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Aspergillus Infections in Transplant Recipients

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INTRODUCTION

The number of patients undergoing transplantation has increased exponentially in recent years. An estimated 15,000 allogeneic and 25,000 autologous stem cell transplants are performed worldwide annually (132). Within the last 5 years (1998 to 2002), a total of 113,682 solid organ transplant surgeries (an average of 22,736 annually) were performed in the United States (319). This represents a 20.1% increase in the numbers compared to those in the previous 5 years, i.e., 1993 to 1997 (319).

Transplant recipients are among the most significant subgroups of immunosuppressed hosts at risk for invasive aspergillosis. *Aspergillus* infections have been reported in 2 to 26% of

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TABLE 1. Epidemiologic characteristics of invasive aspergillosis in transplant recipients[a]

Type of transplant	Incidence range, % (mean)	Mean days to onset (range)	% Due to disseminated aspergillosis	% Mortality
Liver	1–8 (2)	17 (6–1,107)	50–60	87
Lung	3–14 (6)	120 (4–1,410)	15–20	68
Heart	1–15 (5.2)	45 (12–365)	20–35	78
Kidney	0–4 (.7)	82 (20–801)	9–36	77
Pancreas	1.1–2.9	NA[b]	NA	100
Small bowel	0–10 (2.2)	289 (10–956)	66	66
Allogeneic stem cell[c]	5–26 (10)	78 (46–120)	27–30	78–92
Autologous stem cell	2–6 (4.8)	20 (7–456)	10–20	78–92
Nonmyeloblative stem cell	8–23 (11)	107 (4–282)	34	63–67

[a] Data are from references 37, 85, 87, 139, 161, 187, 198, 212, 237, 296, 327, 333, and 335.
[b] NA, not available.
[c] The data presented are for allogeneic myeloablative transplants.

hematopoietic stem cell transplant (HSCT) recipients and in 1 to 15% of organ transplant recipients (Table 1). Historically, the mortality rate in transplant recipients with invasive aspergillosis has ranged from 74 to 92%. An estimated 9.3 to 16.9% of all deaths in transplant recipients in the first year are considered attributable to invasive aspergillosis (237).

Transplantation practices, immunosuppressive regimens, and the characteristics of patients undergoing transplantation have continued to evolve (87, 136, 186, 296). These changes are having a significant effect on the epidemiology of invasive aspergillosis. Since the late 1990s, a rising frequency of isolation of *Aspergillus* species has been noted in large tertiary care centers (52, 187, 296). At one institution, the incidence of invasive aspergillosis in hematopoietic stem cell transplant recipients increased from 7.3% in 1992 to 16.9% in 1998 (187). Although an earlier detection of invasive aspergillosis remains challenging, significant strides have been made in this context.

This review discusses the evolving trends in the epidemiology, advances in diagnostic laboratory assays, and the approach to antifungal treatment and prophylaxis for invasive aspergillosis in transplant recipients.

EPIDEMIOLOGY

Hematopoietic Stem Cell Transplant Recipients

Invasive aspergillosis has been reported in 0.08 to 2.6% of autologous and 3.6 to 10.3% of allogeneic HSCT recipients, with higher rates in those with unrelated or HLA-mismatched than in HLA-matched donors (66, 102, 134, 186, 192, 226, 327). Neutropenia has traditionally been the predominant risk factor, with most infections occurring prior to engraftment, particularly in autologous transplant recipients (210, 327, 335). In recent years, alternative sources of harvested stem cells, newer preparative regimens to decrease rejection and graft-versus-host disease (GVHD), and aggressive therapeutic modalities for GVHD have come to be employed with HSCT recipients (87, 135, 186, 190, 197, 208, 215, 251, 305). These changes have had a dramatic impact on the epidemiology of *Aspergillus* infections in hematopoietic stem cell transplant recipients, both autologous and allogeneic.

The frequency of invasive aspergillosis in autologous transplant recipients has decreased, due largely to more rapid engraftment afforded by the use of hematopoietic growth factors and of grafts with higher stem cell content (102).

The use of peripheral stem cells compared to bone marrow cells for transplantation has been shown to lead to faster hematopoeitic cell repopulation and a reduced potential for disease recurrence (251, 305). Peripheral blood stem cells contain not only more progenitor cells, but also an approximately 1-log-unit-greater number of lymphocytes (23). The larger number of lymphocytes is beneficial in facilitating engraftment and in providing antileukemic effects; however, it may potentially confer a greater likelihood of GVHD (23). While the risk of acute GVHD does not appear to be different, that of chronic GVHD may be higher when peripheral stem cells are used for transplantation (23). Compared to marrow transplantation from an HLA-matched related donor, peripheral blood stem cell transplantation from an HLA-matched related donor was associated with a lower risk of early- but not of late-onset invasive aspergillosis (186).

There has also been an increase in the use of umbilical cord blood stem cells for transplantation, particularly in patients ≤20 years of age (132). The recipients of cord blood are at a notably high risk for invasive aspergillosis early after transplantation (186). Delayed reconstitution of neutrophil function and cellular immunity and the use of antithymocyte globulin as part of the conditioning regimen in these patients may account for these observations (186). Grafts selected for $CD34^+$ progenitor cells have the potential to reduce tumor contamination but may delay lymphocyte reconstitution, particularly of $CD4^+$ cells (215). A trend towards a higher rate of invasive aspergillosis was noted in allogeneic transplant recipients with T-cell-depleted or CD34-selected grafts (186). Invasive aspergillosis was documented in 6% of the patients after CD34-selected autologous peripheral blood stem cell transplantation and in 2% ($P = 0.20$) of those in a contemporaneous cohort who received unselected autologous peripheral stem cell transplantation (225).

Conventionally, the goals of conditioning regimens in HSCT recipients have been to eradicate all host-derived hematopoietic cells in order to minimize graft rejection and to eliminate residual tumor cells. However, severe and protracted neutropenia resulting from these aggressive myeloablative regimens has been a major risk factor for invasive aspergillosis and other opportunistic infections. Nonmyeloablative transplants in which engraftment is achieved with reduced-intensity conditioning regimens have now emerged as an important therapeutic modality in HSCT recipients (87, 135, 197, 208, 329). Between 1998 and 2000, there has been an approximately fivefold

TABLE 2. Variables portending a higher risk for invasive aspergillosis in transplant recipients[a]

Type of transplant and infection onset	Variables portending higher risk
Hematopoietic stem cell	
Early (within 40 days)	Cytomegalovirus disease, delayed neutrophil engraftment, alemtuzumab-containing conditioning regimen
Late (after 40 days)	Cytomegalovirus disease, T-cell-depleted or CD34-selected stem cells, unrelated or mismatched donor grafts, graft-versus-host disease, alemtuzumab for treatment of graft-versus-host disease, corticosteroid dose of >0.5 mg/kg/day
Liver	Retransplantation, renal failure (particularly requiring renal replacement therapy), fulminant hepatic failure as an indication for transplantation
Lung	Single lung transplant, cytomegalovirus infection, rejection and augmented immunosuppression, obliterative bronchitis
Heart	Isolation of *Aspergillus* species in respiratory tract cultures, reoperation, post-transplant hemodialysis, cytomegalovirus disease
Kidney	Graft failure requiring hemodialysis, high level and prolonged duration of corticosteroids

[a] Data are from references 8, 18, 85, 129, 186, 212, 213, 232, 237, 290, 291, and 293.

increase in the number of nonmyeloablative transplants; these less intensive conditioning regimens are now used in about 25% of the allogeneic transplants in some institutions (132).

Nonmyeloablative conditioning comprises a wide spectrum of reduced-intensity regimens. The risk of infection posed may therefore vary for different regimens and between institutions. Generally, these regimens have a reduced potential for causing mucosal and myelopoietic toxicity but are highly immunosuppressive. Invasive aspergillosis has been reported in 11 to 23% of the nonmyeloablative transplant recipients, with most studies reporting rates that are comparable to or somewhat higher than those after conventional myeloablative hematopoetic cell transplantation (87, 137, 329). A trend towards a higher risk of invasive aspergillosis during the first year after transplant was noted among nonmyeloablative compared to myeloablative transplant recipients (hazard ratio, 1.54; 95% confidence interval [CI], 0.96 to 2.47%; $P = 0.09$) (87). GVHD has been identified as the major risk factor for invasive aspergillosis in nonmyeloablative transplant recipients, with most infections occurring postengraftment (8, 87, 329). Severe acute GVHD of grade III or IV, chronic extensive GVHD, and cytomegalovirus (CMV) disease each conferred an independent risk for invasive mold infections in nonmyeloablative transplantation recipients (87). In another study, a higher grade of GVHD and a longer duration of corticosteroid therapy portended a significant risk for invasive aspergillosis in nonmyeloablative transplant recipients (329).

A majority (76%) of the invasive mold infections after nonmyeloablative transplantation occurred between days 40 and 180; only 8% occurred before day 40, and 16% developed later than 6 months posttransplantation (87). A lower risk of severe and protracted neutropenia and the retention of donor T-cell responses through mixed chimerism may have a role in preventing early infections after nonmyeloblative conditioning (137, 183).

Aggressive management of GVHD with potent immunosuppressive agents is also a major contributor to the risk for *Aspergillus* infections (Table 2). Alemtuzumab (Campath-1H), an anti-CD52 monoclonal antibody that depletes peripheral blood T and B cells (without affecting the stem cells), has increasingly come to be used in HSCT recipients. Campath-1H as part of the conditioning regimen conferred a significantly greater risk for early-onset invasive aspergillosis (8). Treatment of GVHD with Campath-1H was likewise associated with a higher risk for late-occurring invasive aspergillosis (8). Infliximab (Remicade) is an anti-tumor necrosis factor alpha antibody used to treat GHVD. Its administration portended a high risk of invasive filamentous fungal infections (193).

Taken together, these data suggest that invasive aspergillosis in HSCT recipients now predominantly occurs late after engraftment in nonneutropenic patients in whom GVHD and its management with increasingly intense immunosuppression have emerged as major risk factors.

Isolation of *Aspergillus* species from the respiratory tract or of *A. fumigatus* from any site has a high predictive value (ranging from 60 to 82) for invasive infection in HSCT recipients (237, 327). *A. niger* appears to be less virulent than *A. fumigatus* and less likely to be associated with invasive infection when detected in clinical specimens (327). Overall, 8.3% of *A. niger* isolates compared to 80% of *A. fumigatus* isolates in one report were associated with invasive infection (327). The fact that *A. niger* was more likely to be recovered from rectal swabs suggests that its acquisition may occur via the gastrointestinal, rather than the respiratory, tract (327). An association between *A. flavus* and sinusitis has been noted in these patients (78, 327).

A notable trend in HSCT recipients is the increasing frequency of isolation of *Aspergillus* species other than *A. fumigatus* (18, 187). *A. terreus* is the most common *Aspergillus* species that is detectable in the bloodstream (151). Overall, *A. terreus* accounts for ~3% of the *Aspergillus* infections. In a recent report, 20% of the invasive mold infections in HSCT recipients were due to *A. terreus* (18). From 1996 to 1998, 33.7% of positive bronchoalveolar lavage (BAL) or biopsy cultures from HSCT recipients yielded non-*A. fumigatus* species of *Aspergillus*, compared with 18.3% from 1993 to 1995 ($P = 0.01$). These data are worrisome given that some molds, e.g., *A. terreus*, are innately resistant to amphotericin B.

The mortality rate in HSCT recipients with invasive aspergillosis ranges from 66.6 to 80% and does not differ for those with early- versus late-onset infections. Invasive aspergillosis is a significant contributor to non-relapse-related mortality in nonmyeloablative HSCT recipients; ~9% of overall mortality and 39% of nonrelapse mortality in these patients were due to in-

vasive mold infections (87). The 1-year infection-related mortality rate did not differ for patients who received standard myeloablative (19%) or reduced-intensity (10%) conditioning regimens after allogeneic peripheral blood cell transplantation (190). For patients with mold infections, the 1-year mortality was 68%, a rate similar to that of the recipients of myeloablative transplantation (87).

Liver Transplant Recipients

Invasive aspergillosis has been reported in 1 to 8% of liver transplant recipients (36, 37, 47, 58, 85, 160, 214, 235, 314). *Aspergillus* infections in these patients typically occur in the early posttransplant period. The median time to onset after transplantation was 17 days in one study (290) and 16 days in another (283). A vast majority of the patients who developed invasive aspergillosis had never left the intensive care unit after liver transplantation surgery (30, 290). Liver transplant recipients are also uniquely predisposed to dissemination of infection beyond the lungs, which occurs in ~50 to 60% of cases (237, 290, 316). Isolation of *Aspergillus* spp. from the respiratory tracts of liver transplant recipients is an infrequent event (~1.5%). However, it has a high positive predictive value, ranging from 41 to 72%, for invasive aspergillosis (237).

Renal dysfunction and retransplantation are among the most significant risk factors for invasive aspergillosis in liver transplant recipients (85, 237, 290). Renal failure, particularly the requirement of renal replacement therapy, has been shown to portend a 15- to 25-fold-greater risk for invasive aspergillosis. Approximately one-fourth of the cases of invasive aspergillosis have occurred after retransplantation (85) Liver transplant recipients undergoing retransplantation have a 30-fold higher risk of invasive aspergillosis (85, 287). Fulminant hepatic failure as an indication for liver transplantation, cytomegalovirus infection, and human herpesvirus 6 infection has also been shown to be a risk factors for invasive aspergillosis in these patients (58, 77, 91, 259, 286).

Two notable changes in the epidemiology of invasive aspergillosis appear to have occurred in liver transplant recipients: the onset of invasive aspergillosis later in the posttransplant period and a decline in the incidence of disseminated and central nervous system infections (125, 291). In a study that compared a cohort of patients with invasive aspergillosis from 1998 to 2002 with those from 1990 to 1995, 55% of the infections in the later cohort compared with 23% in the earlier cohort occurred after 90 days after transplantation (291). The precise reasons for the later occurrence of invasive aspergillosis in liver transplant recipients are unclear. Technical surgical advances and better management have led to a lower frequency of organ system failures and an improved outcome in the early postoperative period. Thus, patients at risk in the early 1990s, who may otherwise not have survived, now constitute a growing group of liver transplant recipients who still develop *Aspergillus* infection, albeit in the later posttransplantation period (291). Cytomegalovirus infection in organ transplant recipients in the present era of ganciclovir prophylaxis is occurring later (167, 288). In this context, a risk factor for invasive aspergillosis may merely have been delayed, thus accounting for the later occurrence of opportunistic infections, such as invasive aspergillosis. In addition, hepatitis C virus infection as an underlying liver disease has become an increasingly common indication for liver transplantation in recent years (291). Indeed, the frequency of hepatitis C virus infection as an underlying liver disease in patients in the later cohort was threefold higher than that in the earlier cohort (291). Significant allograft dysfunction is a known risk for aspergillosis, and because hepatic dysfunction in patients undergoing liver transplantation for hepatitis C virus often occurs later in the posttransplantation period, another risk factor for invasive aspergillosis may now be occurring later.

Whereas disseminated and central nervous system infections were present in 61.5 and 46% of the patients, respectively, in the earlier cohort, only 30% of the patients in the later cohort had disseminated and none had central nervous system infection (291). A potential role of currently used immunosuppressive agents has been proposed as a plausible explanation for these trends. Calcineurin and target-of-rapamycin (TOR) inhibitor agents have potent in vitro activities against *Aspergillus* species (253, 292). These agents (by inhibition of fungal homologs of calcineurin or the target of rapamycin) have been shown to affect a variety of cellular and physiological processes in a number of pathogenic fungi, including *Aspergillus*. Calcineurin regulates hyphal growth and is essential for cell cycle progression in *A. nidulans* (253). Furthermore, calcineurin and TOR inhibitors, in particular, tacrolimus and sirolimus, were found to enhance the activities of antifungal agents in vitro and to attenuate the growth of all *Aspergillus* species tested (150).

The immunosuppressive activities of these agents outweigh their antifungal activities in vivo. Consequently, invasive aspergillosis continues to be observed in organ transplant recipients. However, it is plausible that the currently used calcineurin and TOR inhibitors may have an impact on the clinical manifestations, tissue tropism, or risk of dissemination associated with this fungus (292). Data from animal studies corroborate these observations. In a mouse model of invasive aspergillosis, cyclosporine, tacrolimus, and rapamycin had no impact on survival (119). However, histopathologic examination documented widely disseminated hyphae in the brains of cyclosporine-treated mice, whereas the brains of tacrolimus- and sirolimus-treated mice showed a nearly complete absence of *Aspergillus* hyphae (119).

The mortality rate in liver transplant recipients with invasive aspergillosis has ranged from 83 to 88% (68, 85, 237). More recent studies, however, have reported better outcomes (84, 291). A lower mortality rate in patients receiving transplants between 1998 and 2002 (60%) than in those receiving transplants between 1990 and 1995 (92%) was attributable largely to a lower incidence of disseminated infection and a lesser severity of illness in the current cohort of patients, independent of the use of lipid formulations of amphotericin B as therapy (127).

Lung Transplant Recipients

Aspergillus species can be detected in airway sample cultures from ~25 to 30% of lung transplant recipients (40, 139, 198, 291). Invasive aspergillosis, however, occurs in 3 to 15% (~6% on average) of the patients (27, 34, 40, 101, 104, 121, 129, 139, 144, 157, 195, 198, 218, 221, 293, 333, 341); 58% of these infections are tracheobronchitis or bronchial anastomotic in-

fections, 32% are invasive pulmonary aspergillosis, and 22% are disseminated infections with extrapulmonary involvement (293). *Aspergillus* infection occurs a median of 3.2 months after transplantation, with 51% occurring within 3 months and 72% occurring within 6 months after transplantation (293). Tracheobronchitis or anastomotic infections are the most frequently occurring infections within 3 months after transplantation, whereas invasive pulmonary and systemic infections tend to occur later (293). The median times to onset were 2.7 months for tracheobronchitis or bronchial anastomotic infections, 5.5 months for invasive pulmonary, and 10.6 months for systemic infections.

Host factors, including the underlying lung disease and the type of lung transplantation (single versus bilateral), appear to influence the risk and type of *Aspergillus* infection posttransplantation. Single lung transplant recipients are more likely to develop *Aspergillus* infections later after transplantation and to have a higher incidence of invasive pulmonary aspergillosis (as opposed to tracheobronchitis) and a higher mortality than other lung transplant recipients (293, 299, 333). In the vast majority of the single lung transplant recipients, invasive aspergillosis has been documented in the native lung, suggesting that the infection likely originates from a preexistent focus or a nidus in the native diseased lung (293, 333). Single lung transplant recipients who developed invasive aspergillosis were more likely to have chronic obstructive pulmonary disease as an underlying condition, which is known to predispose patients to airway colonization with *Aspergillus* (293). Using DNA primers for strain typing, a molecular epidemiologic study of lung transplant recipients documented that while the clinical strain of *Aspergillus* in one lung transplant recipient was identical to the one collected from home, the isolates in the other three patients were deemed more likely to be of nosocomial origin (34). A higher mortality rate in single lung transplant recipients may be related to a higher incidence of invasive pulmonary as opposed to tracheobronchial infections.

Bilateral and right lung transplant recipients had a higher incidence of bronchial anastomotic infections in one report (109). Patients undergoing bilateral lung transplantation generally have longer operations and may have a higher risk of ischemia at the site of anastomosis and greater impairment in the cough reflex and muociliary clearance that may confer a higher risk of colonization and subsequent infection of the anastomosis.

Colonization with *Aspergillus* is common in patients with cystic fibrosis before transplantation (221, 232). While these patients have been shown to be at risk for developing bronchial anastomotic infections, particularly within the first month of transplantation, the risk for pulmonary or disseminated infections does not appear to be higher.

Aspergillus infections in lung transplant recipients may be accompanied by fever in only 15% of the patients (293). Fever is generally absent in tracheobronchitis or bronchial anastomotic infections. A characteristic radiographic appearance is also typically lacking. Focal areas of patchy consolidation of infiltrates are the most common lesions on imaging studies. Nodular infiltrates occur in ~27 to 30% of patients, and the halo sign is distinctly unusual (293).

The overall mortality rate from *Aspergillus* infections in lung transplant recipients is 52 to 55% (198, 293). The mortality rate ranges from 23.7 to 29% in patients with tracheobronchial infections and from 67 to 82% in those with invasive pulmonary infections. Since invasive pulmonary as opposed to tracheobronchial infections occur more commonly in single lung transplant recipients and in the later posttransplant period, the outcome has been poorer in single lung transplant recipients with invasive aspergillosis and in those developing late-onset *Aspergillus* infections.

Heart Transplant Recipients

Invasive aspergillosis occurs in 3.3 to 14% (average, 6%) of heart transplant recipients (100, 101, 120, 124, 205, 206, 212, 213, 280, 331). *Aspergillus* infections are the most commonly occurring mycoses in heart transplant recipients, accounting for 69.8% of all invasive fungal infections after heart transplantation (101). The usual time of onset of invasive aspergillosis is 36 to 52 days posttransplantation, with nearly 75% of the cases occurring within 90 days of transplantation. At one institution, the onset of invasive aspergillosis was shown to have been significantly delayed by the introduction of routine ganciclovir prophylaxis for cytomegalovirus infection (205).

Aspergillus can be detected in ~10% of heart transplant recipients after transplantation (212). Recovery of *Aspergillus* species from respiratory tract cultures, particularly of *A. fumigatus*, is highly predictive of invasive aspergillosis in these patients (212). Reoperation, CMV disease, posttransplant hemodialysis, and the existence of an episode of invasive aspergillosis in the institution's heart transplant program 2 months before or after the transplantation date have been shown to be independent risk factors for invasive aspergillosis in heart transplant recipients (213).

The mortality rate in heart transplant recipients with invasive aspergillosis has ranged from 53 to 78% for invasive pulmonary aspergillosis, was 90% for disseminated infections, and was 100% for disseminated infections that involved the central nervous system (205).

Renal Transplant Recipients

In renal transplant recipients, invasive aspergillosis has been reported in ~0.7% and in up to 4% of patients (9, 37, 56, 88, 106, 214, 237, 243, 332). Despite a relatively lower overall incidence compared to other organ transplant recipients, invasive aspergillosis is a significant contributor to morbidity in renal transplant recipients. The national registry of U.S. Renal Data System documented that between1994 to 1997, an estimated 12% of hospitalizations for fungal infections were due to aspergillosis (1). High dose and prolonged duration of corticosteroids, graft failure requiring hemodialysis, and potent immunosuppressive therapy have been shown to be risk factors for invasive aspergillosis after renal transplantation (106, 231, 237). The mortality rate in renal transplant recipients with invasive aspergillosis has ranged from 75 to 80% (88, 237, 243).

PATHOPHYSIOLOGIC BASIS OF INFECTION

Host Response against *Aspergillus*

Immunity against *Aspergillus* is mediated by two types of host responses, innate and adaptive (164, 199, 216, 261, 262, 274).

The innate immunity is comprised of pulmonary alveolar macrophages that ingest and kill the inhaled conidia, primarily by nonoxidative mechanisms, and of circulating polymorphonuclear and mononuclear cells that mediate hyphal damage, mainly by oxidative killing (164, 261, 328). Defects in phagocytic function, neutropenia, and corticosteroid therapy have long been recognized as significant risk factors for invasive aspergillosis (55, 169, 274). High cumulative doses of corticosteroids have been associated with a greater risk for invasive aspergillosis (18, 186). High-dose prednisone (0.5 to 1.0 mg/kg of body weight/day) for GVHD prophylaxis in allogeneic HSCT recipients conferred a sixfold-higher risk of infection than lower dosages (225). Corticosteroids also render antifungal agents less effective as therapeutic agents (152) and are associated with a poorer outcome in invasive aspergillosis (255).

A body of evidence now suggests that T-cell function and adaptive immunity characterized by a dysregulated production of T-helper (Th) cell cytokines play a pivotal role in the pathogenesis of invasive aspergillosis (49, 50, 54, 96, 113, 164, 169, 199, 262–264). T-helper $CD4^+$ cells are the major effector cells responsible for cell-mediated immune responses for the eradication of pathogens (233). Upon recognition of exogenous antigen presented by major histocompatibility complex class II molecules, T-helper cells differentiate into Th1 or Th2 cells, which are morphologically indistinguishable but differ in the pattern of cytokines they produce (233). Th1 cytokines induce a predominantly cell-mediated inflammatory response, whereas Th2 cytokines facilitate antibody production (164, 233).

Th1 responses, e.g., tumor necrosis factor alpha, gamma interferon gamma, interleukin-12 (IL-12), and IL-15, have been shown to confer protection against *Aspergillus*. These cytokines augment superoxide production and enhance the antifungal activity of polymorphonuclear and mononuclear phagocytes against *Aspergillus* species. In a mouse model of invasive pulmonary aspergillosis, exposure of immunocompetent animals to a sublethal inoculum of *Aspergillus* conidia led to the development of resistance to subsequent local and systemic infection (49). This protective Th1 response was characterized by antigen-specific $CD4^+$ T cells that produced gamma interferon and IL-2. Notably, adoptive transfer of *Aspergillus*-specific $CD4^+$ splenic T cells from these animals conferred protection in naive animals challenged with the *Aspergillus* conidia (233).

Th2 responses, e.g., IL-4 and IL-10 production, by comparison have been associated with disease progression, and their neutralization has been associated with an improvement in infection. Th2 cytokines impair the microbicidal activity and hyphal damage mediated by mononuclear cells. IL-10, which is typically a Th2 cytokine, may paradoxically enhance the phagocytic activity against *A. fumigatus* hyphae (262). However, it had no impact on the intracellular conidiocidal activity (262). The net result of Th2 regulatory cytokines, however, is an increased susceptibility to infection.

Signal transduction mediated by Toll-like receptors (TLRs) has been shown to play a key role in immunity against aspergillosis (220). Of 10 human TLRs identified to date, TLR2 and TLR4 are involved in the regulation of cytokines that are important in the pathogenesis of *A. fumigatus* (220). *Aspergillus* conidia stimulated both TLR2 and TLR4 to induce a Th1 cytokine response (220). Germination of hyphae led to the loss of TLR4-mediated signals; however, TLR2-dependent mechanisms remained intact, leading to stimulated production of IL-10 and ultimately a predominant Th2 response (220). Thus, the switch from a proinflammatory to an anti-inflammatory cytokine profile during germination of *Aspergillus* hyphae may represent an escape mechanism by which the fungus evades the host defense (220). These data also suggest that Toll-like receptor-mediated signaling pathways may represent a potential target for immunomodulatory interventions against *Aspergillus* (220).

T-cell responses may have an important role in the host defense against aspergillosis in nonneutropenic hematopoietic stem cell transplant recipients and organ transplant recipients in whom neutropenia is not a major risk factor for invasive aspergillosis. Allogeneic stem cell transplant recipients in the late posttransplant period (median, 134 days) were shown to have suppressed *Aspergillus*-specific T-cell reconstitution (113). These patients also had a low gamma interferon/IL-10 ratio, which is suggestive of a Th2 response that may potentially account for their protracted susceptibility to the development of invasive aspergillosis. By comparison, healthy individuals demonstrated a predominantly Th1-type cellular response.

Finally, dendritic cells pulsed with *Aspergillus* conidia or transfected with conidial RNA can induce Th1-cell priming (33). Upon adoptive transfer, such cells were protective against invasive aspergillosis in mice receiving allogeneic hematopoietic stem cell transplantation (33). Thus, dendritic cells could potentially be employed as one of the vaccine strategies against invasive aspergillosis (33, 302).

Biologic Basis by Which Risk Factors Confer Susceptibility

Corticosteroids suppress several polymorphonuclear functions, e.g., degranulation, oxidative burst, and chemotaxis. Monocyte/macrophage function, e.g., maturation, superoxide production, and migration, are also impaired. A higher risk for *Aspergillus* infections portended by a number of well-recognized risk factors in transplant recipients may also be mediated by their effects on T-cell function. OKT3, an anti-CD3 monoclonal antibody, has been shown to be an independent risk factor for invasive aspergillosis in liver transplant recipients (160). Another risk factor for invasive aspergillosis after liver transplantation is renal dysfunction (85, 287). Renal failure and the initiation of hemodialysis have been shown to impair T-cell proliferative responses and to result in an increase in activation-induced T-cell death (12). A heightened susceptibility of transplant recipients with cytomegalovirus infection to opportunistic mycoses, including invasive aspergillosis, has been reported (91). CMV per se is an immunosuppressive virus, particularly for cell-mediated immune responses (29, 279). Another possible mechanism may be through the effect of CMV on macrophage function (166). Finally, corticosteroids, apart from their effect on neutrophils and monocytes/macrophages, cause Th1/Th2 dysregulation by enhancing the Th2 cytokine response (169). Their use has been associated with a decreased production of IL-2, IL-12, tumor necrosis factor, and gamma interferon and an increase of that of IL-4 and IL-10 (169).

Taken together, these data suggest that approaches targeted towards augmenting cellular immunity, such as neutralization of suppressive cytokines, enhancement of Th1 responses, and

TABLE 3. Performance characteristics of *Aspergillus* galactomannan enzyme immunoassay in selected studies with transplant recipients

Reference	Cutoff value	Type of patients	No. of patients	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	False-positive rate (%)	Days by which test preceded presentation
35	1.0	Hematology and HSCT	41	81.8	95.2				
172	1.0	HSCT	22	60	82				−5 to +21[a]
176	1.0	Hematologic malignancy	191	91	91.5	88.2	93.5	14	Mean, 5 (1–27)[b]
196	1.0	HSCT	67	54.2	99.6				−4.5 to +0.6
	0.5	HSCT	67	83.3	97				
308	1.5	Hematology and HSCT	807	90.6	94			6.3	Mean, 8.4 before radiologic and 6.9 before clinical signs
175	1.0	Hematology and HSCT	100	94.4	98.8	94.4	98.8		Mean, 6 (1–12)[c]
239	1.5	Hematology and HSCT	728	29.4	94.8	57.7	84.9	5	2 (in 2 of 153 patients only)
	1.0	Hematology and HSCT	728	35.1	98.5	87.0	84.4		
83	1.0	Liver transplant	33	55.6	93.9	71.4	88.6		
162	0.5	Liver transplant	154	NA[d]	98.5	NA	NA	13	NA
126	0.5	Lung transplant	70	30	93			20	3 (in 1 of 12 patients)

[a] Clinical signs.
[b] Radiographic signs.
[c] Number of days between positive test and clinical and radiographic signs.
[d] NA, not available.

transfer of adoptive cellular immunotherapy, warrant future investigations as therapeutic modalities for *Aspergillus* infections.

DIAGNOSIS

A substantial delay in establishing an early diagnosis remains a major impediment to the successful treatment of invasive aspergillosis. Cultures of the respiratory tract secretions lack sensitivity. *Aspergillus* is grown from sputum specimens in only 8 to 34%, and from BAL specimens in 45 to 62%, of patients with invasive aspergillosis (237). Thus, confirmation of the diagnosis of invasive aspergillosis has typically required histopathologic evaluation. However, profound neutropenia and thrombocytopenia often preclude the pursuit of biopsies for acquisition of tissue. Characteristic radiographic findings on high-resolution imaging studies e.g., the halo sign, have allowed earlier diagnosis of infection in neutropenic patients. The halo sign, however, is documented in 33 to 60% of patients and is short-lived (41). To be useful for the diagnosis of invasive aspergillosis, the computed tomography scan must be performed within 5 days of the onset of infection, since ~75% of the initial halo signs disappear within a week (41). The "air crescent" sign does not appear until the third week of the illness, and its appearance may be too delayed to be helpful in the diagnosis of invasive aspergillosis (41).

In the recent years, efforts have been directed towards identifying noninvasive markers for rapid and reliable diagnosis of invasive aspergillosis. Those based on the detection of antifungal antibodies have proven to be unreliable in transplant recipients receiving immunosuppressive agents (51). Instead, tests based on identifying fungal antigens or metabolites released into the circulation are potentially promising.

Diagnostic Laboratory Technology

***Aspergillus* galactomannan.** Galactomannan is a polysaccharide cell wall component of *Aspergillus* species that is released into the circulation during fungal growth in the tissues (177, 307). The *A. fumigatus* galactomannan is comprised of a linear mannan core with α-(1,2)-linked mannotetraose units attached with an α-(1,6) linkage (165). The side chains, consisting of an average of 4 to 5 β-(1,5)-galactofuranose units, are linked to the C-6 and C-3 positions of α-(1,2)-linked mannose units of the mannan core (165). Galactomannan is widely distributed among the *Aspergillus* and *Penicillium* species. Subtle chemical differences exist; however, the galactomannan of *Aspergillus* has striking structural similarity to that of *Penicillium*.

Although galactomannan testing using latex agglutination has been available for over a decade, a high detection threshold for galactomannan with this assay has precluded its use as a diagnostic test for invasive aspergillosis (307). The galactomannan concentrations detectable by latex agglutination, radioimmunoassay, and enzyme-linked immunosorbent assay inhibition are 15, 10, and 4 to 5 ng/ml, respectively. The double-sandwich enzyme-linked immunosorbent assay, on the other hand, can detect galactomannan at concentrations of as low as 0.5 ng/ml and has proven to be a potentially promising tool for the early diagnosis of *Aspergillus* infection (307).

(i) Assay performance with various patient populations. Studies evaluating the role of galactomannan assay in the diagnosis of invasive aspergillosis have largely been conducted with patients undergoing cancer chemotherapy or HSCT recipients (Table 3). In these patients a sensitivity of 67 to 100% and a specificity of 86 to 98.8% has been documented (35, 172, 176, 177, 239, 260, 318, 325). When serially monitored, the

galactomannan test preceded the diagnosis of invasive aspergillosis by an average of 6 to 14 days (Table 3). The sensitivity of the test typically has been lower in nonneutropenic patients (15 to 30%) and may be related to lower circulating galactomannan levels, since the ability to clear the fungal mannan from the bloodstream by macrophage mannosyl receptors remains unimpaired in patients without granulocytopenia. Galactomannan testing by enzyme immunoassay (EIA) has also proven to be useful with specimens other than serum e.g., cerebrospinal fluid and BAL fluid. In comparison to that with serum, the galactomannan test with BAL fluid had a higher sensitivity in nonneutropenic (90 versus 38%) as well as neutropenic (85 versus 47%) patients. Urine, on the other hand, appears to be less appropriate than serum for testing galactomannan, given a lower specificity and later diagnosis obtained with urine samples (307).

The performance characteristics of the galactomannan test are less well studied in organ transplant recipients. In liver transplant recipients for whom archived sera were tested, the sensitivity of the test was 55.6% and the specificity was 93.9% (83). A prospective study with 154 liver transplant recipients documented a specificity of 98.5% (162). In lung transplant recipients, the galactomannan test had a specificity of 95% but a relatively low sensitivity (30%) for the diagnosis of invasive aspergillosis (126). Although the test was able to detect the only case of systemic invasive aspergillosis and 29% of the cases of pulmonary invasive aspergillosis, it detected none of the cases of *Aspergillus* tracheobronchitis, a locally invasive form of invasive aspergillosis in lung transplant recipients. Notably, however, colonization without invasive disease was not associated with a positive test (126). Thus, while a positive test in a lung transplant recipient with a clinical illness compatible with invasive aspergillosis may be considered highly suggestive of this infection, a negative test does not rule out the diagnosis of invasive *Aspergillus* infection.

(ii) Other performance characteristics. The use of antifungal agents may lower galactomannan levels by decreasing the fungal load. In animal models of invasive aspergillosis in patients receiving cancer chemotherapy or undergoing HSCT, and in lung transplant recipients, lower galactomannan index values have been noted and may account for false-negative tests (35, 86, 90, 126, 239, 244, 330). The sensitivity of the test was 20% among neutropenic patients receiving antifungal prophylaxis. In lung transplant recipients, 44% of patients with false-negative tests had received an antifungal agent.

False-positive tests have been documented in up to 5 to 14% of patients with hematologic malignancy and HSCT, 13% of liver transplant recipients, and 20% of lung transplant recipients (44, 126, 162, 176, 177, 260). Cytotoxic chemotherapeutic agents and autoreactive antibodies e.g., in chronic graft-versus-host disease, have been shown to cause false-positive tests in patients with hematologic malignancies and in HSCT recipients. Liver transplant recipients undergoing transplantation for autoimmune liver disease and those requiring dialysis were significantly more likely to have false-positive galactomannan tests (162). Galactomannan is renally cleared, with excretion into the urine accounting for 35% of the dose by 24 h in an animal model study (22). However, the effect of renal failure or dialysis on the clearance of galactomannan is not known.

In a report on lung transplant recipients, false reactivity with the *Aspergillus* EIA was documented in 20% (14 of 70) of the patients (126). False-positive tests occurred within 3 days of lung transplantation in 43%, within 7 days in 64%, and within 14 days in 79% of the patients (126). Patients undergoing lung transplantation for cystic fibrosis and chronic obstructive pulmonary disease were more likely to have positive tests in the early posttransplant period (126). Cross-reactivity of galactomannan antibody with the lipoteichoic acid of *Bifidobacterium bifidum* subsp. *pennsylvanicum*, which is found in large inocula in the guts of breast- and formula-fed infants, may account for high false positivity of the test in premature infants and neonates (201).

Cross-reactivity of Platelia *Aspergillus* galactomannan EIA with *Penicillium* spp. has been noted (310) but is deemed to be of little clinical relevance since *Penicillium* spp. are rarely pathogens in humans. Ansorg et al., however, first documented that drugs of fungal origin such as antibiotics may be associated with a false-positive test; galactomannan was detected in a batch of ampicillin-sulbactam and in two batches of piperacillin (13). At least three recent reports from Europe have documented false EIA reactivity related to the administration of piperacillin-tazobactam (2, 309, 326). Over a 10-week period at one institution, receipt of piperacillin-tazobactam was documented in 67.5% (25 of 37) of patients with positive tests, compared to 5.4% (2 of 37) of those with negative tests ($P < 0.001$) (309). In another report, of samples from patients receiving piperacillin-tazobactam, 74% tested positive, compared to 11% of those not receiving this agent (326). Twelve of 15 batches of piperacillin-tazobactam tested yielded a positive test, with a median index value of 4.6 (326). Three of four batches of piperacillin-tazobactam were shown to test positive, with estimated amounts of 10, 2, and 30 μg of the galactomannan antigen per 4-g dose of piperacillin-tazobactam in each of the batches (2).

The reactivities of commonly used antibiotics of fungal origin (penicillins and cephalosporins), nonfungal origin (erythromycin and gentamicin), and synthetic origin (quinolones) with the Platelia *Aspergillus* galactomannan assay were assessed in one study (294). Undiluted samples of piperacillin-tazobactam and piperacillin tested positive, whereas those of amoxicillin, ampicillin-sulbactam, cefazolin, ceftazidime, erythromycin, gentamicin, and levofloxacin tested negative. All lots ($n = 3$) of piperacillin-tazobactam and all bags within each lot tested positive with an index value of greater than 5.168. At achievable concentrations in serum, however, only one of three lots of piperacillin-tazobactam yielded a positive test; concentrations of 75, 150, and 300 μg/ml tested positive, whereas lower concentrations, mimicking the trough levels (5 to 10 μg/ml) tested negative for the galactomannan. Thus, achievable concentrations of piperacillin-tazobactam in serum may potentially result in a false-positive test for galactomannan (294, 309, 326). The timing of collection of the sample may influence the test results, with reactivity being less likely in samples collected at trough levels or prior to the administration of the dose (294).

1,3-β-D-Glucan. 1,3-β-D-Glucan is an integral component the cell walls of a number of pathogenic yeasts and filamentous fungi. A colorimetric assay for its detection has been developed based on the principle that factor G, a coagulation factor for the horseshoe crab, is a sensitive natural detector of this

antigen (202). The results of the assay are expressed in picograms per milliliter. The 1,3-β-D-glucan assay does not detect cryptococcosis, oropharyngeal candidiasis, or fungal colonization. However, in addition to aspergillosis and candidiasis, it detects infections caused by less common fungi, e.g., *Fusarium*, *Trichosporum*, *Acremonium*, and *Saccharomyces*. The sensitivity and specificity of the tests in studies thus far have ranged from 67 to 100% and 84 to 100%, respectively (202, 222, 223, 343). Preliminary data from a multicenter study documented that 8 of 10 patients with invasive aspergillosis were positive at a cutoff of 60 as well as 80 pg/ml with the Glucatell assay for the detection of 1,3-β-D-glucan (229). False-positive tests have been reported in patients undergoing hemodialysis, patients with cirrhosis, recipients of antitumor polysaccharides, and patients immediately following abdominal surgery. An assay that utilized turbidometric readings for the detection of 1,3-β-D-glucan, the Fungitec G test for the diagnosis of systemic mycoses, was shown to have lower sensitivity than the colorimetric assay (122).

***Aspergillus* DNA detection by PCR assays.** PCR-based molecular diagnostic tests for *Aspergillus* are not commercially available and remain largely unstandardized. Such assays, when performed on blood or BAL samples, have shown a negative predictive value for invasive aspergillosis ranging from 92 to 99%. Thus, a negative test would suggest a low likelihood of invasive aspergillosis. However, PCR-based assays performed with BAL samples have shown low positive predictive values that likely reflect the fact that *Aspergillus* may transiently colonize the respiratory tract; ~25% of the BAL samples from healthy subjects were positive for *Aspergillus* by PCR (20). Serum- or plasma-based PCR assays have shown improved specificity without loss of sensitivity. Compared to culture for *A. fumigatus*, PCR was 19.4 times more sensitive (170). A sensitivity of 79 to 100% and a specificity of 81.3 to 93% have been documented, depending on the methodology used (38, 113, 138).

With neutropenic patients at high risk for invasive aspergillosis, the German *Aspergillus* PCR study group documented sensitivity and specificity rates of 63.5 and 93.5%, respectively, with nested PCR and of 14.3 and 98.9%, respectively, with galactomannan for the diagnosis of invasive aspergillosis (39). Quantifying nested PCR results with light cycler-mediated PCR assay did not add to the diagnostic utility of the test (39). When assessed simultaneously with a cohort of patients with leukemia and those undergoing bone marrow transplantation, the sensitivities of PCR, galactomannan assay, and 1,3-β-D-glucan measurement for the detection of invasive aspergillosis were 79, 58, and 67%, respectively, and the specificities were 92, 97, and 84%, respectively (138). Other studies have reported PCR to be less sensitive than the glactomannan assay for the diagnosis of invasive aspergillosis (35, 60). PCR results were usually positive when the galactomannan sample was highly positive; 12 of 20 PCR assays that yielded a positive result were observed in association with galactomannan titers of >5 ng/ml (35). A prospective comparison of real-time PCR, galactomannan, and 1,3-β-D-glucan assays as weekly screening for invasive aspergillosis in patients with hematologic disorders showed that the galactomannan test was most sensitive at predicting the diagnosis (142).

Although the conventional immunoassays and PCR clearly represent an advance compared to the detection of *Aspergillus* by culture, they largely lack optimal sensitivity, specificity, and the speed for the rapid diagnosis of infection. Rider et al. have proposed a novel pathogen sensor system for the identification of specific microorganisms that is based on the rationale that B lymphocytes as mediators of the adaptive immune system have evolved to efficiently identify pathogens (256). A biosensor comprising B-cell lines genetically engineered to express membrane-bound antibodies specific for the pathogen emits light within seconds of exposure to the targeted pathogen. A notable attribute of this pathogen identification system is that it can be tailored to detect a specific species of the microorganism (254, 256). Although this technology has not yet been applied for mycologic diagnosis, it is particularly relevant for the detection of *Aspergillus*, where timely and accurate diagnosis is critically important for the prompt initiation of appropriate therapy.

MANAGEMENT

In Vitro Susceptibility to Antifungal Agents

The interpretation of in vitro susceptibility data for *Aspergillus* species has long been difficult because much data have been based upon nonstandardized testing methods. The methods for testing susceptibility, morphological variations in the fungus, differences in growth rates and optimal growth conditions may all influence determination of the susceptibility of the fungus to antifungal drugs. In view of this, the National Committee for Clinical Laboratory Standards (NCCLS) has proposed a reference method for broth dilution antifungal susceptibility testing of conidium-forming fungi (219). E-test methods have also been utilized (284). However, evaluation of echinocandin susceptibility by using broth dilution testing is difficult, because echinocandin-exposed *Aspergillus* isolates demonstrate growth in every well, making end points of growth and MICs difficult to determine (15). Alternative methods to assess the susceptibility of *Aspergillus* isolates to echinocandins have been proposed (131, 159). One of these is assessment of the minimal effective concentration, i.e., the lowest echinocandin concentration at which the fungi display microscopic morphological changes (159). Imhof and colleagues (131) have developed two additional methods: an agar dilution technique and a method to measure fungal burden by quantifying secretion of galactomannan. It remains to be seen whether these tests can or should be implemented in clinical practice.

Fluconazole is essentially ineffective against *Aspergillus* spp. Amphotericin, the newer azoles, and the echinocandins are generally active against *Aspergillus* spp. (Table 4). However, resistance of *Aspergillus* isolates to amphotericin, itraconazole, voriconazole, and caspofungin has either been observed in isolates from patients or been created in the laboratory (71, 74, 81, 131, 180), It has been suggested that in vitro susceptibility testing of *Aspergillus* spp. is a predictor of clinical outcome in invasive aspergillosis (163). This statement was made on the basis of the finding that nine patients infected with amphotericin resistant-*A. terreus* but treated with amphotericin died (163). Itraconazole resistance in *A. fumigatus* has been correlated with clinical failure in both humans and experimental animal models of infection (71). However, clinical failure in invasive aspergillosis may have many causes other than drug resistance; furthermore, in patients who have died from inva-

TABLE 4. In vitro susceptibility of *A. fumigatus* to antifungal agents[a]

Drug	MIC (μg/ml)		
	50%	90%	Range
Fluconazole	>64	>64	>64
Miconazole	4	16	1–>64
Itraconazole	1	2	0.12–2
Ravuconazole	0.5	0.5	0.25–4
Voriconazole	0.25	0.5	0.12–4
Posaconazole	0.25	0.5	0.03–1
Caspofungin	0.06	0.12	0.06–0.12
Anidulafungin	0.03	0.06	0.004–0.12
Micafungin	≤0.03	≤0.03	
Nystatin	8	16	0.5–>64
Liposomal nystatin	2	2	
Flucytosine	>100	>100	0.5–>64
Rifampin	>1,000	>1,000	>1,000
Terbinafine	>16	>16	4–>16

[a] Data are collated from references 70, 207, and 248.

sive aspergillosis despite amphotericin treatment, emergence of resistance to amphotericin or itraconazole during treatment has not been detected (65, 207). In a review of 11 patients with hematologic malignancies in whom *A. fumigatus* or *A. flavus* was isolated, while on amphotericin, resistance to amphotericin was not detected, but it was thought that poor drug penetration to the site of infection may have been a contributor to failure (238).

At the present time, antifungal susceptibility testing for *Aspergillus* could not be recommended for routine clinical microbiology laboratories in hospitals which have transplant units. However, species identification of *Aspergillus* isolates is indicated; *A. terreus* is usually not susceptible to amphotericin, and *A. niger* is rarely a cause of invasive infections in transplant recipients.

In Vitro and Animal Studies Utilizing Combinations of Antifungal Agents

Amphotericin and azoles. Potentially, one of the most exciting developments in therapy of fungal infections is the opportunity for combination therapy. However, for many years, there have been concerns over the potential risk of antagonism, especially when an azole is administered first with sequential administration of amphotericin. Some of these concerns stem from experimental studies in which ketoconazole has been shown to be antagonistic to amphotericin B in animal models of disseminated aspergillosis (217, 249, 250, 275). Combinations of amphotericin and azoles have had conflicting results when tested in vitro, but the drugs do not appear to reliably add significantly to each other's activity against *A. fumigatus* (Table 5). Amphotericin and itraconazole were synergistic for 14 to 40% of strains tested, additive for 20 to 26%, but antagonistic for 26% of 15 strains in one series (178) and for none of five strains in another series (70). In one isolate from a patient in whom the combination of amphotericin and itraconazole failed, antagonism between amphotericin and itraconazole was demonstrated (273). In a study using E tests as the testing method for assessment of drug combinations, amphotericin and itraconazole were antagonistic, especially when the amphotericin strip was applied after the itraconazole strip (154). This negative interaction is presumed to be secondary to subtle alterations of the sterol composition of the fungal membrane following exposure of the fungus to the azole (154). Voriconazole and amphotericin appear to be an indifferent combination (181). Indifference or antagonism occurs when two azoles are used together (179, 211).

Amphotericin and flucytosine, rifampin, or terbinafine. Combinations of amphotericin and flucytosine, although additive or synergistic in some studies, are more likely to be an indifferent combination in vitro (70, 224). Antagonism has been reported for 23% (6 of 26) strains in one study (70). Combinations of amphotericin and rifampin or rifabutin are frequently synergistic (70, 123, 224). Unfortunately, rifampin is a difficult agent to use in transplant recipients because of its frequent interactions with cyclosporine or tacrolimus. However, addition of rifampin consistently lowers the MICs of amphotericin B by 2- to 10-fold (123). In 92% (36 of 39) *Aspergillus* strains from one study, amphotericin and rifampin were synergistic (70). A single animal model has examined the combination of amphotericin B and rifampin (17), and synergy was reported in the instance studied. Terbinafine and itraconazole were synergistic for all four strains tested in one small study (268) and were additive or synergistic for all nine strains in another study (211). The combination of terbinafine and amphotericin B was synergistic for one of four and indifferent for three of four strains tested in one study (268) and was mostly indifferent or antagonistic in another (211). The synergistic interaction of amphotericin B with terbinafine may vary for different *Aspergillus* species. Antagonism was observed for some strains of *A. terreus*, *A. flavus*, and *A. niger* but not *A. fumigatus* when amphotericin B was combined with terbinafine (63).

Amphotericin and echinocandins. Significant interest has been generated by the potential usefulness of echinocandins in combination with other antifungal classes in the therapy of invasive aspergillosis. The combination of caspofungin and amphotericin B with the checkerboard method was synergistic or synergistic to additive for at least half of the isolates (16). Antagonism was not observed for any of the isolates tested (16). Micafungin and liposomal amphotericin have been investigated in combination in a murine model (95), and while no antagonism was observed, additive effects were seen only in reduction of tissue burden under limited experimental conditions. The survival of mice with invasive pulmonary aspergil-

TABLE 5. Combination therapy and invasive aspergillosis

Combination	In vitro results	Clinical experience
Amphotericin plus azole	Rarely synergistic; sometimes antagonistic	No advantage over monotherapy yet demonstrated
Amphotericin plus echinocandin	Sometimes synergistic	Successful in 35 to 60% of patients, no comparative trials
Echinocandin plus azole	Frequently synergistic	Only case reports and a cohort study documenting success

losis was significantly improved when micafungin was combined with amphotericin than when either micafungin or amphotericin was administered alone (148). On pathological examination, hyphal growth could still be observed at day 6 when monotherapy was used, whereas no hyphal growth was observed when combination therapy was used (217). A study of experimental pulmonary aspergillosis in rabbits showed that survival was achieved in 55% (5 of 9) of the rabbits treated with a combination of micafungin and conventional amphotericin, in 44% (4 of 9) treated with a combination of micafungin and liposomal amphotericin, in 33% (3 of 9) treated with conventional amphotericin alone, in 22% (2 of 9) treated with liposomal amphotericin alone, and in 0% (0 of 20) of the untreated controls (246). No statistically significant differences in outcome could therefore be documented for the combination therapy compared to monotherapy, but the number of mice tested was small.

Echinocandins and azoles. Simultaneous inhibition of fungal cell wall and cell membrane biosynthesis could be hypothesized to result in a synergistic interaction between echinocandins and azoles against *Aspergillus*. In vitro, synergy has been observed between echinocandins and azoles for most (180, 240, 285), but not all (180), combinations assessed. Caspofungin has been combined with voriconazole in a guinea pig model of invasive aspergillosis (145, 240). No mortality occurred among 12 animals treated with both caspofungin and voriconazole, whereas 4 of 12 and 6 of 12 animals treated with caspofungin at 1 and 2.5 mg/kg/day, respectively, died. No animal treated with voriconazole alone died. With the checkerboard method, the combination of caspofungin and voriconazole exhibited a synergistic effect against itraconazole-resistant strains of *A. fumigatus* (63). Posaconazole combined with caspofungin was synergistic against 26.3% of the isolates and additive against 70.2% when tested in vitro (269). No antagonism was observed. Greater efficacy was also demonstrated in an incompetent mouse model (269). When micafungin was combined with ravuconazole in an experimental neutropenic rabbit model of invasive aspergillosis, the combination led to a significant reductions in mortality, residual fungal burden, and serum galactomannan antigenemia compared with either agent alone (245). Combination therapy also resulted in a statistically significant reduction in organism-mediated pulmonary injury and in pulmonary infiltrates assessed by computed tomography (245). Another variation in the concept of combination therapy is sequential therapy. Previous exposure to intraconazole resulted in enhanced effects of caspofungin and vice versa (153).

Therapy of Invasive Aspergillosis

Overview. An optimal approach to the treatment of invasive aspergillosis has yet to be determined, but the availability of new azoles and echinocandins may well revolutionize therapy within the next 5 years. The possibility of combination therapy has opened the way for particularly attractive investigations. Timely initiation of treatment by way of early diagnosis must always play an important role, as do reduction of immunosuppression and recovery from neutropenia. Local lesions should be resected if possible. Therapy should be continued for 10 to 12 weeks or for at least 4 to 6 weeks beyond the resolution of all clinical and radiographic abnormalities, whichever is longer (289). The discussion below concentrates on treatment recommendations for invasive pulmonary aspergillosis but applies, with some modifications, to treatment of other invasive forms of the infection. An additional reference on the topic is "Practice Guidelines for Diseases Caused by *Aspergillus*" from the Infectious Diseases Society of America, published in 2000 (303).

Although amphotericin preparations have been the drugs of choice for invasive aspergillosis for many years, a recent randomized trial (116) comparing amphotericin with voriconazole appears to have placed voriconazole as the treatment of choice for invasive aspergillosis. However, it must be recognized that the clinical appearance of invasive aspergillosis may be mimicked by mucormycosis and some other fungal infections. Voriconazole lacks activity against the zygomycetes, so amphotericin should remain the drug of choice if microbiologic confirmation of *Aspergillus* infection is lacking.

The design of the study comparing voriconazole with amphotericin was as follows. The study was an open, randomized comparison of voriconazole and conventional amphotericin (116). Patients received conventional amphotericin (1 mg/kg/day) or voriconazole (6 mg/kg intravenously for two doses and then 4 mg/kg every 12 h intravenously, which could be followed by 200 mg every 12 h orally). Patients could be switched to other licensed antifungal therapy (for example, lipid preparations of amphotericin or itraconazole) after their initial randomized therapy. A total of 392 patients were enrolled over 3 years in 92 medical centers in 19 countries. Of these, 277 patients had confirmed invasive aspergillosis and received at least one dose of the study drug; 144 of these patients received voriconazole and 133 received conventional amphotericin. Approximately 80% of the patients had a hematologic malignancy or had undergone allogeneic bone marrow transplantation. After 12 weeks of receiving the first dose of study drug, a complete or partial response was seen in 52.8% of those who had received voriconazole and in 31.6% of those who had received amphotericin. These differences were statistically significant. Survival of patients at 12 weeks was 70.8% for those who had received voriconazole versus 57.9% for those who had received amphotericin. Again this difference was statistically significant (hazard ratio, 0.59; 95% CI, 0.40 to 0.88).

Unanswered questions include whether voriconazole versus lipid preparations of amphotericin from the outset of therapy would have provided the same results. Three lipid preparations of amphotericin are now marketed for clinical use: amphotericin liposome for injection (AmBisome; Fujisawa, Deerfield, Ill.), amphotericin colloidal dispersion (Amphotec; Sequus, Menlo Park, Calif.), and amphotericin B lipid complex (Abelcet; The Lipsome Company, Princeton, N.J.). There are conflicting data as to which lipid preparation should be preferred for treatment of invasive aspergillosis. However, a dose of 5 mg/kg/day is currently accepted as appropriate for all of these drugs in the initial treatment of invasive aspergillosis.

A single-center, retrospective study that compared the outcomes for 41 liver transplant recipients who received either conventional amphotericin or amphotericin B lipid complex documented that the 60-day mortality rate was 83% in the patients treated with conventional amphotericin versus 33% in the patients treated with amphotericin B lipid complex (168). Although this was not a randomized analysis, in multivariate

analysis amphotericin B lipid complex therapy was an independent predictor of survival (168). In a randomized, double-blind, controlled trial for the treatment of invasive aspergillosis in immunocompromised patients, of whom 42% were HSCT and 5% were organ transplant recipients, amphotericin B colloidal dispersion was shown to have an efficacy equivalent to that of amphotericin B (32).

For *A. terreus*, voriconazole compared to other antifungal therapies was associated with reduced mortality at 12 weeks (300) and may be a better therapeutic option than a polyene.

Further clinical experience with voriconazole and posaconazole. In a number of small analyses of the use of voriconazole in transplant recipients, the outcome has been similar to that observed in the randomized trial described above (19, 72, 173, 194). In a large series of patients with central nervous system aspergillosis treated with voriconazole, the survival rate was 15% for those with hematologic malignancy, 21% for the HSCT recipients, and 33 to 67% for others, which included organ transplant recipients (317).

Two practical issues arise when utilizing voriconazole in the management of transplant recipients. The first is that there is a significant interaction between voriconazole and cyclosporine, tacrolimus, or sirolimus (265, 323). In human liver microsomes, voriconazole at a concentration of 4 μg/ml inhibited the metabolism of tacrolimus by 50% (323). Indeed, all azole antifungal agents have the potential to increase the levels of cyclosporine, tacrolimus, and sirolimus via inhibition of cytochrome P450 isoenzymes (323). The rank order of potency of the azoles for the inhibition of P450 isoenzymes is ketoconazole > voriconazole > itraconazole > fluconazole (322). The second issue is that the use of intravenous voriconazole is contraindicated in patients with creatinine clearance of less than 50 ml/min. This is because of concerns regarding accumulation of voriconazole's renally excreted carrier, sulfobutyl ether β-cyclodextrin sodium. The consequences of accumulation of sulfobutyl ether β-cyclodextrin sodium in humans are not known. Oral administration of voriconazole in patients with moderate to severe renal dysfunction is safe.

Posaconazole is a new triazole compound that exhibits significant in vitro activity against a number of fungi, including *Aspergillus* (61, 62). The drug is currently in advanced stages of clinical development. It is orally bioavailable and exhibits dose-proportional pharmacokinetics up to a total dose of 800 mg/day; i.e., saturation of absorption occurs at doses of above 800 mg (61). Food increases the relative oral bioavailability of posaconazole by 400% (62). The drug is metabolized by glucoronidation, with only minor amounts of the unchanged drug excreted in the urine (158). Posaconazole inhibits CYP3A4 but not cytochrome P450 enzymes. Since cyclosporine and tacrolimus are substrates for CYP3A4 as well, coadministration of posaconazole with tacrolimus decreased tacrolimus clearance by ~5-fold (272). Concomitant administration of cyclosporine with posaconazole necessitated a 14 to 29% reduction in cyclosporine dosage (62).

Posaconazole was used in the treatment of 25 patients with invasive aspergillosis refractory to conventional therapies (108). Of 15 patients who were still alive 4 weeks after use of the drug had commenced, 53% had a positive clinical response (108). The dose used was 200 mg four times per day as an oral suspension and 400 mg twice per day when the patient was discharged from hospital (108). Of note is that posaconazole, unlike other azoles, has promising activity against zygomycosis (99). In patients with proven or probable zygomycosis who received posaconazole for refractory infection or intolerance to standard therapies, a successful outcome was documented in 70% (99).

Clinical experience with the echinocandins. Caspofungin is licensed for use in the United States and most of Europe, and micafungin is commercially available in Japan (69). Anidulafungin is not yet commercially available. Caspofungin received U.S. Food and Drug Administration approval for the indication of refractory aspergillosis in January 2001. Caspofungin has been evaluated in a multicenter, noncomparative study of 90 patients who failed treatment with or were intolerant to conventional amphotericin, lipid preparations of amphotericin, or azoles (178). The majority of the patients had hematologic malignancies as their predominant underlying disease. Forty-five percent of the patients with pulmonary aspergillosis had a complete or partial response to therapy, compared to 20% of patients with disseminated infection (178). It should be noted, however, that it is difficult to arrive at firm conclusions regarding the efficacy of antifungal drugs from studies of their use as salvage therapy. A potential difficulty with the use of caspofungin in the empirical treatment of patients with suspected invasive aspergillosis is the possibility of unsuspected infection with an organism resistant to the drug. Caspofungin is not active against *Trichosporon* species or *Cryptococcus neoformans*. An allogeneic peripheral blood stem cell recipient who developed tenosynovitis due to *Trichosporon beigelii* while receiving caspofungin for a presumed fungal pneumonia has been reported (92).

Micafungin has been successfully utilized in Japan as salvage therapy for invasive aspergillosis in patients refractory to amphotericin, but the number of published reports are few (342). An open-label multicenter study of micafungin in the treatment of 42 patients with invasive aspergillosis in Japan has been performed (147). The clinical response rate was 60% (6 of 10) in invasive pulmonary aspergillosis, 66.7% (6 of 9) in chronic necrotizing pulmonary aspergillosis, and 54.5% (12 of 22) in pulmonary aspergilloma. Unfortunately, this information has appeared only in abstract form, and the definitions of each infection type are yet to be documented. Coadministration of caspofungin and cyclosporine has resulted in increased concentrations of caspofungin in plasma (a 35% increase in the area under the curve) but no change in the amount of cyclosporine in the blood (69, 270). The mechanism of this interaction is unclear, although it has been speculated that cyclosporine limits caspofungin uptake into the liver. Healthy volunteers who received both cyclosporine and caspofungin developed raised liver function tests (14). However, in a retrospective study of 40 patients treated during market use, significant elevations in transaminases were documented infrequently with the concomitant use of caspofungin and cyclosporine (184). Most patients with liver function enzyme elevations had other causes to account for these, and discontinuations of therapy because of hepatoxicity were uncommon (184). Coadministration of caspofungin with tacrolimus has resulted in slightly reduced tacrolimus levels, but the two can be safely used together as long as tacrolimus levels are frequently monitored (69, 268).

Clinical experience with the combination regimens. In a study of liver transplant recipients with invasive aspergillosis, 50% of patients treated with amphotericin B lipid complex received itraconazole concurrently, and 25% received itraconazole after completion of therapy with amphotericin B lipid complex (168). Multivariate analysis did not show benefit from the addition of itraconazole, but the number of patients with combination therapy was fewer than 10, and the power of this study to show an advantage of combination therapy is very likely lacking.

At least two studies have now described clinical experience with caspofungin in combination with liposomal amphotericin in management of invasive aspergillosis (4, 152). This combination was utilized in 30 leukemic patients who had inadequate responses to amphotericin alone (4). In 60% of these patients a favorable antifungal response was seen when combination therapy was utilized. It should be noted, however, that 20 of 30 patients in this study had possible invasive aspergillosis (4). In another report, the combination of caspofungin and liposomal amphotericin was utilized as salvage therapy in patients who had an inadequate response to liposomal amphotericin monotherapy or as primary therapy (152). The combination was more successful as a primary therapy than as salvage therapy, although the response rates were not statistically significant (53 versus 35%; $P = 0.36$).

In a sequential cohort of 47 patients with aspergillosis who received either voriconazole ($n = 31$) or a combination of voriconazole and caspofungin ($n = 16$) as salvage therapy, the combination was associated with a significantly improved 3-month survival (hazard ratio, 0.42; 95% CI, 0.17 to 1.1; $P = 0.048$) (185). Case reports have assessed the combination of caspofungin and voriconazole (64) or triple combinations with an amphotericin, azole, and an echinocandin (312). Caspofungin and itraconazole were used successfully in combination in the treatment of invasive pulmonary aspergillosis in one case of amphotericin-resistant *A. terreus* infection and in another case of *A. fumigatus* infection in a patient who developed renal impairment with amphotericin B lipid complex (267). We would prefer not to recommend the routine primary use of combination therapy until data from further clinical studies is available.

Therapy in specific situations. (i) *Aspergillus* tracheobronchitis. *Aspergillus* tracheobronchitis is most common in lung transplant recipients but has also been observed in other immunocompromised groups (patients with human immunodeficiency virus infection or hematologic malignancies) and rarely in immunocompetent patients. In lung transplant recipients, this entity may present as a spectrum of disease, with the most dangerous end of the spectrum being ulcerative tracheobronchial aspergillosis at the anastomosis site. The disease may also be a precursor to invasive pulmonary aspergillosis. Descriptions of treatment regimens have been in retrospective, nonrandomized series which evaluated only a small number of patients. A common, although unproven, practice is to combine systemic antifungal therapy (with intravenous amphotericin or voriconazole) and aerosolized amphotericin.

In the initial description of this entity, six patients were treated with itraconazole (200 mg three times a day for 4 days as a loading dose and then 200 mg twice a day) (157). The treatment duration was 4 to 6 months, during which time repeated bronchoscopic examinations were negative (157). Two of the six patients in that report died from invasive or disseminated aspergillosis; both had their course of itraconazole interrupted for a number of days. Other treatment alternatives which have been successfully used include combinations of intravenous amphotericin B and itraconazole (340), aersolized amphotericin B and oral itraconazole (333), and amphotericin B alone (25). Sequential liposomal amphotericin (1.5 to 3.5 mg/kg/day, to a mean total dose of 3.1 g) and then itraconazole (400 mg/day) have been successfully used with at least four patients (203). A single patient who was intolerant of amphotericin B, liposomal amphotericin, and itraconazole and who was successfully treated with terbinafine (250 mg every 12 h by mouth for 3 months) has been described (111). Finally, surgical resection and stent placement may be necessary in conjunction with antifungal therapy if dehiscence of the anastomosis occurs because of tracheobronchial aspergillosis.

(ii) Allergic bronchopulmonary aspergillosis. Allergic bronchopulmonary aspergillosis (ABPA) may sometimes recur following lung transplantation (82). For many years the only treatment available for ABPA has been steroids. However, a randomized, double-blind study of itraconazole (200 mg twice a day for 16 weeks and then 200 mg once a day for 16 weeks) or a placebo for steroid-dependent ABPA showed a significantly greater chance of a response occurring in patients given itraconazole (304); 46% of patients treated with itraconazole were able to achieve at least a 50% reduction in steroid dose, a 25% greater exercise tolerance, and a 25% reduction in serum immunoglobulin E levels. Itraconazole and corticosteroids should now be regarded as the standard of care for most patients with ABPA. Voriconazole has not yet been studied in the context of ABPA, and there is one report of the successful use of nebulized amphotericin in a lung transplant recipient (46).

(iii) Aspergilloma. Pulmonary aspergillomas result from fungal growth within a preexisting pulmonary cavity and have been observed following lung transplantation (333). Occasionally, during the resolution of invasive pulmonary aspergillosis, "fungus balls" are observed in the cavities cause by the initial invasive process. Unfortunately, although the infection is usually localized, erosion of a bronchial artery can lead to life-threatening hemoptysis. Systemic administration of antifungal agents is nearly always ineffective (141). Rare successes with oral itraconazole have been described, although those studies were retrospective and unblinded and did not contain a control group (303). Early surgical resection (lobectomy) is the treatment of choice in those patients with adequate lung function. However, because the surgery may be technically difficult and associated with significant morbidity and sometimes mortality and because the underlying lung disease which predisposed the patient to cavity formation is often severe, lobectomy is not a realistic option for some patients.

In patients for whom lobectomy is precluded, a surgical alternative is cavernostomy performed under local or regional anesthesia. Intracavitary instillation of antifungal agents, in combination with cavernostomy or as an alternative to surgery, has been well described, although it only occasionally cures aspergillomas. Symptomatic relief may be marked, however (339).

(iv) Infections of the sinuses. Sinus infection by *Aspergillus* takes a variety of forms, ranging in severity from fulminant

TABLE 6. Major manifestations of *Aspergillus* spp. in transplant recipients

Manifestation	Transplant group affected	Treatment modalities
Invasive pulmonary aspergillosis	All	Systemic voriconazole, caspofungin, or lipid formulations of amphotericin
Allergic bronchopulmonary aspergillosis	Lung transplant recipients	Itraconazole
Aspergilloma	Lung transplant recipients	Surgical resection
Sinusitis	HSCT and lung transplant recipients more than other solid-organ transplant recipients	Surgery plus systemic agent
Brain abscess	All	Systemic (voriconazole); surgery if possible
Endophthalmitis	All (rare)	Surgery, local amphotericin, systemic agent
Surgical wound infection	Solid organ transplant recipients	Surgery plus systemic agent
Vascular line site	HSCT recipients	Debridement plus systemic agent
Osteomyelitis	All (rare)	Surgery plus systemic agent
Endocarditis	All (rare)	Surgery plus systemic agent

acute invasive sinusitis to allergic sinusitis. Invasive sinusitis may be less common in solid organ transplant recipients than in bone marrow transplant recipients (Table 6). Allergic sinusitis due to *Aspergillus* may manifest as chronic, intractable sinusitis and nasal polyposis. There is no evidence at present that antifungal agents are useful in the management of this condition. The usual management involves endoscopic removal of polyps and inflammatory material followed by long-term intranasal corticosteroids and short-term systemic corticosteroids (75).

In contrast, fulminant invasive *Aspergillus* infection of the sinuses, particularly in neutropenic patients (including stem cell transplant recipients) has a high mortality. Survival in this condition is often determined by early diagnosis, recovery from neutropenia, and possibly the ability to aggressively debride devitalized tissue (75). Although a review in 1990 (73) showed that mortality was higher in those treated with a combination of medical and surgical therapies than in those treated with medical therapy alone, it is possible that the apparent increase in mortality following surgery reflected more severe disease in this group.

Chronic *Aspergillus* infection in the ethmoid sinus may result in bony erosion towards the orbit or the cavernous sinuses, particularly in patients on systemic corticosteroids or with diabetes mellitus. The condition has a poor prognosis and should be treated in a manner similar to that for acute invasive sinusitis.

(v) Infections of the central nervous system. *Aspergillus* infection of the central nervous system is usually manifest as cerebral space-occupying lesions in the context of disseminated disease in transplant recipients. Meningitis and spinal cord involvement are rare.

It is difficult to make conclusions as to the optimal management of cerebral aspergillosis other than to reiterate the role of reduction of immunosuppression and surgical drainage of lesions if possible. The clinical response to voriconazole has been extremely good (67, 72, 173, 182, 282, 324), and this drug may replace amphotericin as treatment of choice. Studies with guinea pigs have shown that voriconazole has excellent penetration into the cerebrospinal fluid and the brain (258). At steady state, drug levels in the central nervous system were double those found in plasma. A case report of successful salvage therapy with voriconazole and caspofungin in the management of cerebral aspergillosis exists (64).

If amphotericin is used, lipid preparations of amphotericin in high doses may be useful. Doses of 6 mg/kg/day (80) and higher (57) have been utilized. Long-term intracavitary administration of amphotericin B via an Ommaya reservoir has been utilized as an adjunct to radical debridement and the use of systemic antifungal therapy (43).

(vi) Endophthalmitis. *Aspergillus* has been documented as a cause of endophthalmitis, usually as part of a disseminated infection, in transplant recipients (276). *Aspergillus* endophthalmitis has a very poor prognosis as a result of delays in diagnosis and its frequent coexistence with disseminated disease. Therapeutic vitrectomy is usually essential. Although intravitreal injection of amphotericin B is said to be hazardous, as much as 5 to 10 mg has been safely injected into the center of the vitreous cavity (97). Most authors have used much smaller doses intravitreally (0.005 to 0.01 mg). Daily administration of subconjunctival amphotericin B (1 to 2 mg) can follow (266). The question of concomitant systemic antifungal therapy is vexed. Penetration of amphotericin into the eye is poor. After intravenous administration, levels of amphotericin B in the human aqueous humor remain less than 0.5 μg per ml. There are few data on penetration of lipid formulations of amphotericin B or echinocandins into the eye. Voriconazole has been successfully used as therapy for endophthalmitis due to other fungi (89), so it may be a potentially useful option.

(vii) Infections of skin and soft tissue. *Aspergillus* may manifest cutaneously as a sign of disseminated infection or may occur as a primary cutaneous infection, including of postsurgical wounds. Primary cutaneous aspergillosis may also be associated with central venous lines. *A. flavus* is more common than *A. fumigatus* in many of these manifestations. Occasionally, rare species such as *A. ustus* may be involved in primary cutaneous infections associated with immunosuppression.

Patients with cutaneous manifestations of documented systemic infections have a poor prognosis despite active therapy. An exception may be in patients with hematologic disorders in which neutropenia may be reversed. Successful treatment of primary cutaneous aspergillosis associated with neutropenia has been associated with reversal of neutropenia by use of granulocyte colony-stimulating factor (G-CSF) (115). Amphotericin preparations or voriconazole would be the preferred initial therapy. Koss et al. (155) described an elderly patient with acute myelogenous leukemia whose cutaneous infection with *A. flavus* failed to respond to amphotericin B lipid complex. Therapy was switched to caspofungin, with dramatic and sustained improvement.

Numerous reports have demonstrated that postsurgical wound infection requires aggressive surgical intervention in addition to antifungal drugs for successful resolution (45, 115). The surgery may be as aggressive as limb amputation or extensive debridement of the kind seen in cases of necrotizing fasciitis. Inadequate debridement can result in deep invasion into subcutaneous tissue, muscle and sometimes deep viscera. Systemic antifungal therapy should accompany surgery.

Cutaneous infection associated with long-standing central venous access devices (especially Hickman catheters) has been observed in neutropenic patients and is sometimes associated with the development of underlying pulmonary aspergillosis. Once *Aspergillus* infection is suspected, the catheter should be removed (7). In the largest series reported, nine patients were treated with intravenous amphotericin (0.75 to 1.25 mg/kg/day) plus 5-flucytosine (3 to 8 g/day), and seven of the nine survived (7). The two patients who died did not recover from their neutropenia. Those authors chose to defer wide debridement of the eschar until neutropenia had resolved (7). An alternative approach is immediate extensive chest wall debridement even while the patient is neutropenic.

(viii) Osteomyelitis. *Aspergillus* bone infection can be part of disseminated or local disease in immunocompromised patients (including transplant recipients, neutropenic patients, and children with chronic granulomatous disease). There appears to be an advantage of surgical therapy in the treatment of bony aspergillosis. Surgery may be important because amphotericin B achieves only low concentrations in bone and joint fluid. Furthermore, the pathology of aspergillosis involves infarction and necrosis, which result in poor drug delivery to tissues.

Although not well studied, it would not appear that lipid preparations of amphotericin enhance penetration of amphotericin into bone. However, an advantage of lipid preparations may be that their comparative lack of nephrotoxicity allows longer courses to be given, such as may be required for treatment of osteomyelitis. Both flucytosine and rifampin penetrate well into bone. Concentrations of itraconazole in bone are two- to threefold higher than those in plasma (118). The entry of voriconazole into bone has not yet been well studied, although there are case reports of success with voriconazole where other agents had failed (306, 311). Adjuncts to surgery and antifungal agents have included hyperbaric oxygen (156) and gamma interferon. Gamma interferon was used only in children with chronic granulomatous disease (133).

(ix) Infections of the heart and vascular system. *Aspergillus* has been reported to cause endocarditis, myocarditis, pericarditis, mediastinitis, septic thrombophlebitis, and infections of aortic grafts. Infection is associated with high mortality despite treatment. In addition, some cases are unsuspected during life and are discovered only at autopsy (236). *Aspergillus* endocarditis may occur as part of disseminated disease, as a complication of cardiac surgery, or rarely de novo (105, 247). Early surgical intervention with valve replacement is the cornerstone of successful management. Only a small number of patients who received medical therapy alone and survived have been reported (174, 252). Amphotericin penetrates poorly into cardiac vegetations but nevertheless should probably be used as adjunctive therapy. High doses (for example, 10 mg/kg/day) of lipid preparations of amphotericin B have been used (252). There is very little or no clinical data on the use of voriconazole, caspofungin, rifampin, or flucytosine in conjunction with amphotericin in this setting.

Adjunctive immunotherapy. A general principle of treatment of invasive aspergillosis in transplant recipients is that immunosuppression should be reduced substantially and preferably discontinued. Very few patients with persistent neutropenia and invasive aspergillosis survive; recovery of functional neutrophils is clearly important. There are anecdotal reports of utilization of granulocyte transfusions from G-CSF-stimulated donors during treatment of invasive aspergillosis (48). In one such study, three of four patients with invasive aspergillosis had a favorable response (76), and in another, five of nine patients responded (242). A randomized study comparing the use of aggressive antifungal therapy with or without granulocyte transfusions is needed to truly determine whether granulocyte therapy is of benefit.

Adjunctive immunotherapy comprising gamma interferon has been utilized. The most established indication for immunotherapy is in chronic granulomatous disease (133). In a randomized controlled trial, patients with chronic granulomatous disease were either given a placebo or gamma interferon (50 $\mu g/m^2$ of body surface area three times a week) as prophylaxis against infection. Four of 65 placebo-treated patients developed *Aspergillus* pneumonia, compared to 1 of 63 given gamma interferon (133). Recent experimental studies suggest that higher doses of gamma interferon than those used in the randomized controlled trial may further enhance protection against *A. fumigatus* (3). A small number of clinical reports have described the use of gamma interferon as an adjunct to antifungal therapy in children with *Aspergillus* osteomyelitis who were not previously given gamma interferon as prophylaxis (114, 146, 234). As far as we are aware, there are no published reports on the use of gamma interferon in transplant recipients. Given the potential role of gamma interferon in the pathophysiology of GVHD (5), the use of gamma interferon in this setting is of concern.

There is some experimental and limited clinical evidence suggesting that administration of G-CSF or granulocyte-macrophage colony-stimulating factor may be useful as immunotherapy against *Aspergillus* infections (301). There is no clear evidence that G-CSF results in improved outcomes; clinical data with granulocyte-macrophage colony-stimulating factor are from case reports and case series only (28, 31, 80). A dendritic cell vaccine against invasive aspergillosis is under development, but no clinical trials have yet been published (33).

Surgery for invasive aspergillosis in transplant recipients. A number of groups have strongly advocated surgery as an important part of the management of invasive pulmonary aspergillosis (24, 93, 107, 191, 271). Surgery was performed on 19 patients with invasive pulmonary aspergillosis (93). These included wedge resections in 7 patients and lobectomies in 12. All but one patient had surgery via a thoracoscopic approach. Nine of the patients were thrombocytopenic (platelet count of less than 60,000) on the day of surgery. There was no intraoperative mortality, and no patient died in the first 30 postoperative days. In another report, 18 patients underwent surgery for invasive pulmonary aspergillosis; surgical resection was a predictor of survival from invasive aspergillosis in this cohort (94).

INFECTION CONTROL MEASURES

Hospital construction (including indoor renovation) is a well-acknowledged risk factor for invasive aspergillosis. Vigorous attempts should be made to prevent airborne transmission of *Aspergillus* from the sites of construction to the location of immunosuppressed patients, by use of physical barriers. Additionally, air filtration systems through high-efficiency particulate air filtration (HEPA) filters for units such as those for bone marrow transplantation have been shown to reduce the occurrence of invasive aspergillosis (228).

It is recommended that all allogeneic hematopoietic stem cell transplant recipients be housed in rooms with more than 12 air exchanges per hour and point-of-use high-efficiency (>99%) particulate HEPA filters with the capacity of removing particles ≥0.3 μm in size (79) For autologous transplant recipients, HEPA-filtered rooms should be considered in the setting of prolonged neutropenia (79). Laminar airflow can provide a higher rate of room air exchanges; however, its high cost and limited availability are potential limitations. Its use is considered optional for HSCT recipients (79). The need for environmental HEPA filtration for organ transplant recipients has not been established. Finally, as noted earlier, there is emerging evidence that hospital water supplies may also be a potential source of *Aspergillus* spp. (10, 11). Precise recommendations on how to monitor hospital water systems have not yet been forthcoming.

PROPHYLAXIS

Although a number of options exist, an optimal approach to prophylaxis for invasive aspergillosis in hematopoietic stem cell as well as organ transplant recipients remains a complex and an unresolved issue. Careful assessment of the risk of infection, adverse effects of the antifungal agent, potential efficacy of the regimen, and impact of prophylaxis on outcome must be considered in determining whether prophylaxis should be administered to all patients or directed towards a subset of patients, the choice of antifungal agent, and its mode of administration.

Organ Transplant Recipients

In liver transplant recipients, low-dose amphotericin B deoxycholate in dosages ranging from 0.1 to 0.5 mg/kg/day not only has been ineffective but has been proposed to increase the risk of invasive aspergillosis. Low doses (1 mg/kg/day) of lipid formulations of amphotericin B have also not been effective in case series (171, 321). Breakthrough infections were documented in 5% of the patients in one study (171) and in 10% in another (321) in which Ambisome at 1 mg/kg/day was used. A lack of cases of invasive aspergillosis precluded meaningful assessment of the efficacy of 1 mg of liposomal amphotericin B (Ambisome)/kg/day as prophylaxis against *Aspergillus* infections in a randomized trial (315).

At least three studies of liver transplant recipients have evaluated the efficacy of lipid formulations of amphotericin B (in dosages of 1 to 5 mg/kg/day) as antifungal prophylaxis (84, 295, 297). Of 31 patients who required ≥5 days in the intensive care unit and who received Abelcet (1 to 5 mg/kg/day) as antifungal prophylaxis, none developed invasive aspergillosis. Antifungal prophylaxis with a lipid formulation of amphotericin B (5 mg/kg/day) targeted towards a cohort of liver transplant recipients requiring dialysis was associated with a significantly lower incidence of invasive fungal infections ($P = 0.0007$), including aspergillosis ($P = 0.02$) (295). In another study, administration of a lipid formulation of amphotericin B (Ambisome or Abelcet at a dose of 100 mg/day) in high-risk liver transplant recipients led to a reduction in the rate of invasive aspergillosis from 23 to 5% (84). In dialyzed patients, the risk of invasive aspergillosis decreased from 32 to 0%. In both studies, the use of antifungal prophylaxis was independently protective against invasive fungal infections. Notably, however, neither study was able to document a reduction in mortality with antifungal prophylaxis.

The efficacy of itraconazole in an oral solution as antifungal prophylaxis has been assessed in two reports on liver transplant recipients. A randomized, controlled trial of itraconazole in an oral solution (200 mg every 12 h) versus intravenous or oral fluconazole (400 mg every 24 h) documented no significant difference in the incidence of invasive aspergillosis (336). Proven fungal infections developed in 9% of 97 patients who received itraconazole (including two cases of aspergillosis), and in 4% of 91 patients who received fluconazole (including one case of invasive aspergillosis). Adverse gastrointestinal effects were documented in a significantly greater number of patients who received itraconazole (336). Although itraconazole in hydroxypropyl-β-cyclodextrin as an oral solution has significantly improved bioavailability compared to the capsule form, critically ill liver transplant recipients who are unable to eat and have altered gastric acidity may have impaired absorption and subtherapeutic levels even with the oral solution (336).

The incidence of invasive aspergillosis in liver transplant recipients, while high relative to that in other transplant patients, ranges from 1 to 2%. If prophylaxis was employed in all patients, a vast majority, while accruing the expense and potential toxicity of the antifungal agent, would be unlikely to benefit from it. The strategy of targeted prophylaxis is particularly well suited for liver transplant recipients in whom the risk factors for invasive aspergillosis are defined rather precisely. Retransplantation, a requirement for dialysis, and fulminant hepatic failure as an underlying disease (Table 3) are readily identifiable and objective criteria that may be used to define the high-risk subgroup.

Far less, however, is known about an optimal choice of antifungal agent for prophylaxis in these patients. Each of the currently available drugs have potential limitations if considered as prophylactic agents. Lipid formulations of amphotericin B require parenteral administration and are expensive. Caspofungin also requires parenteral administration. Despite in vitro data suggesting that caspofungin in combination with calcineurin or TOR inhibitors may have enhanced activity against *Aspergillus*, breakthrough infections in patients on caspofungin have been noted (150). Voriconazole, because of its availability in an intravenous and an oral formulation, may allow a transition to oral therapy and is therefore an attractive option. However, its significant interactions with the immunosuppressive agents are a potential limiting factor. Furthermore, liver transplant recipients in whom prophylaxis is most needed, i.e., those requiring dialysis, are the very patients in

whom voriconazole in an intravenous formulation cannot be employed.

Routine antifungal prophylaxis is not warranted in heart transplant recipients. However, in patients deemed to be at high risk (Table 2), itraconazole may be an option (213). Itraconazole at 400 mg daily administered orally from day 5 after transplantation for 3 to 6 months was associated with a significantly lower incidence of invasive aspergillosis than in an earlier cohort who did not receive antifungal prophylaxis (2 versus 9.6%; $P < 0.05$) (213).

Since most *Aspergillus* infections in lung transplant recipients are pulmonary or tracheobronchial, an aerosolized mode of drug delivery would appear to be a rational form of prophylaxis. It is proposed that amphotericin B may be delivered more efficiently to the lung, attaining higher concentrations in the lung, when administered as an aerosol than by systemic administration (277). In an immunocompromised rat model, amphotericin B deoxycholate at 1.6 mg/kg administered 2 days before infection significantly delayed mortality compared to that of the controls (278). The mean concentration of amphotericin B in the lung after an intravenous dose of 4 mg/kg was 4.3 μg/g of tissue. The same concentration was achieved with only two aerosolized doses of 1.6 mg/kg/day (277). Amphotericin B was detectable in bronchoalveolar lavage samples for up to 24 h after an aerosolization (149). The elimination half-life of amphotericin B from the lungs after aerosolization of a single dose of 3.2 mg/kg was 4.8 days (149). A major limitation of conventional amphotericin B is that deoxycholate tends to foam upon nebulization, with the potential risk of bronchospasm. Since *Aspergillus* conidia that reach the small airways and alveolar spaces have diameters of 2.5 to 3.5 μm, the optimal size of the aerosols generated is 1 to 5 μm. Particles of >5 μm in diameter may be retained in the oropharynx and lead to gastrointestinal side effects and less deposition in the lungs (26).

Unique issues pertaining to the pharmacokinetics and distribution of nebulized amphotericin B deoxycholate were assessed in a study of lung transplant recipients (204). The amount of amphotericin B in the bronchoalveolar lavage fluid (which is reflective of drug concentrations in the distal airways) was significantly greater than that in the bronchoalveolar secretions (which more closely approximates the levels in the proximal airways). The MIC of amphotericin B at which 90% of the isolates are inhibited for most *Aspergillus* species was exceeded in the proximal airways for only 4 h after nebulization (204). It has been proposed that in order to effectively prevent anastomotic infections, a dose of 6 mg/kg administered three times a day may be required for adequate levels in the proximal airway, where bronchial anastomosis is located. Whereas drug deposition in bilateral lung allograft recipients was symmetrical, the distribution in single lung transplant recipients occurred preferentially in the allograft and was erratic and nonuniform in the native lung. Finally, the regional drug distribution correlated closely with lung perfusion. Technitium-labeled amphotericin B was uniformly distributed in 12 of 13 allografts without bronchiolitis obliterans syndrome, compared to 1 of 4 with bronchiolitis obliterans (204).

Aerosolization of the lipid formulations of amphotericin B has been proposed to enhance drug delivery by causing less foaming during nebulization. Concentrations in the lung that were achievable with the lipid formulations were severalfold higher than those with amphotericin B deoxycholate (6, 53). An estimated 23% of the dose of liposomal amphotericin B was retained in a murine pulmonary aspergillosis model, compared to 2.4% retention for amphotericin B deoxycholate, suggesting that the lipid formulation may enhance drug retention (6). Administration of aerosolized liposomal amphotericin B in immunocompromised mice subsequently challenged with intranasal spores of *A. fumigatus* cleared 80% of the lungs, compared to none with amphotericin B deoxycholate (6).

In a prospective, nonrandomized study, the efficacy and safety of aerosolized amphotericin B lipid complex was assessed in 51 lung transplant recipients (230). Pulmonary fungal infections developed in two patients, and both were *Candida* anastomotic infections. Four patients developed extrapulmonary infections that comprised *C. tropicalis* fungemia in one patient, *C. albicans* peritonitis in two patients, and *C. albicans* sinusitis in one patient. No drug was detectable in the sera of all patients who underwent such testing. No significant adverse effects related to prophylaxis were documented in the patients. Fewer than 5% of all treatments were associated with worsening of the pulmonary mechanics by 20% or more posttreatment.

Small case series and reports have documented the efficacy of itraconazole as antifungal prophylaxis in colonized patients (42, 110). However, rigorous clinical trials showing its efficacy in lung transplant recipients are lacking. A survey of 24 North American and 19 European centers documented that antifungal prophylaxis was employed in a vast majority of lung transplant recipients. European centers were more likely to use inhalational amphotericin B, whereas in North America, inhalational amphotericin B plus itraconazole was the more commonly employed form of antifungal prophylaxis (128).

Hematopoietic Stem Cell Transplant Recipients

Low-dose amphotericin B in hematopoietic stem cell transplant recipients not only is of uncertain benefit but has been associated with significant toxicity, particularly renal dysfunction (117, 140, 241, 257, 338). The recently published study of the North American Marrow Transplant group that compared low-dose amphotericin B with fluconazole documented *Aspergillus* infections in 1 of 159 patients randomized to the amphotericin B group and in 2 of the 196 patients in the fluconazole group (338). Drug toxicity leading to discontinuation of prophylaxis was significantly more frequent in the amphotericin B group. Renal toxicity developed in 13 patients who received amphotericin B, 3 of whom required dialysis. Allogeneic compared to autologous transplant recipients were more likely to experience renal toxicity. Liposomal amphotericin B (Ambisome) at a dose of 2 mg/kg thrice weekly was well tolerated in a randomized, placebo-controlled study, although a beneficial effect of prophylaxis against fungal infections could not be shown (143). Severe infusion-related toxicity led to premature discontinuation of the trial that intended to compare the efficacy of amphotericin B colloidal dispersion with that of fluconazole for the prevention of fungal infections in neutropenic patients (313). Given the uncertain benefit, requirement of parenteral administration, and drug expense, it is unlikely that lipid formulations of amphotericin B would have

a significant role as prophylactic agents for aspergillosis in hematopoietic stem cell transplant recipients.

Aerosolization of amphotericin B has been shown to be effective in some but not other reports on hematopoietic stem cell transplant recipients (21, 59, 103, 281). The findings and the conclusions based on these studies, however, are difficult to generalize given the variability in the dose, frequency of administration, mode of delivery, and concomitant use of other antifungal prophylaxis and a low number of cases of documented infections.

The efficacy of itraconazole in an oral solution for antifungal prophylaxis has been evaluated in at least five randomized trials that included hematopoietic stem cell transplant recipients or were conducted exclusively with these patients. Itraconazole compared to fluconazole for 100 days in allogeneic stem cell transplant recipients was associated with significantly fewer proven fungal infections in the first 180 days after transplantation (337). Four percent (3 of 71) of the patients in the itraconazole group and 12% (8 of 67) in the fluconazole group had *Aspergillus* infections ($P = 0.12$). Patients receiving itraconazole, however, had significantly more gastrointestinal side effects (24 versus 9%, $P = 0.02$). The overall mortality did not differ in the two groups. *Aspergillus* infections were documented in 4 of 201 itraconazole recipients and in 1 of 204 patients who received a placebo in a randomized trial with neutropenic patients with hematologic malignancy and autologous transplant recipients (200).

Of 445 patients randomly assigned to receive itraconazole solution versus fluconazole, 40% were bone marrow or peripheral stem cell transplant recipients (209). There were no proven cases of invasive aspergillosis in the itraconazole group ($n = 218$), and there were six cases in the fluconazole group ($n = 227$; $P = 0.038$). Significantly more patients in the itraconazole group withdrew from the study because of adverse events (209). Invasive aspergillosis was documented in 1.8% of 281 patients assigned to receive itraconazole solution, versus 3.3% of 276 patients assigned to the oral amphotericin group ($P = 0.264$) (112), in a randomized trial in which 5% of the sample size accrued were autologous bone marrow transplant recipients.

In allogeneic stem cell transplant recipients, itraconazole compared to fluconazole provided greater protection against invasive mold infections (5 versus 12%; $P = 0.03$) (188). However, toxicity and safety issues led to termination of the trial after 50% of the projected enrollment. Patients who received itraconazole had higher serum bilirubin and creatinine levels, with the highest levels in those who received itraconazole concurrently with cyclophosphamide (189). Azole antifungal agents that act through inhibition of hepatic cytochrome P450 isoenzymes may affect the metabolism of oxazaphosphorine and the taxane class of cytotoxic agents. The use of cylophosphamide with itraconazole may be associated with a higher risk of hepatic dysfunction (189), and that of vincristine plus itraconazole may be associated with a higher risk of neurotoxicity.

A randomized, double-blind trial of micafungin (an echinocandin) versus fluconazole for prophylaxis of invasive fungal infections in hematopoietic stem cell transplant recipients documented invasive aspergillosis in 0.2% (1 of 425) of patients in the micafungin group and in 1.5% (7 of 457) of the patients in the fluconazole group ($P = 0.07$) (320). Fewer patients in the micafungin group discontinued the study drug due to an adverse effect ($P = 0.058$) (320).

The efficacy of newer triazole agents as antifungal prophylactic agents remains to be determined. Benefits of such approaches, if documented, must be balanced against the potential risk associated with the emergence of antifungal resistance or of pathogens that are inherently resistant to the antifungal agent being used. The use of voriconazole for prophylaxis or empirical therapy in allogeneic hematopoietic stem cell transplant recipients correlated with an increase in breakthrough zygomycete infection at one institution (193). In another report, four cases of invasive zygomycosis occurred in HSCT recipients since voriconazole was used as prophylaxis, whereas no cases had been detected in 3 years prior (298). Breakthrough fungal infections occurred in 12 of 139 HSCT recipients who received voriconazole and included six cases of zygomycosis (130).

One-third of patients with a history of aspergillosis will experience a relapse after HSCT transplantation (227). Secondary prophylaxis has been shown to be beneficial in this setting (227). Antifungal prophylaxis with itraconazole, intravenous amphotericin B, or liposomal amphotericin after HSCT transplantation was associated with relapse in 29% (12 of 41) of patients with a prior history of aspergillosis, compared to 59% (4 of 7) in those who received oral amphotericin B or no prophylaxis (227).

The focus of the studies on antifungal propohylaxis in HSCT recipients has largely been the prevention of infections in the conventional high-risk period, i.e., the first 90 to 100 days. A growing proportion of the *Aspergillus* infections, however, are occurring in the late posttransplant period in patients requiring augmented immunosuppression for GVHD. An optimal approach or duration of prophylaxis for these patients has not been defined. Continuation of prophylaxis until the completion of the immunosuppressive treatment course or until immunosuppression is substantially reduced is reasonable (334).

REFERENCES

1. **Abbott, K. C., I. Hypolite, R. K. Poropatich, P. Hshieh, D. Cruess, C. A. Hawkes, L. Y. Agodoa, and R. A. Keller.** 2001. Hospitalization for fungal infections after renal transplantation in the United States. Transplant. Infect. Dis. **3:**203–211.
2. **Adam, O., A. Auperin, F. Wilquin, J.-H. Bourhis, B. Gachot, and E. Chachaty.** 2004. Treatment with piperacillin-tazobactam and false-positive *Aspergillus* galactomannan antigen test results for patients with hematological malignancies. Clin. Infect. Dis. **38:**917–920.
3. **Ahlin, A., G. Eliner, and J. Palmblad.** 1997. Dose-dependent enhancements by interferon-gama on functional responses of neutrophils from chronic granulomatous disease patients. Blood **89:**3396–3401.
4. **Aliff, T. B., P. G. Maslak, J. G. Jurcic, M. L. Heaney, K. N. Catheart, K. A. Sepkowitz, and M. S. Weiss.** 2003. Refractory *Aspergillus* pneumonia in patients with acute leukemia: successful therapy with combination caspofungin and liposomal amphotericin. Cancer **97:**1025–1032.
5. **Allen, R. D., T. A. Staley, and C. L. Sidman.** 1993. Differential cytokine expression in acute and chronic murine graft-versus-host disease. Eur. J. Immunol. **23:**333–337.
6. **Allen, S. D., K. N. Sorenson, M. J. Nejdl, C. Durrant, and R. T. Proffit.** 1994. Prophylactic efficacy of aerosolized liposomal (AmBisome) and nonliposomal (Fungizone) amphotericin B in murine pulmonary apergillosis. J. Antimicrob. Cherother. **34:**1002–1013.
7. **Allo, M. D., J. Miller, T. Townsend, and C. Tan.** 1987. Primary cutaneous aspergillosis associated with Hickman intravenous catheters. N. Engl. J. Med. **317:**1105–1108.
8. **Almyroudis, S., D. Jaffe, K. A. Sepkowitz, E. G. Pamer, E. N. Meier, E. Papadophoulos, T. N. Small, and G. A. Papnicolaou.** 2003. Risk factors for late invasive aspergillosis (IA) after allogeneic stem cell transplantation, abstr. M-1006. Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., Chicago, Ill., 14–17 September 2003.

9. **Altiparmak, M. R., S. Apaydin, S. Trablus, K. Serdengecti, R. Ataman, R. Pztirl, R. Ozturk, and E. Erek.** 2002. Systemic fungal infections after renal transplantation. Scand. J. Infect. Dis. **34:**284–288.
10. **Anaissie, E., and S. F. Costa.** 2001. Nosocomial aspergillosis is waterborne. Clin. Infect. Dis. **33:**1546–1548.
11. **Anaissie, E. J., S. L. Stratton, M. C. Dignani, R. C. Summerbell, J. H. Rex, T. P. Monson, T. Spencer, M. Kasai, A. Francesconi, and T. J. Walsh.** 2002. Pathogenic *Aspergillus* species recovered from a hospital water system: a 3-year prospective study. Clin. Infect. Dis. **34:**780–789.
12. **Ankersmit, H. J., R. Deicher, B. Moser, et al.** 2001. Impaired T cell proliferation, increased soluble death-inducing receptors and activation-induced T cell death in patients underging haemodialysis. Clin. Exp. Immunol. **135:** 142–148.
13. **Ansorg, R., R. van den Boom, and P. M. Rath.** 1997. Detection of *Aspergillus* galactomannan antigen in foods and antibiotics. Mycoses **40:**353–357.
14. **Anttila, V. J., A. Piilonen, and M. Valtonen.** 2003. Co-administration of caspofungin and cyclosporine to a kidney transplant patient with pulmonary *Aspergillus* infection. Scand. J. Infect. Dis. **35:**893–894.
15. **Arikan, S., M. Lozano-Chiu, V. Paetznick, and J. H. Rex.** 2001. In vitro susceptibility testing methods for caspofungin against *Aspergillus* and *Fusarium* isolates. Antimicrob. Agents Chemother. **45:**327–330.
16. **Arikan, S., M. Lozano-Chiu, V. Paetznick, and J. H. Rex.** 2002. In vitro synergy of caspofungin and amphtericin B against *Aspergillus* and *Fusarium* spp. Antimicrob. Agents Chemother. **46:**245–247.
17. **Arroyo, J., G. Medoff, and G. S. Kobayashi.** 1977. Therapy of murine aspergillosis with amphotericin B in combination with rifampin or 5-fluorocytosine. Antimicrob. Agents Chemother. **11:**21–25.
18. **Baddley, J. W., T. P. Stroud, D. Salzman, and P. G. Pappas.** 2001. Invasive mold infections in allogeneic bone marrow transplant recipients. Clin. Infect. Dis. **32:**1319–1324.
19. **Baden, L. R., J. T. Katz, J. A. Fishman, C. Koziol, A. DelVecchio, M. Doran, and R. H. Rubin.** 2003. Salvage therapy with voriconazole for invasive fungal infections in patients failing or intolerant to standard antifungal therapy. Transplantation **76:**1632–1637.
20. **Bart-Delabesse, E. A., A. Marmorat-Khuon, J. M. Cosa, et al.** 1996. Detection of *Aspergillus* DNA in bronchoalveolar lavage fluid of AIDS patients by polymerase chain reaction. Eur. J. Clin. Microbiol. Infect. **15:**24–25.
21. **Behre, G. F., S. Schwartz, K. Lenz, W. D. Ludwig, H. Wandt, E. Schilling, et al.** 1995. Aerosol amphotericin B inhalations for prevention of invasive pulmonary aspergillosis in neutropenic cancer patients. Ann. Hematol. **71:** 287–291.
22. **Bennett, J. E., M. M. Friedman, and B. Dupont.** 1987. Receptor-mediated clearance of *Aspergillus* galactomannan. J. Infect. Dis. **155:**1005–1010.
23. **Bensinger, W. I.** 2000. Blood or marrow? Lancet **355:**1199–1200.
24. **Bernard, A., D. Caillot, J. F. Couaillier, O. Casasnovas, H. Guy, and J. P. Favre.** 1997. Surgical management of invasive pulmonary aspergillosis in neutropenic patients. Ann. Thorac. Surg. **64:**1441–1447.
25. **Bertocchi, M., F. Thevenet, O. Bastien, M. Rabodonirina, J. P. Gamondes, S. Paulus, R. Loire, M. A. Piens, M. Celard, and J. F. Mornex.** 1995. Fungal infections in lung transplant recipients. Transplant. Proc. **27:**1695.
26. **Beyer, J., G. Barzen, G. Risse, C. Weyer, K. Miksits, K. Dullenkopf, D. Huhn, and W. Siegert.** 1993. Aerosol amphotericin B for prevention of invasive pulmonary aspergillosis. Antimicrob. Agents Chemother. **37:**1367–1369.
27. **Birsan, T., S. Taghavi, W. Klepetko, and Vienna Lung Transplant Group.** 1998. Treatment of Aspergillus-related ulcerative tracheobronchitis in lung transplant. J. Lung Heart Transplant. **17:**437–438.
28. **Bodey, G. P., E. Anaissie, J. Gutterman, and S. Vadhan-Raj.** 1993. Role of granulocyte-macrophage colony-stimulating factor as adjuvant therapy for fungal infection in patients with cancer. Clin. Infect. Dis. **17:**705–707.
29. **Boland, G. J., R. J. Hene, C. Ververs, M. A. M. De Haan, and G. C. De Gast.** 1993. Factors influencing the occurrence of active cytomegalovirus (CMV) infections after organ transplantation. Clin. Exp. Immunol. **94:**306–312.
30. **Bonham, C. A., E. A. Dominguez, M. B. Fukui, D. L. Paterson, G. A. Pankey, and M. M. Wagener.** 1998. Central nervous system lesions in liver transplant recipients: prospective assessment of indications for biopsy and implications for management. Transplantation **66:**1596–1604.
31. **Boots, R. J., D. L. Paterson, A. M. Allworth, and J. L. and Faoagali.** 1999. Successful treatment of post-influenza pseudomembranous necrotising bronchial aspergillosis with liposomal amphotericin, inhaled ampotericin B, gamma interferon and GM-CSF. Thorax **54:**1047–1049.
32. **Bowden, R., P. Chandrasekar, M. H. White, L. Xin, L. Pietrelli, M. Gurwith, J. Van Burik, M. Laverdiere, S. Safria, and J. R. Wingard.** 2002. A double-blind, randomized, controlled trial of amphotericin B colloidal dispersion versus amphotericin B for treatment of invasive aspergillosis in immunocompromised patients. Clin. Infect. Dis. **35:**359–366.
33. **Bozza, S., K. Perruccio, C. Montagnoli, R. Gaziano, S. Bellocchio, E. Burchielli, G. Nkwanyuo, L. Pitzurra, A. Velardi, and L. Romani.** 2003. A dendritic cell vaccine against invasive aspergillsois in allogeneic hematopoietic transplantation. Blood **102:**3807–3814.
34. **Brenier-Pinchart, M.-P., B. Lebeau, G. Devouassoux, P. Mondon, C. Pison, P. Ambroise-Thomas, and R. Grillot.** 1998. *Aspergillus* and lung transplant recipients: a mycologic and molecular epidemiologic study. J. Heart Lung Transplant. **17:**972–979.
35. **Bretagne, S., J.-M. Costa, E. Bart-Delabesse, N. Dhedin, C. Rieux, and C. Cordonnier.** 1998. Comparison of serum galactomannan antigen detection and competitive polymerase chain reaction for diagnosing invasive aspergillosis. Clin. Infect. Dis. **26:**1407–1412.
36. **Briegel, J., H. Forst, B. Spill, A. Haas, B. Grabein, M. Haller, E. Kilger, K. W. Jauch, K. Maag, G. Ruckdeschel, and K. Peter.** 1995. Risk factors for systemic fungal infections in liver transplant recipients. Eur, J. Clin. Microbiol. Infect. Dis. **14:**375–382.
37. **Brown, R. S., J. R. Lake, B. A. Katzman, N. L. Ascher, K. A. Sombert, J. C. Emond, and J. P. Roberts.** 1996. Incidence and significance of *Aspergillus* cultures following liver and kidney transplantation. Transplantation **61:** 666–669.
38. **Buchheidt, D., C. Baust, H. Skladny, J. Ritter, T. Suedhoff, M. Baldus, W. Seifarth, C. Leib-Moesch, and R. Hehlmann.** 2001. Detection of *Aspergillus* species in blood and bronchoalveolar lavage samples from immunocompromised patients by means of 2-step polymerase chain reaction: clinical results. Clin. Infect. Dis. **33:**428–435.
39. **Buchheidt, D., M. Hummel, D. Schleiermacher, B. Spiess, H. Skladny, R. Schwerdtfeger, O. A. Cornely, S. Wilhelm, S. Reuter, W. V. Kern, T. Suedhoff, H. Morez, R. Hehlmann, et al.** 2003. Prospective multicenter clinical evaluation of a nested PCR assay, a lightcycler mediated PCR assay and a galactomannan ELISA for detection of invasive aspergillosis in neutropenic high risk patients, abstr. M-2057. Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., Chicago, Ill., 14–17 September 2003.
40. **Cahill, B. C., J. R. Hibbs, K. Savik, B. A. Juni, B. M. Dosland, C. Edin-Stibbe, and M. I. Hertz.** 1997. *Aspergillus* airway colonization and invasive disease after lung transplantation. Chest **112:**1160–1164.
41. **Caillot, D., J. F. Couaillier, A. Bernard, et al.** 2001. Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. J. Clin. Oncol. **19:**253–259.
42. **Calvo, V., J. M. Borro, P. Morales, A. Morcillo, R. Vincente, T. Vincente, F. X. Paris, and Valencia Lung Transplant Group.** 1999. Antifungal prophylaxis during the early postoperative period of lung transplantation. Chest **115:**1301–1304.
43. **Camarata, P. J., D. L. Dunn, A. C. Farney, R. G. Parker, and E. L. Seljeskog.** 1992. Continual intracavitary administration of amphotericin B as an adjunct in the treatment of *Aspergillus* brain abscess: case report and review of the literature. Neurosurgery **31:**575–579.
44. **Cameron, M. L., D. L. Granger, T. J. Matthews, and J. B. Weinberg.** 1994. Human immunodeficiency virus (HIV)-infected human blood monocytes and peritoneal macrophages have reduced anticryptococcal activity whereas HIV-infected alveolar macrophages retain normal activity. J. Infect. Dis. **170:**60–70.
45. **Carlson, G. L., M. M. Mughal, M. Birch, and D. W. Denning.** 1996. *Aspergillus* wound infection following laparostomy. J. Infect. **33:**119–121.
46. **Casey, P., J. Garrett, and T. Eaton.** 2002. Allergic bronchopulmonary aspergillosis in a lung transplant successfully treated with nebulized amphotericin. J. Heart Lung Transplant. **21:**1237–1242.
47. **Castaldo, P., R. J. Stratta, R. P. Wood, et al.** 1991. Clinical spectrum of fungal infections after orthotopic liver transplantation. Arch. Surg **126:** 149–156.
48. **Catalano, L., R. Fontane, N. Scarpato, M. Picardi, S. Rocco, and B. Rotoli.** 1997. Combined treatment with amphotericin B and granulocyte infusion from G-CSF stimulated donors in an aplastic patient with invasive aspergillosis undergoing bone marrow transplantation. Haematologica **82:**71–72.
49. **Cenci, E., A. Mencacci, A. Bacci, F. Bistoni, V. P. Kurup, and L. Romani.** 2000. T cell vaccination in mice with invasive pulmonary aspergillosis. J. Immunol. **165:**381–388.
50. **Cenci, E., A. Mencacci, G. Del Sero, A. Bacci, C. Montagnoli, C. Fe' d'Ostiani, P. Mosci, M. Bachmann, F. Bistoni, M. Kopf, and L. Romani.** 1999. Interleukin-4 causes susceptibility to invasive pulmonary aspergillosis through suppression of protective type I responses. J. Infect. Dis. **180:** 1957–1968.
51. **Centeno-Lima, S., J. M. de Lacerda, J. A. do Carmo, M. Abecasis, and C. Casimiro.** 2002. Follow-up of anti-*Aspergillus* IgG and IgA antibodies in bone marrow transplanted patients with invasive aspergillosis. J. Clin. Lab. Anal. **16:**156–162.
52. **Chandrasekar, P. H., G. Alangaden, and E. Manavathu.** 2000. Aspergillosis: an increasing problem in tertiary care hospitals? Clin. Infect. Dis. **30:** 984–985.
53. **Cicogna, C. E., M. H. White, E. M. Bernard, et al.** 1997. Efficacy of prophylactic aerosol amphotericin B lipid complex in a rat model of pulmonary aspergillosis. Antimicrob. Agents Chemother. **41:**259–261.
54. **Clemons, K. V., G. Grunig, R. A. Sobel, L. F. Mirels, D. M. Rennick, and D. A. Stevens.** 2000. Role of IL-10 in invasive aspergillosis: increased resistance of IL-10 gene knockout mice to lethal systemic aspergillosis. Clin. Exp. Immunol. **122:**186–191.
55. **Clemons, K. V., and D. A. Stevens.** 2001. Overview of host defense mech-

anisms in systemic mycoses and the basis for immunotherapy. Semin. Respir. Infect. **16:**60–66.
56. **Cofan, F., P. Inigo, M. J. Ricart, F. Oppenheimer, J. Vilardell, J. M. Campistol, and P. Carretero.** 1996. Aspergilosis pulmonar invasiva en el trasplante renal y renopancreatico. Nefrologia **XVI:**253–260.
57. **Coleman, J. M., G. G. Hogg, J. V. Rosenfeld, and K. D. Waters.** 1995. Invasive central nervous system aspergillosis: cure with liposomal amphotericin B, itraconazole and radical surgery—case report and review of the literature. Neurosurgery **36:**858–863.
58. **Collins, L. A., M. H. Samore, M. S. Roberts, R. Luzzati, R. L. Jenkins, W. D. Lewis, and A. W. Karchmer.** 1994. Risk factors for invasive fungal infections complicating orthotopic liver transplantation. J. Infect. Dis. **170:** 644–652.
59. **Conneally, E., M. T. Cafferkey, P. A. Daly, C. T. Keane, and S. R. McCann.** 1990. Nebulized amphotericin B as prophylaxis against invasive aspergillosis in granulocytic patients. Bone Marrow Transplant. **5:**403–406.
60. **Costa, C., J.-M. Costa, C. Desterke, F. Botterel, C. Cordonnier, and S. Bretagne.** 2002. Real-time PCR coupled with automated DNA extraction and detection of galactomannan antigen in serum by enzyme-linked immunosorbent assay for diagnosis of invasive aspergillosis. J. Clin. Microbiol. **40:** 2224–2227.
61. **Courtney, R., S. Pai, M. Laughlin, J. Lim, and V. Batra.** 2003. Pharmacokinetics, safety, and tolerability of oral posaconazole administered in single and multiple doses in healthy adults. Antimicrob Agents Chemother. **47:** 2788–2795.
62. **Courtney, R., E. Radwanski, J. Lim, and M. Laughlin.** 2004. Pharmacokinetics of posaconazole coadmnistered with antacid in fasting or nonfasting healthy men. Antimicrob. Agents Chemother. **48:**804–808.
63. **Cuenca-Estrella, M., A. Gomez-Lopez, G. Garcia-Efferon, L. Alcazar, E. Mellado, and J. L. Rodriguez-Tudela.** 2003. Combined activity in vitro of caspofungin plus amphotericin B or plus azole agents against itraconazole resistant clinical isolates of *Aspergillus fumigatus*, abstr. M-991. Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., Chicago, Ill., 14–17 September 2003.
64. **Damaj, G., V. Ivanov, P. E. Le Brigand, E. D'Inean, M. F. Doglio, K. Bilger, C. Faucher, N. Vey, and J. A. Gastaut.** 2003. Rapid improvement of disseminated aspergillosis with caspofungin/voriconazole combination in an adult leukemic patient. Ann. Hematol. 10 Dec. 2003. [Epub.]
65. **Dannaoui, E., J. Meletiadis, A. M. Tortorano, F. Symoens, N. Nolard, M. A. Viviani, M. A. Piens, B. Lebeau, P. E. Verweij, and R. Grillot.** 2004. Susceptibility testing of sequential isolates of *Aspergillus fumigatus* recovered from treated patients. J. Med. Microbiol. **53:**129–134.
66. **De La Rosa, G. R., R. E. Champlin, and D. P. Kontoyiannis.** 2003. Risk factors for the development of invasive fungal infections in allogeneic blood and marrow transplant recipients. Transplant. Infect. Dis. **4:**3–9.
67. **de Lastours, V., A. Lefort, M. Zappa, V. Dufour, N. Blematoug, and B. Fantin.** 2003. Two cases of cerebral aspergillosis successfully treated with voriconazole. Eur. J. Clin. Microbiol. Infect. Dis. **22:**297–299.
68. **Denning, D. W.** 1998. Invasive aspergillosis. Clin. Infect. Dis. **26:**781–805.
69. **Denning, D. W.** 2003. Echinocandin antifungal drugs. Lancet **362:**1142–1151.
70. **Denning, D. W., L. H. Hanson, A. M. Perlman, and D. A. Stevens.** 1992. In vitro susceptibility and synergy studies of *Aspergillus* species to conventional and new agents. Diagn. Microbiol. Infect. Dis. **15:**21–34.
71. **Denning, D. W., S. A. Radford, L. Oakley, E. Hall, E. M. Johnson, and D. W. Warnock.** 1997. Correlation between in-vitro susceptibility testing to itraconazole and in-vivo outcome of *Aspergillus fumigatus* infection. J. Antimicrob. Chemother. **40:**401–414.
72. **Denning, D. W., P. Ribaud, N. Milpied, D. Caillot, R. Herbrecht, E. Thiel, A. Hass, M. Ruhnke, and H. Lode.** 2002. Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. Clin. Infect. Dis. **34:**563–571.
73. **Denning, D. W., and D. A. Stevens.** 1990. Antifungal and surgical treatment of invasive appergillosis: review of 2,121 published cases. Rev. Infect. Dis. **12:**1147–1201.
74. **Denning, D. W., K. Venkateswarlu, K. L. Oakley, M. J. Anderson, N. J. Manning, D. W. Stevens, D. W. Warnock, and S. L. Kelley.** 1997. Itraconazole resistance in *Aspergillus fumigatus*. Antimicrob. Agents Chemother. **41:**1364–1368.
75. **deShazo, R. D., K. Chaplin, and R. E. Swain.** 1997. Fungal sinusitis. N. Engl. J. Med. **337:**254–259.
76. **Dignani, M. D., E. J. Anaissie, J. P. Hester, S. O'Brien, S. E. Vartivarian, J. H. Rex, H. Kantarjian, D. B. Jendiroba, B. Lichtiger, B. S. Andersson, and E. J. Freireich.** 1997. Treatment of neutropenia-related fungal infections with granulocyte colony-stimulating factor-elicited white blood cell transfusions: a pilot study. Leukemia **11:**1621–1630.
77. **Dockrell, D. H., J. C. Mendez, M. Jones, W. S. Harmsen, D. M. Ilstrup, T. F. Smith, R. H. Wiesner, R. A. F. Krom, and C. V. Paya.** 1999. Human herpesvirus 6 seronegativity before transplantation predicts the occurrence of fungal infection in liver transplant recipients. Transplantation **67:**399–403.
78. **Drakos, P. E., A. Nagler, R. Or, E. Naparstek, J. Kapelushnik, D. Engelhard, G. Rahav, D. Neemean, and S. Slavin.** 1993. Invasive fungal sinusitis in patients undergoing bone marrow transplantation. Bone Marrow Transplant. **12:**203–208.
79. **Dykewicz, C. A.** 2001. Summary of the guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. Clin. Infect. Dis. **33:**139–144.
80. **Ellis, M., R. Watson, A. McNabb, M. L. Lukic, and M. Nork.** 2002. Massive intracerebral aspergillosis responding to combination high dose liposomal amphotericin B and cytokine threapy without surgery. J. Med. Microbiol. **51:**70–75.
81. **Espinel-Ingroff, A., M. Bartlett, V. Chaturvedi, M. Ghannoum, K. C. Hazen, M. A. Pfaller, M. Rinaldi, and T. J. Walsh.** 2001. Optimal susceptibility testing conditions for detection of azole resistance in *Aspergillus* spp.: NCCLS collaborative evaluation. Antimicrob. Agents Chemother. **45:**1828–1835.
82. **Fitzsimons, E. J., R. Aris, and R. Patterson.** 1997. Recurrence of allergic bronchopulmonary aspergillosis in the posttransplant lungs of a cystic fibrosis patient. Chest **112:**281–282.
83. **Fortun, J., P. Martin-Davila, M. E. Alvarez, A. Sanchez-Sousa, C. Quereda, E. Navas, R. Barcena, A. Candelas, A. Honsrubia, J. Nufio, V. Pintado, S. Moreno, and the Romon y Cajal Hospital's Liver Transplant Group.** 2001. *Aspergillus* antigenemia sandwich-enzyme immunoassay test as a serodiagnostic method for invasive aspergillosis in liver transplant recipients. Transplantation **71:**145–149.
84. **Fortun, J., P. Martin-Davila, S. Moreno, R. Barcena, E. de Vicente, A. Honrubia, M. Garcia, J. Nuno, A. Candela, M. Uriarte, and V. Pintado.** 2003. Prevention of invasive fungal infections in liver transplant recipients: the role of prophylaxis with lipid formulations of amphotericin B in high-risk patients. J. Antimicrob. Chemother. **52:**813–819.
85. **Fortun, J. P., P. Martin-Davila, S. Moreno, E. de Vicente, J. Nuno, A. Candelas, R. Barcena, and M. Garcia.** 2002. Risk factors for invasive aspergillosis in liver transplant recipients. Liver Transplant. **8:**1065–1070.
86. **Francis, P., J. W. Lee, A. Hoffman, et al.** 1994. Efficacy of unilamellar liposomal amphotericin B in treatment of pulmonary aspergillosis in persistently granulocytopenic rabbits: the potential role of bronchoalveolar D-mannitol and serum galactomannan as markers of infection. J. Infect. Dis. **169:**356–368.
87. **Fukuda, T., M. Boeckh, R. A. Carter, B. M. Sandmaier, M. B. Maris, and D. G. Maloney.** 2003. Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. Blood **102:**827–833.
88. **Gallis, H. A., R. A. Berman, T. R. Cate, J. D. Hamilton, J. Caullie Gunnells, and D. L. Stickel.** 1975. Fungal infection following renal transplantation. Arch. Intern. Med. **135:**1163–1172.
89. **Garbino, J., A. Ondrusova, E. Baglivo, D. Lew, K. Bouchuiguir-Wafa, P. Rohner, and E. Baligvo.** 2002. Successful treatment of *Paecilomyces lilacinus* endophthalmitis with voriconazole. Scand. J. Infect. Dis. **34:**701–703.
90. **George, D., P. Miniter, and V. T. Andriole.** 1996. Efficacy of UK-109496, a new azole antifungal agent, in an experimental model of invasive aspergillosis. Antimicrob. Agents Chemother. **40:**86–91.
91. **George, M. J., D. R. Snydman, and B. G. Werner.** 1997. The independent role of CMV as a risk factor for invasive fungal disease in orthotopic liver transplant recipients. Am. J. Med. **103:**106–113.
92. **Goodman, D., E. Pamer, A. Jakubowski, C. Morris, and K. Sepkowitz.** 2002. Breakthrough trichosporonosis in bone marrow transplant recipient receiving caspofungin acetate. Clin. Infect. Dis. **35:**E35–36.
93. **Gossot, D., P. Validire, R. Vaillancourt, G. Socie, H. Esperou, A. Devergie, P. Guardiola, D. Grunenwald, E. Gluckman, and P. Ribaud.** 2002. Full thoracoscopic approach for surgical management of invasive pulmonary aspergillosis. Ann. Thorac. Surg. **73:**240–244.
94. **Gow, K. W., A. A. Hayes-Jordan, C. A. Billups, J. L. Shenep, F. A. Hoffer, A. M. Davidoff, B. N. Rao, K. P. Schropp, and S. J. Shochat.** 2003. Benefit of surgical resection of invasive pulmonary aspergillosis in pediatric patients undergoing treatment for malignancies and immunodeficiency syndromes. J. Pediatr. Surg. **38:**1354–1360.
95. **Graybill, J. R., R. Bocanegra, G. M. Gonzalez, and L. K. Najvar.** 2003. Combination antifungal therapy of murine aspergillsois: liposomal amphotericin B and micafungin. J. Antimicrob. Chemother. **52:**656–662.
96. **Grazziutti, M. L., J. H. Rex, R. E. Cowart, E. J. Anaissie, A. Ford, and C. A. Savary.** 1997. *Aspergillus fumigatus* conidia induce a Th1-type cytokine response. J. Infect. Dis. **176:**1579–1583.
97. **Green, W. R., R. L. Font, and L. E. Zimmerman.** 1969. Aspergillosis of the orbit. Report of ten cases and review of the literature. Arch. Ophthalmol. **82:**302–313.
98. Reference deleted.
99. **Greenberg, R. N., G. Anstead, R. Herbrecht, A. Langston, K. Marr, K. Mullane, I. Raad, G. Schiller, M. Schuster, J. Van Burik, J. R. Wingard, R. Hare, and G. Corcoran.** 2003. Posazonazole (POS) experience in the treatment of zygomycosis, abstr. M-1757. Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., Chicago, Ill., 14–17 September 2003.
100. **Grossi, P., R. DeMaria, A. Caroli, M. S. Zaina, and L. Minoli.** 1992.

Infections in heart transplant recipients: the experience of the Italian heart transplantation program. J. Heart Lung Transplant. **11:**847–866.
101. **Grossi, P., C. Farina, R. Fiocchi, D. D. Gasperina, et al.** 2000. Prevalence and outcome of invasive fungal infections in 1,963 thoracic organ transplant recipients. Transplantation **70:**112–116.
102. **Grow, W. B., J. S. Moreb, D. Rogue, K. Manion, H. Leather, V. Reddy, S. A. Kahn, K. J. Finiewicz, H. Nguyen, C. J. Clancy, P. S. Mehta, and J. R. Wingard.** 2002. Late onset of invasive *Aspergillus* infection in bone marrow transplant patients at a university hospital. Bone Marrow Transplant. **29:** 15–19.
103. **Gubbins, P. O., J. L. Bowman, and S. R. Penzak.** 1998. Antifungal prophylaxis to prevent invasive mycoses among bone marrow transplantation recipients. Pharmacotherapy **18:**449–564.
104. **Guillemain, R., V. Lavarde, C. Amrein, P. Chevalier, A. Guinvarch, and D. Glotz.** 1995. Invasive aspergillosis after transplantation. Transplant. Proc. **27:**1307–1309.
105. **Gumbo, T., A. J. Taege, S. Mawhorter, M. D. McHenry, B. H. Lytle, D. M. Cosgrove, and S. M. Gordon.** 2000. *Aspergillus* valve endocarditis in patients without prior cardiac surgery. Medicine (Baltimore). **79:**261–268.
106. **Gustafson, T. L., W. Schaffner, G. B. Lavely, C. W. Stratton, H. K. Johnson, and R. H. Hutcheson, Jr.** 1983. Invasive aspergillosis in renal transplant recipients: correlation with corticosteroid therapy. J. Infect. Dis. **148:**230–238.
107. **Habicht, J. M., F. Reichenberger, A. Gratwohl, H. R. Zerkowski, and M. Tamm.** 1999. Surgical aspects of resection for suspected invasive pulmonary fungal infection in neutropenic patients. Ann. Thorac. Surg. **68:**321–325.
108. **Hachem, R. Y., I. I. Raad, C. M. Afif, R. Negroni, J. Graybill, S. Hadley, H. Kantarjian, S. Adams, and G. Mukwaya.** 2000. An open, non-comparative multicenter study to evaluate efficacy and safety of posaconazole (SCH56592) in the treatment of invasive fungal infections refractory or intolerant to standard therapy, abstr. 1109. Abstr. 40th Interscience. Conf. Antimicrob. Agents Chemother., Toronto, Canada, 17–20 September 2000..
109. **Hadjiliadis, D. H., D. N. Howell, R. D. Davis, C. M. Lawrence, J. B. Rea, V. F. Tapson, J. R. Perfect, and S. M. Palmer.** 2000. Anastomotic infections in lung transplant recipients. Ann. Transplant. **3:**13–19.
110. **Hamacher, J., A. Spiliopoulos, A.-M. Kurt, L. P. Nicod, and the Geneva Lung Trnasplantation Group.** 1999. Pre-emptive therapy with azoles in lung transplant patients. Eur. Respir. J. **13:**180–186.
111. **Harari, S., G. F. Schiraldi, E. De Juli, E. Gronda, and A. De Gasperis.** 1997. Relapsing *Aspergillus* bronchitis in a double lung transplant patient, successfully treated with a new oral antimycotic agent. Chest **111:**835–836.
112. **Harousseau, L., A. W. Dekker, A. Stamatoullas-Bastard, A. Fassa, W. Linkesch, J. Gouveia, R. De Bock, M. Rovira, W. F. Seifert, H. Joosen, M. Peeters, and H. Joosen.** 2000. Itraconazole oral solution for primary prophylaxis of fungal infections in patients with hematological malignancy and profound neutropenia: a randomized, double-blind, double-placebo, multicenter trial comparing itraconazole and amphotericin B. Antimicrob. Agents Chemother. **44:**1887–1893.
113. **Hebart, H., C. Bollinger, P. Fisch, J. Sarefati, C. Meisner, M. Baur, J. Loeffler, M. Monod, J.-P. Latge, and H. Einsele.** 2003. Analysis of T-cell responses to *Aspergillus fumigatus* antigens in healthy individual and patients with hematologic malignancies. Blood **100:**4521–4528.
114. **Heinrich, S. D., T. Finney, R. Craver, L. Yin, and M. M. Zembo.** 1991. *Aspergillus* osteomyelitis in patients who have chronic granulomatous disease. J. Bone Joint Surg. Am. **73:**456–460.
115. **Heinz, T., J. Perfect, W. Schell, E. Ritter, G. Ruff, and D. Serafin.** 1996. Soft-tissue fungal infections: surgical management of 12 immunocompromised patients. Plast. Reconstr. Surg. **97:**1391–1399.
116. **Herbrecht, R., D. W. Denning, T. F. Patterson, J. E. Bennett, R. E. Greene, J. W. Oestmann, W. V. Kern, K. A. Marr, P. Ribaud, O. Lortholary, R. Sylvester, R. H. Rubin, J. R. Wingard, P. Stark, C. Durand, D. Caillot, E. Thiel, P. H. Chandrasekar, M. R. Hodges, H. T. Schlamm, P. F. Troke, and B. de Pauw.** 2002. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N. Engl. J. Med. **347:**408–415.
117. **Hertenstein, B., W. V. Kern, T. Schmeiser, M. Stefanic, D. Bunjes, M. Wiesneth, J. Novotny, H. Heimpel, and R. Arnol.** 1994. Low incidence of invasive fungal infections after bone marrow transplantation in patients receiving amphotericin B inhalations during neutropenia. Ann. Hematol. **68:**21–26.
118. **Heykants, J., A. Van Peer, V. Van de Velde, P. Van Rooy, W. Meuldermans, K. Lavrijsen, R. Woestenborghs, J. Van Custem, and G. Cauwenbergh.** 1989. The clinical pharmacokinetics of itraconazole: an overview. Mycoses **32:**67–87.
119. **High, K. P., and R. G. Washburn.** 1997. Invasive aspergillosis in mice immunosuppressed with cyclosporin A, tacrolimus (FK506), or sirolimus (rapamycin). J. Infect. Dis. **175:**222–225.
120. **Hofflin, J. H., I. Potasman, J. C. Baldwin, et al.** 1987. Infectious complications in heart transplant recipients receiving cyclosporine and corticosteroids. Ann. Intern. Med. **106:**209–216.
121. **Horvath, J., S. Dummer, J. Lloyd, B. Walker, W. H. Merrill, and W. H. Frist.** 1993. Infection in the transplanted and native lung after single lung transplantation. Chest **104:**681–685.
122. **Hossain, M. A., T. Miyazaki, K. Mitsutake, H. Kakeya, T. Yamamoto, K. Yanagihara, S. Kawamura, T. Otsubo, Y. Hirakata, T. Tashiro, and S. Kohno.** 1997. Comparison between Wako-WB003 and fungitec G tests for detection of (1–3)-β-D-glucan in systemic mycosis. J. Clin. Lab. Anal. **11:** 73–77.
123. **Hughes, C. E., C. Harris, J. A. Moody, L. R. Peterson, and D. N. Gerding.** 1984. In vitro activities of amphotericin B in combination with four antifungal agents and rifampin against *Aspergillus* spp. Antimicrob. Agents Chemother. **25:**560–562.
124. **Hummel, M., U. Thalmann, G. Jautzke, F. Staib, M. Seibold, and R. Hetzer.** 1992. Fungal infections following heart transplantation. Mycoses **35:**23–34.
125. **Husain, S., B. Alexander, P. Munoz, R. K. Avery, S. Houston, T. Pruett, R. Jacobs, E. A. Dominguez, J. G. Tollemar, C. M. Yu, M. M. Wagener, P. Linden, S. Kusne, and N. Singh.** 2003. Opportunistic mycelial fungi in organ transplant ecipients; emerging importance of non-*Aspergillus* mycelial infections. Clin. Infect. Dis. **37:**221–229.
126. **Husain, S., E. J. Kwak, A. Obman, M. M. Wagener, S. Kusne, J. E. Stout, K. McCurry, and N. Singh.** 2004. Propsective assessment of Platelia *Aspergillus* galactomannan antigen for the diagnosis of invasive aspergillosis in lung transplant recipients. A. J. Transplant. **4:**796–802.
127. **Husain, S., J. Tollemar, E. A. Dominguez, K. Baumgarten, A. Humar, D. L. Paterson, M. M. Wagener, S. Kusne, and N. Singh.** 2003. Changes in the spectrum and risk factors for invasive candidiasis in liver transplant recipients: prospective, multicenter, case-controlled study. Transplantation **75:** 2023–2029.
128. **Husain, S., D. Zaldonis, S. Kusne, and K. R. McCurry.** 2003. Differences in the antifungal prophylaxis strategies among North American and European centers in lung transplantation, abstr. 914. Annu. Meet. Am. Transplant Cong., Washington, D.C., 29 May-3 June 2003.
129. **Husni, R. N., S. M. Gordon, D. L. Longworth, A. Arroliga, P. C. Stillwell, R. K. Avery, J. R. Maurer, A. Mehta, and T. Kirby.** 1998. Cytomegalovirus infection is a risk factor for invasive aspergillosis in lung transplant recipients. Clin. Infect. Dis. **26:**753–755.
130. **Imhof, A., A. Balajee, D. N. Fredricks, J. A. Englund, and K. A. Marr.** 2004. Breakthrough fungal inections in stem cell transplant recipients receiving voriconazole. Clin. Infect. Dis. **39:**743–746.
131. **Imhof, A., A. Balajee, and K. A. Marr.** 2003. New methods to assess susceptibilities of *Aspergillus* isolates to caspofungin. J. Clin. Microbiol. **41:** 5683–5688.
132. **International Bone Marrow Transplant Registry/Autologous Blood & Marrow Transplant Registry**. IBMTR/ABMTR newsletter. http://www.ibmtr.org/newsleter/pdf/2002Feb.pdf.
133. **International Chronic Granulomatous Disease Cooperative Study Group.** 1991. A controlled trial of interferon gamma to prevent infection in chronic granulovatous disease. N. Engl. J. Med. **324:**509–516.
134. **Jantunen, E., V.-J. Amtto, and T. Ruutu.** 2002. Immune reconstitution. Aspergillus infections in allogeneic stem cell transplant recipients: have we made any progress? Bone Marrow Transplant. **30:**925–929.
135. **Junghanss, C., M. Boeckh, R. A. Carter, B. M. Sandmaier, M. B. Maris, D. G. Maloney, T. Chauncey, P. A. McSweeney, M.-T. Little, L. Corey, and R. Storb.** 2002. Incidence and outcome of cytomegalovirus infections following nonmyeloablative compared with myeloablative allogeneic stem cell transplantation, a matched control study. Blood **99:**1978–1985.
136. **Junghanss, C., and K. A. Marr.** 2002. Infectious risks and outcomes after stem cell transplantation: are nonmyeloablative transplants changing the picture? Curr. Opin. Infect. Dis. **15:**347–353.
137. **Junghanss, C., K. A. Marr, R. A. Carter, et al.** 2002. Incidence and outcome of bacterial and fungal infections following nonmyeloablative compared with myeloablative allogeneic hematopoietic stem cell transplantation: a matched control study. Biol. Blood Marrow Transplant. **8:**512–520.
138. **Kami, M., T. Fukui, S. Ogawa, Y. Kazuyama, U. Machida, Y. Tanaka, Y. Kanda, T. Kashima, Y. Yamazaki, T. Hamaki, S. Mori, H. Akiyama, Y. Mutou, H. Sakamaki, K. Osumi, S. Kimura, and H. Hirai.** 2001. Use of real-time PCR on blood samples for diagnosis of invasive aspergillosis. Clin. Infect. Dis. **33:**1504–1512.
139. **Kanj, S. S., K. Welty-Wolf, J. Madden, V. Tapson, M. A. Baz, D. Davis, and J. R. Perfect.** 1996. Fungal infections in lung and heart-lung transplant recipients: report of 9 cases and review of the literature. Medicine **75:**142–156.
140. **Karthaus, M., T. Dooellmann, T. Kimasch, et al.** 2000. Intensive intravenous amphotericin B for prophylaxis of sytemic fungal infections: results of a prospective controlled pilot study in acute leukemia patients. Chemotherapy **46:**293–302.
141. **Kauffman, C. A.** 1996. Quandry about treatment of aspergillomas persists. Lancet **347:**1640.
142. **Kawazu, M., Y. Kanda, Y. Nannya, K. Aoki, M. Kurokawa, S. Chiba, T. Motokura, H. Hirai, and S. Ogawa.** 2004. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1–3)-β-D-glucan test in weekly screening for invasive aspergillsois in patients with hematolgoical disorders. J. Clin. Microbiol. **42:**2733–2741.

143. **Kelsey, S. M., J. M. Goldman, S. McCann, A. C. Newland, J. H. Scarffe, B. A. Oppenheim, and G. J. Mufti.** 1999. Liposomal amphotericin (AmBisome) in the prophylaxis of fungal inections in neutropenic patients: a randomised, double-blind placebo-controlled study. Bone Marrow Transplant. **23:**163–168.
144. **Kessler, R., G. Massard, A. Warter, J. M. Wihlm, and E. Weitzenblum.** 1997. Bronchial-pulmonary artery fistula after unilateral lung transplantation: a case report. J. Heart Lung Transplant. **16:**674–677.
145. **Kirkpatrick, W. R., S. Perea, B. J. Coco, and T. F. Patterson.** 2002. Efficacy of caspofungin alone and in combination with voriconazole in a guinea pig model of invasive aspergillosis. Antimicrob. Agents Chemother. **46:**2564–2568.
146. **Kline, M. W., F. C. Bocobo, M. E. Paul, H. M. Rosenblatt, and W. T. Shearer.** 1994. Successful medical therapy of *Aspergillus* osteomyelitis of the spine in an 11-year-old boy with chronic granulomatous disease. Pediatrics **93:**830–835.
147. **Kohno, S., T. Masaka, and H. Yamaguchi.** 2001. A multicenter, open-label clinical study of FK463 in patients with deep mycosis in Japan, abstr. 834. Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., Chicago, Ill., 22–25 September 2001.
148. **Kohno, S. M. S., J. Iwakawa, Y. Miyazaki, K. Nakamura, H. Kakeya, K. Yanagihara, H. Ohno, Y. Higashiyama, and T. Tashiro.** 2000. Synergistic effects of combination of FK463 with amphotericin B: enhanced efficacy in murine model of invasive pulmonary aspergillosis, abstr. 1686. Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., Toronto, Canada, 17–20 September 2000.
149. **Koizumi, T., K. Kubo, T. Kaneki, M. Hanaoka, T. Hayno, T. Miyahara, et al.** 1998. Pharmacokinetic evaluation of amphotericin B in lung tissue: lung lymph distribution after intravenous injection and airspace distribution after aerosolization and inhalation of amphotericin B. Antimicrob. Agents Chemother. **42:**1597–1600.
150. **Kontoyiannis, D. P., R. E. Lewis, N. Osherov, N. D. Albert, and G. S. May.** 2003. Combination of caspofungin with inhibitors of the calcineurin pathway attenuates growth *in vitro* in *Aspergillus* species. J. Antimicrob. Chemother. **51:**313–316.
151. **Kontoyiannis, D. P., D. Sumoza, J. Tarrand, C. P. Bodey, R. Storey, and I. I. Raad.** 2000. Significance of aspergillemia in patients with cancer: a 10-year study. Clin. Infect. Dis. **31:**188–189.
152. **Kontoyiannis, D. P. R., R. Hachem, R. E. Lewis, G. A. Rivero, A. Torres, J. Thornby, R. Champlin, H. Kantarjian, G. P. Bodey, and I. I. Raad.** 2003. Efficacy and toxicity of caspofungin in combination with liposomal amphotericin B as primary or salvage treatment of invasive aspergillosis in patients with hematologic malignancies. Cancer **98:**292–299.
153. **Kontoyiannis, D. P. R., R. E. Lewis, M. S. Lionakis, N. D. Albert, G. S. May, and I. I. Raad.** 2003. Sequential exposure of *Aspergillus fumigatus* to itraconazole and caspofungin: evidence of enhanced in vitro activity. Diagn. Microbiol. Infect. Dis. **47:**415–419.
154. **Kontoyiannis, D. P. R., R. E. Lewis, N. Sagar, G. May, R. A. Prince, and K. V. Rolston.** 2000. Itraconazole-amphotericin B antagonism in *Aspergillus fumigatus*: an E-test-based strategy. Antimicrob. Agents Chemother. **44:**2915–2918.
155. **Koss, T., B. Bagheri, C. Zeana, M. F. Romagnoli, and M. E. Grossman.** 2002. AmphotericinB-resistant *Aspergillus flavus i*nfection successfully treated with caspofungin, a novel antifungal agent. J. Am. Acad. Dermatol. **46:**945–947.
156. **Kountakis, S. E., Jr. J. V. Kemper, C. Y. Chang, D. J. DiMaio, and C. M. Stiernberg.** 1997. Osteomyeltiis of the base of the skull secondary to *Aspergillus*. Am. J. Otolaryngol. **18:**19–22.
157. **Kramer, M. R., D. W. Denning, S. E. Marshall, D. J. Ross, G. Berry, N. J. Lewiston, D. A. Stevens, and J. Theodore.** 1991. Ulcerative tracheobronchitis after lung transplantation. A new form of invasive aspergillosis. Am. Rev. Respir. Dis. **144:**552–556.
158. **Krieter, P., B. Flannery, T. Musick, R. Courtney, J. Patrick, and M. Laughlin.** 2002. Pharmacokinetics and excretion of 14C-posaconazole following oral administration in male subjects. 42nd Intersci. Conf. Antimicrob. Agents Chemother., San Diego, Calif., 27–30 September 2002.
159. **Kurtz, M. B., I. B. Heath, J. Marrinan, S. Dreikorn, J. Onishi, and C. Douglas.** 1994. Morphological effects of lipopeptides against *Aspergillus fumigatus* correlate with activities against (1,3)-β-D-glucan synthase. Antimicrob. Agents Chemother. **38:**1480–1489.
160. **Kusne, S., J. Torre-Cisneros, R. Manez, et al.** 1992. Factors associated with invasive lung aspergillosis and significance of positive cultures after liver transplantation. J. Infect. Dis. **166:**1379–1383.
161. **Kwak, E., K. Abu-Elmagd, J. Bond, M. F. Zak, M. McHenry, and S. Kusne.** 2002. Invasive fungal infections in adult small bowel transplant recipients, abstr. K-1231. Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., San Diego, Calif., 27–30 September 2002.
162. **Kwak, E. J., S. Husain, A. Obman, L. Meinke, J. Stout, S. Kusne, M. M. Wagener, and N. Singh.** 2004. Efficacy of galactomannan antigen using Platelia *Aspergillus* enzyme immunoassay for the diagnosis of invasive aspergillosis in liver transplant recipients. J. Clin. Microbiol. **42:**435–438.
163. **Lass-Florl, C., G. Kofler, G. Kropshofer, J. Hermans, A. Kreczy, M. P. Dierich, and D. Niederwieser.** 1998. In-vitro testing of susceptibility to amphotericin B is a reliable predictor of clinical outcome in invasive aspergillosis. J. Antimicrob. Chemother. **42:**497–502.
164. **Latgè, J.-P.** 1999. *Aspergillus fumigatus* and aspergillosis. Clin. Microbiol. Rev. **12:**310–350.
165. **Latgè, J.-P., H. Kobayashi, J. P. Desbeaupuis, M. Diaquin, J. Sarfati, and K.-P. Wieruszeski.** 1994. Chemical and immunological characterization of extracellular galactomannan of *Aspergillus fumigatus*. Infect. Immun. **62:**5424–5433.
166. **Laursen, A., S. Mogensen, H. Andersen, P. Andersen, and S. Ellermann-Erksen.** 2001. The impact of CMV on the respiratory burst of macrophages in response to *Pneumocystis carinii*. Clin. Exp. Immunol. **123:**239–246.
167. **Limaye, A. P., L. Corey, D. M. Koelle, C. L. Davis, and M. Boeckh.** 2000. Emergence of ganciclovir-resistant cytomegalovirus disease among solid organ transplant recipients. Lancet **356:**645–649.
168. **Linden, P., K. Coley, D. kramer, S. Kusne, and J. Fung.** 1999. Invasive aspergillosis in liver transplant recipients: comparison of outcome with amphotericin B lipid complex and conventional amphotericin B therapy. Transplantation **67:**S232.
169. **Lionakis, M. S., and D. A. Kontoyiannis.** 2003. Glucocorticoids and invasive fungal infections. Lancet **362:**1828–1838.
170. **Loeffler, J., K. Kloepfer, H. Hebart, L. Najvar, J. R. Graybill, W. R. Kirkpatrick, T. F. Patterson, K. Dietz, R. Bialek, and H. Einsele.** 2002. Polymerase chain reaction detection of *Aspergillus* DNA in experimental models of invasive aspergillosis. J. Infect. Dis. **158:**1203–1206.
171. **Lorf, T., F. Braun, R. Ruchel, A. Muller, B. Sattler, and B. Ringe.** 1999. Systemic mycoses during prophylactical use of liposomal amphotericin B (Ambisome) after liver transplantation. Mycoses **42:**47–53.
172. **Machetti, M., M. Feasi, N. Mordini, M. T. Van Lint, A. Bacigalupo, J. P. Latgè, J. Sarfati, and C. Viscoli.** 1998. Comparison of an enzyme immunoassay and a latex agglutination system for the diagnosis of invasive aspergillosis in bone marrow transplant recipients. Bone Marrow Transplant. **21:**917–921.
173. **Machetti, M., M. Zotti, L. Veroni, N. Mordani, M. T. Van Lint, A. Bacigalupo, D. Paola, and C. Viscoli.** 2000. Antigen detection in the diagnosis and management of a patient with probable cerebral aspergillosis treated with voriconazole. Transplant. Infect. Dis. **2:**140–144.
174. **Maderazo, E. G., N. Hickingbotham, B. Cooper, and A. Murcia.** 1990. *Aspergillus* endocarditis: cure without surgical valve replacement. South. Med. J. **83:**351–352.
175. **Maertens, J., J. Van Eldere, J. Verhaegen, E. Verbeken, J. V. Verschakelen, and M. Boogaerts.** 2002. Use of circulating galactomannan screening for early diagnosis of invasive aspergillosis in allogeneic stem cell transplant recipients. J. Infect. Dis. **186:**1297–1306.
176. **Maertens, J., J. Verhaegen, K. Lagrou, J. Van Eldere, and M. Boogaerts.** 2001. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. Blood **97:**1604–1610.
177. **Maertens, J. J., J. Verhaegan, H. Demuynck, P. Brock, G. Verhoef, P. Vandenberghe, J. Van Eldere, L. Verbist, and M. Boogaerts.** 1999. Autopsy-controlled prospective evaluation of serial screening for circulating galactomannan by a sandwich enzyme-linked immunosorbent assay for hematological patients at risk for invasive aspergillsois. J. Clin. Microbiol. **37:**3223–3228.
178. **Maetens, J., I. Raad, G. Petrikkos, M. Boogaerts, D. Selleslag, F. B. Petersen, C. A. Sable, N. A. Kartsonis, A. Ngai, A. Taylor, T. F. Patterson, D. W. Denning, and T. J. Walsh.** 2004. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. Clin. Infect. Dis. **39:**1563–1571.
179. **Maesaki, S., S. Kohno, M. Kaku, H. Koga, and K. Hara.** 1994. Effects of antifungal agent combinations administered simultaneously and sequentially against *Aspergillus fumigatus*. Antimicrob. Agents Chemother. **38:**2843–2845.
180. **Manavathu, E. K., G. J. Alangaden, and P. H. Chandrasekar.** 1998. In-vitro isolation and antifungal susceptibility of amphotericin B-resistant mutants of *Aspergillus fumigatus*. J. Antimicrob. Chemother. **41:**615–619.
181. **Manavathu, E. K., G. J. Alangaden, and P. H. Chandrasekar.** 2003. Differential activity of triazoles in two-drug combinations with the echinocandin caspofungin against *Aspergillus fumigatus*. J. Antimicrob. Chemother. **51:**1423–1425.
182. **Marbello, L., A. Nosari, G. Carrafiello, M. Anghilieri, C. Cesana, A. M. Cafro, G. D'Avanzo, and E. Morra.** 2003. Successful treatment with voriconazole of cerebral aspergillosis in an hematologic patient. Haematologica **88:**ECR05.
183. **Maris, M., M. Boeckh, B. Storer, M. Dawson, K. White, M. Keng, B. Sandmaier, D. Maloney, R. Storb, and J. Storek.** 2003. Immunologic recovery after hematopoietic cell transplantation with nonmyeloblative conditioning. Exp. Hematol. **31:**941–952.
184. **Marr, K., R. Hachem, G. Papanicolaou, J. Somari, J. M. Arduino, J. Lipka, A. Ngai, J. Chodakewitz, and C. Sable.** 2003. Retrospective study of con-

comitant use of caspofungin with cyclosporin A (CsA) in patients treated during marketed use. Blood **102:**11.
185. **Marr, K. A., M. Boeckh, R. A. Carter, H. W. Kim, and L. Corey.** 2004. Combination antifungal therapy for invasive apergillosis. Clin. Infect. Dis. **39:**797–802.
186. **Marr, K. A., R. A. Carter, M. Boeckh, P. Martin, and L. Corey.** 2002. Invasive aspergillosis in allogeneic stem cell transplant recipients; changes in epidemiology and risk factors. Blood **100:**4358–4366.
187. **Marr, K. A., R. A. Carter, F. Crippa, A. Wald, and L. Corey.** 2002. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin. Infect. Dis. **34:**909–917.
188. **Marr, K. A., F. Crippa, W. Leisenring, M. Hoyle, M. Boeckh, S. A. Balajee, W. G. Nichols, B. Musher, and L. Corey.** 2004. Itraconazole versus fluconazole for prevention of fungal infections in patients receiving allogeneic stem cell transplants. Blood **103:**1527–1533.
189. **Marr, K. A., W. Leisenring, F. Crippa, J. Slattery, L. Corey, M. Boeckh, and G. B. McDonald.** 2004. Cyclophosphamide metabolism is affected by azole antifungals. Blood **103:**1557–1559.
190. **Martino, R., M. D. Caballero, C. Canals, J. San Miguel, J. Sierra, M. Rovira, C. Solano, J. Bargay, J. Pérez-Simon, A. León, J. Sarrá, S. Brunet, R. de la Cámara, et al.** 2001. Reduced-intensity conditioning reduces the risk of severe infections after allogeneic peripheral blood tem cell transplantation. Bone Marrow Transplant. **28:**341–347.
191. **Martino, R., R. Lopez, A. Sureda, S. Brunet, and A. Domingo-Albos.** 1997. Risk of reactivation of a recent invasive fungal infection in patients with hematological malignancies undergoing further intensive chemo-radiotherapy. A single-center experience and review of the literature. Haematologica **82:**297–304.
192. **Martino, R., M. Subira, M. Rovira, C. Solano, L. Váquez, G. F. Sanz, A. Urbano-Ispizua, S. Brunet, R. de la Cámara, et al.** 2002. Invasive fungal infections afater allogeneic peripheral blood stem cell transplantation: incidence and risk fators in 395 patients. Br. J. Hematol. **116:**475–482.
193. **Marty, F. M., S. J. Lee, E. P. Alyea, R. J. Soiffer, J. H. Anten, and L. P. Baden.** 2003. Infliximab use in patients with severe graft-versus-host disease and other emerging risk factors of non-*Candida* invasive fungal infections in allogeneic hematopoietic stem cell transplant recipients: a cohort study. Blood **102:**2768–2776.
194. **Mattei, D., N. Mordani, C. Lo Nigro, D. Ghirardo, M. T. Ferrua, M. Osenda, A. Gallamini, A. Bacigalupo, and C. Viscoli.** 2002. Voriconazole in the management of invasive aspergillosis in two patients with acute myeloid leukemia undergoing stem cell transplantation. Bone Marrow Transplant. **30:**967–970.
195. **Maurer, J. R., D. E. Tullis, R. F. Grossman, H. Velland, T. L. Winton, and G. A. Patterson.** 1992. Infectious complications following isolated lung transplantation. Chest **101:**1056–1059.
196. **McLaughlin, L., A. Balajee, W. Leisenring, M. Tabouret, C. Bentsen, C. Ferrera, and K. A. Marr.** 2002. Bio-Rad Patelia® *Aspergillus* EIA detection of Aspergillus galactomannan antigen in human serum: performance evaluation in a large bone marrow transplant center. Focus on Fungal Infections, Phoenix, Ariz., 20–22 March 2002.
197. **McSweeney, P. A., D. Niedewieser, J. A. Shizuru, B. M. Sanmaier, A. J. Molina, D. G. Maloney, T. R. Chasuncey, T. A. Gooley, U. Hegenbart, R. A. Nash, J. Radich, J. L. Wagner, S. Minor, F. R. Applebaum, W. I. Bensinger, E. Bryant, M. E. D. Flowers, G. E. Georges, F. C. Grumet, H.-P. Kiem, B. Torok-Storb, C. Yu, K. G. Blume, and R. F. Storb.** 2001. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood **97:**3390–3400.
198. **Mehrad, B., G. Paciocco, F. J. Martinez, et al.** 2001. Spectrum of Aspergillus infection in lung transplant recipients: case series and review of the literature. Chest **119:**169–175.
199. **Mehrad, B., R. M. Strieter, and T. J. Standiford.** 1999. Role of TNF-α in pulmonary host defense in murine invasive aspergillosis. J. Immunol. **162:**1633–1640.
200. **Menichetti, F., A. Del Favero, P. Martino, G. Bucaneve, A. Micozzi, C. Girmenia, G. Barbabietola, L. Pugano, P. Leoni, G. Specchia, A. Caiozzo, R. Raimondi, F. Mandelli, and the GIMEMA Infection Program.** 1999. Itraconazole oral solution as prophylaxis for fungal infections in neutropenic patients with hematologic malignancies: a randomized, placebo-controlled, double-blind, multicenter trial. Clin. Infect. Dis. **28:**250–255.
201. **Mennink-Kersten, M. A. S., R. R. Klont, A. Warris, H. J. M. Op Den Camp, and P. E. Verweij.** 2004. Bifidobacterium lipoteichoic acid and false ELISA reactivity in *Aspergillus* antigen detection. Lancet **363:**325–327.
202. **Miyazaki, T., S. Kohno, K. Mitsutake, S. Maesaki, I. Tanaka, N. Ishikawa, and K. Hara.** 1995. Plasma (1,3)-β-D-glucan and fungal antigenemia in patients with candidemia, aspergillosis, and cryptococcosis. J. Clin. Microbiol. **33:**3115–3118.
203. **Monforte, V., A. Roman, J. Gavalda, C. Bravo, L. Tenorio, A. Ferrer, J. Maestre, and F. Morell.** 2001. Nebulized amphotericin B prophylaxis for *Aspergillus* infection in lung transplantation study of risk factors. J. Heart Lung Transplant. **20:**1274–1281.
204. **Monforte, V., A. Roman, J. Gavalda, R. López, L. Pou, M. Simó, S. Aguadé, B. Soriano, C. Bravo, and F. Morell.** 2003. Nebulized amphotericin B concentration and distribution in the respiratory tract of lung-transplanted patients. Transplantation **75:**1571–1574.
205. **Montoya, J. G., S. V. Chaparro, D. Celis, J. A. Cortes, A. N. Leung, R. C. Robbins, and D. A. Stevens.** 2003. Invasive aspergillosis in the setting of cardiac transplantation. Clin. Infect. Dis. **37:**S281–S292.
206. **Montoya, J. G., L. F. Giraldo, B. Efron, E. B. Stinson, P. Gamberg, S. Hunt, N. Giannetti, J. Miller, and J. S. Remington.** 2001. Infectious complications among 620 consecutive heart transplant patients at Stanford University Medical Center. Clin. Infect. Dis. **33:**629–640.
207. **Moosa, M. Y., G. J. Alangaden, E. Manavathu, and P. H. Chandrasekar.** 2002. Resistance to amphotericin B does not emerge during treatment for invasive aspergillosis. J. Antimicrob. Chemother. **49:**209–213.
208. **Morecki, S., Y. Gelfand, A. Nagler, R. Or, E. Naparstek, G. Varadi, D. Engelhard, A. Akerstein, and S. Slavin.** 2001. Immune reconstitution following allogeneic stem cell transplantation in recipients conditioned by low intensity vs myeloablative regimen. Bone Marrow Transplant. **28:**243–249.
209. **Morgentern, G. R., A. G. Prentice, H. G. Prentice, J. E. Ropner, S. A. Schey, and D. W. Warnock.** 1999. A randomized controlled trial of itraconazole versus fluconazole for the prevention of fungal infections in patients with haematological malignancies. Br. J. Haematol. **105:**901–911.
210. **Morrison, V., R. Haake, and D. Weisdorf.** 1994. Non-Candida fungal infections after bone marrow transplantation: risk factors and outcome. Am. J. Med. **96:**497–503.
211. **Mosquera, J., A. Sharp, C. B. Moore, P. A. Warn, and D. W. Denning.** 2002. In vitro interaction of terbinafine with itraconazole, fluconazole, amphotericin B and 5-flucytosine against *Aspergillus* spp. J. Antimicrob. Chemother. **50:**189–194.
212. **Munoz, P., L. Alcala, M. Sanchez Conde, J. Palomo, J. Yanez, T. Pelaez, and E. Bouza.** 2003. The isolation of *Aspergillus fumigatus* from respiratory tract specimens in heart transplant recipients is highly predictive of invasive aspergillosis. Transplantation **75:**326–329.
213. **Munoz, P., C. Rodriguez, E. Bouza, J. Palomo, J. F. Yanez, M. J. Dominguez, and M. Desco.** 2004. Risk factors of invasive aspergillosis after heart transplantation: protective role of oral itraconazole prophylaxis. Am. J. Transplant. **4:**636–643.
214. **Munoz, P., J. Torre, E. Bouza, A. Moreno, A. Echantz, J. Fortun, C. Lumbreras, J. M. Aguado, I. Losada, V. Cuervas, M. Gurgui, J. M. Cisneros, M. Montejo, and C. Farinas.** 1996. Invasive aspergillosis in transplant recipients. A large multicenter study, p. 242. Program Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., New Orleans, La., 1996.
215. **Nachbaur, D., F.-M. Fink, W. Nussbaumer, A. Gächter, G. Kropshofer, and C. Ludescher.** 1997. CD34+-selected autologous peripheral blood stem cell transplantation (PBSCT) in patients with poor-risk hematological malignancies and solid tumors. A single-centre experience. Bone Marrow Transplantation. **20:**827–834.
216. **Nagai, H., J. Guo, H. Choi, and V. Kurup.** 1995. Interferon-γ and tumor necrosis factor-α protect mice from invasive aspergillosis. J. Infect. Dis. **172:**1554–1560.
217. **Nakajima, M. T. S., K. Yoshida, Y. Wakai, T. Nakai, F. Ikeda, T. Goto, Y. Niki, and T. Matsushima.** 2000. Pathologic findings in a murine pulmonary aspergillosis model: treatment with FK463, amphotericin B and a combination of FK463 and amphotericin B, abstr. 1685. Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., Toronto, Canada, 17–20 September 2000.
218. **Nathan, S. D., A. F. Shorr, M. E. Schmidt, and A. B. Burton.** 2000. *Aspergillus* and endobronchial abnormalities in lung transplant recipients. Chest **118:**403–407.
219. **NCCLS.** 2004. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard M38-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.
220. **Netea, M. G., A. Warris, W. M. Van der Meer, M. J. Fenton, J. G. Verver-Janssen, L. E. H. Jacobs, T. Andresen, P. E. Verweij, and B. J. Kulberg.** 2003. *Aspergillus fumigatus* evades immune recognition during germination through loss of Toll-like receptor-4-mediated signal transduction. J. Infect. Dis. **188:**320–326.
221. **Nunley, D. R., N. P. Ohori, W. F. Grgurich, A. T. Iacono, P. A. Williams, R. J. Keenan, and J. H. Dauber.** 1998. Pulmonary aspergillosis in cystic fibrosis lung transplant recipients. Chest **114:**1321–1329.
222. **Obayashi, T., M. Yoshida, T. Mori, H. Goto, A. Yasuoka, H. Iwasaki, H. Teshima, S. Kohno, A. Horiuchi, A. Ito, H. Yamaguchi, K. Shimada, and T. Kawai.** 1995. Plasma (1–3)-β-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. Lancet **345:**17–20.
223. **Obayashi, T., M. Yoshida, H. Tamura, J. Aketagawa, S. Tanaka, and T. Kawai.** 1992. Determination of plasma (1–3)-β-D-glucan: a new diagnosis aid to deep mycosis. J. Med. Vet Mycol. **30:**275–280.
224. **Odds, F. C.** 1982. Interactions among amphotericin B, 5-fluorocytosine, ketoconazole, and miconazole against pathogenic fungi in vitro. Antimicrob. Agents Chemother. **22:**763–770.
225. **O'Donnell, M. R., G. M. Schmidt, B. R. Tegtmeier, et al.** 1994. Prediction of systemic fungal infection in allogeneic marrow recipients: impact of amphotericin prophylaxis in high-risk patients. J. Clin. Oncol. **12:**827–834.

226. **Offidani, M., L. Corvatta, A. Olivieri, S. Rupoli, J. Frayfer, A. Mele, E. Manso, M. Montanari, R. Centurioni, and P. Leoni.** 1999. Infectious complications after autologous peripheral blood progenitor cell transplantation followed by G-CSF. Bone Marrow Transplant. **24:**1079–1087.
227. **Offner, F., C. Cordonnier, P. Ljungman, H. G. Prentice, D. Engelhard, D. De Bacquer, F. Meunier, and B. de Pauw.** 1998. Impact of previous aspergillosis on the outfome of bone marrow transplantation. Clin. Infect. Dis. **26:**1098–1103.
228. **Oren, I., N. Haddad, R. Finkelstein, and J. M. Rowe.** 2001. Invasive pulmonary aspergillosis in neutropenic patients during hospital construction: before and after chemoprophylaxis and institution of HEPA filters. Am. J. Hematol. **66:**257–262.
229. **Ostrosky-Zeichner, L., B. Alexander, D. Kett, J. Vazquez, P. Pappas, F. Saeki, P. A. Ketchum, J. R. Wingard, R. A. Schiff, H. Tamura, M. A. Finkelman, and J. H. Rex.** 2003. Multicenter clinical evaluation of the (1–3) B-Glucan (BG) assay (Glucatell) as an aid to diagnosis of invasive fungal infections (IFI) in humans, abstr. M-1034a. Program Abstr. Addendum 43rd ICAAC, Chicago, Ill., 14–17 September 2003.
230. **Palmer, S., R. H. Drew, J. D. Whitehouse, V. F. Tapson, D. R. Duane, R. R. McConnell, S. S. Kanj, and J. R. Perfect.** 2001. Safety of aerosolized amphotericin B lipid complex in lung transplant recipients. Transplantation **72:**545–548.
231. **Panackal, A. A., A. Dahlman, K. T. Keil, C. L. Peterson, L. Mascola, S. Mirza, M. Phelan, B. A. Lasker, M. E. Brandt, J. Carpenter, M. Bell, D. W. Warnock, R. A. Hajjeh, and J. Morgan.** 2003. Outbreak of invasive aspergillosis among renal transplant recipients. Transplantation **15:**1050–1053.
232. **Paradowski, L. J.** 1997. Saprophytic fungal infections and lung transplantation–revisited. J. Heart Lung Transplant. **16:**524–531.
233. **Parkin, J., and B. Cohen.** 2001. An overview of the immune system. Lancet **357:**1777–1789.
234. **Pasic, S., M. Abinun, B. Pistignjat, B. Vlajic, J. Rakic, L. Sarjanovic, and N. Ostojic.** 1996. *Aspergillus* osteomyelitis in chronic granulomatous disease: treatment with recombinant gamma-interferon and itraconazole. Pediatr. Infect. Dis. J. **15:**833–834.
235. **Patel, R., D. Portelar, A. D. Bradley, W. S. Harmsen, J. J. Larson-Keller, D. M. Ilstrup, M. R. Keataing, R. H. Wiesner, R. A. F. Krom, and C. V. Paya.** 1996. Candida and non-Candida fungal infections after liver transplantation. Transplantation **62:**926–934.
236. **Paterson, D. L., E. Dominguez, F. Y. Chang, D. R. Snydman, and N. Singh.** 1998. Infective endocarditis in solid organ transplant recipients. Clin. Infect. Dis. **26:**689–694.
237. **Paterson, D. L., and N. Singh.** 1999. Invasive aspergillosis in transplant recipients. Medicine **78:**123–138.
238. **Paterson, P. J., S. Seaton, H. G. Prentice, and C. C. Kibbler.** 2003. Treatment failure in invasive aspergillosis: susceptibility of deep tissue isolates following treatment with amphotericin B. J. Antimicrob. Chemother. **52:** 873–876.
239. **Patterson, T. F., P. Miniter, J. L. Ryan, and V. T. Andriole.** 1998. Effect of immunosuppression and amphotericin B on *Aspergillus* antigenemia in an experimental model. J. Infect. Dis. **158:**415–422.
240. **Perea, S., G. Gonzalez, A. W. Fothergill, W. R. Kirkpatrick, M. G. Rinaldi, and T. F. Patterson.** 2002. In vitro interaction of caspofungin acetate with voriconazole against clinical isolates of *Aspergillus* spp. Antimicrob. Agents Chemother. **46:**3039–3041.
241. **Perfect, J. R., M. E. Klotman, C. C. Gilbert, D. D. Crawford, and G. L. Rosner.** 1992. Prophylactic intravenous amphotericin B in neutropenic autologous bone marrow transplant recipients. J. Infect. Dis. **165:**891–897.
242. **Peters, C., M. Minkov, S. Matthes-Mrtin, U. Potschger, V. Witt, G. Mann, P. Hocker, N. Worel, J. Stary, T. Klingebiel, and H. Gadner.** 1999. Leucocyte transfusions from rhG-CSF or prednisolone stimulated donors for treatment of severe infections in immunocompromised neutropenic patients. Br. J. Haematol. **106:**689–696.
243. **Peterson, P. K., R. Ferguson, D. S. Fryd, H. H. Balfour, Jr., J. Rynasiewicz, and R. L. Simmons.** 1982. Infectious diseases in hospitalized renal transplant recipients: a prospective study of a complex and evolving problem. Medicine **61:**360–372.
244. **Petraitiene, R., W. Petraitis, A. H. Groll, et al.** 2001. Antifungal activity and pharmacokinetics of posaconazole (SCH 56592) in treatment and prevention of experimental invasive pulmonary aspergillosis: correlation with galactomannan antigenemia. Antimicrob. Agents Chemother. **45:**857–869.
245. **Petraitis, V., R. Petraitiene, A. Sarafandi, A. M. Kelaher, C. A. Lyman, H. E. Casler, T. Sien, A. H. Groll, J. Bacher, N. A. Avila, and T. J. Walsh.** 2003. Combination therapy in treatment of experimental pulmonary aspergillosis: synergistic interaction between an antifungal triazole and an echinocandin. J. Infect. Dis. **187:**1834–1843.
246. **Petraitis, V. P., R. Petraitiene, R. J. Leguit, M. Candelario, T. Sien, J. Peter, A. Field-Ridley, R. Irwin, T. J. Groll, and T. J. Walsh.** 1999. Combination antifungal therapy with FK463 plus amphotrticin B in treatment of experimental pulmonary aspergillosis, abstr. 2003. Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., San Francisco, Calif., 26–29 September 1999.
247. **Petrosillo, N., A. M. Pellicelli, S. Cicalini, A. Conte, D. Goletti, and F. Palmieri.** 2001. Endocarditis caused by *Aspergillus* species in injection drug users. Clin. Infect. Dis. **33:**e97–99.
248. **Pfaller, M., D. Diekema, S. Messer, L. Boyken, R. Hollis, R. Jones, and the International Fungal Surveillance Participant Group.** 2003. In vitro activities of voriconazole, posaconazole, and four licensed system antifungal agents against *Candida* species infrequently isolated from blood. J. Clin. Microbiol. **41:**78–83.
249. **Polak, A.** 1987. Combination therapy of experimental candidiasis, cryptococcosis, aspergillosis and wangiellosis in mice. Chemotherapy **33:**381–395.
250. **Polak, A., H. J. Scholer, and M. Wall.** 1982. Combination therapy of experimental candidiasis, cryptococcosis and aspergillosis in mice. Chemotherapy **28:**461–479.
251. **Powles, R., J. Mehta, S. Kulkami, J. Treleaven, B. Millar, J. Marsden, V. Shepherd, A. Rowland, B. Sirohi, D. Tait, C. Horton, S. Long, and S. Singhal.** 2000. Allogeneic blood and bone-marrow stem-cell transplantation in haematological malignant diseases: a randomised trial. Lancet **355:** 1231–1237.
252. **Rao, K., and V. Saha.** 2000. Medical management of *Aspergillus flavus* endocarditis. Pediatr. Hematol. Oncol. **17:**425–427.
253. **Rasmussen, C., C. Garen, S. Brining, R. L. Kincaid, R. L. Means, and A. R. Means.** 1994. The calmodulin-dependent protein phosphatase catalytic subunit (calcineurin A) is an essential gene in *Aspergillus nidulans*. EMBO J. **13:**2545–2552.
254. **Relman, D. A.** 2003. Shedding light on microbial detection. N. Engl. J. Med. **349:**2162–2163.
255. **Ribaud, P., C. Chastang, J.-P. Latgè, L. Baffroy-Lafitte, N. Parquet, A. Devergie, H. Epérou, F. Sélimi, V. Rocha, F. Derouin, G. Sociée, and E. Gluckman.** 1999. Survival and prognostic factors of invasive *Aspergillus* after allogeneic bone marrow transplantation. Clin. Infect. Dis. **28:**322–330.
256. **Rider, T. H., M. S. Petrovick, F. E. Nargi, J. D. Harper, E. D. Schwoebel, R. H. Mathews, D. J. Blanchard, L. T. Bortolin, A. M. Young, J. Chen, and M. A. Hollis.** 2003. A B-cell based sensor for rapid identification of pathogens. Science **301:**213.
257. **Riley, D. K., A. T. Pavia, P. G. Beatty, et al.** 1994. The prophylactic use of low-dose amphotericin B in bone marrow transplant patients. Am. J. Med. **97:**509–514.
258. **Roffey, S. J., S. Cole, P. Comby, D. Gibson, S. G. Jezequel, A. N. Nedderman, D. A. Smith, D. K. Walker, and N. Wood.** 2003. The disposition of voriconazole in mouse, rat, rabbit, guinea pig, dog, and human. Drug Metab. Dispos. **31:**731–741.
259. **Rogers, J., N. Singh, D. R. Carrigan, S. Rohal, S. Kusne, K. K. Knox, M. M. Wagener, and J. J. Fung.** 2000. Clinical relevance of human herpesvirus-6 infection in liver transplant recipients: role in pathogenesis of fungal infections, neurologic complications, and impact on outcome. Transplantation **69:**2566–2573.
260. **Rohrlich, P., J. Sarfati, P. Mariani, M. Duval, A. Carol, C. Saint-Martin, E. Bingen, J. P. Latge, and E. Vilmer.** 1996. Prospective sandwich enzyme-linked immunosorbent assay for serum galactomannan: early predictive value and clinical use in invasive aspergillosis. Pediatr. Infect. Dis. J. **15:** 232–237.
261. **Roilides, E., A. Dimitriadou-Georgiadou, T. Sein, I. Kadiltsoglou, and T. J. Walsh.** 1998. Tumor necrosis factor alpha enhances antifungal activities of polymorphonuclear and mononuclear phagocytes against *Aspergillus fumigatus*. Infect. Immun. **66:**5999–6003.
262. **Roilides, E., A. Dimitriadou, I. Kadiltsoglou, T. Sein, J. Karpouzas, P. A. Pizzo, and T. J. Walsh.** 1997. IL-10 exerts suppressive and enhancing effects on antifungal activity of mononuclear phagocytes against *Aspergillus fumigatus*. J. Immunol. **158:**322–329.
263. **Roilides, E., T. Sein, A. Holmes, S. Chanock, C. Blake, P. A. Pizzo, and T. J. Walsh.** 1999. Effects of macrophage colony-stimulating factor on antifungal activity of mononuclear phagocytes against *Aspergillus fumigatus*. J. Infect. Dis. **172:**1028–1934.
264. **Romani, L.** 1997. The T cell response against fungl infections. Curr. Opin. Infect. Dis. **9:**484–490.
265. **Romero, A., P. L. Pogamp, L. G. Nilsson, and N. Wood.** 2002. Effect of voriconazole on the pharmacokinetics of cyclosporine in renal transplant patients. Pharmacol. Ther. **71:**226–234.
266. **Roney, P., C. C. Barr, C. H. Chun, and M. J. Raff.** 1986. Endogenous *Aspergillus* endophthalmitis. Rev. Infect. Dis. **8:**95–98.
267. **Rubin, M. A., K. C. Carroll, and B. C. Cahill.** 2002. Caspofungin in combination with itraconazole for the treatment of invasive apergillosis in humans. Clin. Infect. Dis. **34:**1160–1161.
268. **Ryder, N. S., and I. Leitner.** 2001. Synergistic interaction of terbinafine with triazoles or amphotericin B against *Aspergillus* species. Med. Mycol. **39:** 91–95.
269. **Sabatelli, F.** 2003. In vitro and in vivo interaction of posaconazole and caspofungin against *Aspergillus*, abstr. M-990. Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., Chicago, Ill., 13–17 September 2003.
270. **Sable, C. A., B. Y. Nguyen, J. A. Chodakewitz, and M. J. DiNubile.** 2002. Safety and tolerability of caspofungin acetate in the treatment of fungal infections. Transplant. Infect. Dis. **4:**25–30.
271. **Salerno, C. T., D. W. Ouyang, T. S. Pederson, D. M. Larson, J. P. Shake,**

E. M. Johnson, and M. A. Maddaus. 1998. Surgical therapy for pulmonary aspergillosis in immunocompromised patients. Ann. Thorac. Surg. **65:**1415–1419.
272. **Sansone, A., D. Belle, P. Statkevich, D. Joseph, B. Kantesaria, M. McGlaughlin, and R. Courtney.** 2003. Effect of posaconazole on the pharmacokinetics of tacrolimus in healthy volunteers, abstr. A-1603. Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., Chicago, Ill., 14–17 September 2003.
273. **Schaffner, A., and A. Bohler.** 1993. Amphotericin B refractory aspergillosis after itraconazole: evidence for significant antagonism. Mycoses **36:**421–424.
274. **Schaffner, A., H. Douglas, and A. Braude.** 1982. Selective protection against conidia by mononuclear and against mycelia by polymorphonuclear phagocytes in resistance to *Aspergillus*: observations on these two lines of defense *in vitro* with human and mouse phagocytes. J. Clin. Investig. **69:**617–631.
275. **Schaffner, A., and P. G. Frick.** 1985. The effect of ketoconazole on amphotericin B in a model of disseminated aspergillosis. J. Infect. Dis. **151:**901–910.
276. **Schelenz, S., and D. J. Goldsmith.** 2003. *Aspergillus* edophthalmitis: an unusual complication of disseminated infection in renal transplant patients. J. Infect. **47:**336–343.
277. **Schmidt, H.-J.** 1993. New methods of delivery of amphotericin B. Clin. Infect. Dis. **17:**S501–S506.
278. **Schmitt, H. J., E. M. Bernard, M. Hauser, and D. Armstrong.** 1988. Aerosol amphotericin B is effective for prophylaxis and therapy in a rat model of pulmonary aspergillosis. Antimicrob. Agents Chemother. **32:**1676–1679.
279. **Schrier, R. D., G. P. A. Rice, and M. B. A. Oldstone.** 1986. Suppression of natural killer cell activity and T cell proliferation by fresh isolates of human cytomegalovirus. J. Infect. Dis. **153:**1084–1094.
280. **Schulman, L. L., C. R. Smith, R. Drusin, E. A. Rose, Y. Enson, and K. Reemtsma.** 1988. Respiratory complications of cardiac transplantation. Am. J. Med. Sci. **296:**1–10.
281. **Schwartz, S., G. Behre, V. Heinemann, H. Wandt, E. Schilling, M. Arning, A. Trittin, W. V. Kern, O. Boenisch, D. Bosse, K. Lenz, W. D. Ludwig, W. Hiddenmann, W. Siegret, and J. Beyer.** 1999. Aersolized amphotericin B inhalations as prophylaxis of invasive *Aspergillus* infections during prolonged neutropenia: results of a prospective randomized trial. Blood **93:**3654–3661.
282. **Schwartz, S., D. Milatovic, and E. Thiel.** 1997. Successful treatment of cerebral aspergillosis with a novel triazole (voriconazole) in a patient with acute leukaemia. Br. J. Haematol. **97:**663–665.
283. **Selby, R., C. B. Ramirez, R. Singh, I. Kleopoulos, S. Kusne, T. E. Starzl, and J. Fung.** 1997. Brain abscess in solid organ transplant recipients receiving cyclosporine-based immunosuppression. Arch. Surg. **132:**304–310.
284. **Serrano, M. C., D. Morilla, A. Valverde, M. Chavez, A. Espinel-Ingroff, and R. Claro.** 2003. Comparison of Etest with modified broth microdilution method for testing susceptibility of *Aspergillus* spp. to voriconazole. J. Clin. Microbiol. **41:**2570–2572.
285. **Shalit, L., Y. Shadkchan, Z. Samra, and N. Osherov.** 2003. In vitro synergy of caspofingin and itraconazole against *Aspergillus* spp.: MIC versus minimal effective concentration end points. Antimicrob. Agents Chemother. **47:**1416–1418.
286. **Singh, N.** 2000. Antifungal prophylaxis in organ transplant recipients: seeking clarity amidst controversy. Clin. Infect. Dis. **31:**545–553.
287. **Singh, N.** 2000. Invasive mycoses in organ transplant recipients: controversies in prophylaxis and management. J. Antimicrob. Chemother. **45:**749–755.
288. **Singh, N.** 2002. Delayed occurrence of cytomegalovirus disease in organ transplant recipients receiving antiviral prophylaxis: are we winning the battle only to lose the war? Eur. J. Clin. Microbiol. Infect. Dis. **21:**643–646.
289. **Singh, N.** 2003. Treatment of opportunistic mycoses: how long is long enough? Lancet Infect. Dis. **3:**703–708.
290. **Singh, N., P. M. Arnow, A. Bonham, E. Dominguez, D. L. Paterson, G. A. Pankey, M. M. Wagener, and V. L. Yu.** 1997. Invasive aspergillosis in liver transplant recipients in the 1990s. Transplantation **64:**716–720.
291. **Singh, N., R. K. Avery, P. Munoz, T. L. Pruett, B. Alexander, R. Jacobs, J. G. Tollemar, E. A. Dominguez, C. M. Yu, D. L. Paterson, S. Husain, S. Kusne, and P. Linden.** 2003. Trends in risk profiles for and mortality associated with invasive aspergillosis among liver transplant recipients. Clin. Infect. Dis. **36:**46–52.
292. **Singh, N., and J. Heitman.** 2004. Antifungal attributes of immunosuppressive agents: new paradigms in management and elucidating the pathophysiologic basis of opportunistic mycoses in organ transplant recipients. Transplantation **77:**795–800.
293. **Singh, N., and S. Husain.** 2003. *Aspergillus* infections after lung transplantation: clinical differences in type of transplant and implications for management. J. Heart Lung Transplant. **21:**258–266.
294. **Singh, N., A. Obman, S. Husain, S. Aspinall, S. Mietzner, and J. Stout.** 2004. Reactivity of Platelia *Aspergillus* galactomannan antigen with piperacillin-tazobactam: clinical implications based on achievable serum concentrations. Antimicrob. Agents Chemother. **48:**1989–1992.
295. **Singh, N., D. L. Paterson, T. Gayowski, M. M. Wagener, and I. R. Marino.** 2001. Preemptive prophylaxis with a lipid preparation of amphotericin B for invasive fungal infections in liver transplant recipients requiring replacement therapy. Transplantation **71:**910–913.
296. **Singh, N., M. M. Wagener, I. R. Marino, and T. Gayowski.** 2002. Trends in invasive fungal infections in liver transplant recipients: correlation with evolution in transplantation practices. Transplantation **73:**63–67.
297. **Singhal, S., R. W. Ellis, S. G. Jones, S. J. Miller, C. Fisher, J. G. Hastings, and D. J. Mutimer.** 2000. Targeted prophylaxis with amphotericin B lipid complex iin liver transplantation. Liver Transplant. **6:**588–595.
298. **Siwek, G. T., K. J. Dodgson, M. de Magalhaes-Silverman, L. A. Bartelt, S. B. Kilborn, P. L. Hoth, D. J. Diekema, and M. A. Pfaller.** 2004. Invasive zygomycosis in hematopoietic stem cell transplant recipients receiving voriconazole prophylaxis. Clin. Infect. Dis. **39:**584–587.
299. **Speziali, G., J. C. McDougall, D. E. Midthun, S. G. Peters, J. P. Scott, R. C. Daly, and C. G. A. McGregor.** 1997. Native lung complications after single lung transplantation for emphysema. Transplant. Int. **10:**113–115.
300. **Steinbach, W. J., Jr. D. K. Benjamin, D. P. Kontoyiannis, J. R. Perfect, I. Lutsar, K. A. Marr, M. S. Lionakis, H. A. Torres, H. Jafri, and T. J. Walsh.** 2004. Infections due to *Aspergillus terreus*: a multicenter retrospective analysis of 83 cases. Clin. Infect. Dis. **39:**192–198.
301. **Steinbach, W. J., and D. A. Stevens.** 2003. Review of newer antifungal and immunomodulatory strategies for invasive aspergillosis. Clin. Infect. Dis. **37:**S157–S187.
302. **Stevens, D. A.** 2004. Vaccinate against aspergillosis! A call to arms of immune system. Clin. Infect. Dis. **38:**1131–1136.
303. **Stevens, D. A., V. L. Kan, M. A. Judson, V. A. Morrison, S. Dummer, D. W. Denning, J. E. Bennett, T. J. Walsh, T. F. Patterson, G. A. Pankey, et al.** 2000. Practice guidelines for diseases causes by *Aspergillus*. Clin. Infect. Dis. **30:**696–709.
304. **Stevens, D. A., H. J. Schwartz, J. Y. Lee, B. L. Moskovitz, D. C. Jerome, A. Catanzaro, D. M. Bamberger, A. J. Weinmann, C. U. Tuazon, M. A. Judson, T. A. Platts-Mills, and A. C. DeGraff, Jr.** 2000. A randomised trial of itraconazole in allergic bronchopulmonary aspergillosis. N. Engl. J. Med. **342:**756–762.
305. **Storek, J., M. A. Dawson, B. Storer, T. Stevens-Ayers, D. G. Maloney, A. Kieren, R. P. Witherspoon, W. Bensinger, M. E. D. Flowers, P. Martin, R. Storb, F. R. Appelbaum, and M. Boeckh.** 2001. Immune reconstitution after allogeneic marrow transplantation compared with blood stem cell transplantation. Blood **97:**3380–3389.
306. **Stratov, L., T. M. Korman, and P. D. Johnson.** 2003. Management of *Aspergillus* osteomyelitis: report of failure of liposomal amphotericin B and response to voriconazole in an immunocompetent host and literature review. Eur. J. Clin. Microbiol. Infect. Dis. **22:**277–283.
307. **Stynen, D., A. Goris, J. Sarfati, and J. P. Latge.** 1995. A new sensitive sandwich enzyme-linked immunosorbent assay to detect galactofuran in patients with invasive aspergillosis. J. Clin. Microbiol. **33:**497–500.
308. **Sulahian, A., F. Boutboul, P. Ribaud, T. Leblanc, C. Lacroix, and F. Derouin.** 2001. Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of invasive aspergillosis in two adult and pediatric hematology units during a 4-year prospective study. Cancer **91:**311–318.
309. **Sulahian, A., S. Touratier, and P. Ribaud.** 2003. False-positive test for *Aspergillus* antigenemia related to concomitant administration of piperacillin and tazobactam. N. Engl. J. Med. **349:**2366–2367.
310. **Swanink, C. M. A., J. F. G. M. Meis, A. J. M. M. Rijs, J. P. Donnelly, and P. E. Verweij.** 1997. Specificity of a sandwich enzyme linked immunosorbent assay for detecting *Aspergillus* galactomannan. J. Clin. Microbiol. **35:**257–260.
311. **Swift, A. C., and D. W. Denning.** 1998. Skull base osteitis following fungal sinusitis. J. Laryngol. Otol. **112:**92–97.
312. **Tascini, C., E. Tagliaferri, R. Iapoce, A. Leonildi, and F. Menichetti.** 2003. Caspofungin in combination with itraconazole and amphotericin B for the treatment of invasive aspergillosis in humans, with a method to test ex vivo synergism. Clin. Microbiol. Infect. **9:**901–902.
313. **Timmers, G., S. Zwegman, A. Simoos-Smik, et al.** 2000. Amphotericin colloidal dispersion (Amphocil) vs. fluconazole for the prevention of fungal infections in neutropenic patients; data of a prematurely stopped clinical trial. Bone Marrow Transplant. **25:**879–884.
314. **Tollemar, J., B. G. Ericzon, and J. Andersson.** 1990. The incidence and diagnosis of invasive fungal infections in liver transplant recipients. Transplant Proc. **22:**242–244.
315. **Tollemar, J., K. Hockerstedt, B. G. Ericzon, H. Jalanko, and O. Ringden.** 1995. Lipsomal amphotericin B prevents invasive fungal infections in liver transplant recipients. Transplantation **59:**45–50.
316. **Torre-Cisneros, J., O. L. Lopez, S. Kusne, A. J. Martinez, and T. E. Starzl.** 1993. CNS aspergillosis in organ transplantation: a clinicopathologic study. J. Neurol. Neurosurg. Psychiatr. **56:**188–193.
317. **Troke, P. F., S. Schwartz, M. Ruhnke, P. Ribaud, L. Corey, T. Driscoll, G. Fatkenheur, U. Schuler, E. Thiel, B. Dodell, and I. Lutsar.** 2003. Voriconazole therapy in 86 patients with CNS aspergillosis: a retrospective analysis. 43rd Annu. Meet. Intersci. Conf. Antimicrob. Agents Chemother., Chicago, Ill, 13–17 September 2003.
318. **Ulusakarya, A., E. Chachaty, J.-M. Vantelon, A. Youssef, C. Tancrede, J.-L.**

Pico, J.-H. Bourhis, P. Fenaux, and J.-N. Munck. 2000. Surveillance of *Aspergillus* galactomannan antigenemia for invasive aspergillosis by enzyme-linked immunosorbent assay in neutropenic patients treated for hematological malignancies. Hematol. J. **1:**111–116.

319. **United Network of Organ Sharing**. United Network of Organ Sharing scientific registry, assessed 24 March 2004.
320. **van Burik, J. A., V. Ratanatharathorn, D. E. Stepan, C. B. Miller, J. H. Lipton, D. H. Vesole, N. Bunin, D. A. Wall, J. W. Hiemenz, Y. Satoi, J. M. Lee, and T. J. Walsh for the National Institute of Allergy and Infectious Diseases Mycoses Group.** 2004. Micafungin versus fluconazole for prophylaxis against invasive fungal infections during neutropenia in patients undergoing hematopoietic stem cell transplantation. Clin. Infect. Dis. **39:** 1407–1416.
321. **Varo, E., S. Tome, M. Bustamante, J. Martinez, J. Paredes, R. Conde, A. Brage, F. Segade, J. Punal, E. Otero, C. Galban, C. Portella, and J. Gastroagudin.** 1998. Fungal infection prophylaxis in high-risk liver transplant recipients, abstr. J-138. Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., San Diego, Calif.
322. **Venkataramanan, R., Z. Shimin, T. Gayowski, and N. Singh.** 2004. Voriconazole inhibits tacrolimus metabolism-a study in liver transplant recipients and human microsomes. International Transplant Congress, Miami, Fla.
323. **Venkataramanan, R., S. Zang, T. Gayowski, and N. Singh.** 2002. Voriconazole inhibition of the metabolism of tacrolimus in a liver transplant recipient and in human liver microsomes. Antimirob. Agents Chemother. **46:** 3091–3093.
324. **Verweij, P. E., K. Brinkman, H. P. Kremer, B. J. Kullberg, and J. F. Meis.** 1999. *Aspergillus* meningitis: diagnosis by non-culture-based microbiological methods and management. J. Clin. Microbiol. **37:**1186–1189.
325. **Verweij, P. E., D. Stynen, A. M. M. M. Rijs, B. E. De Pauw, J. A. Hoogkamp-Korstanje, and J. F. G. M. Meis.** 1995. Sandwich enzyme-linked immunosorbent assay compared with Pastorex latex agglutination test for diagnosing invasive aspergillosis in immunocompromised patients. J. Clin. Microbiol. **33:**1912–1914.
326. **Viscoli, C., M. Machetti, P. Cappellano, B. Bucci, P. Bruzzzi, M. T. Van Lint, and A. Bacigalupo.** 2004. False-positive galactomannan Platelia *Aspergillus* test results for patients receiving piperacillin-tazobactam. Clin. Infect. Dis. **38:**913–916.
327. **Wald, A., W. Leisenring, J. A. van Burik, and R. A. Bowden.** 1997. Epidemiology of *Aspergillus* infections in a large cohort of patients undergoing bone marrow transplantation. J. Infect. Dis. **175:**1459–1466.
328. **Waldorf, A. R., S. Levitz, and R. D. Diamond.** 1984. In vivo bronchoalveolar macrophage defense against *Rhizopus oryzae* and *Aspergillus fumigatus*. J. Infect. Dis. **150:**752–760.
329. **Walsh, T. J., M. Roden, L. Nelson, T. Lmidsem, C. Venzon, B. Segal, J. Barrett, and R. Childs.** 2002. Invasive fungal infections complicating nonmyeloablative allogeneic peripheral blood stem cell transplantation, abstr. M-1233. Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., San Diego, Calif., 27–30 September 2002.
330. **Wanger, A., K. Mills, P. W. Nelson, and J. H. Rex.** 1997. Comparison of E test and NCCLS broth microdilution method for antifungal susceptibility testing: enhanced ability to detect amphotericin B-resisant *Candida* isolates. Antimicrob. Agents Chemother. **39:**2520–2522.
331. **Waser, M., M. Maggiorini, A. Luthy, A. Laske, L. von Segesser, P. Mohacsi, M. Opravil, M. Turina, F. Follath, and A. Gallino.** 1994. Infectious complications in 100 consecutive heart transplant recipients. Eur. J. Clin. Microbiol. Infect. Dis. **13:**12–18.
332. **Weiland, D., R. M. Ferguson, P. K. Peterson, D. C. Snover, R. L. Simmons, and J. S. Najarian.** 1983. Aspergillosis in 25 renal transplant patients. Ann. Surg. **198:**622–629.
333. **Westney, G. E., S. Kesten, A. De Hoyos, C. Chapparro, T. Winton, and J. R. Maurer.** 1996. *Aspergillus* infection in single and double lung transplant recipients. Transplantation **61:**915–919.
334. **Wingard, J. R.** 2002. Antifungal chemoprophylaxis after blood and marrow transplantation. Clin. Infect. Dis. **34:**1386–1390.
335. **Wingard, J. R., S. U. Beals, G. W. Santos, et al.** 1987. *Aspergillus* infection in bone marrow transplant recipients. Bone Marrow Transplant. **2:**175–181.
336. **Winston, D. J., and R. W. Busuttil.** 2002. Randomized controlled trial of oral itraconazole solution versus intravenous/oral fluconazole for prevention of fungal infections in liver transplant recipients. Transplantation **74:** 688–695.
337. **Winston, D. J., R. T. Mazlarz, P. H. Chandrasekar, H. M. Lazarus, M. Goldman, J. L. Blumer, G. J. Leitz, and M. C. Territo.** 2003. Intravenous and oral itraconazole versus intravenous and oral fluconazole for long-term antifungal prophylaxis in allogeneic hematopoietic stem-cell transplant recipients: a multicenter, randomized trial. Ann. Intern. Med. **138:**705–713.
338. **Wolff, S. N., J. Fay, D. Stevens, R. H. Herzig, B. Pohlman, B. Bolwell, J. Lynch, S. Ericson, C. O. Freytes, F. LeMaistre, R. Collins, L. Pineiro, J. Greer, R. Stein, and S. A. Goodman.** 2000. Fluconazole vs. low-dose amphotericin B for the prevention of fungal infections in patients undergoing bone marrow transplantation: a study of the North American Marrow Transplant Group. Bone Marrow Transplant. **25:**853–859.
339. **Yamada, H., S. Kohno, H. Kogna, S. Maesaki, and M. Kaku.** 1993. Topical treatment of pulmonary aspergilloma by antifungals. Relationship between duration of the disease and efficacy of therapy. Chest **103:**1421–1425.
340. Reference deleted.
341. **Yeldandi, V., F. Laghi, M. A. McCabe, R. Larson, P. O'Keefe, A. Husain, A. Montoya, and E. R. Garrity, Jr.** 1995. *Aspergillus* and lung transplantation. J. Heart Lung Transplant. **14:**883–890.
342. **Yokote, T., T. Akioka, S. Oka, T. Fujisaka, T. Yamano, S. Hara, M. Tsuji, and T. Hanafusa.** 2004. Successful treatment with micafungin of invasive pulmonary aspergillosis in acute myeloid leukemia, with the renal failure due to amphotericin B therapy. Ann. Hematol. **83:**64–66.
343. **Yuasa, K., H. Goto, M. Iguchi, T. Okamura, and R. Ieki.** 1996. Evaluation of the diagnostic value of the measurement of (1–3)-β-D-glucan in patients with pulmonary aspergillosis. Respiration **63:**78–83.