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Prevalence of voriconazole-resistant invasive aspergillosis and its impact on mortality in haematology patients

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Background: Increasing resistance of *Aspergillus fumigatus* to triazoles in high-risk populations is a concern. Its impact on mortality is not well understood, but rates from 50% to 100% have been reported.

Objectives: To determine the prevalence of voriconazole-resistant *A. fumigatus* invasive aspergillosis (IA) and its associated mortality in a large multicentre cohort of haematology patients with culture-positive IA.

Methods: We performed a multicentre retrospective study, in which outcomes of culture-positive haematology patients with proven/probable IA were analysed. Patients were stratified based on the voriconazole susceptibility of their isolates (EUCAST broth microdilution test). Mycological and clinical data were compared, along with survival at 6 and 12 weeks.

Results: We identified 129 *A. fumigatus* culture-positive proven or probable IA cases; 103 were voriconazole susceptible (79.8%) and 26 were voriconazole resistant (20.2%). All but one resistant case harboured environment-associated resistance mutations in the *cyp51A* gene: TR_{34}/L98H (13 cases) and TR_{46}/Y121F/T289A (12 cases). Triazole monotherapy was started in 75.0% (97/129) of patients. Mortality at 6 and 12 weeks was higher in voriconazole-resistant cases in all patients (42.3% versus 28.2%, P=0.20; and 57.7% versus 36.9%, P=0.064) and in non-ICU patients (36.4% versus 21.6%, P=0.16; and 54.4% versus 30.7%; P=0.035), compared with susceptible ones. ICU patient mortality at 6 and 12 weeks was very high regardless of triazole susceptibility (75.0% versus 66.7%, P=0.99; and 75.0% versus 73.3%, P=0.99).

Conclusions: A very high prevalence of voriconazole resistance among culture-positive IA haematology patients was observed. The overall mortality at 12 weeks was significantly higher in non-ICU patients with voriconazole-resistant IA compared with voriconazole-susceptible IA.

Introduction

Invasive aspergillosis (IA) due to *Aspergillus fumigatus* is a common infectious complication among haematology patients.[1] Case-fatality rates of IA in these patients have decreased in recent years, from 68% (1995–99)[2] to 32%–42% (2003–07)[1,3] and 22%–29% (2009–15),[4,5] probably due to earlier diagnosis and the introduction of more effective and/or less-toxic antifungal drugs, such as voriconazole, which has been clinically

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available since 2002.[6] Voriconazole and isavuconazole are the preferred first-line agents for the treatment of acute IA,[7] but an increasing number of reports of triazole resistance in *A. fumigatus* threatens these treatment options.[8] Most triazole-resistance-associated mutations affect the 14-α-demethylase enzyme (*cyp51A* gene), which converts lanosterol to ergosterol, a key component of fungal cell membranes. [9] Triazole antifungals bind and block the function of this enzyme, leading to membrane instability and subsequent fungal cell death.[10] The most commonly reported resistance mechanisms among IA in haematology patients are the environment-associated mutations TR_{34}/L98H and TR_{46}/Y121F/T289A. The TR_{34}/L98H mutation typically confers pan-azole resistance whereas the TR_{46}/Y121F/T289A mutation is associated with high voriconazole MICs and variable itraconazole and posaconazole MICs.[11–13]

Triazole resistance prevalence in *A. fumigatus* varies between 3.2% and 36.3% depending on the country, centre and underlying condition (e.g. cystic fibrosis is associated with higher triazole resistance prevalence).[12,14–20] Because *Aspergillus* does not grow from culture in >50% of cases,[21] molecular methods are being developed to detect triazole resistance directly in patient samples, but variation of the sensitivity of these assays is an issue.[22] The impact of triazole resistance on mortality is not completely known but rates as high as 50%–100% have been reported in case series of haematology patients with triazole-resistant IA.[15,17,23,24] Since exposure to triazole-resistant conidia in the environment renders every patient at risk of developing triazole-resistant *Aspergillus* disease, an international expert panel recommended that in the case of >10% triazole resistance, primary treatment should be shifted away from triazole monotherapy to liposomal amphotericin B (L-AmB), or triazole and echinocandin combination therapy.[25] As the initial therapy for IA in haematology patients should carefully balance resistance prevalence versus efficacy, there is concern that shifting away from voriconazole or isavuconazole therapy may come at the expense of overall treatment efficacy. Therefore, another approach that has been advocated is the one in which a triazole constitutes part of the initial antifungal therapy until resistance is adequately documented by culture (or PCR).[26,27] To assess the prevalence and mortality of voriconazole-resistant *A. fumigatus* IA, we performed a retrospective multicentre study in a large cohort of haematology patients with IA.

Patients and methods

Study design and patient cohort

We retrospectively studied the prevalence and outcome of IA due to voriconazole-resistant *A. fumigatus* in patients with haematological malignancies in four university medical centres between 2012 and 2017, namely the University Hospitals Leuven (Leuven, Belgium), the Radboud University Medical Centre (Nijmegen, The Netherlands), Leiden University Medical Centre (Leiden, The Netherlands) and Erasmus University Medical Centre (Rotterdam, The Netherlands). Haematology patients diagnosed with probable or proven IA according to the 2008 consensus criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and The National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG)[28] with a positive *A. fumigatus* culture were included. Patients were further stratified by fungal susceptibility phenotype into voriconazole-susceptible and voriconazole-resistant cases.

Data collection

Cases of IA at the University Hospitals Leuven were retrieved through two registered studies (ClinicalTrials.gov NCT01176071 and NCT03004092), previously approved by the local Ethics Committee. Briefly, electronic files from adult patients (>18 years old) with a haematological disease diagnosed between 2012 and 2016 (NCT01176071) and a positive galactomannan test (see Supplementary data available at *JAC* Online) or a positive culture for *A. fumigatus* were reviewed for the presence of IA. For 2017, haematology patients were prospectively screened for IA (NCT03004092). Only cases of proven or probable IA from either trial with a positive *A. fumigatus* culture were included for further analysis. Data from the Dutch centres had been previously collected as part of a multicentre retrospective cohort study on IA regardless of the underlying disease.[29] The hospital-wide cohort included all patients with culture-positive IA,[29] but for the current study only haematology patients with proven or probable IA were included for further analysis (94 out of 196 patients). Data was processed in accordance with the Dutch Personal Data Protection Act (General Data Protection Regulation) and did not fall under the Dutch law on research on human subjects.

In all centres, the antifungal care pathway included a diagnostic-driven approach. Positive serum galactomannan (see Supplementary data), persistent (neutropenic) fever despite broad-spectrum antibiotics, or clinical signs or symptoms suggestive of IA were triggers for performing a chest CT scan, which was followed by bronchoalveolar lavage (BAL) with fungal culture and determination of galactomannan on BAL fluid whenever patients presented with suspected lesions on the CT scan. When clinically indicated, additional fungal cultures besides BAL were taken (sputum, bronchial aspirate, biopsy, etc.).

Day 0 was defined as the day on which mould-active antifungal therapy was started. Patients were included in the ICU subgroup if antifungal therapy was initiated at the ICU and if the duration of admission at the ICU was at least two consecutive days. Survival was recorded at 6 and 12 weeks. Mycological data obtained included cultures performed and results of triazole resistance screening, susceptibility testing and resistance mutation determination in the *cyp51A* gene of triazole-resistant isolates. For comparison between voriconazole-susceptible and -resistant cases, we considered any case with at least one resistant isolate as a voriconazole-resistant case (MIC >2 mg/L). Isolates with an intermediate susceptibility to voriconazole were considered as susceptible cases for this analysis (MIC ≤2 mg/L). Appropriateness of initial antifungal therapy and switch of therapy were determined as well. Initial antifungal therapy was considered as appropriate when a voriconazole-susceptible case received a triazole antifungal as initial therapy. Initial antifungal therapy was considered inappropriate when a voriconazole-resistant case started antifungal treatment with a triazole antifungal (voriconazole or isavuconazole as study medication).

Mycology

All *A. fumigatus*-positive cultures from haematological patients underwent comprehensive mycological analysis, including strain identification through macro- and micro-morphological visual inspection, species confirmation by either growth at 48°C or sequencing of the β-tubulin gene as previously described,[30] susceptibility testing and molecular determination of resistance mutations in the *cyp51A* gene. MICs of itraconazole, voriconazole, posaconazole and amphotericin B were determined using the EUCAST broth microdilution reference method for filamentous fungi; resistance was established based on the EUCAST clinical breakpoints for *A. fumigatus*.[31] In the Dutch centres, triazole resistance screening was routinely performed for all *A. fumigatus* isolates using the commercially available 4-well agar plate dilution method VIPCheck® (MediaProducts, Groningen, The Netherlands), according to the manufacturer's recommendations. Briefly, all *Aspergillus* colonies that grew were swabbed to prepare a suspension (0.5 McFarland standard) in sterile water, of which 25 μL was placed in each

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well and incubated at 36°C. Growth was recorded at 24 and 48 h. Growth on any of the triazole (itraconazole, voriconazole, posaconazole)-containing agars prompted MIC determination (EUCAST). The same routine triazole resistance screening was introduced in the University Hospitals Leuven in 2015. Between 2012 and 2015, positive *A. fumigatus* cultures from haematological patients were directly subjected to susceptibility testing. The presence of mutations within the *cyp51A* gene and its promotor region was assessed as described previously.[32,33]

Statistical analysis

Fisher's exact test was used to compare categorical variables. The Mann–Whitney *U*-test was used to compare continuous variables. Primary endpoints were survival at week 6 (42 days) and week 12 (84 days). Impact of voriconazole susceptibility on survival was assessed using Kaplan–Meier estimators and survival curves were compared using the log-rank test. We performed multivariate Cox regression analysis on 12 week survival by resistance, controlling for ICU admission, EORTC/MSG proven or probable IA group, gender, age and initial triazole antifungal therapy. All analyses were performed using R v3.4.4 (R Foundation for Statistical Computing, Vienna, Austria).

Results

We identified 129 *A. fumigatus* culture-positive proven or probable IA cases including 103 voriconazole-susceptible (79.8%) and 26 voriconazole-resistant (20.2%) cases. Prevalences of voriconazole resistance per centre were: University Hospitals Leuven 17.1% (6/35 cases), Radboud University Medical Centre 10.5% (2/19 cases), Leiden University Medical Centre 34.5% (10/29 cases) and Erasmus University Medical Centre 17.4% (8/46 cases). The patients' baseline characteristics are summarized in Table 1. No gender or age differences were observed between susceptible and resistant cases. The distribution of probable and proven IA classification did not vary between groups ($P=1.00$). Initiation of antifungal therapy in the ICU represented ∼15% of the cases in both groups, 15/103 cases in the voriconazole-susceptible and 4/26 cases in the voriconazole-resistant group ($P=1.00$). No significant difference in voriconazole resistance prevalence was observed in the ICU group (21.0%) compared with the non-ICU group (17.1%). Initial therapy consisted of triazole monotherapy in 75.2% (97/129) of patients; 77.0% (79/103) in voriconazole-susceptible IA and 69.0% (18/26) in voriconazole-resistant cases. Triazole antifungal therapy was more frequently initiated in the non-ICU population (79.0%, 87/110 cases) compared with the ICU population (52.6%, 10/19 cases). In the voriconazole-resistant IA group, 13 patients (72.0%) who started voriconazole monotherapy were switched to L-AmB, with a median time to switch of 11 days after the start of triazole therapy (IQR 7.5–26). In none of the cases was initial triazole treatment modified to a combination therapy. Among all cases, L-AmB or echinocandin therapy with or without combination with a triazole antifungal was initiated in 25.0% (32/129) of the patients; 24 cases among voriconazole-susceptible IA cases (23.3%) and 8 cases among voriconazole-resistant IA cases (30.8%). L-AmB was started more frequently in ICU patients (36.8%, 7/19 cases) compared with non-ICU patients (12.7%, 14/110 cases). Owing to the retrospective design of our study, the reason for initial or switch of initial antifungal therapy was not available for the majority of the cases. Detailed information on the microbiological and clinical characteristics of voriconazole-resistant *A. fumigatus* culture-positive IA haematological patients is shown in Table 2. The majority of voriconazole-resistant isolates originated from BAL cultures (46.2%), followed by sputum cultures (34.6%). A pan-triazole resistance phenotype was observed in 77.0% (20/26) of the voriconazole-resistant isolates. Environment-associated resistance mutations in the *cyp51A* gene were the most common reported mutations: TR_{34}/L98H (13/26, 50.0%) and TR_{46}/Y121F/T289A (12/26, 46.2%), of which one patient had mixed resistant TR_{46}/Y121F/T289A + TR_{34}/L98H infection (patient 6); no mutations were found in the *cyp51A* gene in one isolate (3.8%). All voriconazole-resistant *A. fumigatus* isolates tested were susceptible to amphotericin B (MIC $\leq$1 mg/L).

Within the voriconazole-resistant group, three patients presented with a mixed susceptible/resistant *A. fumigatus* infection, two with initially both a resistant and susceptible isolate (patients 8, 9) and one with an initial susceptible isolate (patient 23). In the latter, the resistant isolate was cultured 6 days after the susceptible isolate (day 0) while on voriconazole treatment. Patient 22 had two independent episodes of IA: a triazole-susceptible and a triazole-resistant episode. Initially, the patient was diagnosed with a susceptible *A. fumigatus* isolate for which voriconazole treatment was initiated with a favourable clinical and mycological response. By day 4 of treatment, total neutrophil counts recovered (>500 neutrophils/μL). By day 49, the patient received a second stem cell donation as treatment for marrow failure. By day 59, while on voriconazole treatment, a breakthrough IA infection was diagnosed due to triazole-resistant *A. fumigatus* and the patient died 22 days after diagnosis of triazole-resistant IA.

Mortality

All-cause mortality among the 129 haematological patients was 31.0% (40/129) at week 6 and 41.1% (53/129) at week 12. Mortality stratified by voriconazole susceptibility in the overall, ICU and non-ICU populations at weeks 6 and 12 is shown in Table 3. In the overall population, we observed a higher but non-significantly different all-cause mortality in the voriconazole-resistant group (42.3%, 11/26 cases) compared with the voriconazole-susceptible group (28.2%, 29/103 cases; $P=0.200$) at week 6 and at week 12 (57.7%, 15/26 cases versus 36.9%, 38/103 cases; $P=0.064$, Figure 1a). In the non-ICU population, mortality was significantly higher among resistant cases (54.5%, 12/22 cases) compared with susceptible ones (30.7%, 27/88 cases) at 12 weeks (24.0% mortality difference between groups, $P=0.035$, Figure 1b). Mortality was highest in the ICU group, with no difference in mortality in voriconazole-resistant cases between week 6 and week 12, which may be partially explained by the already very high mortality of 73.3% in the triazole-susceptible ICU patient group at week 12.

Mortality based on appropriate initial triazole therapy (susceptible cases) or inappropriate initial triazole therapy (resistant cases), showed no significant differences in mortality at 12 weeks in overall cases: 38.0% (30/79 cases) versus 44.4% (8/18 cases, $P=0.609$) respectively. No significant differences in mortality were observed in the non-ICU population either when comparing appropriate initial therapy (33.3%, 23/69 cases) and inappropriate initial therapy (40.0%, 6/15 cases) at 12 weeks ($P=0.637$). Among the triazole-resistant cases, 12 week mortality in patients who switched from initial triazole monotherapy to L-AmB was 46.2% (6/13 cases) compared with 40.0% in patients whose initial triazole therapy was not modified (2/5 cases, $P=0.785$). A trend indicating

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Table 1. Characteristics of haematology patients with culture-positive IA, comparing voriconazole-susceptible and voriconazole-resistant cases

Characteristic	Susceptible (n=103)	Resistant (n=26)	P value	Test
Male gender, *n* (%)	62 (60.2)	16 (61.5)	1.000	Fisher's exact
Age (years), median (IQR)	61.0 (46.5–66.5)	58.5 (51.0–64.0)	0.611	Mann–Whitney *U*
EORTC/MSG proven IA, *n* (%)	21 (20.4)	5 (19.2)	1.000	Fisher's exact
Treatment, *n* (%)			0.628	Fisher's exact
L-AmB	15 (14.6)	6 (23.1)		
echinocandin	4 (3.9)	2 (7.7)		
triazole (VRC + blinded[a])	79 (76.7)	18 (69.2)		
VRC + L-AmB	3 (2.9)	0 (0.0)		
VRC + intrathecal CAS/L-AmB	1 (1.0)	0 (0.0)		
VRC + echinocandin	1 (1.0)	0 (0.0)		
ICU subgroup, *n* (%)[b]	15 (14.6)	4 (15.4)	1.000	Fisher's exact

CAS, caspofungin; VRC, voriconazole.
[a]Randomized double-blinded clinical trial (voriconazole versus isavuconazole, voriconazole versus posaconazole).
[b]Initiation of antifungal therapy in the ICU and stay in the ICU for at least two consecutive days.

a decreased survival at 12 weeks in patients with voriconazole-resistant IA infection was observed by multivariate Cox regression analysis after controlling for possible confounders such as ICU admission, EORTC/MSG proven or probable IA group, gender, age and triazole antifungal initial therapy (HR 1.74, P=0.072; Table S1). Increased HRs were observed for ICU patients (4.00, P=0.001) and proven IA patients (2.37, P=0.007) at 12 weeks.

Discussion

This study revealed a prevalence of 20.2% (26/129 cases) of voriconazole-resistant *A. fumigatus* among culture-positive IA in haematology patients, which varied between centres from 10.0% to 34.0%, and an associated increased mortality in non-ICU patients.

The prevalence of triazole resistance corresponds to previous reports from the Netherlands and the USA, where resistance among culture-positive IA in haematology patients was 16.7% (2/12 cases)[14] and 21.2% (11/52 cases),[16] respectively. However, even higher prevalence of resistance of 30.3% (10/33 ICU cases)[23] and 36.4% (12/33 haematology cases)[18] have been described by other centres. One German study found a resistance prevalence of 29.6% (8/27 cases) in autologous and allogenic stem cell transplant recipients.[15] However, total numbers of IA cases in these studies were low and because small changes in the number of triazole-resistant cases significantly impact the calculated prevalences[34] multicentre studies are required to reliably estimate resistance prevalence. Moreover, the underlying mechanisms that lead to triazole resistance as well as its impact on MICs differ by region. In Europe, triazole resistance is almost entirely driven by *cyp51A* gene mutations while other mechanisms seem to be involved in the study from the USA.[16] Recently, a multicentre study in the Netherlands found a prevalence of voriconazole resistance of 19.0% (37/196) among *A. fumigatus* culture-positive proven, probable or putative IA patients,[29] with 53.0% of the cases having an underlying haematological disease. The resistance prevalence for the Belgian centre (University Hospitals Leuven, 17.1%) in this study was considerably higher than the 5.5% (9/164 cases) detected during a nationwide Belgian surveillance study (April 2011 to April 2012), which targeted all patients with *Aspergillus* disease. Only a single haematology patient with voriconazole-resistant IA was detected during that 1 year survey conducted in 30 hospitals (4.0%, 1/25 cases).[33]

In non-ICU patients with voriconazole-resistant IA, we observed a significantly higher 12 week mortality (54.5%) compared with voriconazole-susceptible cases (30.7%). This is in contrast with the US study in haematology patients in which no correlation between the MIC and the outcome of aspergillosis at week 6 was found. In the US study however, no high-level triazole resistance (MIC $\geq$8 mg/L) was detected among their resistant cases (MIC $\geq$8 mg/L for any triazoles tested)[16] and no *cyp51A* gene resistance-associated mutations were documented. In addition, combination therapy was frequently used in the above-mentioned cohort, which was not further detailed in the publication,[16] but may also have contributed to the lack of correlation between resistance and outcome. In a Dutch multicentre cohort study (with a setting of intensive resistance screening and MIC testing and a resistance prevalence of 19%), voriconazole resistance was associated with an absolute increase in overall mortality of 21.0% on day 42 and 25.0% on day 90.[29] The 12 week all-cause mortality rate (57.7%) among haematology patients with voriconazole-resistant IA in our study is at the lower end of mortality rates reported by other non-comparative case series, which vary between 50.0% and 100.0%.[12,15,23] In agreement with previous studies,[23,29] we observed the highest 12 week mortality in the ICU subgroup regardless of triazole susceptibility, which is a known confounder for mortality.[29] L-AmB antifungal therapy was started more often in ICU patients compared with non-ICU patients (42.0% and 12.8% respectively; P=0.012); however, due to the retrospective nature of the study, the reason for this is not known. We could not find any significant differences in mortality in the ICU subgroup according to triazole susceptibility, which may be partially explained by the already very high mortality of 73.3% in the triazole-susceptible ICU patient group, but also because of the severity of the patients'

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Table 2. Clinical characteristics of voriconazole-resistant *A. fumigatus* culture-positive IA haematology patients

Patient	Centre	Gender	Age (years)	Underlying disease	EORTC/MSG classification[a]	ICU[b]	Sample origin	*cyp51A* gene resistance mutation	MIC (mg/L)			Initial antifungal therapy	Antifungal therapy modification	Outcome week 6 (day 42)	Outcome week 12 (day 84)[d]
									ITC	VRC	POS				
1	1	male	55	alloSCT (AML)	probable	no	sputum	WT	1	4	0.25	L-AmB	no	alive	dead (+52)
2	1	female	59	NHL	proven	no	sinus biopsy	TR_{34}/L98H	>16	4	0.5	L-AmB	no	dead (+15)	
3	2	female	69	MDS	probable	yes	BAL	TR_{46}/Y121F/T289A	>16	>16	1	L-AmB	VRC	alive	alive
4	2	male	51	NHL	probable	no	sputum	TR_{34}/L98H	>16	8	0.5	VRC	L-AmB	alive	alive
5	2	female	64	NHL	probable	yes	BAL	TR_{46}/Y121F/T289A	>16	>16	1	VRC	L-AmB	dead (+25)	
6	2	male	61	NHL	probable	no	sputum	TR_{34}/L98H + TR_{46}/Y121F/T289A	>16	>16	1	L-AmB	no	dead (+11)	
7	2	female	19	NHL	probable	no	sputum	TR_{34}/L98H	>16	8	1	VRC	no	dead (+18)	
8	2	male	54	NHL	probable	no	BAL	TR_{34}/L98H	>16	8	1	VRC	L-AmB	alive	alive
9	2	male	45	NHL	probable	no	bronchial aspirate	TR_{46}/Y121F/T289A	1	>16	0.25	VRC	L-AmB	alive	alive
10	2	female	67	AML	probable	no	bronchial aspirate	TR_{46}/Y121F/T289A	>16	>16	0.25	VRC	L-AmB	alive	alive
11	2	male	62	alloSCT (NHL)	probable	no	tissue biopsy	TR_{46}/Y121F/T289A	0.5	>16	0.5	VRC	L-AmB	alive	alive
12	2	male	70	AML	probable	yes	bronchial aspirate	TR_{34}/L98H	>16	8	1	VRC	L-AmB	alive	alive
13	3	male	46	NHL	probable	no	BAL	TR_{34}/L98H	>16	4	0.5	VRC	no	alive	alive
14	3	male	29	alloSCT (HL)	probable	yes	BAL	TR_{34}/L98H	>16	8	2	VRC	no	alive	dead (+44)
15	3	male	64	haematological malignancy	probable	no	BAL	TR_{46}/Y121F/T289A	>16	4	1	VRC	no	alive	alive
16	3	male	22	alloSCT	probable	no	sputum	TR_{46}/Y121F/T289A	>16	>16	1	L-AmB	no	dead (+9)	
17	3	female	62	haematological malignancy	proven	no	BAL	TR_{46}/Y121F/T289A	2	>16	1	VRC	L-AmB	dead (+10)	
18	3	female	40	alloSCT	proven	yes	BAL	TR_{34}/L98H	>16	4	1	VRC	VRC + echinocandin	dead (+32)	
19	3	male	53	haematological malignancy	probable	no	sputum	TR_{46}/Y121F/T289A	>16	>16	1	VRC	L-AmB	alive	dead (+66)
20	3	female	64	haematological malignancy	probable	no	sputum	TR_{34}/L98H	>16	16	2	VRC	no	alive	alive
21	4	male	75	ALL	probable	no	BAL	TR_{34}/L98H	>16	4	1	L-AmB	no	dead (+3)	
22	4	male	51	autoSCT (HL)	proven	no	sputum	TR_{46}/Y121F/T289A	>16	>16	1	VRC + echinocandin	L-AmB	dead (+22)	
23	4	female	69	alloSCT (AML)	probable	yes	BAL	TR_{34}/L98H	>16	8	1	VRC	L-AmB	dead (+11)	
24	4	male	58	AML	probable	no	BAL	TR_{34}/L98H	>16	4	1	echinocandin	L-AmB + echinocandin	alive	dead (+66)
25	4	female	62	NHL	probable	yes	sputum	TR_{34}/L98H	8	2	1	VRC	L-AmB	alive	alive
26	4	male	57	alloSCT (MM)	proven	no	BAL	TR_{46}/Y121F/T289A	4	>8	1	triazole[c]	L-AmB	dead (+19)	

AutoSCT, autologous stem cell transplant; alloSCT, allogenic stem cell transplant; AML, acute myelogenous leukaemia; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; MDS, myelodysplastic syndrome; ITC, itraconazole; VRC, voriconazole; POS, posaconazole.

[a]IA classification according to the modified EORTC/MSG criteria.

[b]ICU: Initiation of antifungal therapy in the ICU and stay in the ICU for at least two consecutive days.

[c]Patient included in a randomized double-blinded clinical trial (voriconazole versus isavuconazole).

[d]Numbers in parentheses indicate survival (in days) after initiation of antifungal therapy for invasive aspergillosis.

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Table 3. Comparison of all-cause mortality in culture-positive IA haematology patients according to voriconazole susceptibility

Outcome: death	Number	Percentage	*P* value
Week 6 (42 days)			
Overall			0.200
susceptible	29/103	28.2	
resistant	11/26	42.3	
ICU			0.900
susceptible	10/15	66.7	
resistant	3/4	75.0	
Non-ICU			0.160
susceptible	19/88	21.6	
resistant	8/22	36.4	
Week 12 (84 days)			
Overall			0.064
susceptible	38/103	36.9	
resistant	15/26	57.7	
ICU			0.990
susceptible	11/15	73.3	
resistant	3/4	75.0	
Non-ICU			0.035
susceptible	27/88	30.7	
resistant	12/22	54.5	

underlying conditions and the small sample size. We did not observe any significant differences when comparing the characteristics of susceptible and resistant groups (Table 1). Even after controlling for possible confounders such as ICU management, a trend indicating a decrease in survival in patients with voriconazole-resistant IA infection remains (HR 1.74, P=0.072; Table S1).

In the recent hospital-wide cohort study from the Netherlands, mortality in patients who received appropriate initial voriconazole therapy was 24.0% compared with 47.0% in those who received inappropriate therapy, despite switching to appropriate antifungal therapy after a median of 10 days.[29] We, however, did not observe significant differences in mortality among overall and non-ICU haematology patients who received initial inappropriate (44.4% and 40.0%, respectively) or appropriate (38.0% and 33.3%, respectively) triazole therapy or after voriconazole-resistant cases were switched to an appropriate treatment with L-AmB (median time of 11 days). Several hypotheses can be raised that may contribute to this finding. First of all, the subgroup with initial inappropriate therapy, especially when focusing on the non-ICU population, is very small (n=15) and does not allow reliable results with this subanalysis. Secondly, it is possible that more confounding factors play a role in the haematology IA patient population compared with the non-haematology IA patient population. For instance, it is well known that neutrophil recovery and refractory haematological disease are important factors influencing the outcome of invasive fungal disease. On the other hand, our relatively small difference in survival between groups suggests a trend towards increased mortality in patients that received inappropriate initial triazole therapy, yet it may also indicate that initial triazole therapy does not negatively impact the outcome compared with immediate appropriate therapy even in regions with high resistance rates. However, as indicated previously, the number of patients with triazole resistance during the initial antifungal therapy is very small and it is not possible to draw any definite conclusions now. Moreover, several patients were part of clinical trials in which initial therapy was modified within the first 48 h after being assigned to a study medication group, which is a limitation of our study.

Recently, a panel of 21 international experts proposed that regions with a high level of triazole resistance (>10% prevalence in environmental isolates) should consider shifting initial treatment of IA from voriconazole monotherapy to a combination of voriconazole plus an echinocandin or L-AmB,[25] which was also recommended in the recent ESCMID/European Confederation of Medical Mycology (ECMM)/European Respiratory Society (ERS) *Aspergillus* guideline.[35] Given that national resistance surveillance of clinical *A. fumigatus* isolates in the Netherlands indicated that the 10% threshold has been reached for three consecutive years, the Dutch Working Party on Antibiotic Policy (SWAB) recently recommended first-line combination therapy for patients with IA, at least until triazole susceptibility has been established.[26] Initial triazole therapy may be considered for the non-critically ill haematological patient only when early bronchoscopy, together with immediate PCR-based testing for the presence of *cyp51A* gene mutations, is available.[26]

Resistance prevalence data should, however, be interpreted with caution since the prevalence is dependent on the denominator used.[34] The resistance frequency in culture-positive cases might overestimate the true prevalence in the overall IA population, as most IA cases are culture-negative and thus phenotypic resistance assessment cannot be performed. The culture-positive cases may therefore be a specific subpopulation of patients with IA and the cases with highest fungal burden with a different resistance prevalence and mortality compared with non-culture-positive cases. Molecular methods may be helpful to detect triazole resistance directly in clinical samples and help to determine resistance prevalence in culture-negative cases.[24] Montesinos *et al.*[36] evaluated the resistance prevalence in IA patients by comparing two screening strategies, PCR (AsperGenius, PathoNostics, Maastricht, The Netherlands) versus a culture-based strategy, but did not find any difference (prevalence 11.7% versus 10.5%) suggesting that the resistance frequency found in culture may be extrapolated to the entire group of IA cases.

As survival of patients with azole-susceptible IA treated with voriconazole or a combination of voriconazole and an echinocandin is superior to that with L-AmB or echinocandin monotherapy,[37–39] abandoning voriconazole as the initial treatment of choice might be counterproductive, jeopardizing the outcome in non-resistant cases. On the other hand, the increase in 12 week mortality and the high prevalence in patients with culture-positive voriconazole-resistant IA in both the Dutch hospital-wide cohort[29] and in our cohort of haematology patients suggests that adaptation of the policy of using voriconazole as the initial antifungal therapy should be considered, at least in triazole-resistant cases. Early detection of resistance seems critical to retain early triazole therapy and prevent excess mortality, which is the topic of the ongoing Azole-Resistance MANagement Study (AzoRMan, NCT03121235).[27] According to the study protocol, voriconazole is initiated in patients in whom IA is suspected. Treatment is re-evaluated following

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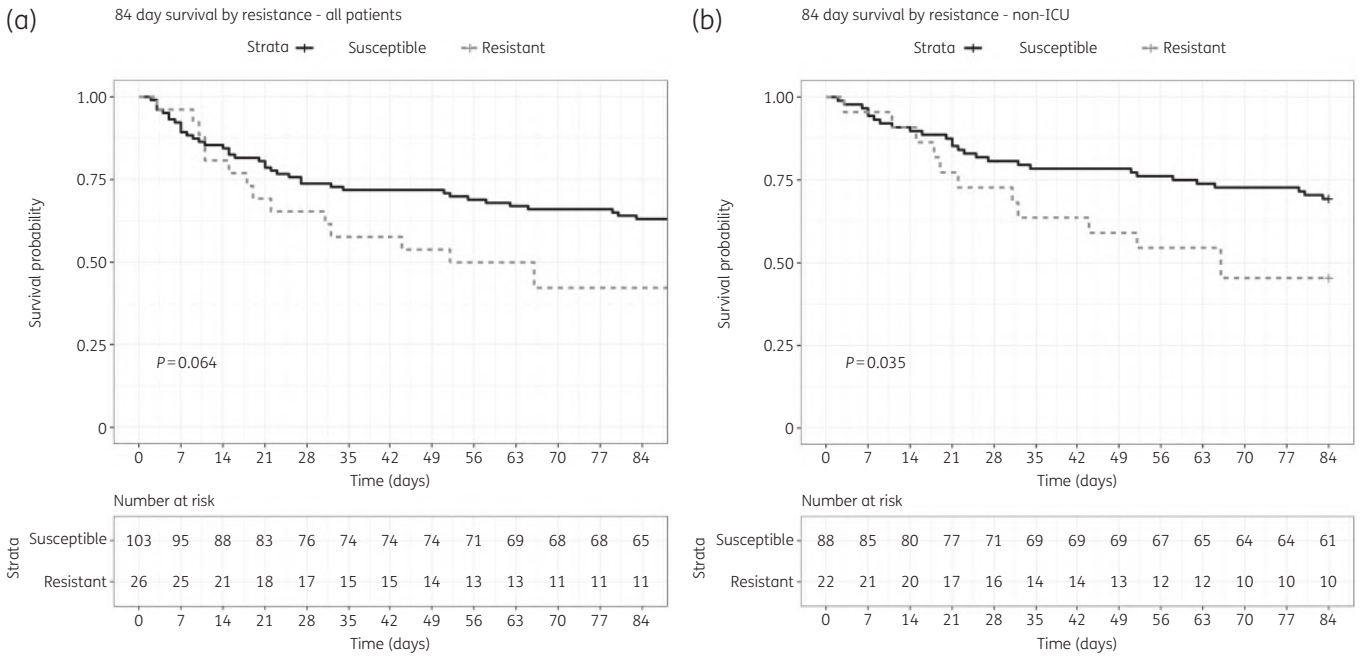

Figure 1. Overall survival of haematology patients with voriconazole-resistant and voriconazole-susceptible IA at 12 weeks (day 84). (a) Survival curve of all IA haematology patients. (b) Survival curve of IA haematology patients who did not start antifungal therapy in the ICU. Solid lines represent patients with voriconazole-susceptible isolates. Dotted lines represent patients with voriconazole-resistant isolates.

results obtained by prompt azole resistance testing by multiplex real-time PCR performed directly on BAL (AsperGenius). If resistance mutations are detected in the BAL sample the treating physician is advised to switch to L-AmB or, if PCR results are inconclusive, to add a second antifungal.[34] This trial may provide evidence that supports retaining voriconazole monotherapy in a setting with >10% environmental resistance, on the condition that a diagnostic strategy that enables early resistance detection is in place.

In conclusion, we found a high prevalence of voriconazole-resistant *A. fumigatus* in culture-positive IA in haematology patients in this multicentre study, with increased mortality in voriconazole-resistant cases in non-ICU patients. Whether the use of voriconazole as initial antifungal therapy for the treatment of IA can be retained or not still needs further investigation.

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Author contributions

The study was designed by K. L., J. M., P. P. A. L., P. E. V., A. R. S. and T. M. Data was collected and analysed by P. P. A. L., T. M., A. F. A. D. S. and A. R. S. First draft of the manuscript was created by A. R. S. and T. M. The final version of the manuscript was critically revised and adapted by A. R. S., T. M., P. P. A. L., J. M., B. J. A. R., A. F. A. D. S., M. T. B., J. J. C., E. K., P. A. V. D. B., P. E. V. and K. L.

Supplementary data

Methods and Table S1 are available as Supplementary data at *JAC* Online.

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