

# ***The role of PET in monitoring therapy in fungal infections***

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## **Abstract**

Positron emission tomography (PET) is a powerful diagnostic nuclear medicine imaging technique. PET allows in vivo detection of a wide variety of physiologic and pathologic phenomena and it offers a noninvasive tool for the monitoring of therapy in various diseases. Invasive fungal infections (IFIs) are a global concern because of the increasing population of patients at risk of IFIs and the high morbidity and mortality. Therapy with antifungal agents is long-standing and expensive. The emerging resistant fungal strains make the management of IFIs challenging. There is an absolute need for a sensitive noninvasive biomarker capable of monitoring the disease activity of IFIs and determining the efficacy of treatment at an early time point. PET imaging with <sup>18</sup>F-fluorodeoxyglucose (FDG) was used to detect and assess disease activity in IFI foci already over 20 years ago. At that time, it was suggested it could be a useful biomarker for monitoring anti-fungal therapy. However, this knowledge has still not been fully exploited for the management of IFIs. The literature reveals an increasing realization of the usefulness of PET in monitoring therapy of IFIs. In this review, we highlight the advantages of nuclear medicine techniques in the management of IFIs with emphasis of the role of PET in monitoring therapy efficacy.

## **Key words**

**Fungal infections; nuclear medicine; FDG PET; therapy monitoring; candida; aspergillus**

## **Introduction**

Nuclear medicine techniques are extensively used in the diagnosis and management of disease. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are the two main nuclear imaging techniques currently used in daily practice. Nuclear imaging, as a functional imaging technique, allows the visualization of physiochemical processes in the body of humans and animals. This allows the imaging of disease at the molecular level. Advances in medicine have resulted in an increased understanding of the molecular basis of diseases; therefore the use of nuclear medicine techniques is becoming more and more relevant in management of disease [1-3]. Despite advances in the diagnosis and management of invasive fungal infections (IFIs), the morbidity and mortality remains high [4]. This is partly due to the lack of sensitivity of the currently available microbiologic diagnostic and management platforms [4,5]. As a result of this inadequacy, new diagnostic and management tools are being developed and other therapeutic strategies are being explored [6-8]. There is an absolute need for a sensitive noninvasive biomarker to be able to monitor and evaluate the traditional and new methods of managing antifungal agents. Up until now, nuclear medicine has played a limited role in the management of IFIs. It has been over 2 decades ago since PET demonstrated the ability to not only detect fungal lesions but also to assess disease activity within these lesions [9]. These findings suggested that PET could be used to monitor IFIs. In spite of this, PET imaging has not been fully exploited in the management of IFIs in general and for the monitoring of therapy in IFIs in particular. In this review paper, we provide an overview of what

nuclear medicine has to offer in patients with IFIs to hopefully make clear that there is an absolute role for PET in therapy evaluation and treatment decision-making.

## **SPECT imaging in infections**

Several SPECT tracers have been used and are still being used in infection imaging. Labeled white blood cells (WBC) are still considered the gold standard for infection imaging [10, 11], although the role of WBC imaging in patients with IFIs is not fully elucidated yet. The process of labeling is however laborious and technically demanding and there is a risk of contamination during handling blood products. The imaging process itself requires four hospital visits for the patient on two consecutive days [12]. A procedure that is not so laborious and technically demanding would be of great benefit to the patients, referring clinicians and nuclear medicine specialists. Other SPECT tracers such as gallium 67 ( $^{67}\text{Ga}$ ) citrate were used in the past for infection; however, this tracer has largely been replaced by labeled WBC except in a few cases such as vertebral osteomyelitis [13, 14]. There are several other SPECT tracers available for imaging microorganisms such as radiolabeled antibiotics, however these tracers are not being used in routine daily practice yet. In IFIs in particular, technetium 99m ( $^{99\text{m}}\text{Tc}$ ) fluconazole has been used and also molecules targeting other molecules or enzymes in the fungi (such as chitin, a component of the fungal cell wall) have been labeled with the aim to localize these infections [15-17], but also their use is still limited. However, these specific tracers for IFIs potentially could be used to monitor fungal infections and therefore deserve to be further explored.

## **PET imaging in infection**

There are several PET tracers available in clinical use for infectious diseases that are able to reveal information at a molecular cell level.  $^{18}\text{F}$ -fluorodeoxyglucose (FDG) is the most commonly used PET tracer in clinical setting. This glucose analogue is taken up by the glucose transporter (GLUT) on the surface of the cells in the body. Once it enters the cell it is phosphorylated by hexokinase into FDG-phosphate, like glucose. However, unlike glucose, it is unable to continue further in the metabolic pathway and therefore remains trapped in the cell thereby allowing it to image the enhance glucose consumption in pathology [18]. The use of FDG in IFIs will be explained further on extensively. There are several other PET tracers available allowing us to image different physiological processes in infectious diseases. For example,  $^{68}\text{Ga}$ -DOTATATE is a somatostatin analogue used for imaging somatostatin receptors that are not only expressed by neuroendocrine tumors [19] but also in granulomatous diseases.  $^{68}\text{Ga}$  citrate is an iron analogue that has already found some applications in infection and inflammation where its corresponding (SPECT) counterpart  $^{67}\text{Ga}$  had a well-established role in the past [20]. However,  $^{18}\text{F}$ -FDG PET is currently used for many roles in infection and inflammation for which  $^{67}\text{Ga}$  was previously used. PET imaging, particularly with FDG, has been validated for monitoring several diseases [3, 21]. Its application has been very prominent in oncology for both hematological and solid tumors. Infectious and inflammatory diseases in which PET is currently used for therapy monitoring include inflammatory bowel disease, infected vascular grafts, endocarditis, and tuberculosis. Research or validation in larger prospective studies is under way in a lot of other diseases [21-25]. We will now focus on IFIs and provide an overview of what FDG PET has to offer in patients with IFIs with special focus on therapy evaluation and decision-making. The increased awareness of the role of PET imaging in invasive fungal infections (IFIs) has been well documented [26].

## **Invasive fungal infections (IFIs)**

IFIs are a cause of high morbidity and mortality worldwide. Their contribution to the global burden of disease is largely unrecognized. Most are opportunistic infections. Although true mortality rates are unknown because of a lack of good epidemiological data, the incidence of invasive fungal infections is rising as a result of modern medical interventions and immunosuppressive diseases, such as AIDS. Despite the high mortality rates of invasive fungal infections, they remain understudied and underdiagnosed as compared with other infectious diseases [4, 27].

### **Risk factors for IFIs**

Risk factors for IFIs include underlying disease such as hematologic or solid malignancies that cause immune suppression, Human Immunodeficiency syndrome (HIV), patients with end organ failure and diabetes mellitus. Medical therapies that cause immune suppression such as chemotherapy for hematologic or solid organ malignancies, hematopoietic stem cell transplantation, solid organ transplantation, invasive and complex surgeries, and immune modulating therapies are also risk factors for IFIs. Patients in intensive care units in whom indwelling catheters remain for a long period of time and in whom the prolonged use of antibiotics changes the gut flora are at risk for acquiring IFIs. Patients with burns and premature babies are also at risk [30-32].

### **Pathogens causing IFIs**

IFIs are not caused by a single organism but by a number of different species of fungi that have been generally divided into yeasts and molds. *Candida* is the most common type of yeast whilst *Aspergillus* is the most common type of mold. Together these two fungi species constitute the most common fungi causing IFIs. There are a number of rarer causes of IFIs such as *Zygomycosis*, *Histoplasmosis*, *Cryptococcus*, *Pneumocystis jirovecii* amongst others, which can also cause IFIs [33, 34]. Some of these are more prevalent in certain population groups. *Cryptococcus* and *Pneumocystis* are more likely to be found in HIV patients. Indeed within the *Candida* species there are many subtypes such as *Candida parapsilosis* and *Candida glabrata*, which vary with geographic location, age of patient, and virulence. The morbidity and mortality varies with the type of fungus, the initial load at the site of infection and even the age of the patient. Furthermore, the length and depth of the immunosuppression state with or without swift adequate diagnostics importantly determines the outcome of IFIs.

### **Candida**

This is the fourth most common blood stream infection in the US and the sixth most common in Europe [30, 35]. The mortality rate of a *Candida* infection is 20-40% [6, 36]. Infants with invasive candidiasis who survive frequently have long-term neurological impairment, including cerebral palsy, blindness, hearing impairment, cognitive deficits, and periventricular leukomalacia [37]. The most predominant species is *Candida albicans* but there is a shift to the less common types like *Candida glabrata*. The susceptibility of the various species to antifungal agent varies [38].

Invasive Candidiasis may present as a candidemia with fever and sepsis. It may also present with a blood culture negative syndrome with disseminated (hepatosplenic) candidiasis or deep seated infections in distant organs in patients with hematologic cancer or other hematological disorders

[30]. Monitoring therapy in the blood culture negative group is a challenging task and DG PET may have added value under such circumstances [39].

There are no prospective data available on the duration of treatment of invasive candidiasis. Recommendations are mainly based on expert opinions in both adults and children [40, 41]. A biomarker that can quantitatively assess disease activity in infectious foci would be of immense benefit in this regard.

## Aspergillus

The mortality rate for an Aspergillus infection is reported to be over 50%-90%, despite the use of antifungal agents [6, 35]. Optimal duration of invasive aspergillosis therapy is not known [40]. Continuation of treatment for 6–12 weeks, or until immune recovery, or resolution of clinical/radiological evidence of disease, is most frequently recommended. The radiological evidence is based primarily on CT or MRI monitoring [42, 43]. The latter recommendation may end up exposing these patients to prolonged and unnecessary therapy and toxicity as there are well known cases in literature in where metabolic resolution of disease was objectified by FDG-PET with anatomical changes (by CT or MRI) lagging behind these metabolic changes (see also table 1).

**Table 1** Published articles on the use of FDG PET in monitoring IFIs

Cause of IFI	Author and year	Age	Underlying condition	Sex	Comment	Finding on FDG PET
<b>Aspergillus</b>	Franzius C et al. 2001. Clin Nucl Med [82]	3 months (twins)	Chronic granulomatous disease	F	IFI was diagnosed in an asymptomatic twin sibling of an active case and both cases monitored by FDG PET	Resolution of pulmonary FDG uptake in both infants after antifungal therapy
<b>Aspergillus</b>	Ozsahin H et al. 1998. Blood [83]	8 years	Chronic granulomatous disease	M	The IFI was unresponsive to antifungal therapy. FDG PET used to monitor and underwent bone marrow transplant (BMT) successfully	FDG avid pulmonary nodules present before (BMT) did not worsen during and after BMT
<b>Aspergillus</b>	Chamilos G et al. 2008. Med Mycol [84]	25 years	Hodgkins disease, HSCT	M	Pulmonary nodules still present on CT after 120 days of antifungal therapy	FDG PET activity in nodules had become negative by day 120
<b>Aspergillus</b>	Chamilos G et al. 2008. Med Mycol [84]	39 years	Non Hodgkins lymphoma, HSCT, GVHD	M	Scarring on CT in IFI lesions after 270 days of antifungal therapy	FDG PET was negative in scarred tissue

						by day 270
<b>Aspergillus</b>	Chamilos G et al. 2008. Med Mycol [84]	61 years	Relapsed esophageal cancer	M	Monitor therapy to determine when to stop antifungal agents	PET positive on day 120 but negative on day 320 of antifungal treatment
<b>Aspergillus</b>	Hot et al. 2011 [85]	33 years	Relapsing Hodgkins disease, HSCT	M	Monitor therapy to determine when to stop antifungal agents	Decrease in FDG uptake on follow up-treatment response
<b>Aspergillus</b>	Hot et al. 2011. Clin Microbiol Infect [85]	6 years	Chronic granulomatous disease	M	Monitor therapy to determine when to stop antifungal agents	Persistence of FDG uptake on follow up
<b>Candida</b>	Xu B et al 2010. Clin Nucl Med [86]	23 years	Acute leukemia (mixed lineage)	M	Led to change of antifungal therapy and helped determine when to stop antifungal	Hepatic, splenic and pelvic uptake due to IFI. Hepatic and splenic lesions progressed and subsequently resolved after antifungal therapy change
<b>Candida</b>	Xu B et al 2010. Clin Nucl Med [86]	57 years	Acute myeloblastic leukemia	M	Detected poor response to antifungal therapy and led to change in antifungal	Pulmonary and hepatic uptake due to IFI which progressed and eventually normalized after antifungal therapy change
<b>Candida</b>	Xu B et al 2010. Clin Nucl Med [86]	30 years	Acute myeloblastic leukemia	F	Detected poor response to antifungal therapy and led to change in antifungal	Hepatic uptake due to IFI worsened and resolved after switch in antifungal therapy
<b>Candida</b>	Avet J Jr et al. 2009. Eur J Nucl Med Mol	16 years	Leukemia	-	Detected poor response to antifungal therapy and led to change	Multiple liver, spleen, kidney, muscle, myocardial and

	Imaging [87]				in antifungal	pulmonary uptake due to IFI which resolved on switching antifungal
<b>Candida</b>	Wallner M et al. 2013. Herz [88]	72 years	Bioprosthetic Aortic Valve	M	Showed good response to antifungal therapy	Complete resolution of increased FDG uptake in the area of prosthetic aortic valve
<b>Candida</b>	Teyton P et al. 2009. Clin Nucl Med [89]	22 years	Acute myeloid leukemia	M	Detected poor response to antifungal therapy and led to change in antifungal	Multiple uptake in liver and spleen not responding to antifungal therapy which resolved after a switch in antifungal therapy
<b>Candida</b>	Hot et al. 2011. Clin Microbiol Infect [85]	27 years	Testicular Cancer	M	Decrease in FDG uptake on follow up- treatment response	FDG uptake in spleen decrease after antitherapy administered
<b>Candida</b>	Hot et al. 2011. Clin Microbiol Infect [85]	24 years	Hodgkins disease, HSCT, GVHD	M	Used to monitor antifungal therapy	Persistence of FDG uptake after 9 months of antifungal therapy
<b>Zygomycosis</b>	Ritz N et al. 2015. Eur J Pediatr [90]	9 years	Burkitts lymphoma	F	Showed extrapulmonary disease and directed biopsy and allowed follow up of antifungal therapy	Gradual resolution of pulmonary uptake and therapy switched because of unresolving splenomegaly
<b>Zygomycosis</b>	Altini C et al. 2015. Clin Nucl	13 years	Acute Lymphoblastic leukemia	F	Assessed response to therapy and	FDG uptake in the rhino-

	Med [91]				correctly predicted progression when MRI did not	orbital-cerebral area which became more intense
<b>Zygomycosis</b>	Liu Y et al. 2013. Clin Nucl Med [92]	23 years	Aplastic anemia	F	Serial scan enabled modification of antifungal therapy	FDG uptake in head and neck due to IFI gradually resolved
<b>Zygomycosis</b>	Chamilos G et al. 2008. Med Mycol [84]	59 years	Relapsed CLL, HSCT, GVHD	M	Persistence of mass after 60 days of antifungal therapy	Complete resolution of FDG uptake in pulmonary IFI after 60 days of antifungal therapy
<b>Zygomycosis</b>	Chamilos G et al. 2008. Med Mycol [84]	42 years	Relapsed CLL, HSCT, GVHD	M	Concordant response of CT and PET findings-resolution	Minimal uptake in regressing pulmonary nodules on CT 90 days after antifungal therapy
<b>Unidentified yeast-like fungi</b>	Miyazaki Y et al. 2011. Ann Hematol [93]	32 years	Relapsed acute myeloid leukemia, BMT	F	Detected response to antifungal therapy earlier than other imaging modalities enabling timely BMT to be undertaken with no recurrence of IFI after BMT	FDG uptake in liver lesions due to IFI had diminished at a time when there was little change in other imaging modalities
<b>Histoplasma</b>	Kasaliwal R et al. 2014. Clin Nucl Med [94]	60 years	None	F	Monitoring of antifungal therapy was consistent with clinical outcome	Reduction in FDG uptake in mediastinal nodes and adrenal glands bilaterally
<b>Histoplasma</b>	Tsai YJ et al. 2013 Clin Imaging [95]	74 years	Diabetes mellitus	M	Monitoring of antifungal therapy was consistent with clinical outcome	Reduction in FDG uptake in adrenal glands bilaterally
<b>Coccidiomycosis</b>	Hot et al. 2011. [85]	61 years	None	F	FDG PET showed treatment	Decreased FDG uptake in lung



					response	and mediastinum after antifungal therapy
<b>Mycetoma</b>	Hot el al. 2011. Clin Microbiol Infect [85]	42 years	None	M	Showed favorable response to antifungal agents	Decreased FDG uptake in the lower limb and fibula after therapy
<b>Phomopsis</b>	Hot el al. 2011. Clin Microbiol Infect [85]	59 years	Diabetes mellitus	M	Showed favorable response to antifungal agents	Decreased FDG uptake in the right ankle after therapy
<b>Scedosporium</b>	Hot el al. 2011. Clin Microbiol Infect [85]	32 years	Cystic fibrosis, chronic liver disease	M	Used to monitor antifungal therapy in vertebral osteomyelitis	FDG uptake revealed more vertebral involvement than MRI that persisted on follow up
<b>Cryptococcus</b>	Dubbioso R et al. 2013. J Neurol Sci [96]	63 years	None	F	Monitored therapy in the choroid plexus	Reduction in uptake in the medial temporal cortex which corresponded to the choroid plexus on co- registered images

## Other species

Morbidity and mortality amongst other species varies with the type. In Cryptococcus, which is a very common IFI in HIV patients, the mortality is 20-70% [6, 44]. The duration of therapy for most of the rarer causes of IFIs is either based on expert opinion or in some cases unclear. In some cases, treatment lasted more than 2 years, thereby exposing the patient to toxicity and drug-drug interactions that frequently accompany antifungal agents [40-46].

## Diagnosis of IFIs

FDG PET is a sensitive functional imaging technique that detects pathology before anatomical changes occur. In IFIs it detects the glucose uptake in the inflammatory cells that are attracted to the site of infection and are activated. This change precedes anatomical changes and so occurs before

imaging methods that rely on anatomical changes will become positive. FDG PET would thus pick up metastatic infective foci at a time when other imaging modalities would be negative. This is important in early diagnosis of IFIs that impacts on the survival the host. Nowadays, hybrid camera systems (PET/CT) exist, leading to better localization of the metabolically active infectious focus and to better diagnostic accuracy.

The detection of metastatic foci in different sites of the body would influence the management of the IFI. The selection of the optimum therapy must take into account the location of IFIs. FDG PET imaging offers this advantage. The antifungal agent penetrates different tissues to a different extent. Cases of IFIs where there is improvement in many sites, but persistent disease activity in a particular site are known to occur (for an example see figure 2). If one monitors treatment efficacy with an imaging procedure limited to one region of the body other potentially persistent locations may be missed and therapy management may be inadequate.

### **PET imaging in invasive fungal infections**

For about 2 decades, IFIs have been known to accumulate FDG [9]. The FDG uptake in these focal lesions was found to correspond to disease activity, making FDG PET a potential candidate for monitoring IFIs. The first publications about IFIs and FDG were focused on the poor specificity of FDG PET, because IFIs were found to cause a false positive uptake in the diagnosis of cancer in patients [47-49]. Only later the potential role of FDG PET in the diagnosis of IFIs was recognized. These publications concentrated on the ability of FDG PET to stage IFIs and highlighted the ability of FDG PET to detect occult foci of IFIs [50, 51]. Recently publications on the topic however have been predominated by FDG's ability to monitor therapy in IFIs, which will be discussed later on.

### **Other diagnostic possibilities**

Identification of the offending fungus with subsequent drug susceptibility testing remains the gold standard in diagnosing IFIs. However as a result of the increased mortality associated with the long turn over times other indirect methods of diagnosing have been employed. These include serological markers such as galactomannan assays for *Aspergillus*, nucleic acid techniques and radiological imaging particularly with high resolution CT scan of the chest. However the definitive diagnosis of IFIs still remains a challenge; it needs biopsy with morphology of invasiveness of the fungus besides culturing. Robust and more sensitive diagnostics are still required [4, 27, 52].

### **Management of IFIs**

The administration of an antifungal drug after the definitive microbiological proof of a fungal infection constitutes what has been defined as targeted antifungal therapy. There is a long turnaround time for culture results with late microbiological documentation in the course of infections [52-55]. A delay in therapy institution in IFIs is associated with increased mortality [56-59]. As a result, different antifungal strategies have been studied for preventing IFIs in non-neutropenic critically ill patients. The administration of any antifungal drug prior to the definitive microbiological evidence of fungal infection constitutes an untargeted antifungal treatment. Recently, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) provided the definitions of three different treatment strategies, classifying them into prophylactic, pre-emptive and empiric

treatments [60]. Prophylaxis has been defined as the administration of antifungal agents in patients without proven or suspected IFIs (i.e. absence of microbiological or radiological evidence) but with risk factors for its development. Pre-emptive treatment (diagnosis-driven approach) has been defined as treatment triggered by imaging with (probable IFI) or without (possible IFI) microbiological evidence of fungal infection, without definitive microbiological proof. Surrogate biomarkers for the presence of fungal infection (e.g. 1-3  $\beta$ -D-glucan, mannan/anti-mannan antibody) have been investigated for this purpose [61, 62]. Empiric treatment (fever-driven approach) has been defined as the antifungal treatment triggered by signs and symptoms of infection in patients at risk for IFI, in the absence of microbiological and imaging evidence of infection at the moment of therapy institution. A recent review of the data provided evidence that the role of untargeted therapy is still not conclusive in non-neutropenic patients [52]. PET imaging, by virtue of the ability to quantify uptake, would be a helpful tool for the early diagnosis in pre-emptive strategies but more importantly it offers objective quantifiable measures of response to therapy in IFIs.

## **Classes of antifungal drugs available**

The classes of antifungals currently in clinical uses for IFIs include the fluoropyrimidines, polyenes, azoles, and the echinocandins [63].

Fluoropyrimidine a synthetic analogue of DNA, has only 5-fluorocytosine as the antifungal agent in use. It is a prodrug possessing no antifungal properties on its own but converted to 5-fluorouracil (the antitumor agent) by a cytosine deaminase enzyme present in fungi and not in humans by which it exerts its antifungal effects. It diffuses rapidly throughout the body after oral administration and is generally safe although it may cause hepatotoxicity and bone marrow suppression. It has broad-spectrum activity particularly potent with *Candida* and other yeasts though its action against *Aspergillus* and other molds are more limited. Its use is declining due to development of resistance and it is mostly used in combination with other antifungal agents [64, 65].

Polyenes are amphiphilic organic compounds known as macrolides that target ergosterol, the main sterol component of fungal membranes. Amphotericin B belongs to this class. It has a broad spectrum covering most fungi. They possess an affinity for human cholesterol giving rise to their toxicity. The toxicity is mainly hepatic and renal and liposomal or lipid complexes minimize these side effects. Resistance has been reported but is a rare clinical event. The major limitation of the use of this drug is its toxicity [66].

Azoles are the most commonly used antifungal drugs in clinical practice and include fluconazole, itraconazole and the more recent new generation of triazoles: voriconazole and posaconazole. The target is a key enzyme in the ergosterol biosynthetic. The azoles, particularly fluconazole, have a broad spectrum of activity though resistance to emerging fungal infections has been reported. Fluconazole has both oral and intravenous formulations and diffuses throughout the body, even to the cerebrospinal fluid. The azoles have specific drug interactions, particularly to some antineoplastic and HIV drugs. Resistance of *Candida* species against fluconazole has developed due to over-prescription for prophylaxis and treatment. The newer generation triazoles have a wide range of activity and are more effective against *Candida* and *Aspergillus*. The side effects and drug interactions are similar to older azoles, but at a greater extent as higher antifungal plasma levels can

be reached. There is also cross-resistance to the newer generation azole in fungi resistant to classical azoles [67-69].

Echinocandins, a new class in the antifungal agents, inhibit enzymes responsible for the synthesis of the cell wall. They include micafungin, caspofungin, and anidulafungin. They possess low toxicity and interaction with other drugs is rare. They have a broad activity range active against both *Candida* and *Aspergillus*. There are however some fungi that show resistance or intermediate susceptibility. The other limitation of these drugs is the absence of an oral formulation. They are particularly useful when other antifungal agents are ineffective. Each drug in this class has special advantages such as the ability to use anidulafungin in patients with hepatic and renal impairment. Their combination with other antifungal agents has a synergistic or at least an additive effect [70-72].

## **Other strategies to combat fungal infections**

In addition to the antifungal agents described above, a number of other therapeutic approaches are employed or being considered to combat fungal infections. The concept of “repurposing” where established medication to treat new diseases has emerged as an approach to expedite drug development in general. In fungal infections, the immune modulator calcineurin and the target of rapamycin inhibitors possess antifungal activity and synergize fluconazole. Antineoplastic and antidepressant agents have been shown to be potential drugs for repurposing in the management of IFIs. Many of these drugs have antifungal activity themselves but also potentiate the action of the traditional antifungal agents [6, 73-75].

The susceptibility to the outcome of IFIs depends on the pathogen and the host. The traditional antifungal agents deal with the pathogen, (the fungus). The efficacy of the immune response and the degree of the immune suppression in the patient are the major host determinants. Modifying the host is a target in the management of IFIs. Strategies to modify the host include cytokine therapy, granulocyte transfusion, antibodies, vaccinations, natural killer cell treatments and adoptive T cell transfer. These strategies are already in use or being explored for the management of IFIs [7, 76-81].

Non pharmacologic therapy such as surgical debridement of infected vertebrae or endoscopic drainage of lung abscesses may also be necessary in addition to antifungal agents for the management of IFIs. Surgery is warranted in cases of zygomycosis.

All these procedures, both pharmacological and nonpharmacological, require monitoring by a sensitive biomarker and PET, as a noninvasive functional imaging modality, has the potential to do so.

## **Monitoring therapy in IFIs**

Despite advances in diagnosis and management IFIs still remain a cause of high morbidity and mortality in immunocompromised patients [4]. This is because the standard diagnostic modalities lack the sensitivity for detection and therapeutic monitoring of IFIs [5, 27,28]. This is of global concern because there is an expanding population of patients at risk for IFIs particularly in the past three decades. The reason for this increase is the advance in medical therapy exposing patient to more immunosuppressed states and complex surgical procedures patients are exposed to [4].

## Monitoring IFIs with FDG PET

The available literature shows that FDG PET is avid irrespective of the pathogen causing the IFI. This provides a sensitive early staging of the IFI and a noninvasive means to follow up the therapy of the patient. The disease activity at various different sites of the body can be adequately evaluated. It can also tell at an early point in the therapy if a particular therapy is not adequate and therefore a modification of antifungal therapy or even a non-pharmacologic therapy such as surgical treatment may be required.

There are relatively few publications on the use of FDG PET for monitoring therapy response in IFIs and these are usually only case reports. Large multicenter studies are lacking. Earlier case reports focused the IFIs as a cause of false positive results in cancer diagnosis, because the scans were primarily performed to evaluate patients with a suspected malignancy. There has been a shift in focus and as a result recent articles on FDG and IFIs more and more address monitoring therapy (table 1). The available publications demonstrate the usefulness of FDG in monitoring both the more common causes of IFIs, *Candida* and *Aspergillus* sp. [82-88], and also the other causes of IFIs such as *Zygomycosis* sp. [84, 90-92], *Histoplasmosis* sp [94, 95], *Coccidimycosis*, *Phomopsis*, *Mycetomas* spp. [85] and *Cryptococcus* sp. [96]. In one report, FDGPET/CT correctly predicted disease progression in IFIs, while MRI erroneously predicted an improvement in disease [91]. These reports included both patients with typical risk factors of immune suppression and those without the typical risk factors. An overview of all the available literature on the use of FDG PET in monitoring IFIs is presented in Table 1.

Again although underrepresented, the literature shows monitoring therapy is effective in children just as well as in adults [82, 83, 85, 87]. High resolution CT scan was found to have an impact on the early diagnosis of IFIs, especially in *Aspergillus*, resulting in including this technique in the guidelines. It has been documented however that there are differences in CT findings between children and adults [97]. The findings of FDG uptake in both the adult and pediatric population suggest it can be used as a tool in both population groups.

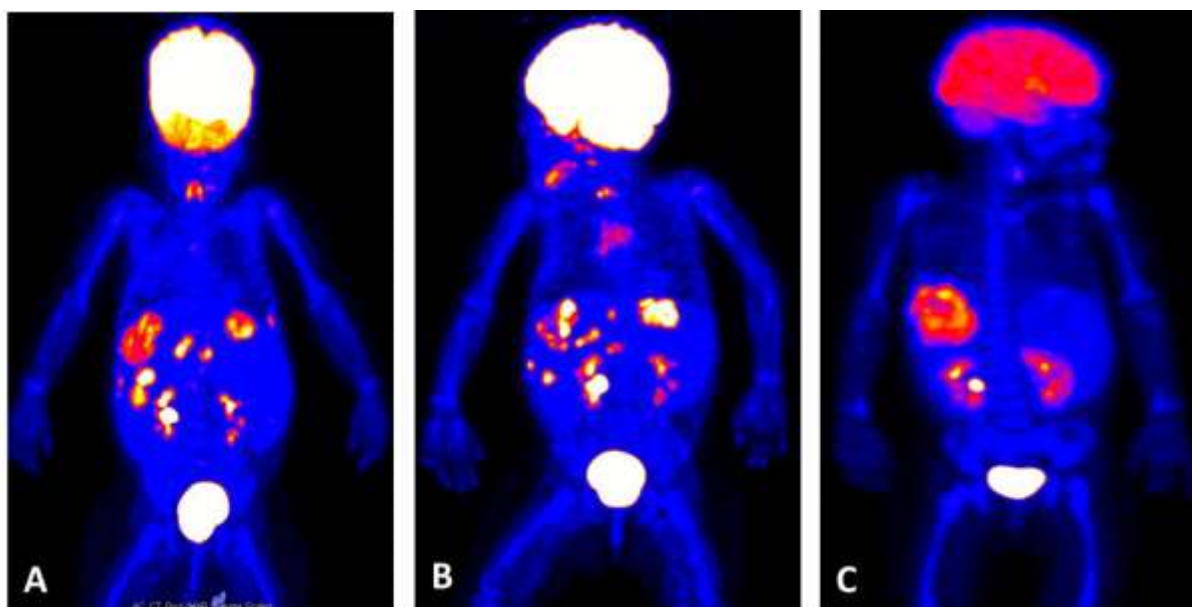
FDG imaging led to a change in antifungal therapy because of the poor response seen on follow up FDG studies in a number of reports [86, 87, 89]. It also helped to extend antifungal therapy in a case in which response was seen but in which there was still some disease activity after 120 days. After another 200 days of antifungal therapy the scan became negative for active disease. FDG PET was also helpful in informing the clinician when to stop antifungal therapy by showing inactive disease at a time point when other imaging modalities had not completely resolved [84].

In another study, FDG was unfortunately not performed before starting the therapy but when the scan was performed during therapy, it not only showed poor response to the antifungal treatment but it also revealed other foci of infection that were previously unknown to the clinicians [87].

In IFIs FDG is taken up by activated inflammatory cells. Some concerns have been raised about the ability of FDG PET to detect IFIs in neutropenic patient that may be the case in patients with hematologic malignancies. Studies conducted in patients with febrile neutropenia showed that infections, including IFIs, were clearly identified. The mechanism is probably due to uptake by other inflammatory cells such as macrophages and not neutrophils, or due to a stress response of the infected host cells [97, 98].

## Our experience in monitoring IFIs with FDG PET

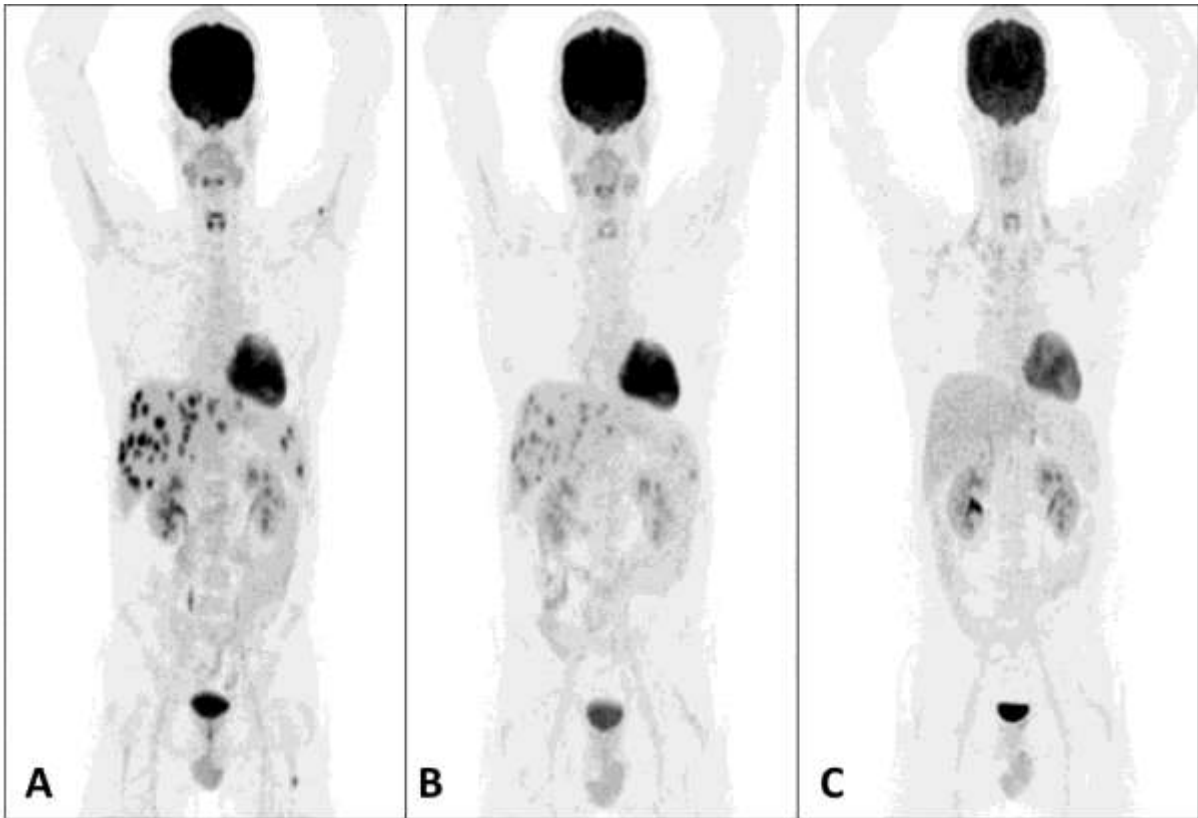
In our hospital, we now already use FDG PET for therapy evaluation in patients with IFIs for a couple of years and in our experience FDG PET helped in decision making in most patients. For example, FDG PET allowed the modification of therapy in one child and in another child with hepatic aspergillosis it helped in deciding to perform surgery for final resolution of all the hepatic lesions. This child showed improvement in all hepatic lesions, except one that had a large necrotic center and became larger during therapy (figure 1). The decision was taken to go to surgery for this progressive lesion and indeed large necrotic areas were found and an encapsulation, the reason why the antifungal drugs were not effective there.



**Figure 1:** Example of use of FDG-PET in therapy monitoring in a 2-year old girl with Langerhans cell histiocytosis and bone marrow transplantation. She was diagnosed (after biopsy) with aspergillus lesions in the liver.

- (A) Baseline FDG-PET scan, MIP image, revealing multiple fungal lesions in the liver.
- (B) FDG-PET scan after 6 months of antifungal therapy, showing a decrease in FDG uptake of some liver lesions, but an increase in other liver lesions. Based on these findings, antifungal treatment was switched.
- (C) FDG-PET scan after therapy switch, revealing disappearance of all liver lesions except one which became larger in time. Eventually this lesion was surgically removed, showing an encapsulated fungal lesion, which could not be reached by the antifungal drugs. Note also the decreased uptake in the brain at the third scan. This scan was performed under sedation.

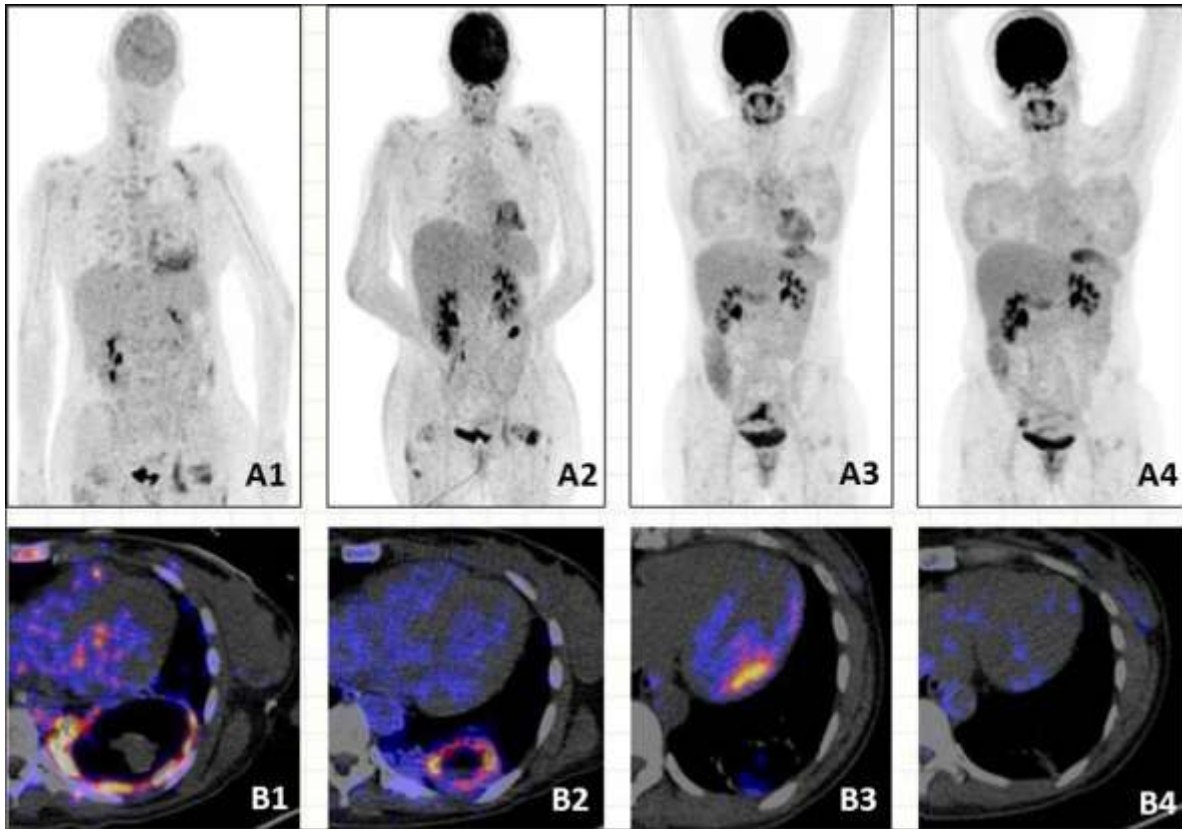
Figure 2 shows an adult patient with disseminated candidiasis in which treatment had to be modified and changed to totally ensure that underlying infection had been eliminated. This is very important when an immunosuppressive therapy such as hematologic stem cell transplant or chemotherapy is being planned.



**Figure 2** Example of use of FDG-PET in therapy monitoring in a 24-year old male on treatment for ALL. He was diagnosed with candidiasis by a biopsy.

- (A) Baseline FDG-PET scan, MIP image, revealing multiple fungal lesions in the liver and spleen.
- (B) FDG-PET scan, MIP image, after 4 months of antifungal therapy, showing a decrease in FDG uptake in some liver lesions (partial response). Based on these findings, antifungal treatment was switched.
- (C) FDG-PET scan, MIP image, 3 weeks after therapy switch, revealing disappearance of almost all hepatic and splenic lesions.

In figure 3 another example of the added value of FDG PET in monitoring IFIs is shown. This is a 42-year old female in remission after treatment for acute myeloid leukemia. She was diagnosed with aspergillus by a combination of clinical presentation, serological markers and a high resolution CT scan of the chest. The initial FDG PET scan was performed to assess the activity and stage infection. The serial scans showed a gradual decrease of FDG uptake till there was complete metabolic response. After this complete response, the chemotherapy could be continued.



**Figure 3:** Example of the use of FDG-PET/CT in therapy monitoring in a 42-year old female in remission after treatment for AML. She was diagnosed with aspergillus by a combination of clinical presentation, serological markers and a high resolution CT scan of the chest. The initial FDG PET scan was done assess the activity and stage infection.

(A1) Baseline FDG-PET scan, MIP image and (B1) corresponding fused PET/CT transverse section through a single large pulmonary lesion with increased glucose metabolism and a cold area in the center consistent with active aspergillosis.

(A2-A4) Other 3 FDG-PET scans, MIP image and (B2-B4) corresponding transverse fusion images: follow up scans while patient was on treatment showing good response and after the 3<sup>rd</sup> follow up scan antifungal therapy was stopped and patient was able to undergo a new cycle of chemotherapy.

### **Therapeutic drug monitoring of antifungal agents**

Some antifungal agents exhibit marked variability in bloodstream concentrations that are difficult to predict on the basis of dosing alone. Therapeutic drug monitoring (TDM) by measuring plasma levels is advocated by some experts and guidelines as an adjunct in routine administration of some antifungal agents [99-101]. TDM may increase the probability of a successful outcome, prevent drug-related toxicity and potentially prevent the emergence of antifungal drug resistance. Much of the evidence that supports TDM is circumstantial [99].

TDM assesses the availability in plasma, thereby monitoring the pharmacokinetics; it does not address the antifungal activity in vivo. FDG PET can provide insight in the total disease activity in the body. The combination of TDM in situations where it is warranted and FDG PET imaging will provide



not only pharmacokinetic information about the drug but would enable visualization of the fungicidal effect and may help to determine if there is resistance to antifungal therapy.

## Limitations

The use of FDG PET imaging in IFIs is not without challenges. FDG PET imaging uses ionizing radiation, which lead to radiation burden to the patient. When FDG PET is combined with contrast enhanced CT the radiation burden is even higher. When diagnostic CT scans are performed the CT component may contribute as much as 81% to combined radiation dose [102]. Multiple studies for follow up would mean an even further increase in the radiation burden. The risk of a devastating and life-threatening outcome of an IFI exceeds in most cases the disadvantages of the radiation exposure used in FDG PET/CT imaging. The principle of “as low as possible achievable (ALARA)” is adhered to during FDG PET/CT imaging to avoid unnecessary radiation to the patient. To reduce the radiation burden we recommend a baseline scan with a diagnostic contrast enhanced CT and all follow up studies with a low dose CT. As PET/MRI becomes more available we would recommend its use, especially in children and in cases where soft tissue definition would be beneficial.

Secondarily, FDG has sites of high physiological uptake in areas such as the brain, urinary tract and heart that may make visualization of IFI foci in these organs difficult. In spite of this FDG visualized IFIs in the brain and was used to follow up IFI in this area [96]. The physiologic uptake may pose difficulties in determining if there is complete metabolic response when FDG is used for monitoring therapy and disease activity is reducing.

## Conclusion and future perspectives

There is an increasing evidence on the use of FDG PET imaging to monitor IFIs. It has been shown, mainly in case reports, to be useful across a wide range of different fungal species. It can have a large impact on management resulting in modification or switch in antifungal treatment. It is especially important in monitoring therapy of blood culture negative IFIs with suspected foci of infection in organs of patients with hematologic malignancies or solid organ transplant. It may also help to determine when antifungal agents may not effectively reach the site and surgery rather than antifungal therapy alone should be recommended. Large multicenter prospective studies are needed to enable this technique to be incorporated into major guidelines.

FDG PET is unable to discriminate fungal infection from other infections such as bacterial infections or malignancy. The clinical setting in which disease occurs but more importantly histopathological finding is needed for definitive diagnosis. The search for other radiopharmaceuticals to give more specific answers to specific problem is an ongoing process. Molecular imaging may provide clinicians with unlimited possibilities and in the future may enable the use of personalized medicine when PET imaging probes are developed that are able to target the specific fungi causing IFIs.

<sup>99m</sup>Tc- fluconazole was successful in imaging Candida infections. <sup>99m</sup>Tc-fluconazole accumulated only in viable Candida infections and its uptake correlated very well with the number of fungi present, suggesting it could be useful for monitoring antifungal therapy. It did not accumulate in bacteria of *Aspergillus fumigates*. The corresponding PET radiotracer <sup>18</sup>F-Fluconazole has not been so successful in imaging Candida infections. There is poor accumulation at infected sites and the amount of background activity in the liver decreases its sensitivity to detect Candida infections. The reason for

the difference between this PET tracer and its SPECT equivalent may be the different labeling methods resulting in <sup>18</sup>F-fluconazole being much more lipophilic than <sup>99m</sup>Tc-fluconazole, resulting in poorer imaging characteristics [15-17]. If the synthesis of a labeled fluconazole analog for PET imaging is optimized, it may provide a radiolabeled probe specific for *Candida*.

Again, in preclinical PET imaging studies, <sup>68</sup>Ga citrate labeled with Triacetylfusarinine C (TAFC) and ferrioxamine E (FOXE) have been shown to be highly sensitive for imaging *Aspergillus*. TAFC and FOXE are common trihydroxamate-type siderophores with relatively low molecular weight produced by fungi, bacteria, and some plants for scavenging iron to make it available to the organism. These tracers appear to be specific for the imaging of *Aspergillus*. Radiotracers that are based on detection of antigens present in fungi but not present in mammals may also provide valuable probe for imaging and monitoring response of antifungal therapy [26].

A new probe for noninvasive detection of *Aspergillus fumigatus* lung infection based on antibody-guided positron emission and magnetic resonance imaging has recently been developed. This [<sup>64</sup>Cu]DOTA labeled *A. fumigatus*-specific monoclonal antibody demonstrated the ability to distinguish invasive pulmonary aspergillosis to bacterial infections in mice. This could potentially be a very useful and specific monitor invasive pulmonary aspergillosis [103].

In summary, PET imaging has the potential to become a sensitive, noninvasive tool to monitor fungal infections in adults and children treated with both pharmacologic and nonpharmacologic therapeutic strategies. The role of PET may even become more important in the future with the development of new specific tracers.

### **Compliance with ethical standards**

*Disclosure statement:* All authors have nothing to disclose.

*Conflicts of interest:* None of the authors have a conflict of interest.

*Ethical approval:* All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and the national regulations and also with the principles of the 1964 Declaration of Helsinki and its later amendments as far as they are required for this type of review study.

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