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Implementation of a clinical decision rule for selecting empiric treatment for invasive aspergillosis in a setting with high triazole resistance

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Abstract

World-wide, emerging triazole resistance increasingly complicates treatment of invasive aspergillosis (IA). In settings with substantial (>10%) prevalence of triazole resistance, empiric combination therapy with both a triazole and liposomal amphotericin B (LAmB) can be considered because of the low yields of susceptibility testing. To avoid toxicity while optimizing outcome, a strategy with monotherapy would be preferable. A newly designed treatment algorithm based on literature and expert consensus provided guidance for empiric monotherapy with either voriconazole or LAmB. Over a four and a half year period, all adult patients in our hospital treated for IA were included and patient data were collected. An independent committee reviewed the attributability of death to IA for each patient. Primary outcomes were 30- and 100-day crude mortality and attributable mortality. In total, 110 patients were treated according to the treatment algorithm. Fifty-six patients (51%) were initially treated with voriconazole and 54 patients (49%) with LAmB. Combined attributable and contributable mortality was 13% within 30 days and 20% within 100 days. Treatment switch to LAmB was made in 24/56 (43%) of patients who were initially treated with voriconazole. Combined contributable and attributable 100-day mortality in this subgroup was 21% and was not increased when compared with patients initially treated with LAmB ($P = 0.38$). By applying a comprehensive clinical decision algorithm, an antifungal-sparing regime was successfully introduced. Further research is warranted to explore antifungal treatment strategies that account for triazole-resistance.

Lay summary

Due to resistance of *Aspergillus* against triazoles, combination therapy with liposomal amphotericin B (LAmB) is applied more often as primary therapy against invasive aspergillosis. This study presents the results of a decision tool which differentiated between triazole or LAmB monotherapy.

Key words: Invasive aspergillosis, liposomal amphotericin B, voriconazole, triazole resistance, antifungal stewardship.

Introduction

Since the introduction of the antifungal drug voriconazole, mold-active triazole formulations are the primary treatment of choice for invasive aspergillosis (IA) in leading European and American guidelines.[1–3] However, over the past decades, emerging triazole resistance has developed as a new important threat to effective prevention and treatment of IA.[4–7] This development has given rise to an increase in the application of liposomal amphotericin B (LAmB), which is the drug of second choice for this indication.[8,9] This is concerning due to decreased efficacy and increased toxicity of Amphotericin B formulations when compared to voriconazole.[1,10–17] The highest incidence rates of IA can be found in patients with a hematological malignancy who receive intensive chemotherapeutic treatment and/or undergo hematopoietic stem cell transplantation (HSCT).[18–21] Despite the use of antifungal chemoprophylaxis, the residual incidences observed in this population often remain 5–10% or more.[21–23] Furthermore, due to the increasing number of patients that survive with temporary or chronic diseases of the immune system and by introduction of new treatment modalities e.g., tyrosine kinase inhibitors like ibrutinib, the population at risk of IA is expanding.[15,24,25]

Traizole-resistance mutations in the fungal DNA, specifically the CYP51A-gene and its promotor-region, are most often associated with pan-triazole resistance and therefore necessitate the use of LAmB. Use of LAmB can be associated with lower rates of treatment success and high rates of toxicity when compared to voriconazole.[10,12,13] Demonstrated resistance against all triazoles is a clear indication for the use of LAmB.[9] Deciding between voriconazole and LAmB as empiric therapy is difficult when the clinician suspects the presence of triazole resistant IA, but definite proof is missing. The background resistance rate, which varies among different populations at risk and different regions, needs to be taken into account when making this decision.

The first clinical isolates with triazole-resistance mechanisms have been recognized in Northern Western Europe.[7,26] At present, resistance against triazoles in environmental as well as clinical isolates has developed to a global problem.[27–32] Results of a nationwide surveillance study in 2014 showed a triazole resistance rate of 5% in the USA, and presence of resistant isolates in the environment has been demonstrated as well.[29,31] In the Netherlands, average resistance rates of *Aspergillus fumigatus* were 14.7% of 764 isolates screened in academic hospitals and 7.8% of 784 isolates screened in non-academic teaching hospitals in 2018.[33] It is complicated to measure the impact of triazole resistance in the clinical setting. Due to the fact that cultures and antifungal susceptibility testing fails in the majority of clinical specimens, the clinician often has to resort to an empirical treatment strategy. Although our ability to determine susceptibility has recently been improved due to the introduction of PCR, the combined results of both culture and PCR are conclusive only in 30–60% of patients with probable invasive aspergillosis.[34–36] The resulting uncertainty about susceptibility easily gives rise to overtreatment with LAmB. When there is a high background resistance rate, any clue that raises the suspicion of resistance could motivate the clinician to opt for the use of LAmB instead of voriconazole. To ensure both the addition of the survival benefit of treatment with voriconazole and covering the risk of triazole resistance, Dutch national guidelines advise to empirically treat IA with combination therapy of both voriconazole and LAmB in case of unknown susceptibility.[37] However, the expected benefits of this strategy need to be weighed against a higher rate of serious adverse events associated with combination therapy, as well as higher costs,[16,38,39] but no randomized study data are available on this topic. Furthermore, no randomized head-to-head comparison between LAmB and voriconazole has been published, leaving some uncertainty about the superiority of voriconazole.[10–13]

To evade unnecessary toxicity while optimizing outcome, a clinical decision rule guiding to monotherapy with either voriconazole or LAmB was designed and validated in our hospital in a region with resistance rates between 16 and 24% reported in the last 10 years.[33]

Methods

Development of a clinical treatment strategy

In 2014, all medical specialties in the Leiden University Medical Center involved in the diagnosis and treatment of patients with IA participated in constructing a treatment algorithm that provided guidance for empiric monotherapy with either voriconazole or LAmB (Figure 1). The algorithm aimed to optimize the balance between the risk of treating triazole-resistant IA with voriconazole and the risk of unnecessarily treating triazole-susceptible IA with LAmB. The treatment protocol was based on literature and guidelines[1,21,23,38,40–42] and information about local resistance rates.[33]

Criteria were formulated that either predispose for a worse outcome (e.g., presence of respiratory insufficiency) or were thought to predispose for infection with a triazole-resistant isolate (development of IA during chemoprophylaxis with a triazole). On the basis of these criteria, a decision about empiric (i.e., awaiting susceptibility testing) treatment was made. Both predicted worse outcome and breakthrough infections were considered indications for empiric treatment with LAmB. Although it is currently not supported by the literature that prophylaxis

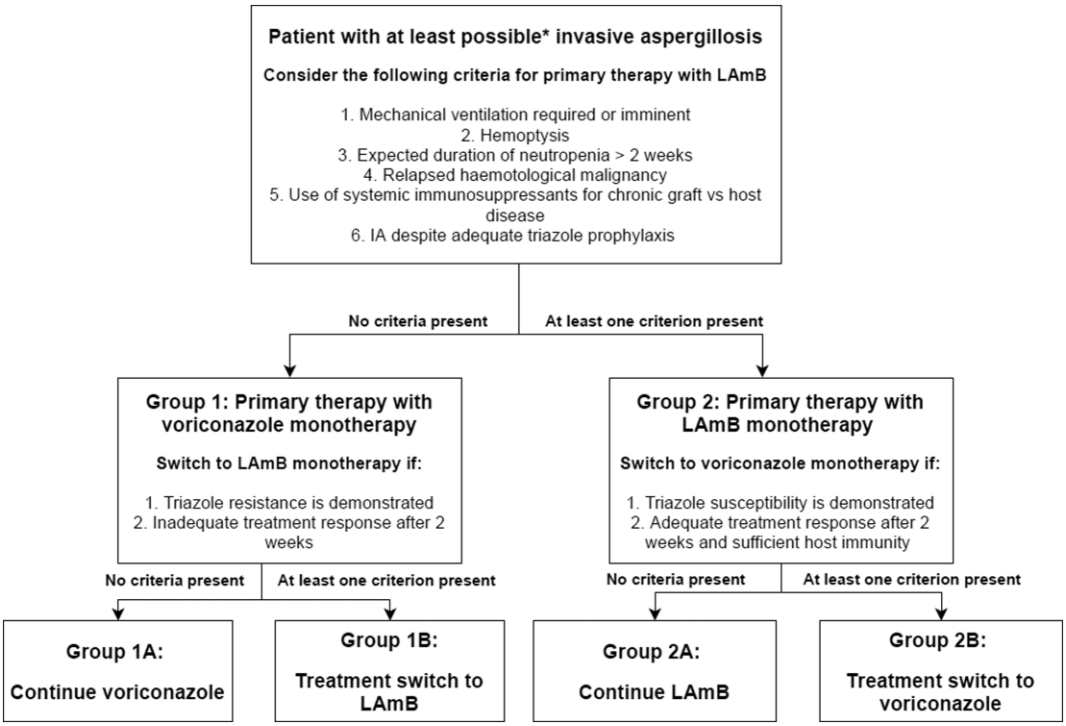

Figure 1. Flowchart of the treatment protocol.
Legend: The treatment protocol was implemented in a setting with high > 10% of triazole resistance. IA denotes Invasive Aspergillosis; LAmB Liposomal amphotericin B. *as defined by the 2008 EORTC/MSG criteria for the diagnosis of invasive fungal infections.[40]

using a triazole predisposes for infection with a triazole-resistant isolate, the committee that constructed the algorithm decided to apply this criterion based on its rational concept. The reason to initially treat patients with a predicted worse outcome with LAmB was to mitigate the risk of applying ineffective treatment to a severely ill patient with potentially triazole-resistant IA. As treatment failure in the first phase of disease is associated with higher mortality, avoiding this risk was considered a high priority.[20,41,43] Combination therapy was not applied to avoid possible toxicity or other complications.

During treatment, results of susceptibility testing were used directly to switch treatment if appropriate. After two weeks of treatment without information on susceptibility, an evaluation of treatment outcome was made. In case of clinical non-response in a patient initially treated with voriconazole, the risk of triazole-resistance was considered high and treatment was switched to LAmB. In case of clinical non-response in a patient initially treated with LAmB, the risk of an (intrinsically) polyene-resistant infectious agent was considered to still be very low. Because host factors were considered to be the most likely cause of non-response in this case, no benefit was expected to switch treatment to voriconazole. In case of response to treatment in patients treated with LAmB, treatment was de-escalated to voriconazole monotherapy under close monitoring.

The designed algorithm was approved by the institution's antimicrobial steering committee and was implemented from January 2015 onwards.

Study protocols and definitions

All patients who were considered to have a high risk of IA routinely received prophylaxis with a triazole with activity against *Aspergillus*. This included patients receiving remission-induction therapy prior to allogeneic HSCT, patient who underwent allogeneic HSCT, patients with prolonged neutropenia (at least two weeks), or other patients receiving high risk chemotherapeutics that induce neutropenia or impaired granulocyte function. Therapeutic drug monitoring was applied in this population.[44]

Protocolized CT-scanning and bronchoalveolar lavage (BAL) were performed upon suspicion of IA. BAL samples were examined by direct microscopy, culture, Galactomannan assay (cut off at 0.5 optical density) and from 2017 onwards also by PCR. All available BAL samples from patients initially not tested by PCR were retrospectively tested. The AsperGenius® PCR assay (Pathonostics, Maastricht, the Netherlands) was used. Triazole resistance was routinely tested by four well agar plate screening (VIP check, Groningen, The Netherlands).

Data collection

All adult patients were retrospectively included if they had been treated according to our treatment protocol between January 2015 and September 2019. Patients who received either LAmB, voriconazole, isavuconazole or posaconazole in our center were identified through the hospital pharmacy database. Of these patients, the electronic medical records were examined to ensure

eligibility for inclusion in our study. Extracted patient characteristics included age, diagnosis of immunocompromising disease, diagnosis of IA, comorbidity and immune status, as well as information about performed diagnostics, triazole susceptibility, dosage of therapy, duration of therapy, treatment history, switch of antifungal therapy, renal function and outcome of IA. IA was classified according to the 2008 revised European Organisation for Research and Treatment of Cancer – Mycoses Study Group criteria for the classification of invasive fungal infections.[40] After retrieval of all relevant information, the data of all participants was pseudonymized.

Definition of attributable mortality

The role of IA with regard to the cause of death was classified as either 'non-attributable to IA', 'contributable', 'attributable', or 'unknown' (see criteria in the **text Box 1**). The last category was introduced because the clinical data at time of death were insufficient for a few cases. Attributable and contributable mortality were the primary outcome measures for this study. The classification was constructed by the investigator group prior to analysis of the data and was based on modification of definitions from literature.[41,43] A committee was instructed to determine attributability in all patients who died within 100 days. The committee consisted of three reviewers who were not directly involved with the study, with experience in the fields of infectious diseases, clinical microbiology and hematology. The reviewers received written instructions to use the medical correspondence, post-mortem reports and laboratory findings as reported in the patient files to categorize the deceased patients according to the above definitions. In case of disagreement between the reviewers, the case was discussed between all three reviewers until consensus was reached.

Box 1. Attributable

The immediate cause of death was defined as the disease process, injury, or complication immediately preceding death. IA was considered the cause of death when the immediate cause of death was due to this infection. Examples are neurological complications of an *Aspergillus* infection that disseminated to the brain, lung bleeding or respiratory insufficiency in a patient with pulmonary aspergillosis

or

IA was judged to have played a major role if death would not have occurred had the patient not had IA, even though another condition was present that also contributed to death. This includes toxicity, interactions and other side effects of antifungal treatment that played a major role in the cause of death. Another example is a *Pseudomonas* bacteremia in a patient with a cavitating pulmonary aspergillosis in which the lungs are considered the most likely source of the bacteremia.

Contributable

IA or treatment of IA was defined as playing a minor role if it was probably not essential in explaining the patient's death but arguably did play some role in the event. Example is a patient with an aspergillus infection as well as severe uncontrolled gastrointestinal GVHD at the time of death

Non-attributable

Mortality was classified as not related to IA if there was a clear other cause of death not related to IA

Unknown:

If insufficient data were present about the circumstances death occurred

Statistics

Survival proportions were calculated and comparative analyses were performed using SPSS version 25 (IBM Corp., Armonk, NY).

Ethics

The study was reviewed by the institutional review board, which deemed that the Dutch law on research on human subjects was not applicable to our study. Data were processed in accordance with the national Personal Data Protection Act.

Results

Protocol adherence and study population characteristics

A total number of 115 patients were treated for IA at our institution from January 2015 to September 2019. Of this total, 110 patients (96%) were treated for IA with either voriconazole or LAmB monotherapy according to the designed algorithm. The remaining five patients received off-protocol treatment regimens (Figure 2). These patients received either combination therapy, or violated the criteria mentioned in the decision tool otherwise. For all results, the remaining 110 patients were used to calculate percentages, with the exception of the triazole resistance rates.

Study population characteristics

Out of 110 included patients, 76 (69%) were male; the median age was 63 (range 20–83), and 100 (90%) patients were treated for an underlying hematologic malignancy or had undergone allogeneic stem cell transplantation (Table 1). Of patients with hematologic malignancy, 14 suffered from a relapsed malignancy and eight from a secondary (treatment related) malignancy. A small subgroup of patients (10%) did not suffer from hematologic malignancy but were recipients of a solid organ transplantation, received chemotherapy for other malignancies, or were immunocompromised for other reasons.

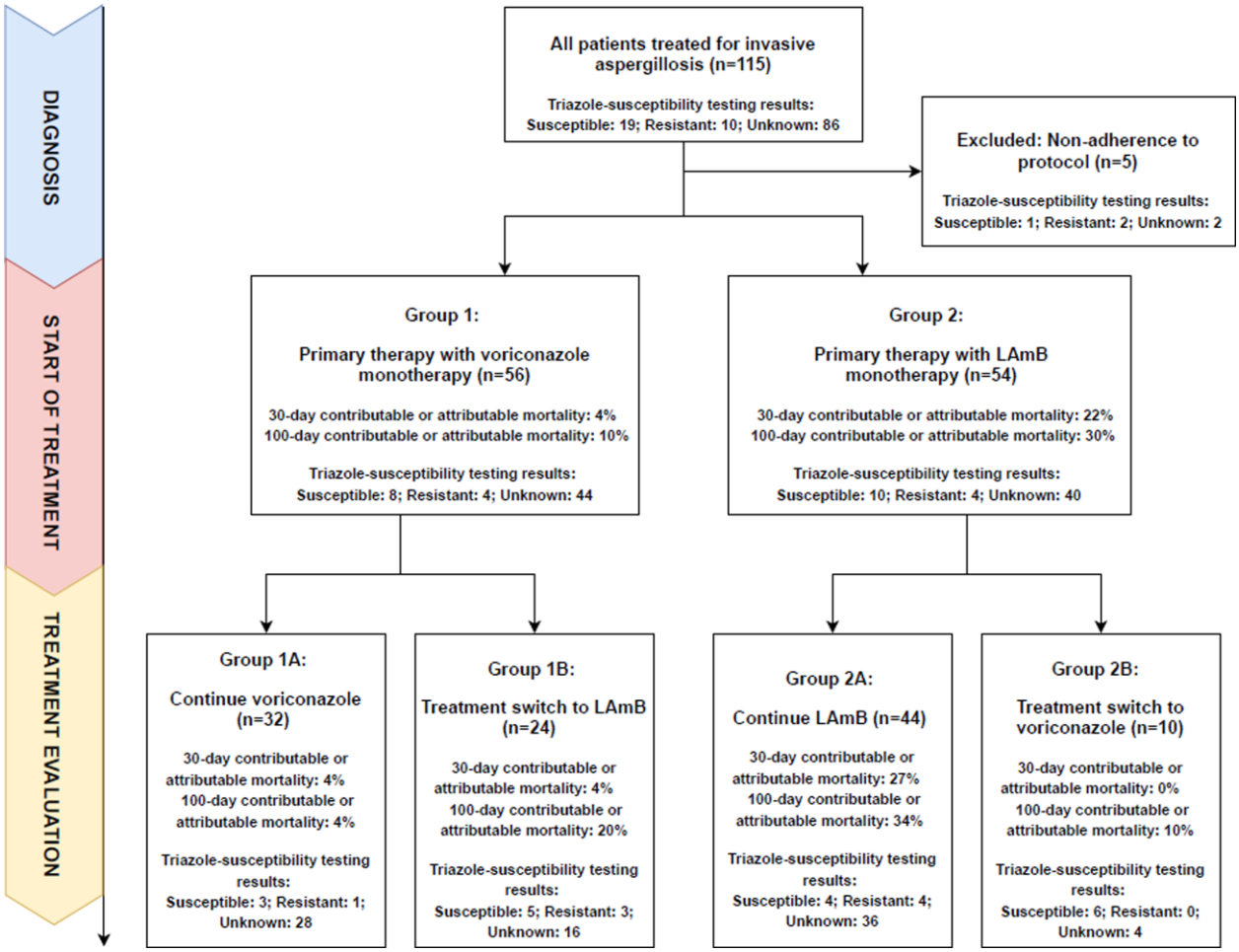

Figure 2. The treatment protocol with numbers of patients in each arm as well as resistance and mortality rates.
Legend: LAmB Liposomal amphotericin B. Susceptibility testing was done by applying both PCR and culture techniques. Resistance data were not yet known at the moment of start of therapy, but were known at the moment of treatment evaluation. Contributable and attributable mortality rates were defined in the process as described in the methods section and can also be found in Table 3. *possible IA was defined according to the 2008 revised definitions for the diagnosis of invasive fungal infections.[40]

Treatment and outcomes

Fifty-six patients (51%) were initially treated with voriconazole and 54 (49%) were initially treated with LAmB. Cultures were positive in 16/115 (14%) patients and phenotypical voriconazole resistance was detected in 5/16 (31%). Overall, susceptibility testing was successful in 29 cases, yielding 10 (34%) triazole-resistant and 19 (66%) triazole-susceptible isolates. Susceptibility was determined phenotypically by culture in 13 clinical isolates, and genetically with PCR in 16 clinical isolates. In one case, triazole resistance was detected by both PCR and culture. Five non-fumigatus *Aspergillus* fungi were identified with PCR or culture, none of which with intrinsic LAmB-resistance (two *Aspergillus flavus*, two *Aspergillus niger*, one *Aspergillus nidulans*).

Upon clinical evaluation of empiric therapy and of resistance data, a switch was made to LAmB in 24/56 (43%) of patients who were initially treated with voriconazole. Table 2 displays the reasons for treatment switch. In the group that started treatment with LAmB, 9 out of 54 (17%) patients switched to voriconazole. Treatment was completed with voriconazole in 42 cases and with LAmB in 68 patients. The flowchart (Figure 2) shows the number of patients in each treatment group.

Therapy-related adverse events occurred in both treatment arms. Hepatotoxicity was a reason to stop treatment with voriconazole in seven patients (13%) and nephrotoxicity was a reason to stop treatment with LAmB in 11 patients (20%). Allergic reaction was a reason to stop treatment with voriconazole in one case. Serious alteration of mental state and/or visual hallucinations was a reason to stop treatment with voriconazole in two cases. Reasons to start treatment with LAmB are displayed in Table 2. The most common reason to initially treat with LAmB was adequate prophylaxis with voriconazole before establishing the diagnosis of IA (28 patients). Of these 28 patients,

Table 1. Characteristics of patients treated for invasive aspergillosis.

Total number of patients	110
Sex, male (%)	76 (69)
Age, median (range)	63 (20–83)
Underlying predisposing disease, number of pts. (%)	
Total with hematologic malignancy or after HSCT	100 (91)
Total who underwent HSCT	54 (49)
Patients with hematologic malignancy:	45 (41)
AML/MDS-RAEB2	29 (26)
ALL	12 (11)
Multiple myeloma	4 (4)
MDS	4 (4)
Aplastic anemia	2 (2)
CLL	2 (2)
Myelofibrosis	1 (1)
CML	1 (1)
Received HSCT for sickle cell disease	
Receiving chemotherapy for solid tumor	2 (2)
Kidney transplantation	3 (3)
Liver transplantation	2 (2)
Other* (not malignant, not transplant-related)	3 (3)

Legend: IA denotes invasive aspergillosis, HSCT hematopoietic Stem Cell Transplantation, LAmB Liposomal Amphotericin B, ALL Acute Lymphoid Leukaemia, AML Acute Myeloid Leukemia, CML Chronic Lymphoid leukemia, CLL Chronic Lymphoid leukemia, MDS Myelodysplastic Syndrome, MDS-RAEB2 Myelodysplastic Syndrome - Refractory Anaemia with Excess Blasts grade 2. *Other underlying diseases: severe anorexia nervosa, badly regulated diabetes type 1, and influenza.

susceptibility testing was successful in four cases, of which only one was triazole resistant.

Crude and attributable mortality rates per treatment stratum are listed in Table 3. The flowchart (Figure 2) shows a combination of attributable and contributable rates only. Contributable or attributable mortality was lower in patients initially treated with voriconazole (30-day contributable or attributable mortality was 6%) than in patients initially treated with LAmB (30-day contributable or attributable mortality was 22%). Mortality was lowest in patients who were only treated with voriconazole monotherapy. Mortality was highest in patients who were only treated with LAmB monotherapy and in patients initially treated with voriconazole and later switched to treatment with LAmB as per the rules of the decision tree. The mortality rates differed only slightly (61 vs 54% crude 100-day mortality, 34 vs 21% combined attributable and contributable 100-day mortality). Attributable mortality was highest in the first period after diagnosis of IA. After 40 days, most mortality was either non-attributable to IA or of unknown cause (Figure 3). Combined contributable and attributable mortality for all groups was 12% after 30 days and 20% after 100 days. Mortality unrelated to IA within 30 days was lower in patients with proven or probable IA only when compared to all patients (4 versus 10% overall).

Table 2. Motivation of treatment decisions.

Number of patients initially treated with voriconazole (group 1)	56
Number of patients initially treated with LAmB (group 2)	54
*Reason to initially treat with LAmB**, number of patients, (% of patients treated with LAmB)	
Mechanical ventilation required or imminent	9 (17)
Hemoptysis	0
Expected duration of neutropenia >2 weeks	2 (4)
Relapsed hematologic disease	14 (26)
Use of systemic immunosuppression for chronic graft versus host disease	0
IA occurred despite adequate prophylaxis with a triazole	28 (52)
Broader antifungal spectrum deemed necessary (e.g., suspicion of mucormycosis)	5 (9)
Intolerance or significant drug interaction with voriconazole	4 (7)
Number of patients who switched from voriconazole to LAmB (group 1B)	**24 (23)**
Reasons to switch, number of patients, (% of patients who switched)	
Resistance to azoles demonstrated	3 (13)
Progression of IA	15 (63)
Intolerance to voriconazole	6 (25)
Number of patients who switched from LAmB to voriconazole (group 2B)	**10 (9)**
Reasons to switch, number of patients (% of patients who switched):	
Susceptibility to azoles demonstrated	6 (60)
Adequate treatment response and sufficient recovery of host immunity	2 (20)
Intolerance to LAmB	2 (20)

Legend: LAmB denotes Liposomal Amphotericin B, IA Invasive Aspergillosis. *More than one reason could be present for one patient.

The calculation of survival rates was repeated for proven and probable cases of IA (defined according to the 2008 revised EORTC criteria[40]) and detailed in supplement A. The results were similar to the results of the overall analysis.

Discussion

By applying a comprehensive clinical decision algorithm in our area with high (>10%) triazole-resistance rates, 51% of patients were empirically treated with monotherapy voriconazole, without indications for excess crude mortality even if a later switch to LAmB was needed. In 29% of patients, therapy with LAmB could be avoided during the entire course of treatment. Our study provides a rationale to effectively account for possible triazole resistance while preventing the negative effects associated with combination therapy. However, due to the way the study has been designed, it is not possible to make direct comparisons

Table 3. Outcomes of patients treated according to the protocol.

	Total	Group 1 (VOR)	Group 2 (LAmB)	Group 1A (VOR)	Group 1B (VOR, LAmB)	Group 2A (LAmB)	Group 2B (LAmB, VOR)
Number of patients	**110**	**56**	**54**	**32**	**24**	**44**	**10**
30-day mortality # *patients (%)*							
Total	**26 (24)**	**7 (13)**	**19 (35)**	**4 (13)**	**3 (13)**	**19 (43)**	**0**
Attributable	3 (3)	2 (4)	1 (2)	1 (4)	1 (4)	1 (2)	0
Contributable	11 (10)	0	11 (20)	0	0	11 (25)	0
Unrelated	11 (10)	5 (9)	6 (11)	3 (9)	2 (8)	6 (11)	0
Unknown	1 (1)	0	1 (2)	0	0	1 (2)	0
100-day mortality # *patients (%)*							
Total	**51 (46)**	**20 (34)**	**31 (57)**	**9 (28)**	**11 (54)**	**27 (61)**	**4 (40)**
Attributable	6 (5)	3 (5)	3 (6)	1 (4)	2 (8)	2 (5)	1 (10)
Contributable	16 (15)	3 (5)	13 (24)	0	3 (13)	13 (29)	0
Unrelated	24 (22)	12 (21)	12 (22)	6 (18)	6 (28)	9 (20)	3 (30)
Unknown	5 (9)	2 (4)	3 (6)	2 (6)	0	3 (7)	0
Azole-resistance # *of patients (%)*	8 (5)	4 (7)	4 (7)	1 (3)	3 (13)	4 (10)	0
Azole-susceptibility # *of patients (%)*	18 (15)	8 (14)	10 (19)	3 (9)	5 (21)	4 (9)	6 (60)
Diagnostic certainty # *of patients (%)*							
Possible	36 (33)	16 (29)	20 (37)	13 (41)	3 (13)	19 (43)	1 (10)
Probable	72 (65)	39 (70)	33 (61	19 (59)	20 (83)	25 (57)	8 (80)
Proven	2 (2)	1 (2)	1 (2)	0	1 (4)	0	1 (10)

Legend: LAmB denotes Liposomal amphotericin B, VOR voriconazole. Diagnostic certainty was defined according to the revised 2008 EORTC/MSG criteria for the diagnosis of Invasive Fungal Infection.[40] Group 1 consists of patient initially treated with voriconazole. Subgroup 1A continued treatment with voriconazole, while subgroup 1B switched to LAmB eventually. Group 2 consists of patients initially treated with LAmB. Subgroup 2A continued treatment with LAmB, while subgroup 2B switched to voriconazole eventually. The treatment rules for the different groups can be found in Figure 1.

between treatment groups; the basis on which the choices for therapy were made were dependent on clinical factors which are also associated with outcomes, thought to be helpful in identifying the patients who would benefit the most from the relevant treatment strategy. This distinction causes factors that are correlated with a worse outcome to not be equally distributed between the treatment groups. Mortality rates of patients initially directed to voriconazole monotherapy were lower when compared to people who were treated with LAmB. However, the group of patients that were initially treated with LAmB were expected to have a worse outcome at baseline.

The treatment decision tree was constructed in accordance with our local antibiotics steering committee, with knowledge about local epidemiology and triazole-resistance rates, current literature and the relevant guidelines. National and international guidelines that describe the optimal management for IA need to rely on low evidence levels, in part due to the relative rarity of IA. Additionally, because of improvements in the management of patients with hemato-oncological disease, the population at risk is becoming harder to define and is ever-changing. Emerging triazole resistance makes it even more difficult to formulate an unambiguous treatment advice.

No data are available that support an increased risk of triazole-resistant IA in patients that develop IA despite receiving adequate prophylaxis with a triazole. We did choose to include this as a criterion to initially treat with LAmB. Nonetheless, no remarkable additional risk of triazole-resistance was found in this subgroup (of four successful susceptibility tests in 28 patients, one isolate showed triazole resistance). A limitation of this study is that detection of resistance was not as successful as reported in literature despite the use of both PCR and conventional culture.[34–36,45] Reported rates of triazole resistant IA in our region are amongst the highest in the Netherlands, and in the world.[32,33] The high local resistance rates have been linked to the extensive use of fungicides in agriculture.[7] All-cause mortality in our cohort was high with a 46% mortality rate within 100 days. In patients with proven and probable invasive aspergillosis only, this rate was similar (41%). In literature, the mortality rates differed greatly between different subpopulations, but were on average lower than in our population.[15,22,23] However, within the total mortality count, a minority of cases was attributable or contributable to IA. The nature of the subpopulation plays a large role in the expected case fatality rate. On average, our population consisted of patients with both a high risk of developing IA and a high risk of mortality due to hematological malignancy. Additionally, T-cell depleted HSCT is standard practice in our center. This method of transplantation is associated with a substantially lower risk of graft versus host disease, but at the cost of more difficulty in the treatment of opportunistic infections due to an initially less effective T-cell mediated immune response.

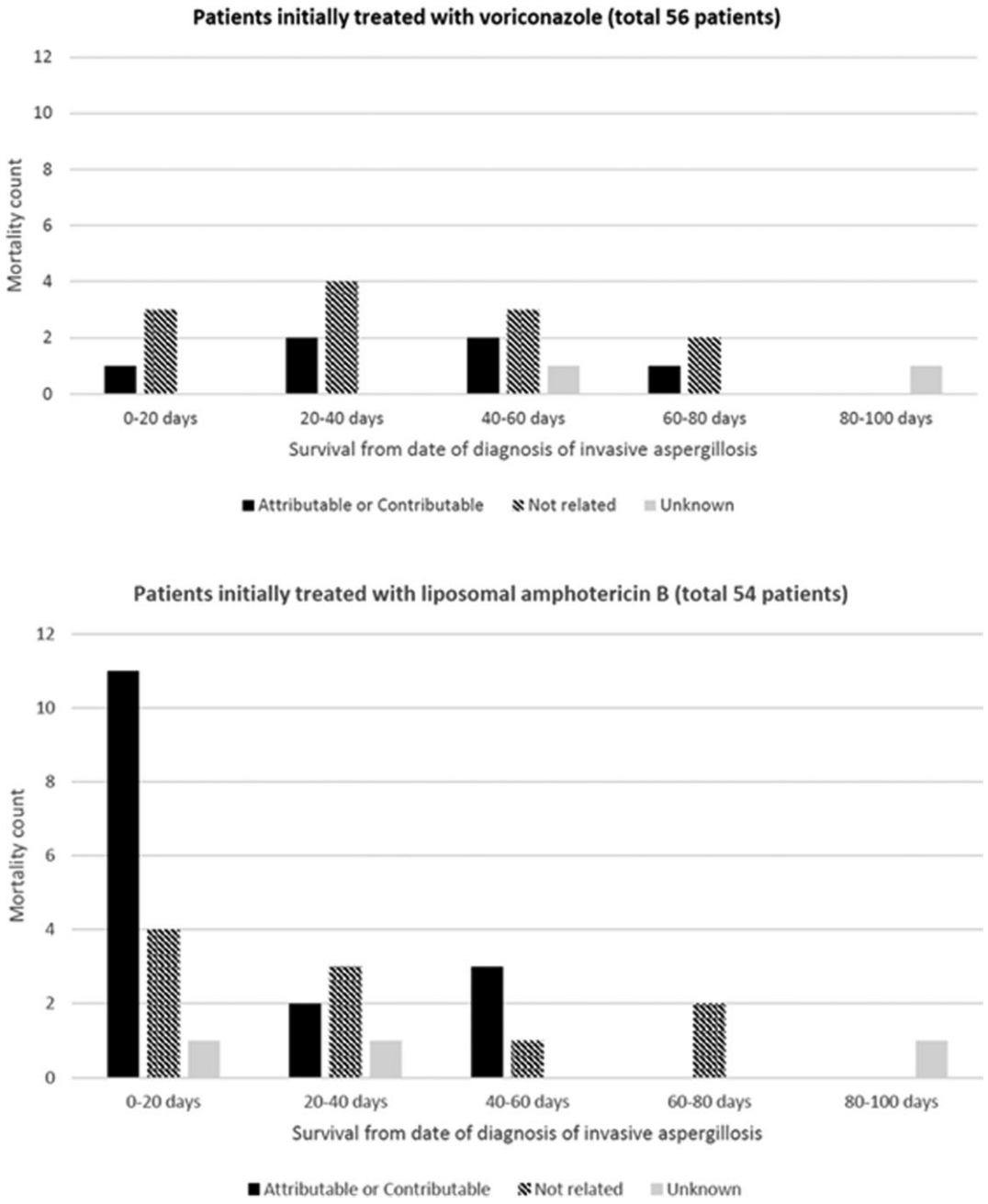

Figure 3: Mortality counts of patients treated for Invasive Aspergillosis.
Legend: IA denotes Invasive Aspergillosis. Attributable or contributable mortality was determined in the procedure as described in the methods section.

In conclusion, the results of our study can provide new insight in the application of an antifungal-sparing clinical decision tool while minimizing the risks of the consequences of undertreatment. The non-randomized approach and heterogeneous population make it difficult to make generalized statements about treatment effectiveness, and future research could further expand on the hypothesis that LAmB can have an important role in the treatment of IA in areas with high triazole resistance without the necessity of combination therapy.

Supplementary material

Supplementary material is available at *MMYCOL* online.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. Patterson TF, Thompson GR 3rd, Denning DW et al. Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the infectious diseases society of america. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2016; 63: e1–e60.
2. Tissot F, Agrawal S, Pagano L et al. ECIL-6 guidelines for the treatment of invasive candidiasis, aspergillosis and mucormycosis in leukemia and hematopoietic stem cell transplant patients. *Haematologica* 2017; 102: 433–444.
3. Ullmann AJ, Aguado JM, Arikan-Akdagli S et al. Diagnosis and management of *aspergillus* diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2018; 24 Suppl 1: e1–e38.
4. Fuhren J, Voskuil WS, Boel CH et al. High prevalence of azole resistance in *aspergillus fumigatus* isolates from high-risk patients. *J Antimicrob Chemother* 2015; 70: 2894–2898.
5. Lestrade PPA, Meis JF, Melchers WJG, Verweij PE. Triazole resistance in *aspergillus fumigatus*: recent insights and challenges for patient management. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2019; 25: 799–806.
6. Rybak JM, Fortwendel JR, Rogers PD. Emerging threat of triazole-resistant *aspergillus fumigatus*. *J Antimicrob Chemother* 2019; 74: 835–842.
7. Verweij PE, Snelders E, Kema GH, Mellado E, Melchers WJ. Azole resistance in *aspergillus fumigatus*: a side-effect of environmental fungicide use? *Lancet Infect Dis* 2009; 9: 789–795.
8. van der Linden JW, Snelders E, Kampinga GA et al. Clinical implications of azole resistance in *aspergillus fumigatus*, the netherlands, 2007–2009. *Emerg Infect Dis* 2011; 17: 1846–1854.
9. Verweij PE, Ananda-Rajah M, Andes D et al. International expert opinion on the management of infection caused by azole-resistant *aspergillus fumigatus*. *Drug Resist Updat* 2015; 21-22: 30–40.
10. Cornely OA, Maertens J, Bresnik M et al. Liposomal amphotericin b as initial therapy for invasive mold infection: a randomized trial comparing a high loading-dose regimen with standard dosing (AmBiLoad trial). *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2007; 44: 1289–1297.
11. Denning DW. Comparison of 2 studies of treatment of invasive aspergillosis. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2007; 45: 1106–1108; author reply 8-10.
12. Herbrecht R, Denning DW, Patterson TF et al. Voriconazole versus amphotericin b for primary therapy of invasive aspergillosis. *N Engl J Med* 2002; 347: 408–415.
13. Herbrecht R, Patterson TF, Slavin MA et al. Application of the 2008 definitions for invasive fungal diseases to the trial comparing voriconazole versus amphotericin b for therapy of invasive aspergillosis: a collaborative study of the mycoses study group (MSG 05) and the european organization for research and treatment of cancer infectious diseases group. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2015; 60: 713–720.
14. Adler-Moore J, Lewis RE, Bruggemann RJM et al. Preclinical safety, tolerability, pharmacokinetics, pharmacodynamics, and antifungal activity of liposomal amphotericin B. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2019; 68: S244–S59.
15. Dragonetti G, Criscuolo M, Fianchi L, Pagano L. Invasive aspergillosis in acute myeloid leukemia: are we making progress in reducing mortality? *Med Mycol* 2017; 55: 82–86.
16. Groll AH, Rijnders BJA, Walsh TJ et al. Clinical pharmacokinetics, pharmacodynamics, safety and efficacy of liposomal amphotericin B. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2019; 68: S260–S74.
17. Kato H, Hagihara M, Yamagishi Y et al. The evaluation of frequency of nephrotoxicity caused by liposomal amphotericin B. *Journal of infection and chemotherapy: official journal of the Japan Society of Chemotherapy* 2018; 24: 725–728.
18. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. *Blood* 2002; 100: 4358–4366.
19. Camargo JF, Husain S. Immune correlates of protection in human invasive aspergillosis. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2014; 59: 569–577.
20. Neofytos D, Treadway S, Ostrander D et al. Epidemiology, outcomes, and mortality predictors of invasive mold infections among transplant recipients: a 10-year, single-center experience. *Transplant infectious disease: an official journal of the Transplantation Society* 2013; 15: 233–242.
21. van de Peppel RJ, Dekkers OM, von dem Borne PA, de Boer MG. Relapsed and secondary disease drive the risk profile for invasive aspergillosis prior to stem cell transplantation in patients with acute myeloid leukemia or myelodysplastic syndrome. *Med Mycol* 2014; 52: 699–705.
22. Pagano L, Caira M, Candoni A et al. Invasive aspergillosis in patients with acute myeloid leukemia: a SEIFEM-2008 registry study. *Haematologica* 2010; 95: 644–650.
23. van de Peppel RJ, Visser LG, Dekkers OM, de Boer MGJ. The burden of invasive aspergillosis in patients with haematological malignancy: a meta-analysis and systematic review. *J Infect* 2018; 76: 550–562.
24. Bercusson A, Colley T, Shah A, Warris A, Armstrong-James D. Ibrutinib blocks Btk-dependent NF-kB and NFAT responses in human macrophages during *aspergillus fumigatus* phagocytosis. *Blood* 2018; 132: 1985–1988.
25. Jain P, Thompson PA, Keating M et al. Long-term outcomes for patients with chronic lymphocytic leukemia who discontinue ibrutinib. *Cancer* 2017; 123: 2268–2273.
26. Snelders E, van der Lee HA, Kuijpers J et al. Emergence of azole resistance in *aspergillus fumigatus* and spread of a single resistance mechanism. *PLoS Med* 2008; 5: e219.
27. Ashu EE, Hagen F, Chowdhary A, Meis JF, Xu J. Global population genetic analysis of *aspergillus fumigatus*. *mSphere* 2017;2.
28. Chowdhary A, Sharma C, Kathuria S, Hagen F, Meis JF. Prevalence and mechanism of triazole resistance in *aspergillus fumigatus* in a referral chest hospital in delhi, india and an update of the situation in asia. *Frontiers in microbiology* 2015; 6: 428.
29. Hurst SF, Berkow EL, Stevenson KL, Litvintseva AP, Lockhart SR. Isolation of azole-resistant *aspergillus fumigatus* from the environment in the south-eastern USA. *J Antimicrob Chemother* 2017; 72: 2443–2446.
30. Jensen RH, Hagen F, Astvad KM et al. Azole-resistant *aspergillus fumigatus* in denmark: a laboratory-based study on resistance mechanisms and genotypes. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2016; 22: 570 e1–9.
31. Pham CD, Reiss E, Hagen F, Meis JF, Lockhart SR. Passive surveillance for azole-resistant *aspergillus fumigatus*, united states, 2011–2013. *Emerg Infect Dis* 2014; 20: 1498–1503.
32. van der Linden JW, Arendrup MC, Warris A et al. Prospective multicenter international surveillance of azole resistance in *aspergillus fumigatus*. *Emerg Infect Dis* 2015; 21: 1041–1044.
33. NethMap: Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands 2019 [Available from: www.rivm.nl/documenten/nethmap 2019].
34. Chong GM, van der Beek MT, von dem Borne PA et al. PCRbased detection of *aspergillus fumigatus* Cyp51A mutations on bronchoalveolar lavage: a multicentre validation of the aspergenius assay(R) in 201 patients with haematological disease suspected for invasive aspergillosis. *J Antimicrob Chemother* 2016; 71: 3528–3535.
35. Dannaoui E, Gabriel F, Gaboyard M et al. Molecular diagnosis of invasive aspergillosis and detection of azole resistance by a newly commercialized PCR kit. *J Clin Microbiol* 2017; 55: 3210–3218.
36. Guegan H, Robert-Gangneux F, Camus C et al. Improving the diagnosis of invasive aspergillosis by the detection of *aspergillus* in broncho-alveolar lavage fluid: comparison of non-culture-based assays. *J Infect* 2018; 76: 196–205.
37. SIFIG C. SWAB guidelines for the management of invasive fungal infections 2017 [Available from: www.swab.nl/richtlijnen].
38. van de Peppel RJ, van der Beek MT, Visser LG, de Boer MGJ, Wallinga J. Managing invasive aspergillosis in hematologichematological patients in the era of resistance polymerase chain reaction and increasing triazole resistance:

a modelling study of different strategies. *Int J Antimicrob Agents* 2019; 53: 284–293.
39. Botero Aguirre JP, Restrepo Hamid AM. Amphotericin b deoxycholate versus liposomal amphotericin B: effects on kidney function. *Cochrane Database Syst Rev* 2015; CD010481.
40. De Pauw B, Walsh TJ, Donnelly JP et al. Revised definitions of invasive fungal disease from 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) consensus group. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2008; 46: 1813–1821.
41. Garcia-Vidal C, Peghin M, Cervera C et al. Causes of death in a contemporary cohort of patients with invasive aspergillosis. *PLoS One* 2015; 10: e0120370.
42. Tonin FS, Steimbach LM, Borba HH et al. Efficacy and safety of amphotericin b formulations: a network meta-analysis and a multicriteria decision analysis. *J Pharm Pharmacol* 2017; 69: 1672–1683.
43. Nivoix Y, Velten M, Letscher-Bru V et al. Factors associated with overall and attributable mortality in invasive aspergillosis. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2008; 47: 1176–1184.
44. Ashbee HR, Barnes RA, Johnson EM et al. Therapeutic drug monitoring (TDM) of antifungal agents: guidelines from the british society for medical mycology. *J Antimicrob Chemother* 2014; 69: 1162–1176.
45. Postina P, Skladny J, Boch T et al. Comparison of two molecular assays for detection and characterization of *aspergillus fumigatus* triazole resistance and Cyp51A mutations in clinical isolates and primary clinical samples of immunocompromised patients. *Frontiers in Microbiology* 2018; 9: 555.