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Computed Tomography Guided Percutaneous Lung Biopsies and Suspected Fungal Infections in Pediatric Cancer Patients

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Background. The spectrum of potential fungal pathogens known to cause invasive pulmonary infections has grown as a result of intensified immunosuppressive therapy and the emergence of antifungal resistance. ***Procedure.*** In a retrospective single center study, we investigated computed tomography guided percutaneous lung biopsies in 16 childhood patients with suspected fungal infections. Microbiological analysis consisted of microscopic examination, culture, and a broad-range fungal polymerase chain reaction for detection of either *Aspergillus* or *Mucorales* species. ***Results.*** In 14 patients (88%), invasive fungal infection with *Aspergillus* species including *A. terreus*, *Mucormycetes*, and *Saccharomyces cerevisiae* being the main pathogens was confirmed, including patients with a double infection (19%). In two cases, the most likely diagnosis of primary bronchiolitis obliterans organizing pneumonia was established based on the results of typical histopathologic features, negative culture results, and symptoms resolved after treatment with high-dose cortisone. Diagnosis of invasive fungal pneumonia led to an immediate interruption of antineoplastic treatment in 100%, reduction of antibiotic drugs in 76%, and change of empirical to targeted antifungal therapy in 63%. The safety of lung biopsy was guaranteed by lack of any complications, such as bleeding or pneumothorax. ***Conclusions.*** The increased detection of rare fungal infections by computed tomography guided biopsy supports the need for a rapid and precise diagnosis, as most of the fungal pathogens are at least partially resistant to available antifungal therapy and proper treatment is essential for best practice in patient management. Pediatr Blood Cancer 2014;61:1620–1624.

Key words: biopsy; cancer; childhood; fungal

INTRODUCTION

The burden of invasive fungal infections (IFIs) continues to increase as a result of more intensified therapy, particularly among patients treated for cancer. In a large multicenter prospective surveillance analysis of patients who underwent hematopoietic stem cell transplantation, a constant rise in the 1-year cumulative incidence of IFIs has been reported [1]. In children treated for acute myeloid leukemia, the cumulative risk for developing IFIs can reach 15% [2]. In terms of costs, antifungal drugs are among the most expensive in health care. Moreover, affected patients have a high rate of morbidity, resulting in longer hospital stays [2], and significant risk of disease related mortality [3]. Considering the impact of this issue, most effort is required to significantly ameliorate this problem.

One of the key elements in optimizing the care for patients with suspected IFIs consists of an early and reliable diagnosis, which in turn leads to an immediate and targeted therapy [4]. In general, diagnosis of IFIs depends on a variety of actions, including clinical investigations, culture, and non-culture based tests [5]. Non-invasive methods such as tests for detection of cell wall components and fungus-specific nucleic acids in serum as well as in BAL material represent a major advance in the diagnosis of patients at risk for IFI [6]; however, utility is limited by long assay times, low sensitivity, and/or poor specificity. Along with the fact that rare fungal pathogens, such as *Mucormycetes*, have increasingly been reported, most attention is directed toward the recovery of the organism from clinical specimens. Although computed tomography (CT) guided biopsy has been reported in adult patients [7], relatively little is known about this procedure in the pediatric population [8]. The purpose of this study is to review a 10 years' pediatric experience with CT-guided biopsy of parenchymal and pleural-based lesions in the thorax in patients with suspected fungal infections.

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METHODS

Patient Population

This study included 402 patients treated for childhood cancer or immunodeficiency in which 16 consecutive patients underwent CT-guided percutaneous transthoracic biopsy of a parenchymal or pleura-based lesion at the pediatric oncologic ward of the university hospital Innsbruck. Biopsies of mediastinal masses were excluded from this study. Data were collected from October 15, 2001 to October 14, 2011. There were 7 female and 9 male patients, ranging in age from 1.1 to 16.7 years (mean 11.5, SD 5.7). Intense medical work-up was obtained; clinical details are given in Table I. Early high resolution CT-scan of the lungs was performed in all febrile neutropenic patients who did not respond to a 3-day broad-spectrum antibiotic therapy. Fever was defined as a single episode of body temperature of 38.5°C or higher or as a temperature between 38.0 and 38.4°C persisting for at least 6 hours. Neutropenia was defined as an absolute neutrophilic count (ANC) $< 500 \times 10^6$/L. Escalating efforts of antimicrobial therapy were previously described by our group [9], and included the sequentially use of meropenem,

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gentamycin, and vancomycin. Complete blood count, blood (5-ml blood drawn) and urine cultures, and the level of C-reactive protein were performed as laboratory screening in all patients daily. If a possible fungal infection was identified by the presence of multiple small nodules, halo sign, air crescent sign, or cavity within an area of consolidation, CT-guided percutaneous lung biopsy with fine-needle aspiration for cytology was performed. Written informed consent for the procedure was obtained from all patients or their parents by the interventional radiologist. This study was performed under a protocol approved by the local ethics committee.

Biopsy Technique

All patients with suspected fungal infection underwent previous diagnostic CT of the thorax that was reviewed to determine the best approach for percutaneous biopsy. A normal DCO or FVC, a count of $>$50,000 thrombocytes/μl and a normal partial thromboplastin time were requested before performing the biopsy. All procedures were initiated under general anesthesia and assisted by mechanical ventilation; respiration was suspended at a constant point in the respiratory cycle at end expiration in order to obtain a consistent image that decreases inaccuracy from respiratory motion. In our study, an outer coaxial needle with a 19-gauge diameter and an inner biopsy needle with a 20-gauge diameter have been used. After an appropriate skin entry site was chosen, a radiopaque marker was placed on the skin at the side of the lesion with the use of the axial laser light and confirmed with 3-mm incremental imaging. The needle was placed in the skin in the appropriate plane of the access tract and its position was confirmed with CT imaging. Lesions that had a diameter of $>$1 cm and that were most easily accessible were chosen for biopsy. When the lesion was penetrated, the tip of the needle was checked and aspiration specimens were obtained. An on-site cytologist confirmed the adequacy of the sample. In case of an inadequate specimen, the pass was repeated. The mean number of passes taken through the pleura was 1.13 (range 1–3).

Sample Preparation

Biopsy specimens were transferred to 2 ml of normal saline and prepared as previously described by our group [7]. All samples were examined for the presence of fungi by application of the Calcofluor White (CFWS) solution (Polysciences Europe GmbH, Eppelheim, Germany) and by broad-range Aspergillus polymerase chain reaction (PCR); selected samples that showed unseptated hyphae by CFWS and that yielded negative results of Aspergillus PCR were evaluated by a PCR specific for *Mucormycetes*.

PCR Assay

DNA extraction of 5 ml of EDTA-anticoagulated blood was performed using recombinant lyticase (Sigma, Vienna, Austria) and a QIAmp tissue kit (QIAGEN, Vienna, Austria) as previously described [7]. In addition, biopsy specimens were treated with lyticase and proteinase K and beaten with glass beads. A highly conserved sequence of the multicopy 18S rRNA of various fungal pathogens was amplified by PCR using specific primers. Primers (Roth, Graz, Austria) bind to conserved regions of this 18S rRNA. A total of 34 cycles of repeated denaturation (94˚C for 30 seconds), annealing (62˚C for 1 minutes), and extension (72˚C for 2 minutes) were applied. Amplicons were detected by PCR-enzyme-linked immunosorbent assay using species-specific digoxin-labeled oligonucleotides (Roth) and antidigoxigenin antibodies conjugated with alkaline phosphatase (Boehringer Mannheim, Vienna, Austria) as described earlier [7]. Five Aspergillus species (A. fumigatus, A. flavus, A. niger, A. terreus, and A. nidulans) and seven Mucorales species (Mucor heimalis, M. racemosus, M. cercinelloidea, Rhizopus arrhizus, R. microsporus, R. pusillus, and Absidia corymbifera) were detected with these genus-specific oligonucleotide probes. Negative and positive probes were routinely used for quality control.

Patient Follow-Up

A post-biopsy CT examination was performed after the needle was removed to check for complications. A chest radiograph was obtained 2–6 hours after the procedure to exclude pneumothorax and bleeding.

Data Analysis

Retrospectively gathered procedural data were reviewed for medical history, indications for the procedure, admission status, previous imaging, lesion size and number, histology, and immediate complications. Assay performance characteristics and patient demographics were assessed using descriptive statistics.

RESULTS

Patient's Characteristics

CT of the chest was performed in all neutropenic patients treated previously empirically with a standardized 3-day broad-spectrum antibiotic regimen without clinical improvement and no evidence of bacterial infection. In 15 patients with childhood cancer and in 1 patient with immunodeficiency, CT findings were highly suggestive for invasive fungal pneumonia. As per EORTC/MSG definition [4,10], presence of multiple nodules was noted in 11, opacities (including ground glass opacities) in 4, halo signs in 6, crescent signs, and/or cavitations in 7 patients. At the time of CT scan, 81.1% of patients had been treated prophylactically with either oral fluconazole or itraconazole. Upon diagnosis of fungal pneumonia, empiric treatment with either liposomal amphotericin B (3 mg/kg once daily) or voriconazole (7 mg/kg twice daily) was initiated. The median age of all patients enrolled was 11.5 years (SD 5.7) and 43.8% of the patients were female. The majority of patients was undergoing treatment for either acute lymphatic (50%) or myeloid leukemia (25%), while four patients were enrolled undergoing treatment for acute graft versus host disease (GvHD) after allogeneic hematopoietic stem cell transplantation.

The number of patients with a negative result on CT scans was 41. Blood cultures yielded Gram-positive bacteria in 17% of patients, most frequently staphylococci and in 19% Gram-negative bacteria within 5 days of culture. In 5% of febrile episodes, Clostridium difficile or its toxin was isolated. In the remaining cases fever of unknown origin was assumed and broad-spectrum antibiotic treatment was continued until 48 hours after resolution of symptoms. Fatal episodes occurred in 4 of 41 patients with a negative CT scan. Two patients succumbed to viral infections: CMV-associated pneumonitis following allogeneic bone marrow transplantation and one case of fatal measles. In two patients death was a direct consequence of bacterial infection: one patient with Pseudomonas and one patient with Enterococcal septicaemia.

TABLE I. Characteristics of 16 Patients With Suspected Invasive Fungal Infections

Patient	Disease	Age	EORTC criteria	Empiric therapy	Targeted therapy	Underlying Pathology
1	ALL	16.7	+	AMB	VOR	*A. flavus*
2	AML	14.1	++	VOR	CAS + AMB	*Aspergillus* species + *Candida*
3	ALL	15.5	+	AMB	AMB + POS	*Aspergillus* species
4	ALL	14.7	+	AMB	AMB	*Saccharomyces cerevisiae*
5	NB	1.1	++	AMB	AMB	*A. fumigatus*
6	ALL	15.0	+	AMB	AMB	*Aspergillus* species
7	ALL	9.3	+	AMB	AMB + VOR	*Aspergillus* species
8	ES	14.9	+	VOR	AMB + POS	*A. fumigatus* + *Rhizopus*
9	ALL	15.0	+	VOR	AMB + CAS	*Aspergillus* species + *Rhizopus*
10	ALL-R	18.0	++	VOR	VOR	*A. terreus*
11	AML	2.4	+	AMB	VOR	*A. terreus*
12	NB	14.9	+	AMB	VOR	*Aspergillus* species
13	ALL	1.8	+	VOR	CAS	*A. terreus*
14	AML	6.7	+	AMB	AMB	*Mucor*
15	ID	8.9	++	AMB	COR	BOOP
16	AML-R	15.2	+	AMB	COR	BOOP

EORTC, European Organization for Research and Treatment of Cancer; ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; NB, neuroblastoma; ES, Ewing sarcoma; ALL-R, ALL-relapse; AML-R, AML-relapse; ID, immunodeficiency; AMB, amphotericin B; VOR, voriconazole; POS, posaconazole; CAS, caspofungin; COR, cortisone.

Biopsy Microscopy and Culture

Percutaneous CT-guided biopsies were obtained within 24 hours after diagnosing suspicious findings in the CT for 44% (n = 7) of patients, in additionally 25% (n = 4) within 48 hours. Cumulatively, in 88% of patients (n = 14) biopsies were performed within 72 hours and immediately sent for microscopic analysis and culture. For the remaining two patients, biopsies were obtained at 7 and 9 days after CT scan, respectively, and the critical clinical condition was stabilized. Microscopic examination was performed in all samples: in 14 cases (88%) samples tested positive for septated or unseptated fungal hyphae; however, two samples were without fungi. In both cases, further histological examination by the pathologist revealed marked intraluminal proliferation of fibrous tissue, consistent with bronchiolitis obliterans organizing pneumonia (BOOP) as the underlying pathology.

Pan-Aspergillus and Mucormycoses-PCR was performed in all samples, specificity was high (100%), confirming the results obtained previously with microscopy. Culture was positive for five *Aspergillus* species (three *A. terreus*, one *A. flavus*, and one *A. fumigatus*), one for *Candida albicans* and *Aspergillus fumigatus* and one for *Saccharomyces cerevisiae*.

Minimum inhibitory concentrations (MICs) of the antifungal drugs amphotericin, voriconazole, posaconazole, and fluconazole were determined and compared to our previous results. Overall, we noted similar activities (Table II); however, for *Aspergillus flavus* the MIC values for amphotericin were higher, compared with those of voriconazole and posaconazole.

To determine the impact that percutaneous CT-guided biopsy findings have on decisions regarding changes in the treatment strategies, we identified two patients in whom the possible diagnosis of IFI by EORTC/MSG criteria was abrogated for a specific diagnosis of primary BOOP, and treatment was immediately switched to high dose cortisone. These patients were regarded as having chronic GvHD 9 and 17 months, respectively, after allogeneic stem cell transplantation; symptoms disappeared after 3–4 weeks and patients are still alive without sequelae at the most recent follow-up examination, after 4.3 and 8.6 years, respectively. In three patients culture of *Aspergillus terreus* was obtained and treatment was modified from empirical liposomal amphotericin-B

TABLE II. Susceptibility of Cultured Fungal Pathogens Against Common Antifungal Agents

Patient	Fungus	AMB	VOR	POS	FLU	CAS	ITRA
1	*A. flavus*	−	+	+	−	+	n.d.
4	*Saccharomyces cerevisiae*	−	−	−	−	−	+
5	*A. fumigatus*	+	+	+	−	+	n.d.
8	*A. fumigatus*	+	+	+	−	+	n.d.
10	*A. terreus*	+	+	+	−	+	n.d.
11	*A. terreus*	−	+	+	−	+	n.d.
13	*A. terreus*	−	+	+	−	+	n.d.

+, high activity rate; −, mostly resistant; n.d., not determined; AMB, amphotericin B; VOR, voriconazole; POS, posaconazole; FLU, fluconazole; ITRA, itraconazole.

to the more active antifungal voriconazole. Also, for one patient with positive culture of *A. flavus* and a high MIC for amphotericin-B, treatment was switched to voriconazole. In three patients with isolates of Mucormycoses, treatment with liposomal amphotericin B was continued. In one patient with acute myeloid leukemia and identification of *R. microsporus*, severe acute (adult) respiratory distress syndrome developed and treatment with extracorporeal membrane oxygenation was needed. The patient recovered fully and is free of complaints at 8.7 years after the diagnosis. Cumulatively, for all patients changes in treatment included the immediate subtraction of one (44%) or more (32%) antibiotics and temporarily discontinuation of antineoplastic drugs (100%).

In summary, diagnosis of fungal pneumonia, identification of the fungal pathogen and analysis of antifungal susceptibility, resulted in cumulative change of empirical antifungal treatment in 63% of patients, including two patients identified with BOOP and for whom treatment was switched to high-dose cortisone. Excluding patients who died from relapse, the mortality attributable to IFIs in our study population was 19%. Additionally, no single patient was detected with any complications resulting from the CT-guided biopsy, including infection, hematoma, or pneumothorax.

DISCUSSION

These results demonstrate that percutaneous CT-guided biopsy can obtain reliable histological material in childhood patients affected with malignancy and suspected for pulmonary IFI. The incidence of infectious complications, such as IFIs, has been noted and been attributed as a major treatment-related complication [11]. This increase is likely the result of several factors, including the length and intensity of immunocompromised periods, widespread use of broad-spectrum antibiotics, use of central lines, but also increased awareness and optimization of diagnostic tools [11,12]. Since opportunistic mycoses have grave consequences, including high mortality rates for immunocompromised patients, the reliable detection of a fungal pathogen is indispensable for the effective clinical management.

Most commonly, identification of a fungal infection is based on phenotypical and physiologic tests, including the Platelia *Aspergillus* EIA Galactomannan (GM) assay, β-D Glucan test and several DNA-based methods. Although improved performance of different tests has continuously been reported and has been routinely adopted in therapeutic algorithms, there are still debates over the appropriate cut-off values, the specificity of the results and the best strategy for its use in specific populations. In the few studies that have been performed in children, false-positive results have been reported at 4–10 times more commonly than in adults [13,14]. On the other hand, false-negative results have also been reported and are associated with prophylactic or preemptive therapy with mold-active antifungals [15]. This is particularly true, if GM testing is performed in a serial fashion [16]. Alternatively, several PCR based methods for quantitative analysis of pathogenic fungi have recently been developed, but are also limited by its low level of detection and in that they either identify only single species or a few kinds of fungi at a time or require time-consuming and costly probe hybridization procedures [17]. Our attempt to perform a CT-guided lung biopsy revealed a culture with a highly resistant strain of *A. flavus*, for which treatment was successfully changed to voriconazole. We also identified two very likely cases, in which the patients have met criteria for presence of fungal pathogens, but after biopsy the diagnosis was revised to BOOP, associated with GvHD, and the treatment changed from antimycotic treatment to immunosuppressive therapy. Although biopsies yielded a specific, reliable, and clinically helpful diagnosis of IFI and the results had a high impact on treatment, the high cumulative incidence (48%) of *A. terreus* and *Mucormycoses* reflects the relative lack of susceptibility to therapeutic interventions and underlines the need for specific diagnosis. Considering the poor outcome after non-*Aspergillus* mould infection, an early adequate therapy is highly recommended and a suitable prophylactic intervention may be warranted.

Even with a good rationale in favor of performing lung biopsy in patients with suspected IFI, it is critical to address the safety and risks associated with this procedure. There were several publications addressing this question over the past decade, most of them retrospective cohorts of adult patients and based on single center experiences. Hoffer et al. [8] reported 28 children who had a rate of 46% of bleeding complications, including 12 patients with evidence of blood in the endotracheal tube and 1 patient with pleural collection of blood.

Our approach to percutaneous lung biopsy differs in the presence of adverse events (18% vs. 54%). We believe at least part of the differences in our complication rate relates to our practice of preparing the patients for the procedure (normal PTT values and normal lung function), of limiting the number of passes required (1.13 vs. 3.5) and of performing the intervention under general anesthesia with paralysis. Our experience supports the low incidence of complications (one single patient with tension pneumothorax requiring a chest tube drainage) reported by Cahill et al. [18] in 75 lung biopsies for children—the majority with suspected malignancy or lymphoproliferative disorder.

In conclusion, the accuracy and safety of CT-guided transthoracic biopsy clearly supports the application of this method in children suspected with IFIs. The relevance of specific histological and microbiologic diagnoses in the care of immunocompromised individuals cannot be over-emphasized. Beyond its value for specific patient management, our results identify the array of rare and emerging fungal pathogens, and need to be considered for future prophylactic and empiric management of suspected fungal infections in affected children.

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