world is increasing and so is infection in these devices. Cardiac device-associated infection requires urgent diagnosis and

management. It is also important to differentiate superficial wound infection from an actual device infection as both types of infections are managed differently. Whereas deep infection affecting the device requires removal of the device with its attendant risk for mortality and morbidity, superficial wound infection only requires local wound care and parenteral antibiotics (11). Clinical assessment is often not sufficient to make this distinction (12). In a group of 70 patients with inconclusive diagnosis of infective endocarditis using Duke criteria, a study applying F-18 FDG PET/CT to these patients was able to reclassify these patients as having infective endocarditis in 18 patients, no IE in 45 (13).

F-18 FDG PET/CT is highly useful in the evaluation of suspected vascular graft infection. It is also essential to differentiate a superficial wound infection from a deep infection involving the vascular graft. In a prospective study of 32 patients with suspected vascular graft infection, 12 of whom were already on antimicrobial treatment, F-18 FDG PET/CT correctly identify 27 patients with infection and ruled out infection in six patients (14). Overall, diagnostic performance of F-18 FDG PET/CT in the study using a five-point visual grading system was: Sensitivity of 100%, specificity of 86%, positive predictive value of 96%, negative predictive value of 100% and accuracy of 97%. Imaging prior to commencing antimicrobial treatment improved the diagnostic sensitivity. This was however not confirmed in a more recent study with a larger patient population (15). It is important to know that non-infected prosthetic vascular grafts also demonstrate F-18 FDG uptake. A comprehensive description of this physiologic uptake is presented in the study by Keidar et al (16).

Fever of unknown origin (FUO) is commonly a result of infection, inflammatory non-infectious conditions or malignancy. F-18 FDG accumulate in all of these conditions making FDG

PET/CT suitable for the evaluation of patients with FUO. In a recent study of 76 patients with FUO, F-18 FDG PET/CT led to the final diagnosis of infection as the cause of FUO in 21% of patients, malignancy in 22% of patients, inflammatory noninfectious diseases in 12% of patients and miscellaneous diagnoses in 5% of patients *(17)*. A 56% diagnostic yield was found for F-18 FDG PET/CT in the evaluation of FUO in a meta-analysis of 18 eligible studies including 905 patients *(18)*.

Tuberculosis is an infectious disease that has plagued the human population for many millennia. Despite extensive research into this disease, significant challenges still exist in its diagnosis, treatment and therapy response assessment *(19)*. The diagnostic gold standard for tuberculosis is by culture of the tubercle bacilli, an endeavor that is difficult to achieve due to the difficulty with obtaining the bacilli in a sputum sample or other body fluid or tissue from an infected site. In endemic regions, the diagnosis often relies on suggestive clinical findings and imaging. F-18 FDG PET/CT complements the low yield of bacteriological confirmation in the initial diagnosis of tuberculosis and follow-up (Fig. 1). In a report of 35 patients evaluated for tuberculosis, F-18 FDG PET/CT was true positive in 34 patients *(20)*. Both PET and CT components of this study were false negative in one patient with microbiologically confirmed tuberculosis. Overall, PET performed better than CT in identifying disease in 34 patients as against 23 identified by CT. Both modalities performed equally with regards to identifying pulmonary tuberculosis while PET performed better in identifying sites of extra-pulmonary disease. Identification of sites of extra-pulmonary tuberculosis involvement is crucial in management because most sites of extra-pulmonary disease require longer duration of anti-tuberculous therapy.

The tuberculosis granulomatous lesions are highly heterogeneous and undergo changes as they mature (21). They behave differently in response to treatment in the same patient. While some lesion may demonstrate response to therapy, other lesions may progress on treatment. F-18 FDG PET has been shown to be a useful modality in therapy response assessment and outperforms diagnostic CT in this setting (20). In a study of 18 patients with tuberculous lymphadenitis, F-18 FDG PET/CT correctly identified cure in nine patients (no FDG uptake on post treatment PET scan) and in two patients with treatment failure (22). A recent study showed non-reliability of clinical and microbiological methods in the assessment of cure following successful completion of anti-tuberculous therapy (23). In this study, the investigators performed an end-of-treatment F-18 FDG PET/CT in patients considered cured based on microbiology assessment and found residual or persistent F-18 FDG uptake in lesions. In these patients, persistence of microbial replication was shown by demonstrating *Mycobacterium tuberculosis* mRNA and DNA in respiratory samples obtained by bronchoalveolar lavage.

The presence of Human immunodeficiency virus (HIV) infection introduces different dynamics into tuberculosis diagnosis and management. HIV-associated immunosuppression is a common cause of reactivation of latent infection. Patients with latent tuberculosis have isoniazid for chemoprophylaxis. It is however important to identify those patients whose disease is rather subclinical and may progress to clinical tuberculosis following HIV infection. A study evaluated the role of F-18 FDG PET/CT in 35 HIV-positive, anti-retroviral therapy-naïve patients with latent tuberculosis and identified 10 patients with pulmonary findings suggestive of sub-clinical tuberculosis disease (24). Findings suggestive of subclinical tuberculosis were (1) pulmonary infiltrates and/or fibrotic scars suggestive of bronchogenic reactivation of

tuberculosis and (2) active nodules suggestive of hematogenous spread of tuberculosis. Four of 10 patients with these findings required full course of anti-tuberculous therapy within 6 months of follow-up.

Imaging Antibiotic Binding to Microbes: Radiolabeled Antimicrobial Agents

Antimicrobial peptides have been successfully radiolabeled and tested for infection imaging in animal models as well as in humans. The first radiolabel antimicrobial agent evaluated for human use was Tc-99m ciprofloxacin. Disappointing results from its application in humans led to its withdrawal from the market. Many other antibiotics including fluoroquinolones, cephalosporines and anti-tuberculous drugs have since been successfully labeled with suitable radionuclide and tested in preclinical studies *(25,26)*.

A radiolabeled antimicrobial peptide that has gained popularity in the clinic is radiolabeled Ubiquicidin-UBI (Fig. 2). UBI is a human antimicrobial peptide present in the respiratory epithelium. Its fragments have been successfully labeled with Gallium-68 for PET imaging and Tc-99m for SPECT imaging *(27)*. The basis of the use of UBI fragment – UBI 29-41 is in its ability to be attracted to the negatively charged bacterial cell wall, itself being positively charged. UBI also accumulates in activated macrophages and in colonic epithelial cells. In an analysis of the diagnostic performance of Tc-99m UBI in infection imaging from studies published between 2004 and 2010, a pooled sensitivity and specificity of 94.5% (95% CI: 91.2-96.8%) and 92.7% (95% CI: 80-100%) were reported *(28)*.

Zn-DPA, a positively charged metal complex that is attracted to the negatively charged bacterial cell wall, is another agent that has been studied in animal models of bacterial

infection. It has been coupled to In-111 for imaging and to PSVue®794 for florescent imaging in a study where both forms of Zn-DPA were found to accumulate at the site of *Streptococcus pyogenes* infection intensely but only mildly at the site of sterile inflammation *(29).* The specificity of Zn-DPA for infection imaging remains a challenge as it is also attracted to the negatively charged membrane of cells undergoing apoptosis or necrosis *(30)*.

Successful treatment of HIV infection reduces the viral load to an undetectable level. Viral replication however continues in sanctuary organs. As efforts for HIV cure continues, the need to identify persistence viral replication in aviremic patients is crucial. A study using immunoPET demonstrated the utility of 7D3-PEG-^{64}Cu-DOTA which targets the simian immunodeficiency virus envelop protein gp120 in identifying residual viral replication sanctuary sites of aviremic antiretroviral-treated monkeys *(31)*. This holds a great promise for the evaluation of the effectiveness of therapies directed at HIV cure.

Imaging Microbial Iron Metabolism: Radiolabeled Siderophores

Iron is essential for many metabolic processes including respiration in eukaryotes and many prokaryotes. Siderophores are iron-binding molecules which bacterial and fungi use to scavenge extracellular iron for their metabolism. Several siderophores exist in nature and many of them have been successfully chelated to radiometals such Ga-67 and In-111 (for SPECT imaging) or Ga-68 and Zr-89 (for PET imaging) *(32).* Ga-67 used to be popular for infection imaging. It has however fallen out of favor owing to its poor image quality, long imaging period and high radiation burden to the patients. It has been replaced by Ga-68 Citrate, a PET tracer with better image quality and favorable dosimetry properties. Vorster et al. recently described

its use in imaging infection and inflammation *(33)*. A derivative of deferoxamine was successfully labeled to Ga-67 and was shown to localize to the site of *Staphylococcus aureus* infection *(34)*.

In another study, the use of radiolabeled siderophore in animal model of invasive fungal infection due to *Aspergillus fumigatus* was investigated. Ga-68 was successfully labeled to triacetylfusarinine C (a siderophore produced by *A. fumigatus*) and ferrioxamine E *(35)*. Both agents showed good uptake at the site of pulmonary aspergillus infection with intensity of uptake correlating with severity of the infection. No abnormal uptake of either agent was seen in animals without the infection. Identification of organism-specific siderophore and its labeling with a radiometal holds promise for organism-specific molecular imaging of infection in humans.

Imaging Bacterial Specific Carbohydrate Metabolism: F-18 Fluorodeoxysorbitol (F-18 FDS)

Enterobacteriaceae are a group of gut-inhabiting gram-negative bacilli capable of causing serious human infection. They are different from other microbial or mammalian cells in their ability to metabolize sorbitol. F -18 FDS has been successfully synthesized from F-18 FDG and it showed specific uptake in a cultures of *Escherichia coli* and *Klebsiela pneumoniae (36).* No uptake of F-18 FDS was seen in gram positive organisms, human or cancer cells. Heat killed *Escherichia coli* did not accumulate F-18 FDS. The probe was able to differentiate infection due to Enterobacteriaceae from sterile inflammation and the PET signal disappeared following successful treatment. The safety and biodistribution of F-18 FDS has been demonstrated in a human study *(37)*.

Imaging Microbial Replication: Radiolabeled Fialuridine

Fialuridine is a nucleoside analogue which is a substrate for the bacterial thymidine kinase enzyme but is not acted upon by the human form of the enzyme. This is its basis for its use as a potential molecular probe for infection imaging. The initial exciting results obtained from imaging skeletal infection has not been replicated in recent human studies. Paterson et al. could not demonstrate incorporation of C-14 Fialuridine into *Pseudomonas aeruginosa* DNA in a liquid scintillation counter because Pseudomonas is one of the bacteria without thymidine kinase *(38)*.

Imaging Microbial Bioluminescence: Radiolabed Fluorophores

Optical imaging has been extensively utilized in animal models of infection. Its application in humans has faced many challenges including toxicity, scattering and absorbance of light within tissue and poor signal to noise ratio of fluorophores within the human body *(39)*. Using fluorophores such as indocyanine green which fluoresce near the infrared region has been helpful in overcoming tissue absorption and scattering of light hence making imaging deep infectious processes possible at the skin surface. The feasibility of multi-agents in infection imaging using antimicrobial peptide conjugated to a fluorophore and a radionuclide has been demonstrated *(40)*. Mills et al. have recently reported on an array of optical probes that have been tested in infection imaging *(39)*.

Perspective

Metabolic imaging of infection holds a great promise in infection imaging. The focus of its application is shifting from mere diagnosis of infection to include prognostication where response to treatment can be predicted, identification of resistant strains, response assessment and identification of at-risk patients for prevention. Introduction of CT for complementary morphologic imaging has improved the diagnostic performance of metabolic imaging for infection. It is hoped that when hybrid SPECT/PET and MRI imaging achieve greater clinical utility these hybrid system may have even more applications in infection imaging. It is also hoped that hybrid molecular probes for multi-modality imaging may gain clinical relevance for infection imaging in the near future. Focused research is pointing towards a time when molecular probes shall not only be able to not only detect infection but also identify the offending organism and its biological characteristics.

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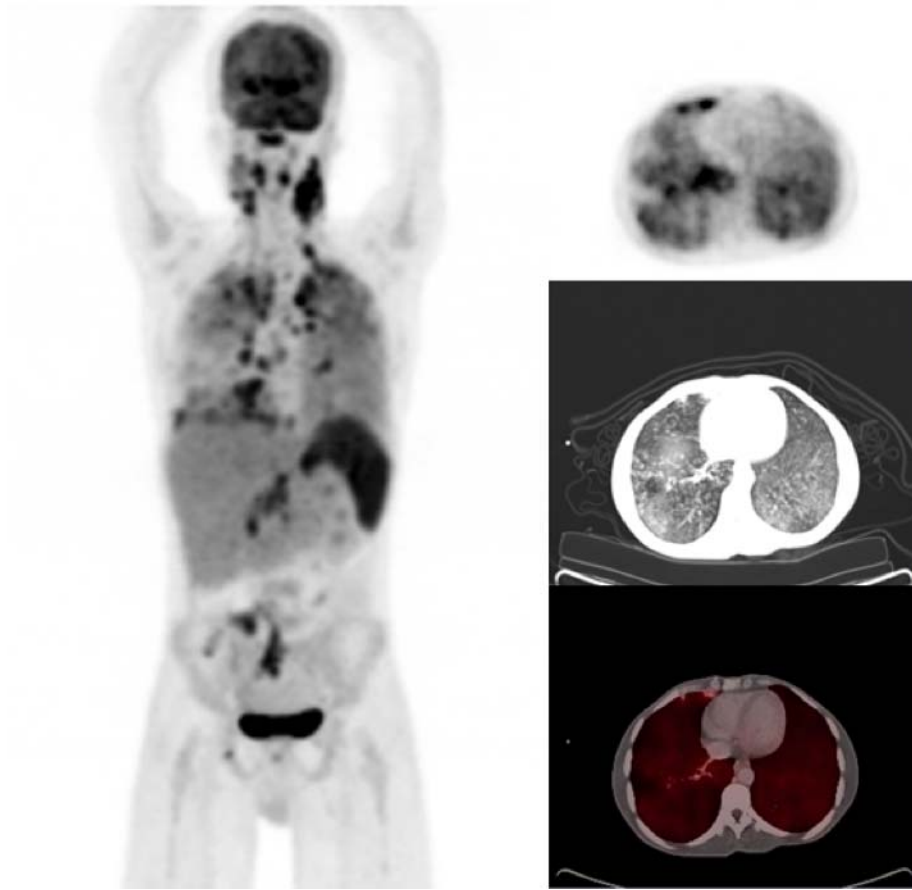
Figure 1.
A 27 year old HIV positive patient with suspected recurrence of TB 3 years post therapy. (A) Maximum-intensity-projection PET and axial fused PET/CT demonstrated diffuse FDG-avid lymphadenopathy in the cervical, hilar, mediastinal and abdominal nodes. With intense pulmonary parenchymal splenic uptake. FDG PET/CT could demonstrate that TB is active and also up-staged the patient to show extra-pulmonary involvement
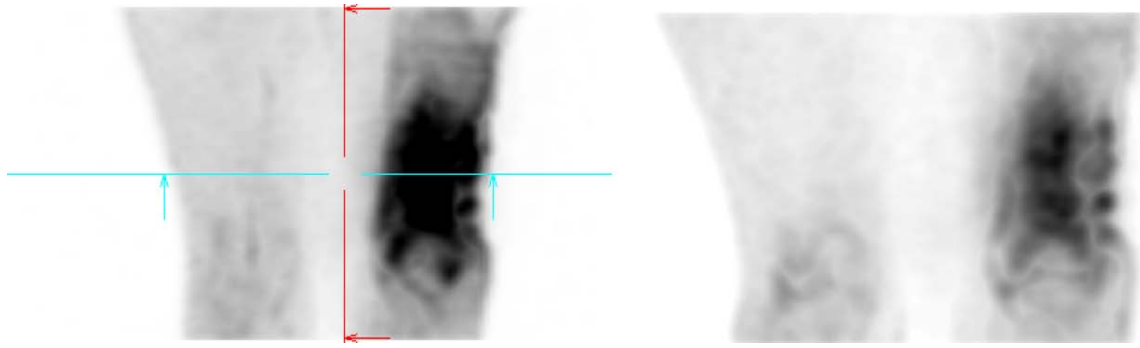
Figure 2.
99mTc-UBI 29-41 scintigraphy demonstrated intense uptake in the left distal femur that corresponded to the confirmed osteomyelitis by culture. Significantly reduced uptake was noted on the second scan (3 weeks apart), consistent with partial response and improvement on clinical assessment. This indication may become an important role of 99mTc-UBI 29-41 in the management osteomyelitis. Courtesy of Dr Enrique Estrada Lobato, IAEA.