Imaging of Invasive Fungal Infections- The Role of PET/CT

Alfred O. Ankrah, MD, PhD,[1,2,3,*] Ismaheel O. Lawal, MD, PhD,[2,4] Rudi A.J.O. Dierckx, MD, PhD,[3] Mike M. Sathekge, MD, PhD,[2] and Andor W.J.M. Glaudemans, MD, PhD[3]

[1]National Centre for Radiotherapy Oncology and Nuclear Medicine, Korle Bu Teaching Hospital, Accra GA, Ghana.

[2] Department of Nuclear Medicine, University of Pretoria, Steve Biko Academic Hospital, Pretoria, South Africa.
[3] Medical Imaging Center, University Medical Center Groningen, University of Groningen, RB Groningen, The Netherlands.

[4] Department of Radiology and Imaging Sciences, Emory University, Atlanta, GA.

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*Corresponding author: Alfred Ankrah, National Centre for Radiotherapy and Nuclear medicine, KBTH: Korle Bu Teaching Hospital, Accra, Ghana E-mail: ankrah.alfred@gmail.com

Over the last decades, the population at risk for invasive fungal disease (IFD) has increased because of medical therapy advances and diseases compromising patients' immune systems. The high morbidity and mortality associated with invasive fungal disease in the immunocompromised present the challenge of early diagnosis of the IFD and the need to closely monitor the infection during treatment. The definitive diagnosis of invasive fungal disease based on culture or histopathological methods often has reduced diagnostic accuracy in the immunocompromised and may be very invasive. Less invasive and indirect evidence of the fungal infection by serology and imaging has been used for the early diagnosis of fungal infection before definitive results are available or when the definitive methods of diagnosis are suboptimal. Imaging in invasive fungal disease is a non-invasive biomarker that helps in the early diagnosis of invasive fungal disease but helps follow-up the infection during treatment. Different imaging modalities are used in the workup to evaluate fungal disease. The different imaging modalities have advantages and disadvantages at different sites in the body and may complement each other in the management of IFD. Positron emission tomography integrated with computed tomography with [18F]Fluorodeoxyglucose (FDG PET/CT) has helped manage IFD. The combined functional data from PET and anatomical data from the CT from almost the whole body allows noninvasive evaluation of IFD and provides a semiquantitative means of assessing therapy. FDG PET/CT adds value to anatomic-based only imaging modalities. The nonspecificity of FDG uptake has led to the evaluation of other tracers in the assessment of IFD. However, these are mainly still at the preclinical level and are yet to be translated to humans. FDG PET/CT remains the most widely evaluated radionuclide-based imaging modality in IFD management. The limitations of FDG PET/CT must be well understood, and more extensive prospective studies in uniform populations are needed to validate its role in the management of IFD that can be international guidelines.

Introduction

More than 5 million fungal species and newer species cause disease in humans being described or identified. 123 The human body is constantly exposed to fungal molecular sequences from spores or fungal parts. Fungi are ubiquitous organisms that survive in almost every environment in soil, air, and water on living organisms such as plants, animals, and humans. Fungi can adapt to harsh environmental conditions, including extremes of temperature, pH, humidity, or unfavorable conditions created by the human immune system. 345 Fungi occur as either unicellular yeasts or multicellular molds with hyphae structures. Some fungi are dimorphic fungi that can assume the form of either mold or yeast depending on the environmental conditions. The immune system of most humans constantly eliminates the fungal elements trying to invade human tissue. A compromised immune system renders the human host susceptible to fungal infections. While over a million fungi species are in the environment, less than 2% of this cause human disease. [5,6]

Fungal Disease in Humans

Fungi invade human tissue causing diseases that cause symptoms which may vary from mild or even asymptomatic to severe disease. Fungi may cause cutaneous, mucosal, and deep fungal infections. Superficial fungal infections usually involve the skin and nails that may result in severe dermatologic infections. A fungal infection may affect mucus membranes and cause esophagitis, vulvovaginitis or keratitis that may be associated with significant morbidity. When a fungal infection is established in the body's deeper tissue, it gives rise to an invasive fungal disease (IFD) which may cause severe morbidity and mortality. [7]

Epidemiology of IFD

IFD causes high morbidity and mortality in the immunocompromised population. The annual mortality of IFD before the coronavirus (COVID-19) pandemic was more than 1.6 million, which was more than the mortality of tuberculosis, a known global pandemic. [8,9] The COVID-19 pandemic increased the population of patients at risk of IFD with an increased incidence of some IFD. [10,11] More than 150 million people have severe fungal infections.[9] The number of IFD cases may be much higher than the documented cases, as IFD is underestimated due to the complexity of the diagnosis.[12]

Population at Risk for IFD

The population of patients at risk for IFD have increased in the past few decades due to various disease and advances in medical procedures that result in immunosuppression. In patients with immunosuppression caused by neutropenia, hematologic malignancies, solid and hematopoietic stem cell transplant, chemotherapy, or congenital immunodeficiency disorders, candidiasis and aspergillosis are the most common IFD.[13] In patients with human immunodeficiency virus (HIV), IFDs caused by *Pneumocystis jiroveci* and *Cryptococcosis sp*. are common.[14] Patients with diabetes mellites are predisposed to several IFD, including mucormycosis, some IFD like coccidioidomycosis, histoplasmosis, and blastomycosis are endemic and are prevalent in some geographic locations. The epidemiology of IFD has been changing over the last few decades. *Candida albicans* and *Aspergillus fumigatus* used to be the most common species in immunocompromised patients; however, the percentage of non-albicans *Candida sp*., non-fumigatus *Aspergillus sp.*, and molds other than *Aspergillus sp* .

have been increasing. Other fungi like *Candida auris* may cause severe outbreaks in intensive care units and nosocomial infections that are difficult to treat. They may be resistant to antifungal therapy and are associated with higher mortality.[15]

PET/CT Imaging and IFD

Imaging has played an essential role in IFD. Imaging can help in the early diagnosis of IFD, staging the disease, and monitoring antifungal therapy. Radiological imaging especially computed tomography has been the mainstay in diagnosing and managing IFD.[16] Functional imaging with nuclear medicine imaging has also been used to manage IFD. Single-photon emission computed tomography (SPECT) imaging was used in the past, particularly in HIV patients.[17] PET/CT imaging has been evaluated in IFD associated with patients with immunocompromised immune systems due to neutropenia, hematologic malignancies, solid and hematopoietic stem cell transplant, chemotherapy, or congenital immunodeficiency disorders.[18] Functional imaging provides pathophysiologic data from diseased tissue deep within the body. The combination of functional data from PET and anatomical data from CT allows for anatomical localization and attenuation correction of the PET data collected during image acquisition. As physiological changes precede anatomical changes, early changes in pathology due to disease or response to therapy may occur earlier for physiologic data compared to morphological changes.[19] [18F]Fluorodeoxyglucose (FDG) is the most common tracer used to evaluate IFD. FDG, a glucose analogue, accumulates in the inflammatory cells, which increases their glucose utilization during the respiratory burst in response to the IFD. FDG is taken up by the glucose transporters on the cell membrane and is phosphorylated but does not proceed further in the glycolytic pathway. The phosphorylated FDG remains trapped in the IFD lesion and generates an FDG signal that a PET camera can image. FDG accumulates in patients with IFD, including patients with severe neutropenia, which can be a limiting factor in other radionuclide imaging.

PET/CT is a whole-body imaging procedure; thus, a single study provides information from the head to the midthigh. This allows whole-body imaging and helps examine most organs in one study to detect disseminated IFD. The acquisition protocols for FDG PET/CT imaging have been standardized to compare the results of scans acquired on different scanners from different centres.[20]

The evidence for the use of FDG PET/CT in IFD has been building up over the last few decades through case reports and some retrospective single-centred studies.[18,21,22,23,24,25,26] Initial studies performed when malignancies were the main indications FDG PET/CT revealed that IFIs could be a cause of false-positive for malignancy. [27,28] FDG PET/CT was subsequently realized to potentially play a role in infectious and inflammatory diseases, including IFD. Some comprehensive reviews have discussed the role of FDG PET/CT in IFD in the immunocompromised host, in children and monitoring therapy.[18,29,30] This review discusses the added value of FDG PET/CT to other imaging modalities used in evaluating FDG PET/CT.

Clinical Manifestation of IFD in the Immunocompromised

The clinical manifestation of IFD depends on the type of fungi, the cause of the immunosuppression, degree of immune suppression, comorbid disease, or treatments used. IFD frequently occurs in critically ill and immunocompromised patients, and the symptoms

may be confounded by the patient's state. [31,32] The lung is the most common portal of entry of fungi into the human body. Inhaled fungal spores or mycelia are not efficiently eliminated by the immune system of patients with immunosuppression. The body's immune system recognizes fungal-associated molecular patterns on the fungi by receptors on the immune cell and epithelial cells. On recognition of fungi, both the innate and adaptive immune system of the human body is activated to eliminate the invading fungi. [32,33] In patients with compromised immune systems, the fungi are not properly cleared, and a pulmonary IFD ensues. Fungal infection of the upper respiratory tract may be severe or life-threatening, such as sinusitis with potential bone destruction or intracranial extension.[34] A lower respiratory fungal infection may cause bronchopneumonia or pneumonia and may present with cough, dyspnea, chest pain, and fever. The infection may disseminate to other organs, which results in poor clinical outcomes if not identified and treated early. Fungi may also cause noninvasive diseases; they may colonize and grow in lung cavities from previous respiratory diseases. In some patients, the fungi may create allergic disease in the lung to produce asthma-like symptoms with frequent recurrent episodes of dyspnea and respiratory distress.[35]

HIV may give rise to distinct clinical manifestations of IFD. One of the most common IFD seen in HIV patients is caused by *Pneumocystis jiroveci. Pneumocystis jiroveci* pneumonia in HIV patients may lead to progressive dyspnea, which can be fatal if not diagnosed and treated. Another IFD that causes severe disease in HIV patients is cryptococcosis which enters through the respiratory system and can cause pulmonary disease. In addition, *Cryptococcus sp*. has the propensity to disseminate to the central nervous system, where it can cause life-threatening meningitis or intracranial space-occupying lesions.[36]

Challenges in IFD Diagnosis

The diagnosis of IFD can be challenging, especially in the immunocompromised susceptible to other viral and bacterial infections that may present with a similar clinical picture. In cases of severe immunosuppression, the clinical picture may be unremarkable with the lack of typical signs and symptoms.[37] Again, IFD frequently occurs in patients with other underlying non-infective conditions, which may confound the overall clinical picture. Early diagnosis and prompt initiation of treatment are necessary to reduce morbidity and mortality of IFD. The recovery of fungi by culture remains the gold standard for diagnosis of IFD but has very low sensitivity depending on the fungal species. The yield of fungi from bronchoalveolar lavage may represent contamination rather than invasive fungi in patients who have undergone lung transplantation or with chronic lung disease. The time for the results of culture varies with fungal species. Most fungi will be recovered within 2 weeks, but incubation for at least four is recommended for slow-growing fungi.[38] Cultures allow the growth of fungi, and sensitivity tests to be conducted. The prolonged culture time would delay diagnosis and the initiation of treatment. The use of histopathology and microscopy can allow direct visualization of hyphae or fungi from tissue from sterile sites and produce results earlier than culture. Microscopy cannot do sensitivity testing to antifungal agents and cannot identify organisms beyond the species level. The sensitivity of microscopy in IFD is suboptimal.[39]

Response to Diagnostic Challenge of IFD

In response to the difficulty in the early diagnosis of IFD, laboratory and radiological studies that provide indirect evidence of the presence of IFD in humans are used to help identify patients with the infection. International guidelines have been developed to help identify such patients. These guidelines were developed for clinical trials to evaluate diagnostic and therapeutic interventions for IFD. The European Organization for Research and Treatment of Cancer Mycosis Study Group Education and Research Consortium (EORTC/MSGERC) developed a criterion that helps in the early diagnosis of IFD. The EORTC/MSGERC definitions of IFD are proven, probable and possible IFD and have been revised and updated since the initial classification as more diagnostic platforms and evidence-based definitions evolve. The proven criteria of IFD rely on the recovery of fungi by culture or histological identification of fungi from sterile sites in the body. The proven criteria are used to diagnose IFD in all populations, while the possible and probable criteria are used for patients with immunosuppression. The probable and possible criteria rely on host factors and indirect evidence of the presence of fungi by serologic and radiologic studies. The host factors include prolonged granulocytopenia ($<0.5\times10^{9}$ /L for ten days), allogeneic stem-cell transplantation, treatment with immunosuppressive drugs, congenital immunodeficiency, and use of corticosteroids. The guidelines have been revised and updated to include more of the population at risk for IFD, such as patients on immunomodulating drugs.[40] Newer diagnostic platforms such as PCR-based methods such as T2Candida have been included.[41]

Laboratory Platforms for IFD

Laboratory and radiological methods which give indirect evidence of the presence of fungi have been deployed to allow help early diagnosis and management of IFD. The serologic markers used include galactomannan and beta-D-glycan. Galactomannan provides indirect evidence for aspergillosis, but it has several limitations in the indirect identification of aspergillosis. The limitations include cross-reactivity with other IFD like histoplasmosis and different cut-off value for different causes of immune suppression. Recent guidelines have set out various cut-off values for different conditions and determined that galactomannan testing can be applied to different body fluids, including serum, plasma, bronchoalveolar lavage and CSF.[42] β-D-glucan can be used for invasive candidiasis and invasive aspergillosis but also has its limitations. β-D-glucan has been validated for use in serum and shown promising results for cerebrospinal fluid, but evidence for its use in other fluids is lacking. β-D-glycan is helpful in diagnosing invasive aspergillosis, invasive candidiasis, and other fungi except for a few fungi like Mucorales and Cryptococcus species. β-D-glucan is used to exclude IFD or to determine when antifungals must be stopped. However, the recommendation varies for patients with hematologic malignancy, solid organ transplant recipients, patients on long-term immunosuppressive therapy and patients in the intensive care unit.[43] Other laboratory tests for IFD have been developed, which use other methods such as polymerase chain reaction tests. Lateral flow devices which produce results in less than 15 minutes have also been developed.[44] The difficulty in diagnosing IFD and the many indirect and direct methods with varying sensitivity means a combination of host factors and laboratory platforms are used in diagnosing IFD. The laboratory-based platform used in diagnosing IFD imaging also plays a significant role in the indirect methods of diagnosing IFD and in the management of IFD.[45]

Imaging in IFD

Early diagnosis and the prompt initiation of appropriate treatment are essential for favorable outcomes for the treatment of IFD. Imaging has the advantage of being a non-invasive and relatively rapid means of supporting a diagnosis of IFD. Imaging of IFD has been performed with chest radiographs (CXR), computed tomography scan (CT scan), including high-resolution computed tomography (HR CT), magnetic resonance imaging (MRI), ultrasound (US) and positron emission tomography integrated with computed tomography (PET/CT).[46] Single-photon emission tomography (SPECT) imaging was previously used, especially in evaluating IFD associated with HIV/AIDS. The advantages and limitations of these methods in evaluating invasive fungal infection must be understood to maximize the use of the methods in invasive IFD.[47]

Early Diagnosis of IFIs With Imaging

HR CT is the most useful imaging modality in the early diagnosis of pulmonary IFD. The role of HR CT in the early diagnosis of IFD is well established. [45] The HR CT findings for IFD have been better defined, and the latest EORTC/MSGERC guidelines have criteria for an increased likelihood of the early diagnosis of early IFD caused by fungi that cause angioinvasion such as aspergillosis and mucormycosis.[40,45] There are several specific signs of HR CT, which in the presence of the appropriate host factor, increases the likelihood of an IFD. The CT features are not exclusive to IFD but have a high likelihood for IFD in the appropriate circumstance. The signs of early IFD caused by fungi associated with angioinvasion on radiologic imaging include one or more of the following: dense, well-circumscribed lesion or lesions with or without a halo sign, air crescent sign, cavity, and a wedge-shaped segmental or lobar consolidation. The nodules are the most common radiological features of pulmonary IFD on HR CT.[49]

Nodules on HR CT in Early IFD

On radiological imaging, nodules are areas of opacification less than 3 cm in their longest diameter. Nodules may be rounded or irregular. Different nodules are encountered in IFD, which are often named according to appearance.

Halo Sign

A nodule of IFD on HR CT surrounded by ground-glass appearance is called a “halo sign.” The “halo sign” is due to a pulmonary infarct surrounded by pulmonary hemorrhage. The sensitivity of the halo sign for IFIs is variable but depends on the degree of immunosuppression. [48] In patients with hematologic malignancy and neutropenia at baseline, the halo sign is present in more than 70% with IFIs and decreases with time to less than 20% in 2 weeks.[45,50,51,52,53] The sensitivity of the halo sign is higher for patients with neutropenia and less sensitive for patients without neutropenia. The specificity of the halo sign for IFD has been reported as high (>90%) in several studies; however, various other studies reported lower specificity. 5354555657 The halo sign is associated with a better prognosis, probably related to the ability to start early treatment and is less common in children.[58]

Reverse Halo Sign

The reverse halo sign is another sign that HR CT detects in pulmonary aspergillosis and pulmonary mucormycosis. The reverse halo sign is an area of ground-glass opacification surrounded by an area of consolidation (Fig. 1 A). The reversed halo sign was found to be more specific for pulmonary mucormycosis compared to pulmonary aspergillosis.[59] The reversed halo sign was first described in patients with cryptogenic organizing pneumonia, and the reverse halo sign occurs in other diseases like pulmonary infarction due to thromboembolism, sarcoidosis, and tuberculosis.[60,61,62,63,64,65]

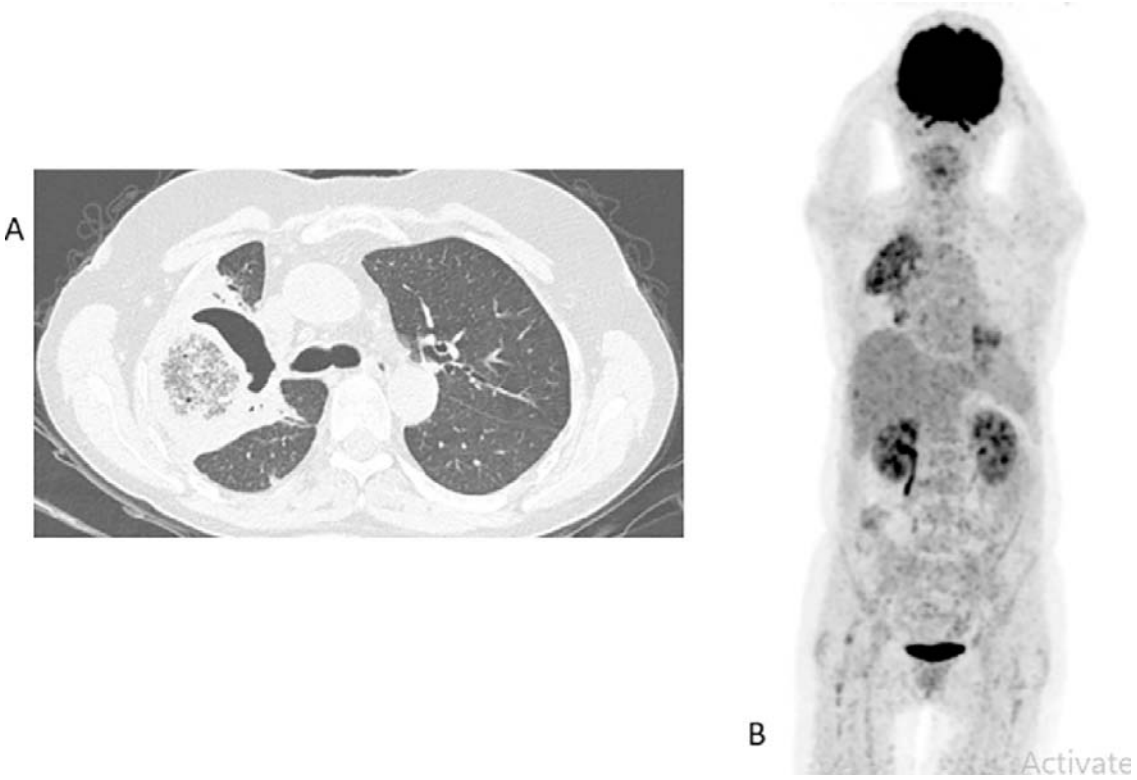

Figure 1. Sixty-year-old female patient with acute myeloid leukemia on chemotherapy with prolonged neutropenia and clinical signs of pneumonia which is not responding to antibiotics. HR CT and FDG PET/CT were performed, and the patient started on antifungal and improved.

Air Crescent Sign

The air crescent sign, as the name implies, is a crescent shape of air that appears in a mass on HR CT (Fig. 1 A). It usually appears about two weeks after initial diagnosis and thus has limited value in early diagnosis of IFD.[45] The air crescent sign appears before cavitation and is associated with recovery from neutropenia.[66] Although it is seen in almost half two-thirds of adult patients, it is rarely seen in the pediatric population.[51,52,67]

Hypodense Sign

The hypodense sign is a central area of decreased attenuation present in an area of consolidation or a mass on a non-contrast-enhanced CT of the lung. It corresponds to a zone of infarction on a CT scan better identified on the soft tissue window rather than the lung window. The hypodense sign usually occurs a week after diagnosis of invasive pulmonary

aspergillosis. In contrast to the reverse halo sign, the hypodense sign is more common in patients who do not have neutropenia and less common in patients with immunosuppression. The hypodense sign was reported in 38% of patients with invasive pulmonary aspergillosis and totally absent from patients with pneumonia not due to IFD.[68,69] Another study found the hypodense sign on the HR CT in 68% of patients with invasive pulmonary aspergillosis patients who had had a liver transplant.[70]

HR CT and FDG PET/CT in IFD

FDG PET/CT combines PET data which occurs before anatomical changes with anatomical changes from CT. The earlier occurrence of FDG PET data made the early diagnosis of IFD with FDG PETCT a favorable prospect. However, the underlying pathology which allows the early diagnosis of IFD on HR CT is the angioinvasion with pulmonary infarction and hemorrhage. The body's response at the cellular level to pulmonary infarct or hemorrhage is inflammation with activation and infiltration of immune cells to infective foci. The activated immune cells will produce an FDG signal. There is no distinction between the FDG uptake due to an angioinvasion caused by fungi from other pathology. As a result, although FDG uptake may be seen early in the cause of IFD, the uptake is nonspecific and cannot be attributed to IFD. The nonspecificity of FDG uptake is a well-recognized limitation of FDG PET/CT. The integrated CT of FDG PET/CT improves the specificity of FDG uptake in infectious diseases. [71] In early pulmonary IFD, however, the relatively thicker slice of the CT of the FDG PET/CT may not detect the subtle changes seen on the thin slices of HR CT due to angioinvasion and pulmonary infarction. Thus, FDG PET/CT cannot provide the required specificity needed to diagnose IFD even if FDG uptake is detected. HR CT remains the imaging method of choice for evaluating early disease for invasive pulmonary aspergillosis and pulmonary mucormycosis. [45] FDG PET/CT cannot replace HR CT in the early diagnosis of IFD. FDG PET/CT may help in the early diagnosis of IFD when PET-directed biopsy of a patient with febrile neutropenia or fever of unknown origin leads to IFD diagnosis.[18,72]

CT scans are useful in evaluating IFD at most other sites in the body and are used to stage IFD and detect occult infection.[26] CT may help assess complications such as bone destruction that could complicate IFD and is used to monitor treatment with antifungals. [73,74,75,76] FDG PET/CT can detect these CT changes, and in addition, it has the advantage of the FDG PET uptake, which can give information on the disease activity of the pathology recognized on CT. [72] Douglas et al. compared FDG PET/CT and CT in assessing disseminated IFD. They found that FDG PET/CT detected IFD dissemination in more patients compared to CT.[26] In another study from the Netherlands, FDG PET/CT detected IFD lesions beyond the site imaged by CT in 40.4% of FDG PET/CT studies done within two weeks of the CT.[47]

A. HR CT shows a large area of opacification with features of an air-crescent sign and reverse halo sign.

B. Maximum intensity projection image showing intense uptake in the pulmonary lesion

Chest Radiograph in IFD

The chest radiograph may detect IFD lesions at a later stage of the infection but is so sensitive in the early stage. Techniques that remove the bones of the thoracic cage to visualize lung

parenchyma on chest radiograph better have been evaluated in IFD diagnosis. These bone suppressing techniques showed improvement in the evaluation of IFD; however, the sensitivity and specificity are still inferior to HR CT.[77] The advantage of the chest radiograph is the ease of performing it with a portable chest. FDG PET/CT detected lesions in IFD patients that were not visualized on 7.5 % of chest radiographs and detected lesions beyond the chest in 42.3% of the chest radiographs in one study. [47]

Magnetic Resonance Imaging

The lung consists of air-filled tissue, which reduces the signal-to-noise ratio of MRI imaging. MRI is not the preferred imaging modality for the assessment of pulmonary IFD. MRI using high-speed gradient systems and sequences with short echo times can improve diagnostic accuracy to be comparable to CT and, therefore, can be used as an alternative method in assessing pulmonary IFD. The sequences needed for the use of MRI may require numerous breath-holding episodes over at least 20 minutes which may be difficult for patients to do. [78,79,80,81,82] MRI has excellent soft-tissue resolution and is very useful for evaluating and detecting IFD of the central nervous system (brain and meninges).[83,84] The early sign of IFD in the brain usually involve cerebritis without abscess formation. Later pus develops around IFD lesions with inhomogeneous ring enhancement with decreased diffusion due to fungal pus's increased cellular content and viscosity.[85] MRI does not use ionizing radiation and has a superior soft-tissue resolution in solid organs. Patients with claustrophobia and metal artefacts may not tolerate or be unable to have an MRI.[86] In a study to determine the added value of PET/CT, MRI detected more lesions than FDG PET/CT in 21.1% of IFD. This was related to the high FDG uptake, which limited the sensitivity of detection of cerebral IFD by FDG PET /CT. In that same study, FDG PET/CT detected lesions beyond the site imaged by the MRI in 71.4% of the scans.[47]

Ultrasound Imaging

Recent guidelines have been published on the role of ultrasound in diagnosing pulmonary pathology.[87] Pulmonary ultrasound was comparable to CT and more sensitive and specific than the chest radiograph in evaluating community-acquired pneumonia. Pulmonary ultrasound has not been evaluated for the early diagnosis of IFD, and further research would be required. Ultrasound studies have been used to evaluate IFD especially involving abdominal organs.[88,89] Due to the lack of ionizing radiation in ultrasound imaging, it is frequently requested in the pediatric population. Ultrasound is useful for the evaluation of abdominal IFD but has been reported to be overutilized in IFD with important false-positive and false-negative diagnoses of IFD. There is a need to develop guidelines for ultrasound in IFD imaging to improve diagnostic accuracy.[90] In a study to determine the added value of FDG PET/CT, FDG PET/CT detected more lesions in 9.1% of ultrasound imaging done within two weeks. The earlier detection was considered to be related to the earlier detection of pathology by FDG PET. FDG PET/CT in that study same study detected IFD lesions outside the region imaged by the ultrasound in 66.7% of cases.[47]

FDG PET in IFD

IFD Lesion Activity

A prospective monocentric center found IFD lesions accumulated FDG across all the different fungi species in all lesions that had been noted by anatomic-based lesions. [91] IFD lesions are very FDG avid, much higher than most non-malignant pathology and can cause false-positive during oncologic evaluation by FDG PET/CT.[27,28] The mean SUVmax from different IFD lesions from different organs was found to be 7.4 ± 4.9.[92] Another study found the median SUVmax of pulmonary IFD was 11.5 (3.7-24.9); the SUVmax values were higher than all other nonmalignant pulmonary lesions and were closer to the SUVmax of malignant lesions.[94] The intense FDG avidity of IFD is used to assess the disease activity in the residual lesion as inflammation associated with a healed lesion is not expected to be that intense.[26,94] The intense uptake helps stage IFD, and the resolution of the uptake as the IFD heals forms the basis of monitoring therapy with IFD. Figure 1 shows intense FDG uptake in the fungal lesion noted on HR CT.

Staging of IFD With FDG PET/CT

IFD is a systemic disease and often occurs in patients with immunosuppression. IFD may be disseminated, and the disease may occur at any site in the body. FDG PET/CT is a whole-body imaging modality that can stage IFD by providing information from different body sites. This provides a metabolic map correctly defining the disease burden and identifying occult IFD lesions. This may have an implication on the dose of antifungal to be used as some sites, such as osteomyelitis IFD, may require higher doses of antifungal than the dose used for abdominal IFD.[94] Leroy-Freshchini et al. used FDG PET/CT to stage IFD in patients who had not started antifungals. They found FDG PET/CT was superior to other imaging modalities that were done at the time of FDG in 10 out of 19, 53% of patients with IFD. FDG PET/CT more accurately defined the disease extent in the same organ or detected in organs beyond the site imaged by the other study. The authors reported that the primary staging influenced the diagnostic workup in 53% of patients. [92] Douglas et al. found FDG PET/CT to be better at staging disease in IFD than CT with more dissemination sites (35% to 5% $p < 0.001$) in 40 patients that had both FDG PET/CT and CT scans. In that same study, FDG PET/CT localized clinically occult infection in 40% of patients with IFD and disseminated disease was found in 38% of patients with IFD.[26] In another study from the Netherlands, FDG PET/CT detected lesions outside the site imaged by all other imaging modalities in almost 50% of the studies performed close to the FDG PET/CT.[47] All these studies demonstrate the ability of FDG PET/CT to accurately stage IFD.

Monitoring of Antifungal Therapy

Antifungal drugs are expensive and toxic. Newer antifungal drugs have been added to antifungal drugs, but the number of drugs remains limited.[30] It becomes imperative the monitoring of treatment of IFD with antifungal therapy to ensure the most effective use of antifungal therapy. Therapy monitoring by FDG PET/CT in IFD is perhaps the most potent use of PET/CT in IFD (Fig. 2). FDG PET/CT is a non-invasive marker of disease and standard uptake value, and other metabolic indices provide a semi-quantitative measure of the disease activity within lesions. This allows longitudinal follow-up of IFD lesions over time,[19,29,30,72] Again, the earlier physiological FDG PET data changes may detect response to antifungal therapy earlier

than other morphological images in response to antifungal treatment. In a study from a single Medical Centre in the Netherlands, FDG PET/CT was found to have altered the treatment of IFD in 50% of patients leading to the cessation, switch in antifungal therapy or increase in the dose of antifungal therapy. [95] Another study showed that FDG PET/CT led to an increase or change in the antifungal drug that was being monitored in eight of the 54, 15% of the patients that were being monitored for IFD. Again, FDG PET/CT led to reduction or cessation of the antifungal therapy being monitored in 17 out of 54, 31% of patients. [93] A study from Australia showed that 11 out of 18 patients, 61% of patients who had baseline and follow-up FDG PET/CT showed normalization of FDG uptake where residual CT lesions were still present on CT, suggesting resolution of infection. [26] The anatomic lesions' persistence could have led to unnecessary prolongation of antifungal therapy when IFD had resolved.

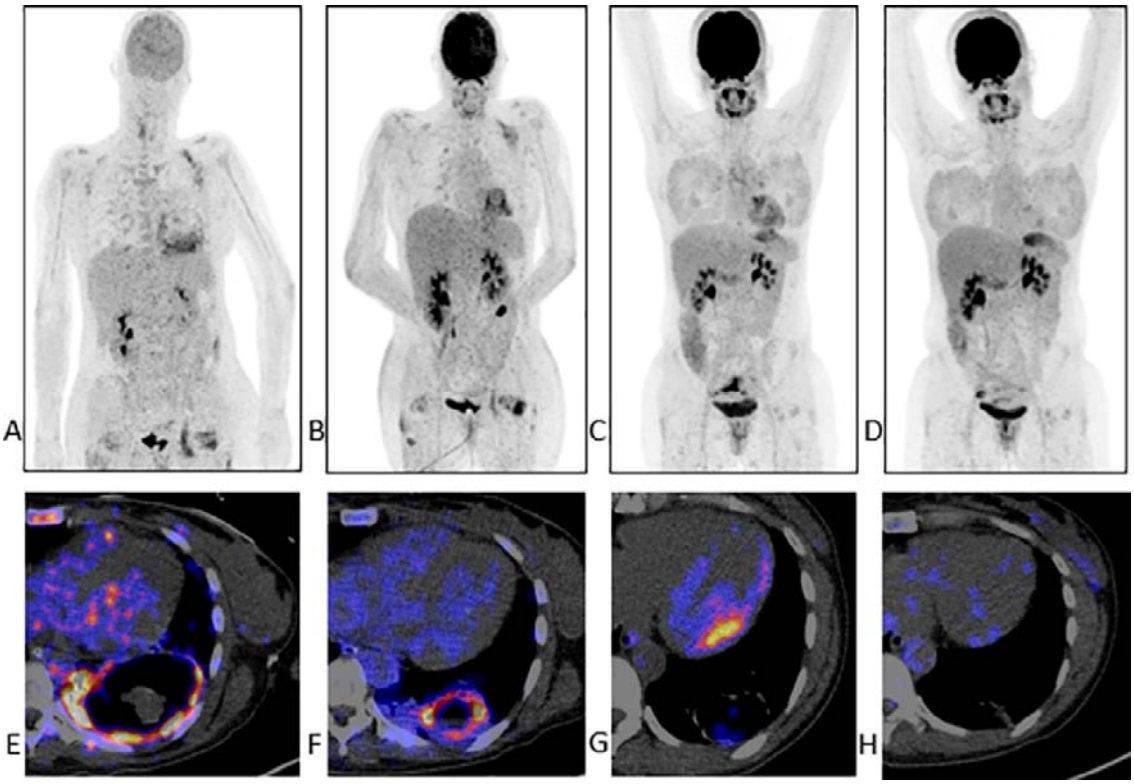

Figure 2. Forty-two-ear-old male with acute myeloid leukemia who presented with pancytopenia respiratory distress. Initial HR CT revealed a large cavity suspected to be aspergilloma. Disease activity was found in the wall of the cavity, and the patient was treated and monitored with antifungal therapy before the patient had allogeneic stem cell transplantation. (A-D) Maximum intensity projection FDG images demonstrating how FDG was used to monitor the disease activity. There is a gradual reduction with complete resolution of FDG uptake in the chest lesion.

The FDG MIP showed a large cavity with an intensely avid cavity wall, which was considered an active disease. The patient was started on antifungal therapy, which was monitored with FDG PET/CT, which eventually showed a metabolic response (Left to tight). The last images (D and H} show residual anatomic with a resolution of metabolic activity.

Assessment of Response With IFD

To monitor antifungal treatment with FDG PET/CT, at least two FDG PET/CTs performed at different times while the patient is on antifungal is required.[19] FDG PET/CT finding should always be correlated with the clinical findings and other laboratory markers of IFD. Normalizing FDG uptake in an area with increased uptake due to IFD suggests a metabolic response of that IFD lesion. The interpretation of the response of antifungal therapy in a patient with multiple IFD foci is much more complex. A complete resolution of all initial IFD lesions indicates a metabolic response of the IFD and usually suggests a response to antifungal treatment even when residual anatomic lesions are present.[92,94] The resolution of FDG uptake in some IFD lesions with a partial decrease in other lesions suggests the IFD is resolving, and a continuation of the antifungal therapy is recommended. If there is the appearance of new FDG avid IFD lesions or the previous lesions are more intense, the IFD is not responding to therapy and may suggest the need to change the antifungal treatment or increase the dose. However, it may represent a new pathology, not due to IFD. Whenever possible, histological evaluation of the appearance of a new lesion should be done as FDG PET/CT is not specific, and the new lesion may be due to uptake due to different pathology.[19] When FDG uptake is persistent in an IFD lesion with the resolution of FDG uptake in most other lesions, tissue diagnosis is recommended. This may represent residual active IFD or residual molecular sequences of fungi as reported in other microorganisms.[96] The persistent activity may be due to a host immune reaction attempting to eliminate fibrosis in a healed IFD or may be due to a different pathology.[91] Some challenges and questions exist in the use of FDG PET/CT in IFD monitoring antifungal therapy. IFD are caused by different fungi in different conditions in patients with different causes and degrees of immunosuppression. There is often a lack of a baseline FDG PET/CT, and the timing of the follow-up FDG PET/CT has not been standardized as with solid tumors and PERCIST.[97] The role of metabolic parameters is yet to be thoroughly investigated. These different challenges need to be addressed in large multicenter prospective trials. Figure 2 demonstrates the use of FDG PET/CT to follow up on a patient with metabolically active aspergilloma.

Metabolic Indices and IFD Monitoring

Metabolic parameters have been used in assessing response to therapy in other pathology. Ankrah et al. evaluated different metabolic parameters in assessing response to therapy in IFD, and the global total lesion glycolysis and metabolic volume were found to correlate to the clinical outcome better than other parameters such as the mean maximum standardized uptake value and mean peak standardized uptake value.[95] Defining the region of interest of infective FDG uptake in a patient with widespread disease and multiple FDG uptake due to IFD is almost impossible in routine clinical work. However, software that automatically defines these lesions is available and may help translate the use of metabolic indices into the clinic.[98] The role of FDG PET/CT metabolic indices in IFD must be further researched in larger prospective studies. Other radiomic derived indices from PET and CT data may also be evaluated to determine how they can help in IFD evaluation.

Diagnostic Accuracy of FDG PET/CT in IFD

Leroy-Freschini et al. evaluated the diagnostic accuracy of FDG PET/CT in IFD in 51 immunocompromised patients with IFD. They determined that the sensitivity and specificity were 93% and 81%, respectively. They found the positive predictive value, negative predictive

value and global accuracy to be 95%, 92%, and 90%.[92] These results suggest a high sensitivity for FDG PET/CT in IFD. Although the sensitivity is high, it is not 100%. In the evaluation of IFD by FDG PET/CT, some situations may result in a false-negative study. FDG PET/CT in the presence FDG PET/CT detects lesions when the fungi settle in tissue. A patient who has a blood-borne fungal infection with no deposit in tissue, like candidemia, would return a negative FDG PET/CT scan even though the IFD may be proven. Again, the high physiologic uptake of FDG PET/CT in some organs such as the brain and kidneys may obscure a lesion due to IFD and give a false negative. On the other hand, the low specificity of FDG PET/CT may result in other pathologies such as tuberculosis or cancer, giving rise to a false positive.[22,28,71]

Pattern of FDG Uptake in IFD

Different patterns of FDG PET/CT can be found in IFD. In pulmonary aspergillosis, FDG patterns include absent, isointense, or very intense, corresponding to a pulmonary nodule noted on HR CT has been described.[99] These patterns are nonspecific, and histology is required for a definitive diagnosis.

Candidiasis may present as a chronic disseminated disease where multiple small uniform and focal lesions are scattered through the liver and the spleen. Similar small, well-defined, circumscribed lesions have been described in the FDG PET/CT in other organs like the muscles or the kidneys.[21,100] FDG uptake in the lung in disseminated candidiasis, but the lung lesions are not usually as well-defined and as circumscribed as found in other organs. Histoplasmosis and cryptococcosis may cause pulmonary disease because they enter by inhalation but tend to disseminate to the adrenal gland with bilateral or unilateral adrenal uptake on FDG PET/CT.[22,101,102] A pattern of diffuse lung uptake has been described in patients with pneumocystis imaged with FDG PET/CT and is often bilateral.[102,103]

Assessment of Disease Activity Before Therapy That Can Potentially Cause IFD to Disseminate

Some clinicians have used FDG PET/CT as a gatekeeper before patients undergo therapies that may potentially result in the dissemination of IFD. In patients with previous IFD and residual anatomic lesions, FDG PET/CT is done to exclude active disease in the residual lesions or occult sites before patients are subjected to procedures such as hematologic stem cell or solid organ transplantation.[47,93,104] FDG PET/CT may serve as a gatekeeper before procedures that result in immunosuppression.

Noninvasive Pulmonary Aspergillosis and FDG PET/CT

Non-invasive aspergillosis may present as allergic bronchopulmonary aspergillosis, aspergilloma, and chronic pulmonary aspergillosis. Non-invasive aspergillosis is important; the morbidity and mortality can be high, and there is the risk of the infection becoming invasive. FDG PET/CT was found to be useful in distinguishing invasive pulmonary aspergillosis and non-invasive pulmonary aspergillosis. Invasive pulmonary aspergillosis predominantly had a hypermetabolic pattern (75%) compared to noninvasive aspergillosis, which had an isometabolic pattern.[105]

Limitation of FDG PET uptake in IFD

The physiologic accumulation of FDG PET/CT in some anatomic sites may limit the sensitivity of detection of IFD lesions in certain parts of the body.[47] In some cases, modification to the acquisition protocol of FDG may enhance the study. The uptake of FDG in the brain may limit the detection of IFD lesions in the brain, and small septic foci may be easily missed. MRI is preferred in the evaluation of brain lesions due to the excellent soft-tissue resolution.

The variable uptake of FDG by the heart may also limit the detection of IFD in the myocardium or the heart valves. If an IFD involving the heart is suspected, the study must be modified by suppressing myocardial FDG uptake by using intravenous heparin 50 IU/kg 15 minutes before FDG administration and fasting for at least 6 hours to try and improve the sensitivity of FDG detection of cardiac IFD lesions.[106]

The tracer excretion of FDG by the kidney may affect the evaluation of renal IFD. Methods encouraging faster renal excretion, such as proper hydration and delayed diuresis, have been evaluated for gynecologic pathology and upper and lower urinary tract FDG PET/CT studies. A few studies have evaluated delayed diuresis for evaluation of upper urinary tract pathology with promising results. 107108109 This may be used to improve the evaluation of IFD renal lesions by FDG PET/CT, but this must be validated in larger studies.

Other PET/CT Imaging in IFD

The non-specificity of FDG PET/CT imaging prompted the search for a more specific FDG tracer for IFD imaging. Most of these are still in the preclinical stage but have shown promise for IFD imaging. The tracers used include siderophores, antibodies and antifungal agents.

Siderophores in IFD Imaging

Fungi have an elaborate system to trap iron needed for fungi bio-metabolism. In the human body, proteins such as transferrin and ferritin sequester iron and making it unavailable for microorganisms. For microorganisms to survive in the human body, they utilize siderophores, proteins that trap iron to make them available to the microorganism. In *Aspergillus fumigatus*, once iron binds to the siderophores, they are taken up by the fungi by siderophore transporters. Gallium 68 is (^{68}Ga) an analogue of iron and which can be bound to siderophores just like iron. In vivo studies in mice and in vitro studies using ^{68}Ga-siderophores complexes were found to accumulate in *Aspergillus fumigatus* infectious foci in a dose-dependent manner suggesting a potential role in therapy monitoring.[110,111] Two siderophore complexes, desferri-triacetylfusarinine C (TAFC) and [68Ga]Ga-ferrioxamine E (FOXE), were evaluated for their specificity in IFD. There was no significant accumulation in both tracers for cancer cells, gram-negative and mycobacteria bacteria that were tested. The two siderophores complexes showed lower levels of accumulation in *Aspergillus sp.* other than *Aspergillus fumigatus*. In the gram-positive bacteria evaluated (*Staphylococcus aureus*), FOXE showed accumulation while TAFC did not.[112,113] The results indicate that the siderophores may be useful in imaging IFD and have good specificity for IFD. However, siderophores imaging is yet to be translated to human studies.

Radiolabeled Antifungal in IFD Imaging

Fluconazole

Antimicrobials have been labelled with radiopharmaceuticals and used to image IFD in vivo and in vitro.[114,115] Fluconazole is an antifungal agent that belongs to the azole group of antifungals. Fluconazole is active against predominantly yeast with limited activity against molds. The mechanism of action of azoles is the inhibition of the enzyme 14-α-demethylase. 14-α-demethylase inhibits ergosterol synthesis. Ergosterol is a critical component of the fungal cell membrane, and inhibition by fluconazole leads to reduced fungal growth and fungal death. Fluconazole was radiolabeled with both ^{99m}Tc and ^{18}F. The ^{99m}Tc labelled fluconazole was found to bind to accumulate in *Candida albicans* with relatively poor uptake in *Aspergillus fumigatus* consistent with the antifungal spectrum.[116,117] Fluconazole was also labelled with ^{18}F to take advantage of the better spatial resolution of PET imaging and the more validated semi-quantification radiotracer uptake. The PET labelled ^{18}F tracer was more lipophilic and had high hepatic excretion. The hepatic excretion of ^{18}F labelled PET/CT drawback as hepatic involvement of IFD may need to be excluded. Fluconazole has a narrow spectrum of activity against fungal agents, and resistance among *Candida albicans* and other fungi that are susceptible to fluconazole has been increasing. This would be a limitation if fluconazole were to be used in the treatment of IFD. Fluconazole was radiolabeled with radioisotopes more than two decades ago and has not been translated into the management of IFD in humans. ^{18}F labelled fluconazole has been used for in vivo biodistribution of the drug in humans and was able to determine that much higher doses were needed for osteomyelitis compared to hepatosplenic and renal candidiasis.

Amphotericin B

Amphotericin B is an antifungal that has broad-spectrum antifungal activity. Amphotericin B exerts its antifungal effect by binding to the ergosterol in the fungal cell membrane and disrupting the membrane by forming pores which leads to fungal cell death. Amphotericin B has been radiolabeled with ^{99m}Tc and ^{68}Ga. In vitro studies found that radiolabeled amphotericin B demonstrated the accumulation of radiolabeled amphotericin in the hyphal form of molds but not the spores.[117] This distinction in molds is vital as the hyphae represent active growing mold in invasive disease while the spores may be present in the non-invasive disease. The accumulation of Amphotericin B in fungi is not affected by the resistance of fungi to the drug, with accumulation reported in fungi species known to be resistant to the drug. The presence of ergosterol, irrespective of antifungal activity, is sufficient to cause the accumulation of the drug. The results of the in vitro studies were similar for both the PET and SPECT labelled Amphotericin B. [118] In vivo studies using mice demonstrated the accumulation of radiolabeled Amphotericin B with *Aspergillus fumigatus* and *Candida albicans* with no accumulation noted in sterile accumulation.[116] There are currently no human studies of Amphoteric B available.

Caspofungin

Caspofungin is a member of the echinocandin class of antifungal agents. The echinocandins work by inhibiting the enzyme β-(1,)3-D-glycan synthase needed for building the fungal cell wall. A complex of Caspofungin has been radiolabeled with ^{99m}Tc and demonstrated accumulation in *Candida albicans,* and *Aspergillus fumigatus* infected mice. No accumulation

was noted in sterile sites of accumulation. No equivalent PET tracer has been investigated. This tracer needs further studies for translation to humans for either evaluation of IFD or determining the distribution of tracer and studying the dose of the antifungal.[119]

Radiolabeled Monoclonal Antibodies

A monoclonal antibody, JF5, against *Aspergillus* mannose protein has been radiolabeled with copper 64 (^{64}Cu). The mannose protein is expressed on the hyphae of growing *Aspergillus spp* . but not on spores and helps distinguish the invasive disease from noninvasive forms. The radiolabeled monoclonal antibody showed uptake in mice lungs infected with *Aspergillus fumigates* but not in gram-positive and gram-negative bacteria that were tested.[120,121] There was also high uptake seen in the blood pool, liver, spleen, and kidneys. The use of ^{64}Cu, which has a long half-life of 12.7 hours, allows delayed images to be acquired, which may reduce some of the high uptake noted in the organs. The monoclonal antibody derived from mice may result in a human anti-mouse antibody (HAMA) reaction, which prevents repeated use of radiolabeled tracer. A humanized radiolabeled antibody has been developed that can reduce the risk of developing HAMA.[122] The monoclonal antibody has not been translated to human studies.

Radiolabeled Fungal Components

Chitinase

The enzyme chitinase, which degrades chitin, a component of the fungal cell wall, has also been radiolabeled with SPECT tracers. The chitinase was initially labelled with Iodine 123 (^{123}I) and ^{99m}Tc. [123,124] The ^{123}I labelled chitinase demonstrated intense uptake in *Aspergillus fumigatus* and *Candida albicans* with no significant uptake in bacterial or human cells. The delayed images of the ^{123}I chitinase showed high uptake in the thyroid and stomach breakdown of the radiopharmaceutical in vivo. No equivalent PET tracer is available, and no translation of the radiolabeled chitinase studies to humans.

Oligonucleotide Probes

Specific oligonucleotide probes that recognize fungi' ribosomal patterns have been developed to identify fungi. The genetic sequence for various microorganisms has been sequenced and known. Genetic probes which form complementary sequences for the identification of bacteria have been radiolabeled with ^{99m}Tc and have been used to image IFD in mice. [125] The radiolabeled antisense imaging agents are deoxyribonucleic acid oligomers that bind to complementary deoxyribonucleic acid and ribonucleic acid sequences. The ^{99m}Tc labelled oligonucleotide showed high specific uptake in *Aspergillus fumigatus, Aspergillus flavus*, and *Candida albicans*. No PET tracers have been used for IFD imaging, although PET-based probes have been synthesized. [126] No translation to human studies has been done.

Nonspecific Tracer in IFD

Antimicrobial Peptides

In addition to the specific tracers, some tracers have broad-spectrum activity against infectious agents, including fungi but are more specific than FDG PET/CT. Ubiquicidine 29-41 (UBI 29-41) have been labelled with SPECT and PET.[127,128] UBI 29-41 is a fragment of

antimicrobial peptides. Antimicrobial peptides are naturally occurring peptides that are part of the innate immune system. *Aspergillus fumigatus* and *Candida albicans* were found to radiolabeled UBI 29-41. Radiolabeled UBI also accumulates in bacterial infection, reducing its specificity for IFD; it does not accumulate in malignant cells. This tracer could potentially play a role in monitoring antifungal therapy.

Gallium Citrate- iron Analogue

Another nonspecific tracer that was previously used extensively in IFD is the SPECT tracer [67Ga]Gallium citrate. This was used for HIV-associated fungal infections such as *Pneumocystis jiroveci* pneumonia, where it was used in diagnosis when other imaging modalities were equivocal and used to monitor treatment. [129,130]

Conclusion and Future Perspective

FDG PET/CT is the most validated tracer in the imaging of IFD. It is useful in staging IFD and complementary to other imaging methods by detecting previously undiagnosed IFD sites. Monitoring therapy remains the most important use of FDG PET/CT. The lack of specificity and high physiologic uptake are significant limitations. FDG uptake in the brain limits the use for intracerebral lesions, and PET/MRI may be beneficial; however, the lack of widespread availability may limit its use. A PET tracer which does not show brain uptake may be better at defining brain lesions, and such a tracer can be explored. [131] Large prospective multicenter studies are required to address certain issues, such as the best time to perform FDG PET/CT follow-up scan for monitoring IFD. Some IFD-specific tracers have been evaluated, but most of these tracers have remained at the preclinical stage. The specificity achieved by the specific tracer for IFD creates the potential for treating resistant IFD with radionuclide therapy by applying theragnostic principles. [132] Multimodality imaging with optical tracers has also been explored in IFD. The backbone of the tracer used for PET can be chemically modified and attached to optical imaging agents. Imaging with an optical probe can allow real-time visualization of infected tissue during procedures such as surgery or bronchoscopy. When PET imaging is combined with optical imaging, deep tissues can be examined, and the extent of infection can be assessed in real-time. The specific siderophore tracer, which is labelled with ^{68}Ga, has also been labelled with optical probes for imaging of aspergillosis.[133] This multimodality imaging can improve management in cases requiring both preoperative assessment of deep tissues by PET and intraoperative assessment of infection by optical imaging.

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