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Voriconazole Resistance and Mortality in Invasive Aspergillosis: A Multicenter Retrospective Cohort Study

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Background. Triazole resistance is an increasing problem in invasive aspergillosis (IA). Small case series show mortality rates of 50%–100% in patients infected with a triazole-resistant *Aspergillus fumigatus*, but a direct comparison with triazole-susceptible IA is lacking.

Methods. A 5-year retrospective cohort study (2011–2015) was conducted to compare mortality in patients with voriconazole-susceptible and voriconazole-resistant IA. *Aspergillus fumigatus* culture-positive patients were investigated to identify patients with proven, probable, and putative IA. Clinical characteristics, day 42 and day 90 mortality, triazole-resistance profiles, and antifungal treatments were investigated.

Results. Of 196 patients with IA, 37 (19%) harbored a voriconazole-resistant infection. Hematological malignancy was the underlying disease in 103 (53%) patients, and 154 (79%) patients were started on voriconazole. Compared with voriconazole-susceptible cases, voriconazole resistance was associated with an increase in overall mortality of 21% on day 42 (49% vs 28%; $P = .017$) and 25% on day 90 (62% vs 37%; $P = .0038$). In non-intensive care unit patients, a 19% lower survival rate was observed in voriconazole-resistant cases at day 42 ($P = .045$). The mortality in patients who received appropriate initial voriconazole therapy was 24% compared with 47% in those who received inappropriate therapy ($P = .016$), despite switching to appropriate antifungal therapy after a median of 10 days.

Conclusions. Voriconazole resistance was associated with an excess overall mortality of 21% at day 42 and 25% at day 90 in patients with IA. A delay in the initiation of appropriate antifungal therapy was associated with increased overall mortality.

Keywords. invasive aspergillosis; *Aspergillus fumigatus*; voriconazole resistance; mortality.

Triazoles are the mainstay of therapy for invasive aspergillosis (IA) and have led to a substantial improvement in overall survival. However, triazole resistance has become a concern for the management of infections caused by *Aspergillus fumigatus*. Through culture-based surveillance studies, the number of countries that report azole resistance continues to increase, although resistance frequencies vary considerably between different geographic regions [1]. Resistance rates as high as 29% have been observed in specific patient populations, such as critically ill patients [2]. Variations in resistance frequencies may reflect true geographic differences or might be due to other variables, including study design, patient populations, and laboratory practices [3, 4].

Triazole resistance may develop through therapy of individual patients with *Aspergillus* disease, which primarily occurs in patients with chronic pulmonary aspergillosis [5]. More important, triazole resistance may develop in the environment following exposure to azole fungicides [6]. Patients inhale *A. fumigatus* spores resistant to medical triazoles, which may evolve into triazole-resistant IA. The environmental route is characterized by an apparent lack of patient risk factors, as the majority of patients who present with triazole-resistant IA have not been previously treated with medical triazoles [7]. The optimal management of patients suspected of IA in regions with environmental resistance remains unclear, and an expert panel recommended considering moving away from triazole monotherapy when regional resistance frequencies exceed 10% [8]. This 10% threshold has been the subject of debate given the toxicity, costs, and lack of oral formulations and of comparative clinical trials of non-triazole antifungals such as liposomal amphotericin B (L-AmB) and echinocandins or antifungal

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combination therapies. Animal experiments consistently show that the efficacy of triazoles in infection with *A. fumigatus* with elevated triazole minimum inhibitory concentrations (MICs) is reduced compared with wild-type infection [9, 10]. This has been shown for itraconazole, voriconazole, posaconazole, and isavuconazole. Furthermore, several small case series reported mortality rates of 50%–100% in patients with triazole-resistant IA [11]. These rates are higher than those reported in recent clinical trials, where mortality rates in triazole-treated aspergillosis patients were below 30%. However, selection bias may partially explain the very high mortality; therefore, the exact impact of triazole resistance remains to be defined as direct comparisons between triazole-susceptible and triazole-resistant infection are lacking [7, 12]. To investigate the characteristics and outcome of voriconazole-susceptible IA and voriconazole-resistant IA, we conducted a retrospective, multicenter study in a large cohort of *A. fumigatus* culture-positive patients.

METHODS

Study Design

A retrospective cohort study was performed at 3 tertiary care university medical centers in the Netherlands: Radboud University Medical Center in Nijmegen, Leiden University Medical Center in Leiden, and Erasmus University Medical Center in Rotterdam.

General Management of IA

Diagnostic work-up in patients suspected of invasive pulmonary mold disease, typically included chest computed tomography (CT) and, if possible, bronchoscopy and bronchoalveolar lavage (BAL). In patients with acute myeloid leukemia or myelodysplastic syndrome and hematopoietic stem cell transplant recipients, a diagnostic-driven strategy was used, including monitoring of serum galactomannan (GM) during neutropenia or in febrile patients. Chest CT was performed in patients with positive serum GM, in those with persistent fever despite 3–5 days of broad-spectrum antibacterial therapy, and in patients with progressive respiratory failure. If CT confirmed the presence of pulmonary infiltrates, BAL was performed for fungal culture and GM measurement. Voriconazole was the first-choice treatment option for patients with IA. During the study period, no hospital treatment guidelines were available for documented voriconazole-resistant IA. However, when resistance was documented or suspected in critically ill patients, treatment was changed to either triazole and echinocandin combination therapy or L-AmB.

Data Collection

The microbiology database was searched for positive *A. fumigatus* cultures of patients admitted between January 2011 and December 2015. In order to select patients with IA, the clinical records of culture-positive patients needed to meet the following 3 conditions: antifungal therapy was started within 1 month before or after a positive culture, the patient had received at least 2 days of antifungal therapy, and the patient could be classified as probable or proven IA according to European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group; National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) definitions or putative or proven IA according to criteria of Blot et al for the subgroup of patients admitted to the intensive care unit (ICU) [13, 14].

Patient characteristics included age, gender, underlying diseases, ward/ICU admission, and antifungal prophylaxis or therapy. ICU admission was defined as initiation of antifungal therapy in the ICU and stay in the ICU for at least 2 consecutive days. In addition, patients who were admitted to the ICU during their hospitalization were analyzed separately in a Cox regression model. Furthermore, the appropriateness of initial antifungal therapy was assessed for patients treated with voriconazole. Antifungal therapy was considered appropriate if voriconazole was started in patients with voriconazole-susceptible disease and inappropriate in those with voriconazole-resistant IA. Switch to appropriate antifungal therapy and time to switch were determined.

The study was reviewed by the institutional review boards of the 3 medical centers, which confirmed that the study did not fall under the Dutch law on research on human subjects. Data were processed after encoding and in accordance with the Dutch Personal Data Protection Act.

Mycology

Fungal cultures were routinely performed if a patient underwent bronchoscopy with BAL and if ordered for other respiratory specimens. *Aspergillus fumigatus* was identified by macroscopic and microscopic morphology and growth at 48°C. *Aspergillus fumigatus* isolates were routinely screened for the presence of triazole resistance using an agar-dilution method (VIPcheck, MediaProducts bv, Groningen, the Netherlands) [15]. The method relies on agar wells supplemented with itraconazole, voriconazole, and posaconazole and a growth control. Fungal growth on any triazole-containing well was considered indicative of resistance, and these isolates were sent to Radboud University Medical Center for MIC testing according to the European Committee on Antimicrobial Susceptibility Testing reference method [16]. Infection was considered to be voriconazole resistant if 1 or more cultured *A. fumigatus* isolates exhibited a voriconazole MIC above the clinical breakpoint of 2 mg/L. If a patient had more isolates cultured within 1 month of initiation of antifungal therapy, the most resistant isolate was used to classify the patient. In addition, the presence of a resistance mutation in cyp51A was determined by *Cyp51A* gene sequencing, which is specific for *A. fumigatus* sensu strictu, excluding sibling species from the *A. fumigatus* species complex [17, 18].

Data Analyses

The primary endpoints were day 42 and day 90 mortality in voriconazole-resistant IA compared with voriconazole-susceptible IA cases. Day zero was set at day of initiation of antifungal therapy. Other factors with possible impact on survival were also investigated, including choice of first-line antifungal therapy, ICU admission, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, and appropriateness of initial antifungal therapy.

Statistical Analyses

Statistical analyses on the relation of voriconazole resistance and mortality was performed in SAS 9.4 and SPSS 24 with survival analysis (Kaplan-Meier) and the log-rank method. Confidence intervals were calculated with the Kaplan-Meier method. Possible confounders, that is, ICU admission, underlying hematological disease, and center, were analyzed for each comparison with Cox regression survival analysis, Kaplan-Meier survival (log-rank), and Fisher exact. Other differences were compared with Fisher exact.

RESULTS

Demographics

In the 5-year period, 2266 patients with a positive *A. fumigatus* culture at the 3 centers were eligible for the study. Overall, 196 (8.6%) patients met our case definition, that is, received antifungal therapy within 30 days of a positive culture, received at least 2 days of antifungal therapy, and could be classified according to the EORTC/MSG or AspICU criteria (Figure 1). A proven infection was documented in 43 (22%) patients, a putative diagnosis in 36 (18%) patients, and a probable diagnosis in 117 (60%) patients (Table 1). Hematological malignancy was the most frequent underlying disease, diagnosed in 103 of 196 (53%) patients. Eighty-five (43%) patients were admitted to the ICU during hospital admission, while 59 (30%) patients first received antifungal therapy in the ICU. Voriconazole was the initial therapy in 154 (79%) patients. Further details regarding the demography for individual centers and the total patient population are provided in Table 1.

Voriconazole Susceptibility

Voriconazole resistance was observed in 37 of 196 (19%) patients, but the resistance frequency varied from 10% to 31% at individual centers (Table 1). Voriconazole-resistant IA was diagnosed in 14 of 59 (24%) ICU patients and in 23 of 137 (17%) non-ICU patients. Voriconazole resistance corresponded with resistance to isavuconazole for all 14 patients where isavuconazole susceptibility was determined (Table 2). In 30 of 37 (81%) patients, the *A. fumigatus* isolate showed a pan-triazole–resistant phenotype; in 7 patients, the susceptibility to itraconazole, voriconazole, and posaconazole varied. Analysis of triazole-resistant *A. fumigatus* isolates showed resistance mutations that are associated with the environmental route of resistance selection in 32 of 37 (87%) patients; TR_{34}/L98H in 18 patients and TR_{46}/Y121F/T289A in 13 patients (Table 2). In 5 voriconazole-resistant isolates, no mutations were found in the *Cyp51A* gene, suggesting that other uncharacterized resistance mutations might be present. In 7 patients (19%), a mixed infection was diagnosed; this consisted of an infection with a triazole-resistant and triazole-susceptible *A. fumigatus* in 6

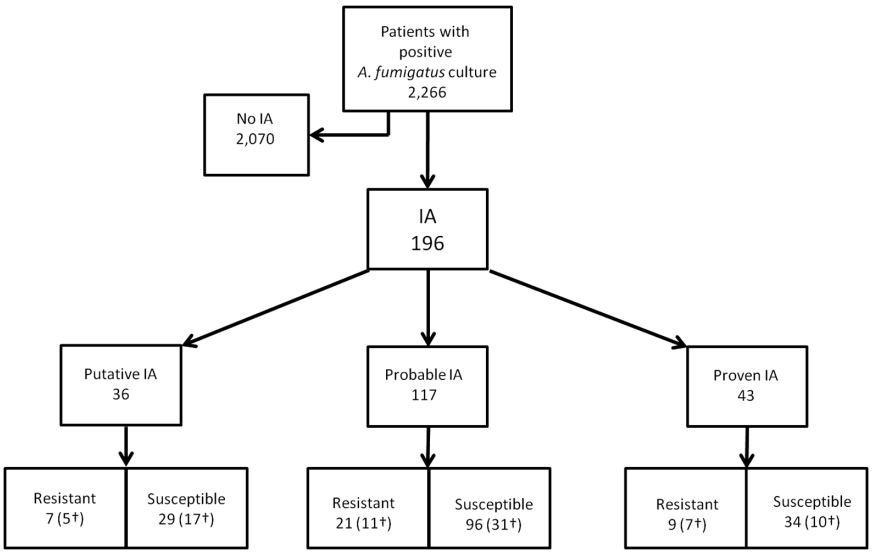

Figure 1. Inclusion of patients with positive *Aspergillus fumigatus* cultures from lower respiratory tract or sterile specimens between January 2011 and December 2015. A total of 2,266 patients had one or more positive cultures, and 196 patients could be classified according to the definitions of AspICU and the European Organization for Research and Treatment of Cancer and the National Institute of Allergy and Infectious Diseases Mycosis Study Group. Abbreviation: IA, invasive aspergillosis; †Mortality.

Table 1. Demographics, Invasive Aspergillosis (IA) Classification, Voriconazole Susceptibility, Management, and Outcome of 196 Patients With Culture-positive IA

Parameter		Center 1 (60 Cases)	Center 2 (59 Cases)	Center 3 (77 Cases)	Total (196 Cases)
Patient	Male/Female	34/26	36/23	45/32	115/81
	Median age, years	64 (3–79)	61 (4–80)	61 (2–83)	62 (2–83)
	Hematological malignancy	23 (38%)	34 (58%)	46 (60%)	103 (53%)
	Autoimmune disease	12 (20%)	6 (10%)	6 (8%)	24 (12%)
	Solid organ transplant	8 (13%)	5 (8%)	11 (14%)	24 (12%)
	Structural lung disease	5 (8%)	6 (3%)	3 (4%)	14 (7%)
	Solid tumor	3 (5%)	…	3 (4%)	6 (3%)
	Congenital immune disorder	4 (7%)	…	…	4 (2%)
	Other	4 (7%)	8 (4%)	2 (3%)	14 (7%)
	None	1 (2%)	…	6 (8%)	7 (4%)
	ICU admission	20 (33%)	23 (39%)	16 (21%)	59 (30%)
	Acute Physiology And Chronic Health Evaluation II (APACHE II) score	21	25	21	22
Invasive aspergillosis	Putative	15 (25%)	14 (24%)	7 (9%)	36 (18%)
	Probable	24 (40%)	39 (66%)	54 (70%)	117 (60%)
	Proven	21 (35%)	6 (10%)	16 (21%)	43 (22%)
	Voriconazole susceptible	54 (90%)	41 (69%)	64 (83%)	159 (81%)
	Voriconazole resistant	6 (10%)	18 (31%)	13 (17%)	37 (19%)
ICU	Voriconazole susceptible	18 (90%)	17 (74%)	10 (63%)	45 (76%)
	Voriconazole resistant	2 (10%)	6 (26%)	6 (37%)	14 (24%)
Non-ICU	Voriconazole susceptible	36 (90%)	24 (67%)	54 (89%)	114 (83%)
	Voriconazole resistant	4 (10%)	12 (33%)	7 (11%)	23 (17%)
Management	Triazole prophylaxis	…	16 (27%)	4 (5%)	20 (10%)
	Voriconazole	42 (70%)	45 (76%)	67 (87%)	154 (79%)
	Itraconazole	1 (2%)	-	-	1 (0.5%)
	Posaconazole	1 (2%)	-	2 (3%)	3 (2%)
	L-AmB	13 (22%)	13 (22%)	1 (1%)	27 (14%)
	Echinocandin	…	…	1 (1%)	1 (0.5%)
	VCZ+L-AmB	…	…	4 (5%)	4 (2%)
	VCZ+Ecand	3 (5%)	1 (2%)	1 (1%)	5 (3%)
	VCZ+intrathecal caspofungin/L-AmB	…	…	1 (1%)	1 (0.5%)
	Inappropriate therapy/appropriate therapy	3/57	15/44	12/65	30 /196
Outcome	42-day mortality	13 (22%)	26 (65%)	23 (30%)	62 (32%)
	90-day mortality	23 (38%)	29 (85%)	29 (38%)	81 (42%)
	42-day mortality ICU	11 (55%)	18 (78%)	7 (44%)	36 (61%)
	90-day mortality ICU	13 (65%)	20 (87%)	7 (44%)	40 (68%)
	42-day mortality non-ICU	2 (3%)	8 (14%)	15 (19%)	25 (18%)
	90-day mortality non-ICU	10 (17%)	9 (15%)	22 (29%)	41 (30%)

Abbreviations: ICU, intensive care unit; L-AmB, liposomal-amphotericin B; VCZ, voriconazole.

patients, while in 1 patient, isolates with 2 different resistance mutations were recovered (Table 2). All cultured *A. fumigatus* isolates were susceptible to amphotericin B.

Mortality

The overall mortality in the 195 patients with IA was 62 (32%) at day 42 and 81 (42%) at day 90 (Table 1). One patient was discharged to a hospice on day 25, but the exact day of death was not known; therefore, his survival was censored at 25 days. Comparing the patients infected with voriconazole-susceptible and voriconazole-resistant *A. fumigatus*, a 21% higher overall mortality was observed in patients infected with a resistant isolate; 44 of 158 (28%; 95% confidence interval [CI], 21% to 35%) patients with voriconazole-susceptible infection had died at day 42 vs 18 of 37 (49%; 95% CI, 34% to 66%; log-rank test, $P = .017$) of those with voriconazole-resistant IA. At day 90, the absolute difference in mortality had increased to 25% (58 of 158; 37%; 95% CI 30% to 45% and 23 of 37; 62%; 95% CI 47% to 77%, respectively; log-rank test, $P = .0038$; Figure 2A). As expected, the cumulative survival rates were much lower for 59 patients who first received antifungal therapy in the ICU; mortality was 26 of 45 (58%) for patients with voriconazole-susceptible IA and 10 of 14 (71%) for those with voriconazole-resistant IA at day 42 (log-rank test, $P = .37$; Figure 2B). For 136 patients who first received antifungal therapy on the ward (non-ICU group), a 19% lower survival rate was observed for patients with

Table 2. Underlying Condition, Invasive Aspergillosis (IA) Classification, *Aspergillus fumigatus* Genotype and Phenotype, and Outcome in 37 Patients With Voriconazole-resistant IA

Sex, Age (years)	Center	Underlying Disease	Intensive Care Unit	IA Classification	Sample With Resistant Culture	Cyp51A-Resistance Mutation	Minimum Inhibitory Concentration (mg/L)				Initial Antifungal Therapy	Outcome at Day 90 (Day of Death)
							ITZ	VCZ	POS	ISA		
M, 49	1	Diabetes, necrotizing pancreatitis	Yes	Proven	Lung biopsy	TR_{34}/L98H	>16	16	1	8	L-AmB	Alive
M, 71	1	Kidney transplant	No	Proven	Bronchial aspirate	TR_{46}/Y121F/T289A S/R[a]	0.5	>16	0.25	>16	VCZ	Died (+50)
M, 55	1	AML, alloHSCT	No	Probable	Sputum	WT	1	4	0.25	…	L-AmB	Died (+52)
M, 70	1	COPD, lung fibrosis	Yes	Putative	BAL	WT	>16	>16	1	…	VCZ	Died (+14)
F, 59	1	T-cell lymphoma	No	Proven	Sinus biopsy	TR_{34}/L98H	>16	4	0.5	…	L-AmB	Died (+15)
F, 65	1	Kidney transplant	No	Proven	Brain biopsy	TR_{34}/L98H S/R[a]	>16	8	0.5	8	VCZ	Died (+90)
F, 69	2	Myelodysplastic syndrome	Yes	Probable	BAL	TR_{46}/Y121F/T289A	>16	>16	1	…	L-AmB	Alive
M, 51	2	B-cell lymphoma	No	Probable	Sputum	TR_{34}/L98H	>16	8	0.5	…	VCZ	Alive
F, 64	2	B-cell lymphoma	Yes	Probable	BAL	TR_{46}/Y121F/T289A	>16	>16	1	…	L-AmB	Died (+25)
M, 59	2	AML	Yes	Putative	BAL	TR_{34}/L98H	16	8	1	…	VCZ	Died (+27)
F, 39	2	Liver transplant	Yes	Putative	BAL	TR_{34}/L98H	>16	16	1	…	VCZ	Died (+62)
F, 44	2	Autoimmune hepatitis	Yes	Putative	BAL	TR_{46}/Y121F/T289A	>16	>16	1	…	VCZ	Died (+7)
M, 61	2	B-cell lymphoma	No	Probable	Sputum	TR_{46}/Y121F/T289A R/R[a]	>16	>16	1	…	L-AmB	Died (+11)
					BAL	TR_{34}/L98H	>16	8	1	…		
M, 15	2	Severe aplastic anemia	Yes	Probable	BAL	TR_{34}/L98H	>16	8	1	…	L-AmB	Died (+4)
F, 53	2	Lung carcinoma	Yes	Probable	Drain fluid	TR_{34}/L98H	>16	4	0.5	…	VCZ	Died (+7)
M, 67	2	COPD	Yes	Proven	Lung autopsy	TR_{34}/L98H	>16	8	1	…	VCZ	Died (+8)
F, 19	2	B-cell lymphoma	No	Probable	Sputum	TR_{34}/L98H	>16	8	1	16	VCZ	Died (+18)
M, 54	2	B-cell lymphoma	No	Probable	BAL	TR_{34}/L98H S/R[a]	>16	8	1	8	VCZ	Alive
M, 45	2	B-cell lymphoma	No	Probable	Bronchial aspirate	TR_{46}/Y121F/T289A S/R[a]	1	>16	0.25	>16	VCZ	Alive
F, 67	2	AML	No	Probable	Bronchial aspirate	TR_{46}/Y121F/T289A	>16	>16	0.25	>16	VCZ	Alive
M, 62	2	B-cell lymphoma, alloHSCT	No	Proven	Tissue biopsy	TR_{46}/Y121F/T289A	0.5	>16	0.5	>16	VCZ	Alive
M, 71	2	Influenza	Yes	Putative	Sputum	TR_{34}/L98H S/R[a]	>16	>16	2	>16	VCZ	Alive
M, 70	2	AML	Yes	Probable	Bronchial aspirate	TR_{34}/L98H	>16	8	1	8	VCZ	Alive
F, 58	2	Follicular lymphoma	Yes	Putative	BAL	WT S/R[a]	>16	>16	1	>16	VCZ	Alive
M, 22	3	alloHSCT	Yes	Probable	Sputum	TR_{46}/Y121F/T289A	>16	>16	1	…	L-AmB	Died (+9)
M, 46	3	Kidney transplant, Marginal zone lymphoma	No	Probable	BAL	TR_{34}/L98H	>16	4	0.5	8	VCZ	Alive
M, 53	3	Hematological malignancy	No	Probable	Sputum	TR_{46}/Y121F/T289A	>16	>16	1	…	VCZ	Died (+66)
F, 64	3	Hematological malignancy	No	Probable	Sputum	TR_{34}/L98H	>16	16	2	…	VCZ	Alive
M, 64	3	Hematological malignancy	No	Probable	BAL	TR_{46}/Y121F/T289A	>16	4	1	…	VCZ	Alive
M, 29	3	Hodgkin lymphoma, alloHSCT	Yes	Probable	BAL	TR_{34}/L98H	>16	8	2	…	VCZ	Died (+44)
F, 63	3	Lung transplant	Yes	Probable	Sputum	TR_{34}/L98H	>16	16	2	16	VCZ	Died (+40)
F, 64	3	Neurosarcoidosis	Yes	Probable	Sputum	WT	0.25	4	0.25	…	VCZ	Died (+4)
F, 40	3	alloHSCT	Yes	Proven	BAL	TR_{34}/L98H	>16	4	1	…	VCZ	Died (+32)
M, 56	3	Autoimmune disease	Yes	Proven	Sputum	TR_{46}/Y121F/T289A	2	>16	1	…	VCZ	Died (+32)
F, 60	3	SOT	Yes	Proven	Sputum	TR_{46}/Y121F/T289A	16	>16	0.5	…	VCZ	Alive
F, 62	3	Hematological malignancy, influenza	Yes	Proven	BAL	TR_{46}/Y121F/T289A	2	>16	1	…	VCZ	Died (+10)
M, 38	3	Poly trauma	Yes	Putative	BAL	WT	>16	4	0.5	8	VCZ	Died (+24)

Abbreviations: AlloHSCT, allogeneic hematopoietic stem cell transplant; AML, acute myeloid leukaemia; CLL, chronic lymphoblastic leukemia; COPD, chronic obstructive pulmonary disease; ISA, isavuconazole; ITZ, itraconazole; L-AmB, liposomal amphotericin B; POS, posaconazole; SOT, solid organ transplant; VCZ, voriconazole; WT, wild type.

[a]Mixed infection: S/R, voriconazole-susceptible and voriconazole-resistant; R/R, 2 different voriconazole-resistant genotypes.

voriconazole-resistant IA compared with voriconazole-susceptible infection, with a mortality rate of 8 of 23 (35%) and 19 of 113 (16%) at day 42, respectively (log-rank test, $P = .045$; Figure 2C). The mortality for 18 patients infected with TR_{34}/L98H was not different from that of 13 patients infected with TR_{46}/Y121F/T289A (Supplementary Figure S1).

At the discretion of the treating physician, 27 of 196 (14%) patients received initial antifungal therapy with L-AmB. Eight of these 27 patients were infected with voriconazole-resistant *A. fumigatus*. The survival at day 42 of L-AmB–treated patients was 55% compared with 71% for voriconazole-treated patients (log-rank test, $P = .04$; Figure 3). However, the proportion of ICU

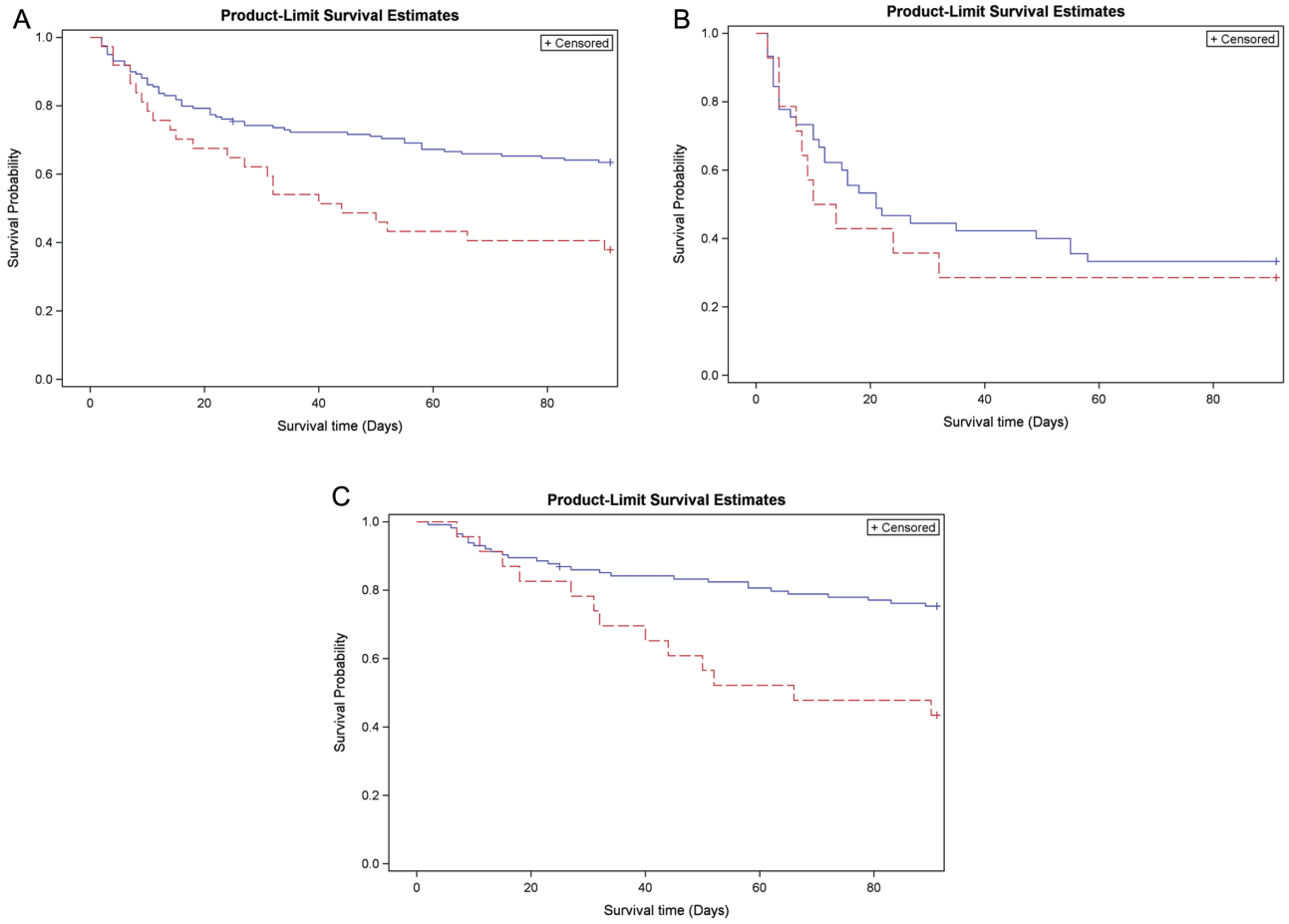

Figure 2. Cumulative survival of patients with voriconazole-susceptible and voriconazole-resistant invasive aspergillosis (IA). *(A)* Cumulative survival of all patients with IA. *(B)* Cumulative survival of patients who started antifungal therapy in the intensive care unit (ICU). *(C)* Cumulative survival in non-ICU patients with IA. Blue lines represent patients with IA due to voriconazole-susceptible *Aspergillus fumigatus*. Red lines represent patients with IA due to voriconazole-resistant *A. fumigatus*. One patient was discharged to a hospice after 25 days, and his survival was, therefore, censored at day 25.

patients in the L-AmB–treated group was significantly higher compared with voriconazole-treated patients (13 of 27 [48%] vs 43 of 154 [28%]; Fisher exact test, $P = .04$), indicating that confounding by indication at least partly explained this difference.

The mortality of patients who received appropriate and inappropriate therapy was compared for 154 patients with initial voriconazole therapy. Thirty patients (81%) with voriconazole-resistant IA initially received voriconazole therapy and were classified to have received inappropriate antifungal therapy (Table 2). Therapy was switched to appropriate therapy in 18 patients after a median of 10 days (range, 1 to 39 days). Inappropriate voriconazole therapy corresponded with reduced survival at day 42 compared with appropriate therapy (76% and 53%, respectively; log-rank test, $P = .016$; Figure 4). Six patients presented with mixed infection (Table 2).

Cox Regression Analysis

ICU admission, underlying hematological disease, and center were analyzed as possible confounders for mortality. ICU admission contributed significantly to mortality, whereas the presence of hematological disease had no effect (see Supplementary Table 1). Comparison of the centers indicated that the resistance frequency was significantly higher in center 2 compared with centers 1 and 3 ($P = .009$). The hazard ratio at day 42 for patients who started voriconazole therapy on the ICU was 7.7 (95% CI, 3.9 to 15.3; $P < .001$), while a hazard ratio of 1.4 was found for voriconazole resistance (95% CI, 0.8 to 2.4; $P = .272$; Supplementary Table S1). In patients where voriconazole therapy was initiated on the ward, voriconazole-resistance frequency was higher in patients who required ICU admission compared with those who completed treatment on the ward (8 of 16 [50%] compared with 15 of 111 [14%] patients, respectively; $P = .044$).

DISCUSSION

Our retrospective cohort study showed a higher mortality in patients with voriconazole-resistant IA compared with voriconazole-susceptible IA. In a setting of primary therapy with voriconazole, the absolute difference in day 42 and day 90 mortality was between 21% and 33%, respectively, for the overall

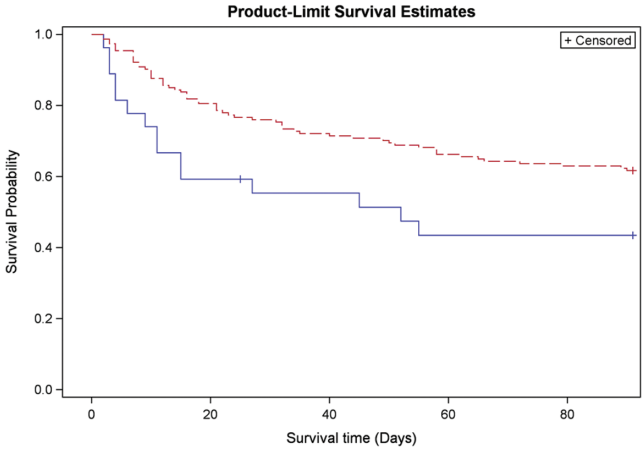

Figure 3. Cumulative survival of liposomal amphotericin B (L-AmB)-treated invasive aspergillosis (IA) patients compared with voriconazole-treated patients. The red line represents patients with IA who were treated with voriconazole. The blue line represents patients with IA who were treated with L-AmB. One patient was discharged to a hospice after 25 days, and his survival was, therefore, censored at day 25.

patient group and for non-ICU patients. These observations are in line with results from in vivo models of resistant infection and case series [7, 9, 10, 12, 19]. However, these case series included a small number of IA patients and were, therefore, prone to selection or publication bias. In the subset of patients admitted to the ICU, no significant difference in survival between voriconazole-resistant and voriconazole-susceptible IA was found. However, the smaller sample size of this subgroup as well as the high mortality of 67% in voriconazole-susceptible IA patients in the ICU makes this analysis severely underpowered.

L-AmB is considered alternative treatment for IA; however, a randomized comparison with voriconazole has never been performed and, therefore, its efficacy relative to voriconazole remains unclear [20]. In our study, the survival of L-AmB–treated patients was not better than voriconazole-treated patients with IA. However, patients who received L-AmB were more often admitted to the ICU compared with patients on voriconazole and, therefore, had an a priori higher probability of dying. Although the very small number of patients in this subanalysis makes any definite conclusions premature, this may indicate that in critically ill patients and those with advanced IA, clinical deterioration could not be reversed by polyene-based therapy. Indeed, preclinical studies showed that L-AmB, even at a dose of 10 mg/kg, was ineffective when treatment was delayed until 48 hours post-infection [21], underscoring the need for early intervention. Treatment delay was also found to be associated with poorer outcome of IA in clinical studies [22], which is supported by our observation of lower survival when the initial antifungal therapy was inappropriate.

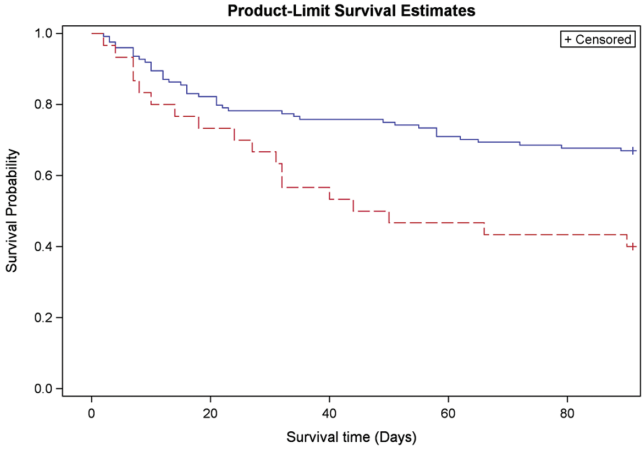

Figure 4. Cumulative survival of patients who initially received voriconazole therapy. Patients who received appropriate initial voriconazole therapy were compared with those who received inappropriate therapy. The blue line represents patients with invasive aspergillosis (IA) who received appropriate initial voriconazole therapy. The red line represents patients with IA who received inappropriate initial voriconazole therapy.

Voriconazole resistance was dominated by mutations associated with environmental resistance selection, accounting for 87% of resistance mutations [7, 11, 12]. The majority of isolates were pan-azole resistant, and there was 100% cross-resistance between voriconazole and isavuconazole. There are no known risk factors that can help to identify patients at high risk for triazole-resistant IA. In our study, all cases of inappropriate antifungal therapy were due to voriconazole therapy in voriconazole-resistant IA.

Our study has several limitations, including its retrospective design. Many factors may have an impact on the outcome of IA, and some of these could act as a confounder as they may not be well balanced between voriconazole-susceptible and voriconazole-resistant patient groups. We identified possible confounders in our cohort. As expected, ICU admission was associated with significantly higher mortality. However, when ICU patients were excluded, mortality in voriconazole-resistant IA remained significantly higher compared with voriconazole-susceptible IA. Furthermore, patients with voriconazole-resistant IA were more likely to require ICU admission, suggesting that initial therapy was not successful. Cox regression analysis indicated that the hazard of death due to voriconazole resistance was 1.4 times higher than in voriconazole-susceptible infection.

Our study relied on *Aspergillus* culture as this enabled reliable resistance screening and in vitro susceptibility testing. Agar-based resistance screening through VIPcheck was found to be highly sensitive and specific to identify resistant *A. fumigatus* colonies in cultures. Also, unlike polymerase chain reaction (PCR)-based resistance detection, VIPcheck allows detection of a broad range of resistance mutations, including uncharacterized mechanisms [15]. However, sensitivity of culture is low; thus, our cohort represents a small subset of IA cases and may not be directly translatable to culture-negative cases of IA. A recent study that used PCR cyp51A resistance testing directly on BAL of hematology patients with IA showed a 31% difference in overall mortality, similar to what we observed [19].

As 79% of patients received initial therapy with voriconazole, our study represents an escalation strategy, that is, initial voriconazole and escalation when resistance is documented. The Infectious Diseases Society of America (IDSA) recommends an escalation strategy when MIC testing is advocated in patients suspected of resistance or failing primary antifungal therapy [20]. In our study, a higher mortality was observed if patients with voriconazole-resistant IA started on voriconazole despite intensive resistance screening. Treatment was switched after a median of 10 days, which did not prevent poor clinical outcome. A management strategy based on less intensive resistance testing, as recommended by the IDSA, might result in excess mortality in those patients with voriconazole-resistant IA. Direct detection of resistance mutations by molecular techniques in BAL fluid may reduce the time to resistance detection, and PCR-based strategic studies are currently ongoing.

As appropriate initial antifungal therapy was found to be critical, up-front combination antifungal therapy may be required to increase the probability of survival of patients at risk for IA in geographic regions with high resistance rates. Combination therapy includes voriconazole or isavuconazole combined with an echinocandin or L-AmB, but clinical evidence supporting these treatment options is lacking. However, the 10% threshold recommended by an expert panel was met in our centers, and the Dutch treatment guideline has been revised to recommend routine triazole-resistance testing and combination therapy for patients suspected of IA, at least until the presence of resistance has been ruled out [23]. In most countries, resistance rates are lower than reported in the Netherlands, which does not justify a deescalation strategy [1, 24].

Our findings underscore the need for rapid resistance tests and antifungal drugs based on new targets. As azole fungicide use appears to be an important driver for resistance in *A. fumigatus* and new resistance mutations continue to emerge in the environment [25], strategies that are aimed at overcoming resistance selection in the environment need to be developed. However, antimicrobial resistance action plans and "One-Health" research are generally restricted to bacterial resistance [26]. Governments, medical research councils, and public health organizations are called to action to prioritize fungal research and help to overcome the problem of triazole resistance.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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