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Pediatric Invasive Gastrointestinal Fungal Infections: Causative Agents and Diagnostic Modalities

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Authors' contributions

This work was carried out in collaboration between all authors. Author MHFES specified the topic of the research. Author LAM designed the study, managed the literature research and wrote the first draft of the manuscript. Authors NK and KV wrote the subsequent drafts. Author MHFES revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Invasive gastrointestinal fungal infections are posing a serious threat to the ever-expanding population of immunocompromised children, as well as some healthy children at risk. In this narrative review, we collate and explore the etiologies and diagnostic modalities of these overlooked infections. Currently, the conventional diagnostic approaches of histopathologic examination and culture are still considered the gold standard for diagnosis. However, these approaches may be time-consuming and have low sensitivities, which emphasizes the need for new diagnostic modalities in such life-threatening infections. Meanwhile, biomarkers that detect fungal antigens e.g. galactomannan and beta-D-glucan have been established and implemented in various clinical settings. On the other hand, novel molecular techniques have been developed and are currently

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subjected to further evaluation and validation. Other promising approaches such as the matrix-assisted laser desorption ionization (MALDI) and surface-enhanced resonance Raman scattering (SERRS) have proved reliable in clinical trials but still require standardization before widespread clinical application. The incorporation of standardized novel diagnostic tools would provide the necessary guidance to therapeutic approaches. Prompt treatment of IFD necessitates surgical intervention together with systemic anti-fungal agents. The most widely used agents include amphotericin B, voriconazole, and caspofungin. A high index of suspicion coupled with prompt diagnosis and judicious management can tremendously improve the survival of the vulnerable pediatric population.

Keywords: Invasive fungal infections; immunosuppression; candidiasis; aspergillosis; basidiobolomycosis; mucormycosis; MALDI-TOF MS; SERRS.

1. INTRODUCTION

With an alarming rise in the immunocompromised pediatric population, the once-rare invasive mycoses are now escalating to worrisome levels. Advances in the care of debilitated children suffering prematurity, malignancy, human immune-deficiency virus (HIV) infection, or uncontrolled diabetes mellitus have improved the survival of these fragile patients. Often times, these children have impaired immune defenses and disrupted natural barriers, and are thus at risk for invasive fungal disease (IFD) [1].

Pediatric patients with hematological malignancies are particularly at high risk. An IFD can occur when the child develops neutropenia due to the malignancy itself or the cytotoxic chemotherapy. Noteworthy, 20-50% of patients dying from hematological malignancies show evidence of IFD at autopsy. On the other hand, fungal infections occur in 5–45% of all solid organ transplant recipients. For instance, a transplanted lung can actually be a reservoir for pathogenic fungi, which may have been dormant in the donor but may well be pathogenic to the immunosuppressed recipient child. Meanwhile, children with chronic granulomatous disease (CGD), an inherited disorder of the neutrophils that serve as an important defense against fungi, are also at risk for IFD. Furthermore, even immune-competent children are not exempted from IFD. The intact skin and mucosal surfaces are natural barriers against micro-organisms, but if these barriers are breached (e.g. during surgery or catheterization), fungi can gain access into various tissues to initiate an invasive disease [2]. IFD of the gastrointestinal tract (GIT) have been recently increasingly reported with quite heterogenous clinical spectrum in both immunocompromised and immunocompetent children, with the most common culprits being *Candida, Aspergillus, Basidiobolus,* and *Mucor* [1]. Moreover, mixed fungal infections have also been reported, with grave sequelae and often poor outcomes [3].

In this study, we conducted a comprehensive research review in Google Scholar and PubMed to find out the etiologic agents and diagnostic modalities of pediatric invasive gastrointestinal fungal infections.

2. CANDIDIASIS

Although *Candida* spp. is a normal commensal of the GIT, only slight alterations can transform it into a pathogenic organism with a potential to cause invasive disease [4]. *Candida* spp. can overgrow the normal flora due to an altered microbiome as a consequence of antibiotic therapy, neutropenia, diabetes mellitus, or burn injuries. These factors can predispose to invasive candidiasis [5]. Noteworthy, *Candida* spp. accounts for 10-15% of infections in children receiving chemotherapy for treatment of leukemia [6].

C. albicans has earlier been the predominant species; however, nowadays the non-*albicans* species account for 50% of invasive candidiasis cases [7].

Children with gastrointestinal candidiasis may present with prolonged antibiotic-refractory fever, abdominal pain, and weight loss, associated with hepatic and/or splenic enlargement.[2] Gastrointestinal candidiasis may also manifest as diffuse pancreatitis [8] or may eventually lead to gastric perforation.[9] In some children, particularly premature infants, gastrointestinal candidiasis may result in intestinal perforation with necrotizing enterocolitis, where the fungus may not only be found in the intestinal lumen, but also intravascular [10].

A rare complication of candidiasis is vasculitis [11,12] and subsequent pseudo-aneurysms [13]. In such settings, the resulting gastrointestinal hemorrhage would be difficult to manage due to the diffuse involvement of the GIT [14].

Unexpectedly, gastrointestinal candidiasis has also been reported in immune-competent children. One of these children was a 3-year-old child who presented with acutely perforated gastric ulcer, and *C. tropicalis* was retrieved by blood culture [15]. Another case was an 11-months-old infant who presented with tender abdomen and protracted diarrhea, and subsequently developed intestinal perforation [16].

3. ASPERGILLOSIS

An immunocompromised child can readily contract fungal infections from the environment if standard safety precautions are not followed [17]. Infection with *Aspergillus* spp. occurs following inhalation of the airborne spores of this ubiquitous mold [9]. As in adults, the most frequently encountered species in children are *A. fumigatus*, followed by *A. flavus* and *A. terreus* [18].

Aspergillus spp. may invade the GIT following disruption of the gastrointestinal mucosal barrier as a result of chemotherapy [19,20]. Alternatively, the fungus may disseminate from the lungs to the GIT *via* hematogenous spread [21].

Being angiotropic, *Aspergillus* hyphae may adhere to and invade the vascular endothelial cells. The hyphal fragments then traverse the endothelial cells to disseminate into distal sites and invade the deep tissues [22].

The clinical spectrum of gastrointestinal aspergillosis is quite variable, where some children may have subclinical infection [23], while others may present with gastrointestinal ulcers and abscesses [2]. Other children may present with acute gastritis that may be complicated with ischemic gastric perforation (Fig. 1) [9]. It has also been reported that some children have presented with hematemesis and bloody diarrhea [24]. Disseminated aspergillosis is a fatal complication that may affect debilitated children on prolonged treatment with broad-spectrum antibiotics, corticosteroids, or cytotoxic drugs [2]. A child with cancer is the most predisposed host to aspergillosis due to neutropenia resulting from chemotherapy [9].

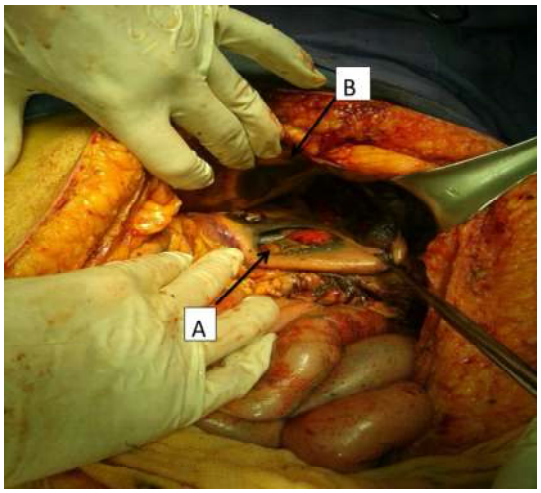

Fig. 1. Gastric perforation in a 13-year-old female with invasive aspergillosis. (A) Loss and necrosis in 80% of anterior stomach wall. (B) Necrotic lesion of the inferior face in the gastric pit of the left lobe of the liver. Figure reprinted with permission from the Journal of Infection in Developing Countries

4. BASIDIOBOLOMYCOSIS

The genus *Basidiobolus* belongs to the order *Basidiobolales* (class *Basidiobolomycetes*) within the subphylum *Entorophthoromycotina,* phylum *Entomophthoromycota* [25]. *Basidiobolus* spp. include *B. ranarum, B. meristosporus,* and *B. haptosporus*. According to antigenic analysis and restriction enzyme studies, all human-pathogenic isolates belong to *B. ranarum* [26,27].

Basidiobolomycosis is typically considered to be a rare chronic subcutaneous disease and may affect both immunocompromised and immunocompetent children [28]. It has just recently been recognized as a distinct fungal infection which has to be classified apart from the entomophthoromycoses which the basidiobolomycosis was formerly ascribed to [29]. Basidiobolomycosis has long been identified as a disease of tropical and subtropical regions, however, the disease incidence has broadened nowadays to affect other parts of the world including the United States [28,30].

Although this fungus has a predilection for subcutaneous tissues, gastrointestinal and retroperitoneal basidiobolomycoses have been

increasingly reported [31]. The fungus may gain access to the GIT through two main portals, either *via* ingestion of contaminated food or *via* inoculation of a minor rectal abrasion followed by hematogenous spread [30].

Meanwhile, in some previous studies, the infection was reported years after a surgical procedure, hence, implantation of the fungus following surgery is another less likely portal [26]. Another proposed theory is a history of ingestion of ranitidine, an H2 receptor antagonist used as a medication that diminishes gastric acidity [32], thus permitting fungal survival [33].

The affected sites in the GIT include the stomach, duodenum, terminal ileum, liver, pancreas, colon, and rectum. The most frequently reported manifestations include fever, indolent abdominal pain, distension, fatigue, vomiting [30], constipation, and bloody diarrhea which may lead to bowel perforation [28,34]. Some children have presented with concomitant hepatic and intestinal masses [33]. Male children appear to be more predisposed to develop basidiobolomycoses if compared with female children [28] as elucidated using common laboratory diagnostic tools [35].

5. MUCORMYCOSIS

Apart from basidiobolomycoses and entomophthoromycoses, both caused by members of the subphylum *Entomophthoromycotina*, the *Mucoromycotina* also serves as a large reservoir of causative agents of fungal infections which are commonly summarized as mucormycoses [36]. *Mucor* and its relatives are ubiquitous fungus belonging to the subphylum *Mucoromycotina* [37]. With 26 potentially human pathogenic species, *Mucoromycotina* represent the most common diverse source of uncommon but emerging fungal infections [38]. Whilst all of the species cause acute disease in humans, one species, which is *Mucor irregularis,* has been reported to cause chronic disease [39]. Mucormycosis has been identified as an opportunistic fungal infection frequently associated with uncontrolled diabetes mellitus, hematologic malignancies and immunosuppressive therapy secondary to transplantation [40]. In neonates, prematurity seems to be the most important risk factor of mucormycosis. The immune system in preterm infants is usually immature and their fragile skin may permit fungal entry. Added to these factors, the warmth and high humidity found in the enclosed incubators could favor fungal growth [41]. *Mucor* may gain entry to the human body *via* inhalation of spores, ingestion, or *via* percutaneous routes [42].

Mucormycosis exhibits a wide array of clinical manifestations including rhino-cerebral, intestinal, pulmonary, and disseminated mucormycosis. Although the disease afflicts mainly immunosuppressed adults, it has also been described in children, of which one fifth have presented with intestinal mucormycosis [43].

A child with gastrointestinal mucormycosis may present with fever and abdominal pain, or even gastrointestinal abscess. In addition, mucormycosis has been reported to cause gastric perforation and necrosis of the bowel. The disease may also involve the liver, spleen, and kidneys [42].

The vascular supply of the GIT, including the hepatic and renal arteries may be affected. A case of rupture of both hepatic and renal arteries due to mixed infection with *Mucor* and *Aspergillus* has previously been reported [3].

6. DIAGNOSIS OF IFD

Despite the improved diagnostic means, early accurate diagnosis of IFD still constitutes an enormous challenge [44]. In fact, diagnosis can be a frustrating experience and sometimes remains unconfirmed until autopsy [45].

6.1 Clinical and Radiologic Diagnosis

The high morbidity and mortality linked to IFD renders timely diagnosis of pivotal importance [46,47]. However, the problem stems from the fact that many clinicians may be unaware of the possible manifestations of IFD. In addition, the invasive gastrointestinal mycoses may occur in an immunocompetent child, which is quite a remote possibility for many clinicians [33].

Hence, invasive gastrointestinal mycoses are often misdiagnosed as gastrointestinal neoplasms or chronic granulomatous diseases including tuberculosis, Crohn's disease, or schistosomiasis [48]. A misdiagnosis can delay the definitive diagnosis and subsequently proper management, which in turn aggravates morbidity and mortality [31]. Meanwhile, the clinical symptoms are often non-specific and resemble those of bacterial and viral infections [2].

For radiologic diagnosis of fungal gastrointestinal mycoses, computed tomography (CT) and magnetic resonance imaging (MRI) have been recommended [49]. However, it seems that preoperative diagnosis of invasive gastrointestinal mycoses is relatively impossible when relying solely on clinical and radiologic features. The most common radiologic findings have been concentric intestinal wall thickening, gastric wall thickening or polypoid mass which are all non-specific criteria [50]. Meanwhile, advances in radiologic diagnosis have included the use of angio-CT that capitalizes on the angio-invasive nature of IFD and has shown promising results for early diagnosis [51]. Likewise, positron emission tomography/computed tomography (PET/CT) capitalizes on the fact that fungal lesions have high avidity for fludeoxyglucose (FDG) and this can be used in some cases to determine the extent of infection and monitor the response to treatment [52]. Although FDG PET/CT can raise the suspicion of fungal infection, histopathologic examination and culture are still required for a specific diagnosis [53].

6.2 Conventional Laboratory Diagnosis

A definite diagnosis of IFD necessitates histological and/or cultural evidence from a biopsy. However, this may not be quite feasible at early stages of infection [49].

It has been recommended that tissue biopsies should be microscopically examined using periodic acid–Schiff, Grocott's methenamine silver, or optical brighteners (e.g., calcofluor white) [54].

Thrombosed vessels in a biopsy should always be meticulously analyzed by the pathologist, and several serial sections should be examined. Direct microscopic examination of filamentous fungi can provide vital data e.g., the presence or absence of septa, the hyphal diameter, the ramification pattern, thus yielding valuable diagnostic clues [39]. The microscopic differentiation between *Mucor* and *Aspergillus* can be more feasible using immune-histochemical techniques [55].

In the meantime, all samples from children at high risk for IFD must be cultured for fungi. Usually, isolation of molds from clinical samples is more difficult than isolation of *Candida* spp. [56,57]. For detection of fungaemia, examination of sequential blood cultures (i.e. two pairs from peripheral veins and central lines) is considered the method of choice. To ensure the highest detection level, at least two aerobic/anaerobic blood culture bottles should be used, with 10 ml of blood in each [49].

Some techniques e.g., the lysis centrifugation system (Dupont Isolator) may enhance the detection of fungi but with a risk of false-positive results [58].

Noteworthy, definite diagnosis *via* culture may be hindered by the presence of commensal fungi, or ubiquitous fungi in the environment, thus yielding false-positive results. Furthermore, sample collection often necessitates an invasive procedure, which may not be advisable in critically ill children. On the other hand, serological tests for antibody detection have low sensitivity and specificity, because many children with IFD are immunocompromised and, thus, have impaired antibody response. Even in immunocompetent children, the delay between the onset of infection and the development of antibodies diminishes the diagnostic value of these tests [2].

6.3 Biomarkers (Antigen-based Tests)

The use of biomarkers for diagnosis of IFD has become a reality in some centers. Although false positive results may be obtained [59]; yet, the detection of galactomannan (GM) and beta-D-glucan (BDG) as markers for *Aspergillus* and *Candida*, respectively, has permitted applying more preemptive and less empiric therapeutic regimens [60].

In addition to qualitative testing of BDG, tracking quantitative BDG values can provide a prognostic marker for patient's response. Consistently decreasing BDG levels during anti-fungal therapy have been proven to result in favorable therapeutic outcomes among patients with IFDs [61,62].

Meanwhile, narrow progress has been made in the application of biomarkers for diagnosis of mucormycosis and basidiobolomycosis [63].

Issues of concern include the inconsistent sensitivities and specificities yielded in different studies. Sensitivity and specificity values, irrespective of the invasive organism, can range from 38% to 100% and 45% to 99%, respectively, with similar results observed for the positive predictive value (PPV; 30% - 89%) and

the negative predictive value (NPV; 73% - 97%) [64].

Overall, it is highly probable that the increasing reliability of biomarkers may reduce the need for invasive procedures (e.g., biopsy) in these high-risk children [65].

6.4 Molecular Diagnosis

In the current era of novel diagnostic tools, molecular techniques can settle a disputed diagnosis of IFD [28]. Molecular identification of fungal pathogens in fresh frozen or paraffin-embedded sections can confirm the diagnosis and identify the fungus to the species level. The commonly used techniques include: DNA probes that target the 18S ribosomal subunit, PCR using pan-fungal primers followed by sequencing, as well as real-time PCR [28,31,34].

In fact, most commercial PCR assays utilize real-time technology, which yields rapid results for a single target or a limited number of targets. On the other hand, using a surface-enhanced resonance Raman scattering (SERRS) assay has shown superior sensitivity and multiplexing capacity compared to real time PCR [66]. This method involves specific sensors to detect the scattered light produced by DNA-coupled with a certain dye and placed against roughened metal surfaces of silver or gold [67]. SERRS can be used to detect multiple targets simultaneously. The SERRS technique can differentiate among multiple etiological targets by detecting specific oligonucleotides for each agent. With high sensitivity and multiplexing capacity, SERRS has the potential for an ideal screening assay [68]. Moreover, a recent SERRS spectroscopy platform, RenDx (Renishaw Diagnostics Ltd, UK), has been developed to detect target nucleic acids, and is capable of detecting up to 10 targets per reaction. This platform encompasses semi-automated post-amplification processing, and multiplex detection with Raman spectroscopy [66].

Using this approach, an assay was developed (RenDx Fungiplex) to diagnose invasive aspergillosis using a generic *Aspergillus* probe, as well as invasive candidiasis by combining a broad-range *Saccharomycetales* sample with a specific sample for *Candida* spp. This assay has the added value of identifying potentially antifungal-resistant species (*Aspergillus terreus, Candida glabrata*, and *Candida krusei*) using specific probes. Preliminary clinical evaluations of the RenDx Fungiplex assay have shown a promising performance [66].

Other varieties of molecular techniques have been tested over the past years. Among these techniques, the fluorescence *in situ* hybridization (FISH) has been used combined with culture [69] or PCR [70]. This method has shown a promising performance even when culture hasn't been performed [67].

Another method, nucleic acid sequence-based amplification (NASBA) differs from PCR in being isothermal and in that it amplifies mRNA instead of DNA, The ability to detect mRNA has the advantage of detecting an active disease, rather than a latent or old infection [71].

Meanwhile, in spite of the tremendous progress in molecular diagnosis, some issues still have to be resolved, including concerns about affordability, sensitivity, accuracy, and reproducibility [72]. Of note, clinical reports of the sensitivities and specificities of molecular methods have exhibited considerable variations; from 43 to 100% and 64 to 100%, respectively [66]. For a comprehensive overview of the current possibilities to perform molecular diagnosis of IFD, it's recommended to refer to Lieu, 2011 [73].

6.5 Other Promising Diagnostic Tools

Traditional microscopic examination and culture techniques constitute the 'gold standard' against which novel tests are judged [74].

In this scope, a miniaturized magnetic resonance nanotechnology [75] has been introduced with rapid and amenable results. In addition, the explosive efficiency of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectroscopy (MALDI-TOF MS) has proven rapid and accurate for identification of yeasts and molds [65].

These approaches can be used as complementary tests to the conventional methods, since standardization is required before recommending a widespread implementation [74]. Such promising techniques have the potential to revolutionize fungal identification methods in the microbiology laboratory [76].

7. TREATMENT

Delayed treatment of IFD may cause life-threatening outcomes. The complications include

gastric or intestinal perforation, fistula formation or massive necrosis of the GIT [33]. A complicating factor when adjusting appropriate antifungal treatment is that some children may be simultaneously infected by more than one fungal agent [77]. Fortunately, broad-spectrum antifungal drugs are becoming increasingly available, which can provide better therapeutic choices [78-80].

Prompt treatment of IFD consists of combined surgery and systemic anti-fungal agents to eradicate the disease and prevent recurrence. The most commonly used antifungal agents include amphotericin B, voriconazole, itraconazole, posaconazole, isavuconazole, and caspofungin [33]. Noteworthy, the Infectious Diseases Society of America recommends preemptive therapy with amphotericin B, voriconazole, itraconazole, or caspofungin in high-risk patients with prolonged neutropenia who suffer from persistent fever despite broad-spectrum antibiotics [9].

On the other hand, trials have been pursued to use probiotics as supplements, in order to reduce *Candida* colonization of the GIT in critically ill children [81].

8. CONCLUSION

The rapid emergence of IFD among the pediatric population has been accompanied by considerable morbidity and mortality. Although immunocompromised children are more commonly involved; yet, IFD may also afflict an immunocompetent child.

The clinical signs are often non-specific and may well be misleading. In most cases of pediatric IFD, a definite diagnosis has been problematic and prognosis has been dismal. Meanwhile, a delayed management in such fragile hosts can severely undermine the child's survival. This in turn necessitates higher degrees of alertness by the clinicians, pathologists, and microbiologists.

Histopathological examination and culture have long been the mainstay of diagnosis in IFD. However, these methods may lack rapidity and high sensitivity. Novel serologic and molecular methods have been rapidly evolving with promising results. Nonetheless, the lack of standardization of these methods is currently hindering large-scale implementation. This entails that more studies should be pursued to standardize these methods, which will ultimately be reflected on the children's survival and therapeutic outcomes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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