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ORIGINAL ARTICLE

Zygomycosis and other rare filamentous fungal infections in solid organ transplant recipients

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Summary

Fungi cause severe infections in solid organ transplant (SOT) recipients. Recently, a shift towards non-Aspergillus filamentous fungal infections (nAFFI) was noticed. In a series of 2878 SOTs (kidney, pancreas, islets, liver, heart, lung, and bowel) performed between January 1995 and December 2006 at the Innsbruck medical university, eleven cases of nAFFI were diagnosed. The encountered species included Zygomyzetes ($n = 8$), and *Alternaria alternate, Pseudallescheria boydii, Trichoderma* spp. (one each); there were three liver and three heart, one intestinal, pancreas, lung, bilateral forearm and renal recipient each. Five patients died from nAFFI (zygomycosis: 4, *Pseudallerichia boydii*: 1); four were diagnosed postmortem. In five cases infection was surgically treated in combination with antifungals. Risk factors for nAFFI were renal failure (73%) and intensified immunosuppression (73%); two cases were associated with post-transplant lymphoproliferative disorder, one with graft versus host disease. An increase in the incidence of nAFFI was observed parallel to introduction of caspofungin and voriconazole (three cases until 12/2003, seven cases thereafter). NAFFI are increasingly found in SOT recipients. If diagnosed in time, the outcome seems acceptable. Intensified immunosuppression and exposure to antifungals not active against zygomycetes may be risk factors. Surgical therapy may play an important role in these infections.

Introduction

Survival following solid organ transplantation (SOT) has significantly improved during the past decade [1–3]. With rejection rates dramatically declining due to the development of powerful immunosuppressants, the incidence of some opportunistic infections has increased [4–6]. Fungal infections have gained importance and remain to be associated with a high mortality rate [7–11]. *Candida* and *Aspergillus* spp. are the most important invasive fungi in SOT recipients; however, a shift towards more uncommon fungi including non *Candida albicans* species and non-*Aspergillus* filamentous fungi (nAFF) including Zygomycetes, *Fusarium*, *Pseudallescheria boydii* and many others has been observed [7,12–18]. In 1975, Hammer *et al.* reported the first case of rhinocerebral mucormycosis in a renal transplant patient who died despite surgical and antifungal treatment [19]. Two decades later, another case

was described and 46 additional cases reported in solid organ recipients were summarized [20]. The rhinocerebral form still was the most common manifestation at that time and mortality remained high [20]. The recently published series by Almouyroudis includes ten cases from a single centre collected over a 10 years period (1989–1999) and in addition they reviewed 106 cases reported between 1970 and 2002, which is prior to the availability of several new antifungals [21]. Clinical presentation of these rare infections can be unspecific mimicking immunological complications or graft dysfunction, radiological findings may be normal or unspecific and mycological diagnosis remains difficult to be established [16–18,21–25]. Only if timely diagnosis is established and adequate antifungal therapy is initiated, outcome may be acceptable [19–22].

For many decades, amphotericin B had remained the only active agent against many fungi, however, recently several new agents have been introduced [7,8,23–26]. The echinocandins micafungin, anidulafungin and caspofungin and the new azole voriconazole have good activity against *Aspergillus*, however, they lack activity against zygomycetes [27–30]. Posaconazole is the first agent with reliable activity against Aspergillus and zygomycetes [31–36]. Azoles interact with the metabolism of calcineurin inhibitors (CNI) and mammalian target of rapamycin inhibitors (mTOR inhibitors), requiring significant dose reduction of immunosuppressive agents [37]. The new antifungals are far less nephrotoxic than amphotericin B and less expensive than the lipid formulations of amphotericin B [38]. Treatment of filamentous fungal infections in most cases is based on a combination of reduction in the intensity of immunosuppression together with targeted antifungal agents [25]. Surgical interventions are considered for selected cases only [20,25,39]. For invasive aspergillosis ample data on this strategy is available, whereas, for other filamentous fungal infections data are scarce [40–42].

Established risk factors for development of these rare fungi are anti-rejection therapy, high dose immunosuppression, diabetes mellitus, renal failure and graft dysfunction [18,43–45].

The aim of this retrospective study was to systematically analyze all cases of nAFF infections in SOT recipients from a single centre.

Patients and methods

Data collection and statistical analysis

Data from clinical charts of all patients admitted to the Department of General and Transplant Surgery at Innsbruck Medical University in Austria, who underwent solid organ transplantation between January 1995 and December 2006, were enrolled in this study. According to the availability of voriconazole and caspofungin, the study population was divided into the period before (1995–2002) and after (2003–2007) introduction of these agents. Clinical data included age, gender, co-morbidities, type of fungal infection, surgical intervention(s), antifungal therapy and clinical outcomes. The transplant database was cross linked with the database of our mycological laboratory. This study was conducted according to the requirements of the ethics committee of the Innsbruck medical university. Statistical analysis was carried out using MS excel and SPSS. Data are reported as median with minimum/maximum range and/or mean with standard deviation.

Recipient demographics

During the 12 years study period (January 1995–December 2006), 2875 solid organ transplants were performed including 1467 kidney, 671 liver, 305 pancreas, 25 islet, 250 heart, 124 lung, four heart–lung, 10 multivisceral, 16 small bowel and three double hand transplants. Surgical techniques and perioperative management were performed according to standard methods or have been published in detail [3,46,47].

Immunosuppression

Prophylactic immunosuppression comprised Cyclosporine A (CsA) or Tacrolimus (TAC), rapidly tapered steroids and azathioprine or Mycophenolate mofetil (MMF). Patients classified as immunologically at high risk additionally received induction therapy with antithymocyte-globuline (ATG) (Thymoglobulin, Sangstat or ATG Fresenius), basiliximab (Simulect® Roche, Switzerland) or daclizumab (Zenapax®, Novartis, Switzerland). Within the last 4 years alemtuzumab was used for induction therapy and for treatment of steroid resistant rejection as part of study protocols. First and second rejection episodes were treated with high-dose steroids, steroid-resistant rejections with ATG or alemtuzumab. Whole plasma exchange was carried out in renal recipients for predominantly vascular rejection.

Antifungal prophylaxis

Systemic perioperative antifungal prophylaxis was only given to all patients considered at significant risk for either *Candida* infection (fluconazole) or aspergillosis (conventional or liposomal Amphotericin B).

Bacterial/fungal monitoring

Specimens were collected perioperatively from preservation solutions of all grafts, donor bile in liver grafts, donor ureter in renal grafts, bronchial secretions from

donor lungs, intraluminal swabs from intestinal grafts and from the duodenal segment from pancreatic grafts. Post-transplant, tips of removed urinary or intravascular catheters as well as those of all intra-abdominal or intrathoracic drains were sent for microbiological investigation.

Patients that developed severe pneumonia or did not respond to our routine antibiotic regimen for pneumonia underwent bronchoalveolar lavage (BAL). Lung biopsies (transbronchial or open) were performed to ascertain the diagnosis in patients with atypical pneumonia, nodular lung lesions or suspected fungal infection.

Diagnosis

Specimens were obtained by percutaneous CT – guided biopsies, surgery or postmortem material and investigated for the presence of fungal elements.

Histopathology

Routinely formalin-fixed biopsy and resection material (4% buffered paraformaldehyd) was dehydrated and deparaffinated as well as stained with hematoxylin and eosin (H&E) in an automated standard procedure. The periodic acid-Schiff reaction (PAS) was performed automated. The Grocott stain was performed following a standard protocol (10% chrom-4-oxyd, 1% natriumbisulfat, hexamethylen silvernitrat- solution).

Mycology

Biopsy specimens were transferred to 2 ml NaCl and were minced aseptically and investigated for fungi by application of the Fungi-Fluor™ (Calcofluor White staining solution, Polysciences, Warrington, PA, USA); aliquots were transferred on Sabouraud dextrose agar and Sabouroud Broth and incubated at 30 °C for 15 days. Species identification was done according to standard mycology procedures.

For fast and easy investigation to exclude infections due to *Aspergilli*, tissues samples were homogenised aseptically, kept at room temperature for 30 min and centrifuged. An *Aspergillus*-PCR and the galactomannan enzyme immunoassay (GM EIA; BioRad, Vienna, Austria) were assessed from the supernatants and the various tissues. GM EIA assays were performed according to the instructions of the company and a 0.5 cut off was used to define positivity for GM EIA. For PCR genes of the 18S rRNA were amplified and the QIAmp Tissue Kit (Qiagen, Wien, Austria) was applied as previously described [48,49]. Selected samples which showed unseptate hyphae by CFWS and remained negative in GM EIA, *Aspergillus*-PCR and culture were evaluated by specific PCR for mucormycoses [50].

Definitive non-*Aspergillus* fungal infection was defined as positive tissue biopsy with typical unseptate (sparsely), broad and irregular hyphae (CFWS) with or without positive culture for any suitable fungus.

Results

During the study period, 2878 SOTs were performed at our transplant centre and 11 cases (0.4%) of nAFF infections were identified including three liver, one renal, one pancreas/kidney, one intestinal, one lung, one hand and one cardiac and two combined heart/kidney recipients. There were four women and seven men with a median age of 49.8 (range 23.1–67.5) years. Clinical data are depicted in detail in Table 1. The dominant pathogens belonged to the group of Zygomycetes with eight patients; the remaining three species included *Pseudallescheria boydii* and *Alternaria alternata* in one case each and in one patient *Trichoderma* species together with *Absidia corymbifera* was isolated. Median onset of fungal disease was 2.4 (0.3–26.4) months post-transplant.

The first documented case in this series was observed in 1995 and this patient is still alive. Additional single cases were identified in the years 1998, 1999 and 2000 and no cases in 2001 and 2002. From 2003 to March 2007, seven cases occurred. Therefore, during the first 8 years of this study (1896 transplants) the incidence of these nAFF infections was 0.2%, whereas during the last 4 years (982 transplants) the incidence rose to 0.7%.

A short case report is given for each patient according to fatal cases and survivors. Demographic data of the cohort are given in Table 2, clinical data in Table 3 and mycological data in Table 4. Histological presentations of the various fungi from our patients are shown in Figs 1 and 2.

Fatal cases

Case 2

The postoperative course of a heart/kidney recipient was complicated by multiple infections. Piperacillin/Tazobactam and amphotericin B were administered for pneumonia. He also developed ischemic colonic perforation with peritonitis and metronidazole was added. He died 29 days post-transplant due to sepsis with multi organ failure. On postmortem invasive pulmonary zygomycosis (*Rhizopus* sp) was diagnosed.

Case 3 [43]

A former renal recipient underwent liver transplantation (LT) for hepatitis C virus (HCV) associated liver disease using the left lobe of a split graft. Poor initial graft function indicated high urgency reLT. She developed multiple infectious episodes and graft dysfunction post reLT. From

Table 1. Summary of study population.

Transplanted organs (1995–2006)	2878
1995–2002	1896
2003–2006	982
Patient data	
Total number of patients	11 (0.4%)
Increase in overall incidence	
1995–2002	4 (0.2%)
2003–2006	7 (0.7%)
Median age (range) years	50.0 (23.1–67.5)
Median onset of infection month (post-Tx)	2.4 (0.3–26.4)
Female/male	4F/7M
Filamentous fungal infection/organ	*n* (prevalence)
Liver	3 (0.3%)
Kidney	1 (0.2%)
Pancreas	1 (0.3%)
Heart	3 (1.2%)
Lung	1 (0.8%)
Bowel	1 (6.3%)
Composite tissue	1 (33.3%)
Microbiological data	
Zygomycosis	9
Mucor species	2
Rhizopus oryzae	1
Rhizomucor	1
Absidia corymbifera	4
Cunninghemella	1
Pseudallerischia boydii	1
Alternaria alternate	1
Diagnosis of infection	
Postmortem	4
Biopsy	4
Surgical resection specimen	3
Clinical data	
Rejection	8 (72.7%)
Intensified immunosuppression	8 (72.7%)
Prior antifungal treatment	6 (54.5%)
No/inadequate therapy	4 (36%)
Antifungal treatment only	1 (9.1%)
Combined antifungal/surgical treatment	5 (45.5%)
Survival	6 (54.5%)
Death	5 (45.5%)

a superficial wound infection *Pseudallerischia boydii* was isolated and topical treatment with amphotericin B but no systemic antifungal agents was given. The patient rapidly deteriorated and finally liposomal amphotericin B was started. However, she died 101 days after first split-liver transplantation. Disseminated *Pseudallerischia boydii* infection was found postmortem involving the lung, kidneys, abdominal cavity and abdominal wall.

Case 5

A female kidney–pancreas recipient developed septic thromboembolism of the renal artery with coagulase negative staphylococcal deriving from endocarditis 33 days post-transplant. She underwent graft nephrectomy and mitral valve reconstruction together with aorto-coronary-bypass. The patient developed multiple infectious complications and received caspofungin for *Candida glabrata* intra-abdominal infection but progressively deteriorated. She died 91 days post-transplant due to multiorgan failure. On postmortem disseminated zygomycosis (*Rhizomucor* sp) was diagnosed.

Case 8

A 25-year-old woman had an uneventful early course after isolated small bowel transplantation. She was readmitted due to incompliance and graft dysfunction and developed small bowel graft perforation in the course of post-transplant lymphoproliverative B-cell lymphoma, for which rituximab was given. Caspofungin and fluconazole were given for *Candida* peritonitis. The patient died 95 days post-transplant due to sepsis and multi organ failure. On postmortem, disseminated zygomycosis within the spleen, liver and lung was found.

Case 9

On day 28 postliver transplant a male patient developed generalized exanthema, diarrhea and oesophageal ulcers. Skin, oesophageal and colonic biopsies revealed acute graft-versus-host disease (GvHD). Immunosuppressive therapy was intensified including bolused steroids, cyclophosphamide, alemtuzumab and increase in TAC trough levels. Antibacterial and antiviral prophylaxis and in addition voriconazole was given. The patients died from progressive GvHD, sepsis and multiorgan failure. On postmortem, disseminated infection with *Trichoderma* and *Absidia corymbifera* involving the liver, gastrointestinal tract, lung, heart, kidneys and skin was found.

Survivors

Case 1

A 50-year-old men underwent LT for alcoholic liver disease. Post-transplant he presented with fever and cough. Bronchoscopy revealed a large ulceration at the tracheal bifurcation. Microscopy of biopsies suggested zygomycosis and liposomal amphotericin B was given. CT scan revealed pulmonary infiltration and decision was made to perform lobectomy for suspected invasive zygomycosis, however, on histopathology no invasive fungal infection was found. Instead, necrotizing pneumonia was diagnosed and specimens grew *Legionella pneumophila*. Erythromycin was started and the patient recovered and is alive after 12 years.

Case 4 [44]

A 23-year-old male patient underwent a second kidney retransplantation. Within 3 weeks he presented with the classical picture of rhinocerebral zygomycosis, which was

Table 2. Demographic data.

No	Gender	Date of tranplant	Age at transplant	Transplanted organ	Underlying disease	Induction therapy	Immunosuppressive therapy
1	M	4/3/1995	50.3	Liver	Alcoholic liver disease	No	CsA, Aza, prednisolone
2	M	11/18/1998	67.5	Heart/kidney	Ischemic cardiomyopathy	ATG	CsA, Aza, prednisolone
3	F	4/26/1999	58.8	Liver	Hepatitis C associated liver disease	No	CsA, MMF, prednisolone
4	M	12/24/1999	23.1	Kidney	Graft failure	No	TAG, MMF, prednisolone
5	F	20/12/2003	49.8	Pancreas/kidney	Diabetic nephropathy	ATG	TAG, Sirolimus, prednisolone
6	M	3/2/2004	44.9	Heart	Cardiomyopathia	ATG	TAG, MMF, prednisolone
7	M	2/17/2003	41.1	Bilateral forearm	High voltage accident	ATG	TAG, MMF, prednisolone
8	F	5/1 1/2005	25.3	Small bowel	Motility disorder	ATG	TAG, Aza, prednisolone
9	M	4/20/2006	62.6	Liver	Alcoholic liver disease	Basliximab	TAG, Sirolimus, prednisolone
10	F	4/18/2006	52.5	Lung	COPD, MZ	Basliximab	CsA, MMF, prednisolone
11	M	11/7/2006	47.0	Heart/kidney	Goodpasture syndrome, cardiomyopathy	Alemtuzumab, Rituximab	TAG, MMF, prednisolone

COPD, chronic obstructive pulmonary disease; MZ, alpha-1-antitrypsin phenotype; CsA, Cyclosporine A; TAC, tacrolimus; ATG, antithymocyte globuline; MMF, mycophenolate-mofetil; Aza, azathioprine.

confirmed by biopsy. Maxillary sinus mucosectomy and temporal lobe resection was performed and liposomal amphotericin B started. He is still alive but lost the renal graft after discontinuing immunosuppression.

Case 6

11 months after cardiac transplantation a pulmonary nodule was found in a 50-year-old man on CT scan (Fig. 3b). Zygomycosis (*Rhizopus*) of the lung was diagnosed by biopsy and this patient was successfully treated by pulmonary wedge resection together with liposomal amphotericin B.

Case 7 [45]

In a recipient of a double forearm graft, who experienced multiple rejection episodes and Cytomegalovirus (CMV) disease, a tumorous lesion of the right thigh was surgically removed. *Alternaria alternate* was isolated and liposomal amphotericin B (2 weeks) followed by itraconazole (3 months) was administered with complete remission.

Case 10

Zygomycosis with *Absidia corymbifera* of the lung was diagnosed 9 months after lung transplantation in a 53 old woman who had been successfully treated for PTLD. She received antifungal combination of posaconazole, amphotericin B liquid complex, which was switched to amphotericin B colloidal dispersion (Figs. 3c and 4a,b). She was able to clear the fungal infection but died 8 months later from recurrent PTLD.

Case 11

Cunninghamella infection of the lung was diagnosed in renal/heart recipient 3 months post-transplant (Fig. 3d). Treatment consisted of pulmonary wedge resection together with posaconazole. The patient is currently under antifungal treatment, and remaining pulmonary nodules are shrinking.

Clinical course, diagnosis, therapy

Six patients are currently alive with stable graft function in five, one renal graft was lost. Of the five deaths, four were due to invasive fungal infection and one was associated with PTLD, fungal infection and bacterial sepsis. In four cases, diagnosis was made postmortem and patients had not received any treatment (*Trichoderma* $n = 1$, zygomycosis $n = 3$). Only in one liver recipient with *P. boydii* wound infection, which disseminated, appropriate antifungal treatment was initiated but failed [43].

Excluding the postmortem identified cases, diagnosis for the seven cases was made from biopsy in six patients and fungal culture in one patient. In all patients immunosuppressive therapy was reduced. Combined surgical and antifungal treatment was employed in five patients including lung resection in three cases, resection of rhinocerebral mucormucosis in one and resection of an inflammatory tumor at the right thigh [42]. Concerning pulmonary infections ($n = 5$), only one patient with zygomycosis in the lung graft received antifungal treatment alone without surgery. Antifungal therapy consisted of liposomal amphotericin B in five cases, liposomal amphotericn B in combination with itraconazole in one case, liposomal amphotericin B together with posaconazole in the remaining two cases.

Identified risk factors

Intensified immunosuppression was identified in nine patients including induction with ATG (5), alemtuzumab

Table 3. Clinical data.

No	Renal failure	Rejection	Other infections	Risk factors	Clinical presentation	Pretreatment	Treatment
1	Yes	Yes	Oral Herpes simplex infection, Legionellosis	None	Chest pain	No	Liposomal amphotericin B, surgery
2	Yes	No	Citrobacter pneumonia, pleural empyema, sepsis, ischemic colon necrosis with peritonitis	Multiple infections, excessive immunosuppression	Pneumonia	Fluconacole, amphotericin	Liposomal amphotericin B (only 48 h prior to death)
3	Yes	Yes	CMV disease, infective hepatic artery aneurysma, ESBL + Enterobacter cloacae sepsis	Renal transplant, rejection, small for size graft, multiple infections neutropenia (Valganciclovir),	Multiorgan failure	No	Liposomal amphotericin B
4	No	Yes	Localized Herpes simplex infection	Positive cross-match, retransplant, excessive immunosuppression	Rhinocerebral infection	No	Liposomal amphotericin B, surgery
5	Yes	Yes	Endocarditis	Renal graft loss, endocarditis, excessive immunosuppression	Coma, multiorgan failure	Caspofungin	No therapy
6	Yes	No	No	Retransplant	Fever, dyspnea	No	Liposomal amphotericin B, surgery
7	No	Yes	Localized Herpes simplex infection, cytomegalo virus disease	Recurrent rejection, excessive immunosuppression (alemtuzumab)	Tumorous lesion on right thight	No	Liposomal amphotericin B, itraconozol, surgery
8	Yes	Yes	post-transplant lympho proliverative disorder, peritonitis	PTLD, neutropenia, excessive IS	Multiorgan failure	Caspofungin	No therapy
9	Yes	Yes	Graft-versus-host, lung, liver, gastrointestinal tract	GvHD, chemotherapy, alemtuzumab, excessive immunosuppression	Skin, multiorgan failure	Voriconazole	No therapy
10	No	No	Post-transplant lympho proliverative disorder	PTLD, neutropenia, excessive immunosuppression	Dyspnea	Voriconazole	Posaconazole, amphotericin B liquid complex, amphotericin colloidal dispersion
11	Yes	Yes	Urinary tract infection, erysipel	Rejection, retransplant, excessive immunosuppression, neutropenia	Pneumonia	Caspofungin, amphotericin	Posaconozole, surgery

TLD, post-transplant lymphoproliferative disorders; GvHD, graft versus host disease.

Table 4. Mycological data.

No.	Tranplant to infection (months)	Transplant to death (days)	Infection to death (days)	Infection site	Specimen	Histopathology	Microscopy calcofluor white Stain	Genus	Species	PCR
1	0.9	Alive	Alive	Tracheal ulcer	Biopsy	Tissue invasion	Unseptate hyphae	Zygomycosis	*Mucor* species	nd
2	0.4	29	16	Lung	Postmortem	Tissue invasion	Unseptate hyphae	Zygomycosis	*Rhizopus* species	nd
3	1.1	101	65	Wound	Culture	Tissue invasion	Unseptate hyphae	Pseudoallescheriosis	*Pseudallescheria boydii*	nd
4	0.3	Alive	Alive	Rhinocerebral	Biopsy	Tissue invasion	Unseptate hyphae	Zygomycosis	*Mucor* species	nd
5	2.4	91	19	Disseminated	Postmortem	Tissue invasion	Unseptate hyphae	Zygomycosis	*Rhizomucor* species	Yes
6	11	Alive	Alive	Lung	Biopsy	Tissue invasion	Unseptate hyphae	Zygomycosis	*Rhizopus oryzae*	Yes
7	26.4	Alive	Alive	Right thigh	Biopsy	Tissue invasion	Unseptate hyphae	Altemariosis	*Alternaria alternata*	nd
8	2.7	95	13	Disseminated	Postmortem	Tissue invasion	Unseptate hyphae	Zygomycosis	*Absidia corymbifera*	Yes
9	1.4	44	2	Disseminated	Postmortem	Tissue invasion	Unseptate hyphae	Zygomycosis/ Trichoderma	*Absidia corymbifera, Trichoderma species*	Yes
10	8.5	360	110	Lung	Biopsy	Tissue invasion	Unseptate hyphae	Zygomycosis	*Absidia corymbifera*	Yes
11	2.8	Alive	Alive	Lung	Biopsy	Tissue invasion	Unseptate hyphae	Zygomycosis	*Cunninghamella* species	Yes

Nd, not done.

(1) and basiliximab (2), treated rejection episodes ($n = 8$) and two patients suffering from PTLD receiving chemotherapy and one having GvHD, which was treated with cyclophosphamaide and alemtuzumab. Renal failure was found in eight patients, whereas diabetes and cytomegalovirus disease were found in only two patients each. Multiple bacterial or viral infections were present in ten patients. Six patients received antifungal treatment prior to acquisition of nAFF infection Pretreatment with voriconazole (2) or caspofungin (3) was found in five of seven patients, who suffered from the infection after 2003, when these agents were introduced.

Discussion

Our retrospective study demonstrates that rare filamentous fungal infections should be considered important pathogens in solid organ recipients [6–8,17,24]. The spectrum has become more diverse and includes not only zygomycetes, but also other rare fungal species [15,16]. Therefore, identification to the species level and antifungal susceptibility testing is highly warranted [49,50]. An important risk factor in our cohort was intensified immunosuppression [18,29]. Pretreatment with the new antifungal agents caspofungin and voriconazole was a common characteristic in patients who developed these infections after 2003 [27]. The clinical and radiological presentation has become more diverse than a decade ago and only a single patient in this series had rhinocerebral infection [44]. Surgical therapy definitely plays an important role in the treatment. The last two patients in this series received the new agent posaconazole [31–33]. Overall, the mortality rate is not as high as in invasive aspergillosis, however, the fact that four cases were diagnosed postmortem illustrates a lack of awareness [17,18,25].

Infections with opportunistic fungi including nAFF are increasingly diagnosed in SOT recipients [8,12]. In our study population, zygomycetes were the predominant organisms. Traditionally, zygomycosis was a disorder affecting diabetics; however, today this infection is more frequently diagnosed in transplant recipients [18–20,26]. Rhinocerebral or pulmonary infections are most common but disseminated infection is associated with an excessive fatality rate [25,51]. Rare angioinvasive tissue infections are characterized by necrosis, granulomas and abscess formation and have been reported to involve unusual sites such as the genital area (18). In case of localized infection, surgical debridement is treatment of choice together with antifungal therapy [25,52].

At our centre, only sporadic cases were diagnosed until 2002 but from 2003 to 2007 these infections became more frequent parallel to an increased use of more powerful immunosuppressive agents such as alemtuzumab and the

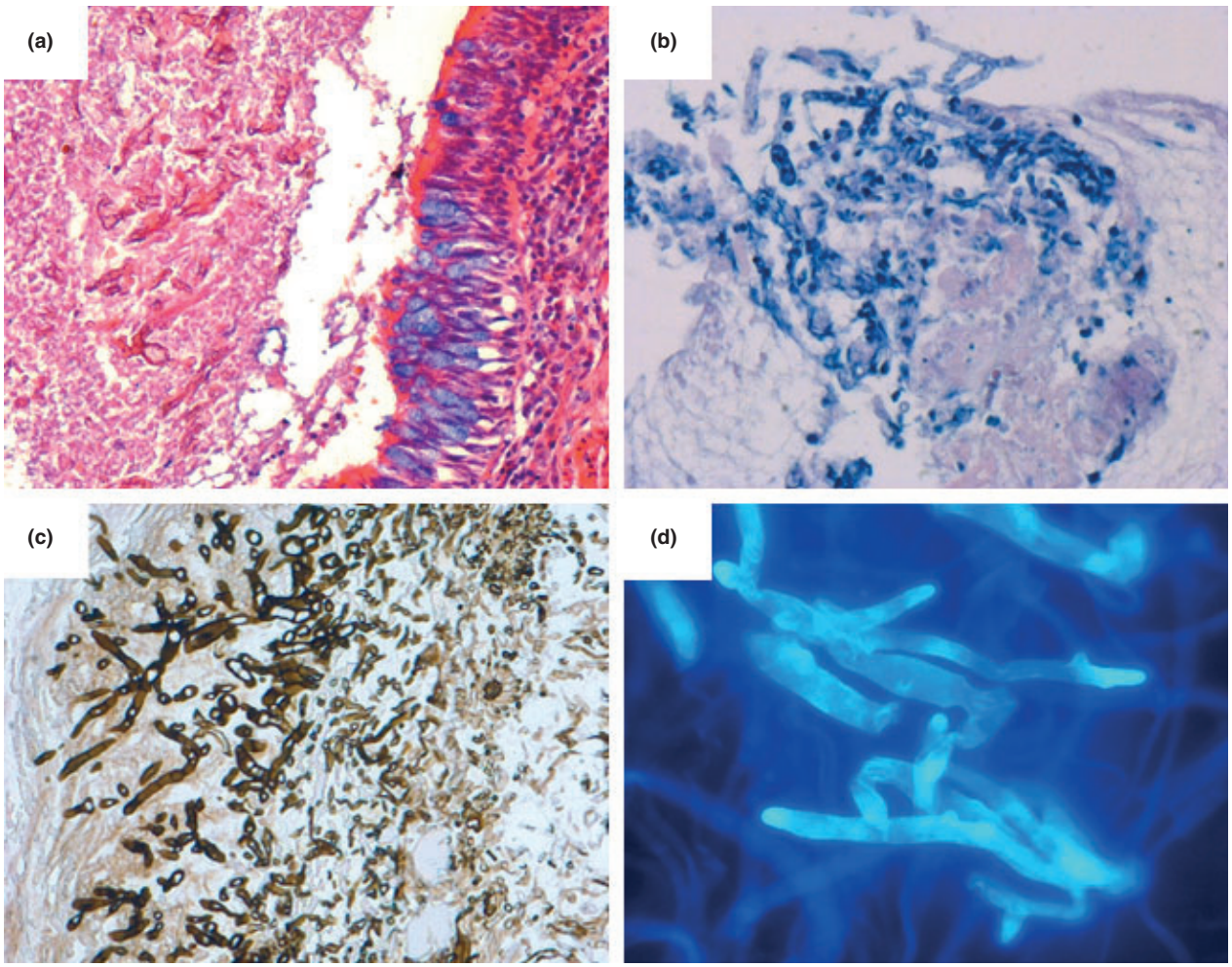

Figure 1 Histology: survivors. (a) Patient no. 1: biopsy of tracheal ulcer; Grocott; Mucormucosis. (b) Patient no. 11: lung wedge resection: hematoxillin/eosin: *Cunninghamella.* (c) Patient no. 6: lung wedge resection; hematoxillin/eosin: submucose lymphocellular infiltrate; debris in bronchial lumen with filamentous fungal infiltration. (d) Patient no. 10: culture: Calcofluor White Staining: *Absidia corymbifera*.

introduction of voriconazole and caspofungin lacking activity against zygomycetes [10,27,32,53]. Five of six patients with zygomycosis diagnosed since 2003 had received these agents prophylactically against aspergillosis. At our institution amphotericin B resistant *Aspergillus terreus* is a significant problem and this has lead to an increase in the use of the new antifungals [53]. It is currently unclear, weather the use of voriconazole and caspofungin truly favor infection by zygomycetes or if patients survive aspergillosis and die later from zygomycetes or other rare pathogens [10,27]. Two patients had PTLD and one GvHD, five patients received ATG and three antiCD25 antibodies, which demonstrates that impaired immune defense represents the most important risk factor for these infections [54]. In the series of Almyroudis only 7.3% of patients received OKT3 (muromonab-CD3 AK) (Orthoclone OKT3, Ortho Biotech, Bridgewater, NJ, USA) and 3.1% ATG for steroid resistant rejection and 28.4% had induction immunosuppression. [21]. Also the vast majority in their series and the 106 cases from the literature were renal recipients and the rhinocerebral and orbital from accounted for more than one third of cases.

In our series 30% of cases were caused by rarer pathogens than zygomycetes and one was a mixed infection with *Trichoderma* with a zygomycete outlining the importance of a dedicated mycological back up to be available for transplant centers [55]. *Pseudallescheria boydii* has been involved in fatal infections of the lung, thorax and intra-abdominal organs [43]. Similar to zygomycosis, the reported high mortality rate is due to delayed diagnosis and limited treatment options. In our case, the wound infection was not taken seriously and no systemic anti-

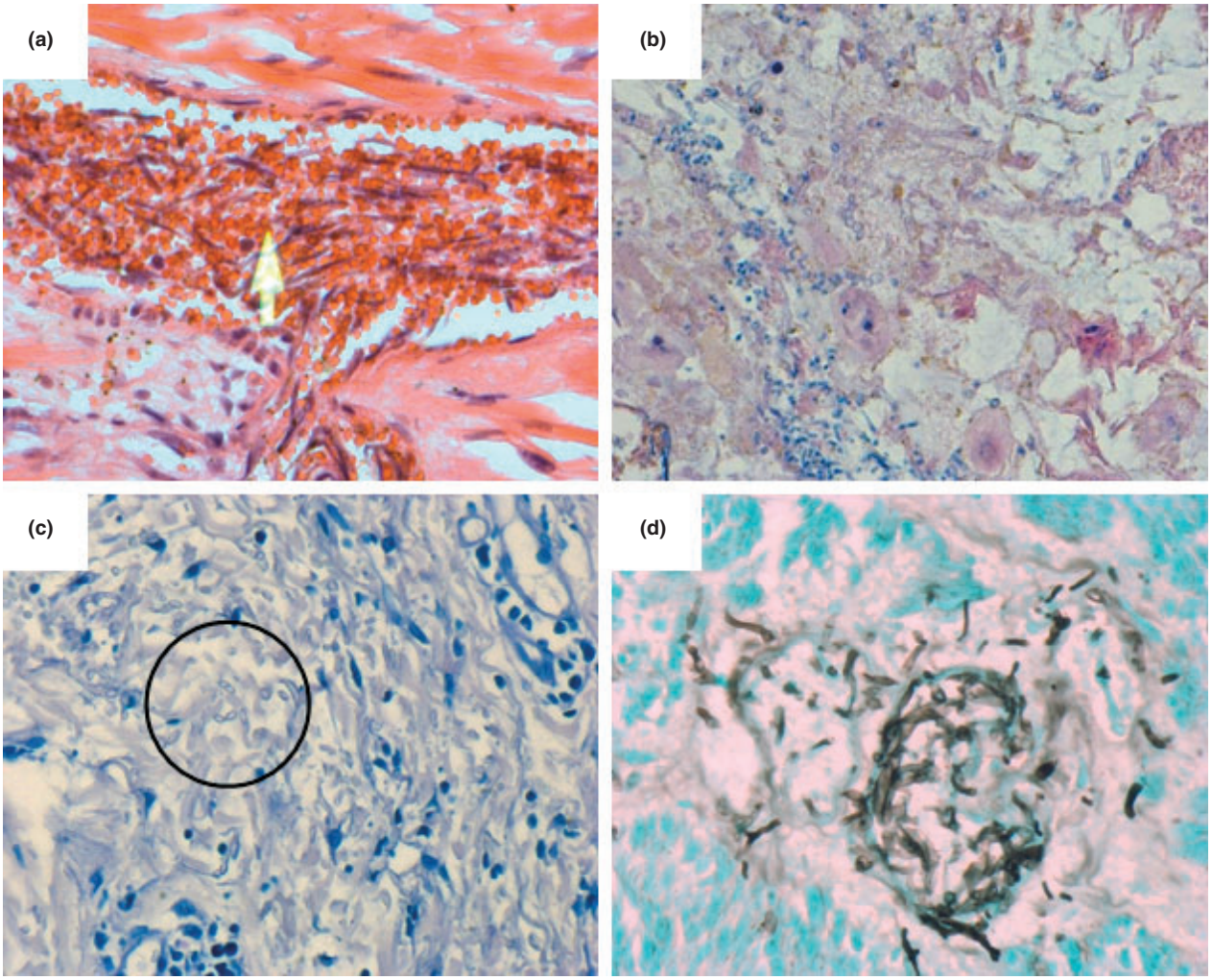

Figure 2 Histology: fatal cases. (a) Patient no. 5: postmortem: hematoxillin/eosin; cardiac muscle: occlusion of blood vessel by fungi, infiltration of perivascular tissue: *Rhizomucor*. (b) Patient no. 8: postmortem: hematoxillin/eosin; necrotic intestinal graft; infiltration by filamentous fungi: *Absidia corymbifera*. (c) Patient no. 9: skin biopsy: hematoxillin/eosin; graft versus host disease and infiltration with filamentous fungi: *Absidia corymifera*. (d) Patient no. 9: postmortem; muscle biopsy; Grocott: *Trichoderma*.

fungal therapy was initiated at an early stage. Once the infection had disseminated, liposomal amphotericin B had no impact on the fatal course [43]. *Trichoderma* is another rare and frequently fatal pathogen. In our case *Trichoderma spp.* was isolated together with *Absidia corymbifera*. The clinical presentation with fever, diarrhea, exfoliating exanthema and multiorgan failure was also compatible with ongoing GvHD. Therefore, diagnosis was not established and no appropriate antifungal treatment was initiated and the patient died due to disseminated infection. This patient had received prophylactic voriconazole as it was well recognized that the patient was at excessive risk for aspergillosis; however, *Trichoderma* and *Absidia corymbifera* were not covered [55–57]. Little data are available on *Alternaria* infection in solid organ recipients [58,59]. Our patient was a double forearm recipient and had received multiple treatment courses for steroid resistant rejection including alemtuzumab [45,58]. In this case surgical resection of the tumorous lesion was performed in time, which allowed early diagnosis and subsequent adequate antifungal therapy with liposomal amphotericin B followed by itraconazole [45].

Lack of specific clinical signs was an important factor in delay of diagnosis and initiation of treatment in our series [18,60]. Awareness of the possibility of a rare fungal infection should lead to utilization of the full range of diagnostic tools including CT scan or MRI [61–63]. CT-guided biopsies of any lesions suspicious for these infections should be deployed [61]. Bronchoalveolar lavage with transbronchial biopsy is an alternative in the case of pul-

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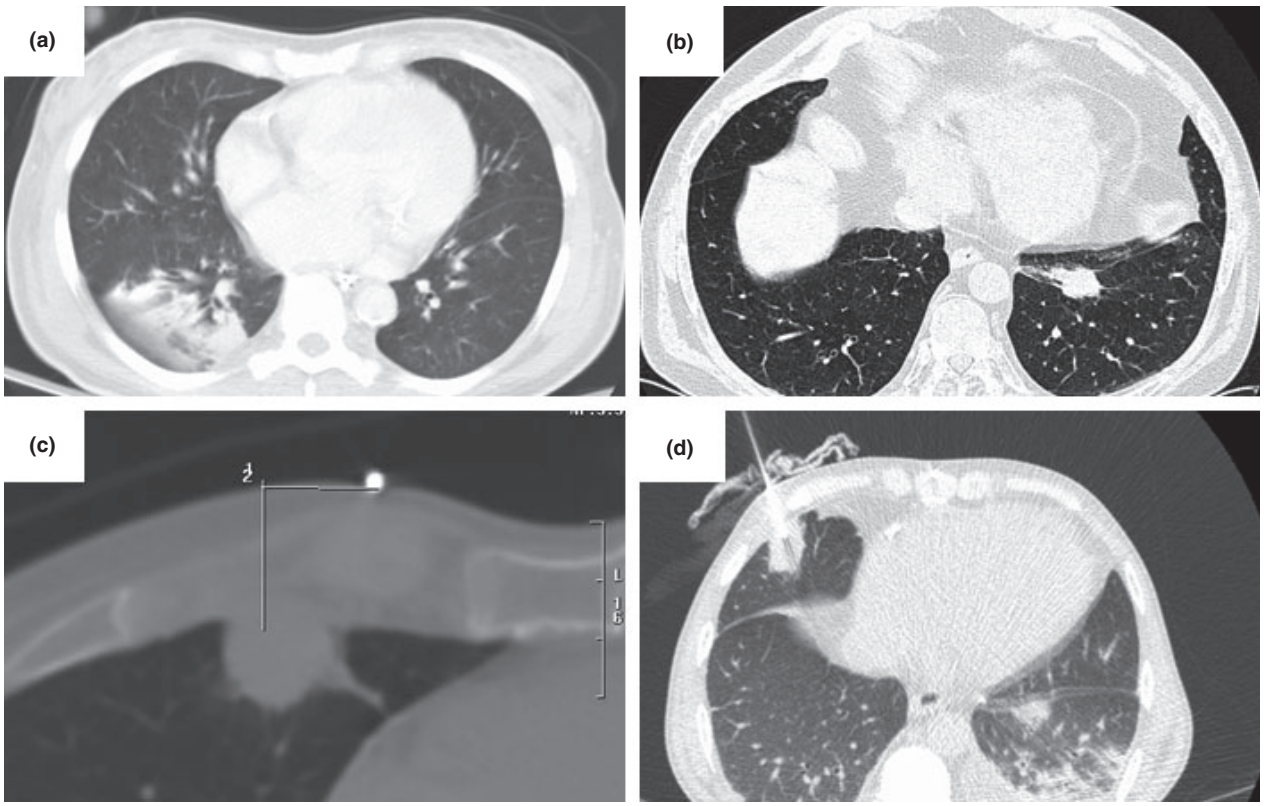

Figure 3 CT scans. (a) Patient no. 5: kidney/pancreas recipient: pulmonary infiltrate right lung. (b) Patient no. 6: cardiac recipient: pulmonary nodule left lung. (c) Patient no. 10: lung recipient: pulmonary nodule right lung: close up (CT-navigation for percutaneous biopsy). (d) Patient no. 11: cardiac recipient: pulmonary nodule right lung (CT-guided percutaneous biopsy) and multiple infiltrates left lung.

monary lesions [61]. If evidence for infection is present, most reliable specimens for rapid diagnosis and differentiation from *Aspergillus* infection can be obtained by biopsies of infected tissue with Calcofluor-white staining [64,65].

Therapeutic strategies in treatment of nAFF infections include surgical intervention together with antifungal therapy based on sensitivity testing [26,44,45]. Five patients in this cohort (71% of diagnosed cases) under-

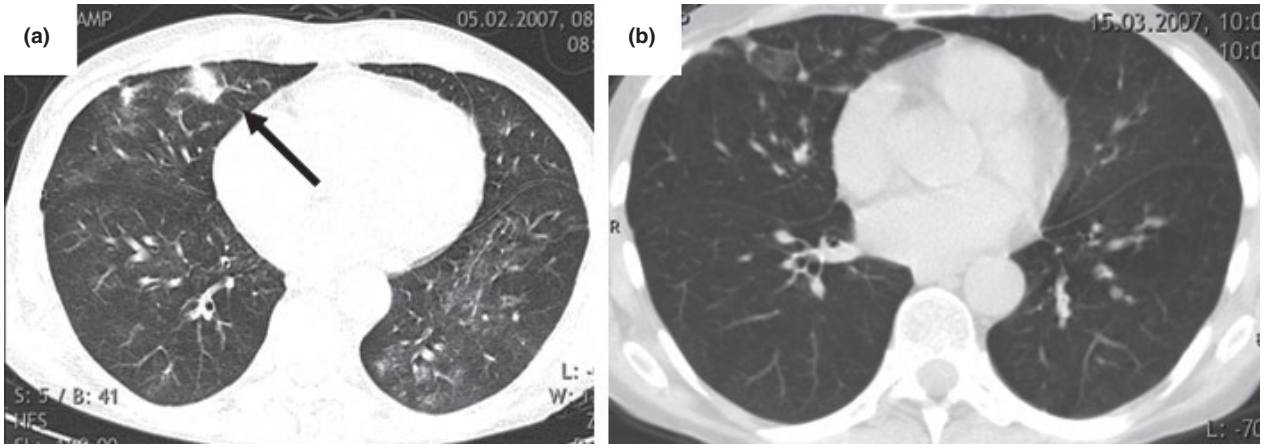

Figure 4 Pulmonary zygomycosis after lung transplantation: Patient no. 10:. (a) pre treatment: nodule right lung (arrow). (b) after 6 weeks antifungal treatment: significant regression of the lesion.

went surgery. Pulmonary lesions may be approached through thoratocomy or video assisted thoracoscopic surgery [66–68]. Amphotericin B and posaconazole are antifungal treatment options for disseminated zygomycosis; voriconazole and itraconazole may have useful activity against some other filamentous fungi, [9,69]. Greenberg *et al.* demonstrated a 79% success rate of posaconazole in patients with zygomycosis refractory to standard therapy and 80% success rate in case of intolerance to standard therapy [34]. In our study, two patients with pulmonary zygomycosis were successfully treated with oral posaconazole.

From this study we conclude that rare filamentous fungal infections in transplant recipients are an increasing problem. The intensified immunosuppression, exposure to antibiotics and antifungals and other factors may be important co-factors [70–74]. Awareness of this issue in patients presenting with specific and nonspecific symptoms should be followed by proper diagnostic approach, biopsy and identification of the causing organism to the species level and antifungal susceptibility testing. Combined surgical and antifungal treatment seems to be the best therapy. If these steps are undertaken, the prognosis is favorable.

Authorship

IS, CG, IG, SS, AH, HB: data collection. CLF, BZ, NS: mycology, pathology. IS, CLF, LM, NS, TLP, RM, HB: manuscript writing.

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