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ORIGINAL ARTICLE

WILEY mycoses Diagnosis, Therapy and Prophylaxis of Fungal Diseases

The diagnosis and treatment of invasive aspergillosis in Dutch haematology units facing a rapidly increasing prevalence of azole-resistance. A nationwide survey and rationale for the DB-MSG 002 study protocol

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Summary

Patients with haematological malignancies are at risk for invasive fungal diseases (IFD). A survey was conducted in all Dutch academic haematology centres on their current diagnostic, prophylactic and therapeutic approach towards IFD in the context of azole-resistance. In all 8 centres, a haematologist and microbiologist filled in the questionnaire that focused on different subgroups of haematology patients. Fungal prophylaxis during neutropaenia was directed against *Candida*

and consisted of fluconazole and/or amphotericin B suspension. Mould-active prophylaxis was given to acute myeloid leukaemia patients during chemotherapy in 2 of 8 centres. All centres used azole prophylaxis in a subset of patients with graft-versus-host disease. A uniform approach towards the diagnosis and treatment of IFD and in particular azole-resistant *Aspergillus fumigatus* was lacking. In 2017, all centres agreed to implement a uniform diagnostic and treatment algorithm regarding invasive aspergillosis with a central role for comprehensive diagnostics and PCR-based detection of azole-resistance. This study (DB-MSG 002) will re-evaluate this algorithm when 280 patients have been treated. A heterogeneous approach towards antifungal prophylaxis, diagnosis and treatment was apparent in the Netherlands. Facing triazole-resistance, consensus was reached on the implementation of a uniform diagnostic approach in all 8 centres.

KEYWORDS
azole-resistance, IFD, invasive aspergillosis, management

1 | INTRODUCTION

Invasive fungal disease (IFD) occur in 5%-40% of patients with haematological malignancies. Approximately 95% of the IFD are caused by *Aspergillus* and *Candida* species.[1] IFD is associated with a very significant morbidity and mortality that is explained by the difficulties in diagnosing IFD rapidly.[1] In addition, the presence of an IFD leads to a delay in subsequent anti-leukemic therapy, and therefore also indirectly affects the outcome of the patient.[2]

Antifungal prophylaxis prevent IFD during acute myeloid leukaemia (AML) therapy or during graft-versus-host disease (GVHD). These benefits have to be weighed against risks of drug toxicity, interactions, selection of resistance and costs. Different opinions on the preferred antifungal strategy in these patients exist and the approach varies considerably from institution to institution.

Over the last 10 years resistance of *A. fumigatus* against triazoles, has become a significant problem in the Netherlands but has recently also been reported in other countries.[3-5] Triazole-resistance can develop through long-term azole therapy in patients with chronic pulmonary aspergillosis. However, the selection of tri-azole resistance in the environment by the use of azole fungicides is far more important. This in agreement with the observation that the majority of triazole-resistant *A. fumigatus* strains contain the environmental TR_{34}/L98H or the TR_{46}/Y121F/T289A mutation pattern in their *Cyp51A* gene.[6] This gene encodes for the target enzyme of triazoles.[7] Infections with a triazole-resistant *A. fumigatus* result in a high mortality and the best diagnostic and treatment approach is uncertain.[5,8] We conducted a survey on fungal diagnostics, antifungal prophylaxis and treatment in all Dutch academic haematology centres. The survey facilitated the development of a consensus approach towards the management of invasive aspergillosis (IA) in a context of rising azole-resistance.

2 | MATERIALS AND METHODS

A questionnaire was sent to a haematologist and a microbiologist with special interest in supportive care and medical mycology respectively and both parties were asked to answer as a team for their centre. The questionnaire focused on (i) primary prophylaxis during AML chemotherapy, during allogeneic hematopoietic stem cell transplantation (allo-HSCT) and at the time of GVHD. (ii) How was screened for IFD and which diagnostic tests were performed. (iii) The current antifungal treatment for different clinical scenarios. The results were processed and during a consensus meeting the protocol for The Azole-Resistance MANagement (AzoRMan) Study was developed and implemented.

3 | RESULTS

3.1 | Prophylaxis

3.1.1 | Prophylaxis directed against *Candida*

Fluconazole is given during neutropaenia of >10 days in 4/8 centres at very different dosages and amphotericin B oral suspension was used in 2 (Table 1). One centre also uses amphotericin B lozenge. One centre starts fluconazole when surveillance cultures grow *Candida*. If surveillance cultures show *Candida* species resistant to fluconazole, some centres switch to amphotericin B suspension and one centre adds amphotericin B suspension to fluconazole. Finally, one centre stops fluconazole and no other prophylaxis is initiated.

3.1.2 | Mould-active prophylaxis

Only one centre applies mould-active prophylaxis (itraconazole) during chemotherapy induced neutropaenia of >10 days and during myeloablative allo-HSCT. Therapeutic drug monitoring of itraconazole is performed and when no effective plasma concentrations are reached, a switch to voriconazole is made. In another centre nebulised liposomal amphotericin B (L-AmB) at 15 mg QD, twice weekly is used for this purpose. All centres start mould-active prophylaxis when corticosteroids are given for GVHD but the drugs of choice differ (Table 1).

TABLE 1 Prophylactic strategies used against Candida and Aspergillus

	Antifungal agent	Dosage	Number of centres
Candida prophylaxis during longstanding chemotherapy-induced neutropaenia	Fluconazole	50 mg/24 h	1
		200 mg/24 h	2
		400 mg/24 h	1
	Amphotericin B suspension	500 mg/6 h	2
		200 mg/12 h	1
	Fluconazole when surveillance cultures grow *Candida*		1
Anti-mould prophylaxis in AML/MDS/AlloTx during longstanding chemotherapy-induced neutropaenia	Itraconazole suspension	Start with 200 mg bid, dose increased based on TDM results	1
	L-AmB aerosols	15 mg twice weekly	1
	None		6
AlloTx with GVHD treated with systemic corticosteroids	Itraconazole	Start with 200 mg bid, dose increased based on TDM results	1
		2,5 mg/kg/12 h	1
	Voriconazole	200 mg/12 h	1
	Posaconazole	300 mg/24 h tablets	5

AlloTx, Allogeneic stem cell transplantation; AML, Acute Myeloid Leukaemia; GVHD, Graft-versus-Host disease; MDS, Myelodysplastic syndrome; TDM, Therapeutic Drug Monitoring. L-AmB, liposomal amphotericin-B.

3.2 | Diagnosis

3.2.1 | Diagnostic procedures

A chest CT is routinely performed in all centres after 3 to 5 days of neutropenic fever without an infectious focus despite antibiotic therapy (Table 2). When the chest CT scan shows pulmonary infiltrates a broncho-alveolar lavage (BAL) with galactomannan (GM) detection and fungal culture is performed in all centres (if clinically feasible). Twice weekly serum GM monitoring as a screening tool is performed in one centre only. Two centres perform an *Aspergillus* DNA PCR on BAL routinely; in one centre this is done only when BAL GM is positive or when an EORTC compatible radiological finding is suggestive of an IFD.

3.2.2 | Susceptibility testing

Different *Aspergillus* susceptibility testing methods are used: VIPcheck™ or Etest followed by confirmation with testing according to the European Committee on Antibiotic Susceptibility Testing (EUCAST) method when resistance is suspected based on the screening assay (Table 3). The EUCAST method is operational in the mycology reference laboratory (RefLab). Resistance screening is done in all but one centre with a 4-well plate (VIPcheck™) in which three of the four wells contain agar supplemented with an azole (voriconazole, itraconazole and posaconazole) and the fourth functions as a growth control. The other centre uses the Etest (bioMérieux) for resistance

TABLE 2 Diagnostic strategies used in patients at risk for or suspected of having an IFD

Diagnostic procedure	Possibilities	Nr of centres
Screening with serum GM (twice weekly) during prolonged neutropaenia	Yes	1
	No	7
Chest CT-scan when 3-5 days neutropaenic FUO despite broad-spectrum antibiotic treatment	Yes	8
	No	0
Bronchoscopy with BAL (when no evident cause for infiltrative lesions on imaging)	Yes	8
	No	0
GM measurement on BAL fluid sample, if BAL sampling is performed	Yes	8
	No	0
Aspergillus species PCR on BAL fluid	Yes, always	2
	Yes, if GM is positive	1
	No	5

BAL, Bronchoalveolar lavage; GM, Galactomannan; FUO, Fever of unknown origin; IFD, Invasive Fungal Diseases.

TABLE 3 Diagnostic tests done on BAL fluid samples

Susceptibility assay	Possibilities	Nr of centres
Aspergillus species: Screening for azole-resistance with VIP™ check-testing	Yes	7
	No	1 Sends *Aspergillus* strain directly to RefLab
Phenotypic azole-resistance testing (EUCAST) of cultured *Aspergillus* strains	Directly sent to RefLab for EUCAST testing	4
	Send to RefLab only if VIP™ screening is positive	2
	Send to RefLab only if E-test is positive	1
	EUCAST testing on site=RefLab	1
Testing for RAM on cultured *Aspergillus* strains	Yes, in-house	4
	Yes, not in-house	1
	No	3
Testing for RAM (*CYP51A*) directly on BAL fluid	Yes	2
	No	4
	On indication (if BAL culture is negative and patient is not doing clinically well)	1
	Sends BAL sample to the RefLab	1

EUCAST, The European Committee on Antimicrobial Susceptibility Testing; GM, Galactomannan; VIP™ testing, resistance assay (explanation: see text); RAM, Resistance associated mutations (TR_{34}/L98H, TR_{53}, and TR_{46}/Y121F/T289A); RefLab, National mycology reference laboratory in Nijmegen (The Netherlands); BAL, Broncho-alveolar lavage.

screening. Simultaneous to the screening test, 4 centres send the *Aspergillus* strain directly to the RefLab for MIC testing. PCR testing for the presence of TR_{34} and TR_{46} directly on cultured *A. fumigatus* colonies is performed on-site in 4 centres to speed up resistance detection. A PCR-based resistance assay is performed directly on BAL in 3 centres. For this purpose, a commercially available qPCR (AsperGenius®) or an in-house PCR is used. One centre sends BAL samples to the RefLab for PCR testing.

3.3 | Treatment

Suspected invasive fungal infection:

All centres use voriconazole as the initial treatment for patients in a respiratory stable condition suspected of having an IFD while waiting for the microbiological tests (Table 4). One centre frequently uses posaconazole as well and another centre with a high local azole-resistance prevalence prefers L-AmB if the patient is very ill. The feasibility of BAL fluid sampling is the decisive factor in another centre to guide therapy and voriconazole is given if a BAL is obtained and therefore, the detection of azole-resistance becomes more likely. If BAL is not feasible, this centre gives L-AmB as antifungal.

Proven or probable IA

Voriconazole is the treatment of choice for all centres when a BAL-GM assay is positive in a respiratory stable patient and the lesions on chest CT are not widespread, fungal culture remains negative and no susceptibility PCR is performed or the test was not successful. In the same clinical situation with a patient in respiratory distress or with extensive pulmonary infiltrates, five centres would still start voriconazole. Two centres would start L-AmB and one centre posaconazole.

Proven or probable IA and documented voriconazole resistance

If voriconazole resistance is demonstrated with one of the phenotypic susceptibility tests or by a resistance PCR, all centres give L-AmB.

3.4 | Therapeutic drug monitoring

3.4.1 | Voriconazole

Two centres do not perform therapeutic drug monitoring (TDM). Two centres do TDM when toxicity or treatment failure is suspected. The other centres routinely perform TDM.

3.4.2 | Posaconazole

Three centres always perform TDM and two centres do not. The other three centres perform TDM on indication only.

3.5 | Triazole resistance data

In 2016, *A. fumigatus* isolates from 784 clinical patients were screened for triazole resistance using a 4-well agar plate (VIPcheck™). Isolates that grew on the triazole-containing agar have a high probability of resistance and were sent to the Reflab for phenotypic and genotypic characterisation. 101 isolates (12.9%) were triazole-resistant, which

TABLE 4 Treatment of invasive aspergillosis

Presentation	Clinical condition	Treatment options	Nr of centres
Chest CT: suspected IFD but microbiological results pending	Respiratory and clinically stable	Voriconazole	8
	Respiratory and clinically instable	Voriconazole	6
		L-AmB	1
	+BAL possible	Voriconazole	1
	+BAL impossible	L-AmB	
BAL GM pos, Culture/PCR neg	Respiratory and clinically stable	Voriconazole	8
	Critically ill	Voriconazole	5
		L-AmB	2
		Posaconazole	1
Resistance detected by culture or PCR	Respiratory and clinically stable/instable	L-AmB	8
TDM voriconazole		No	2
		Sometimes*	2
		Always	4
TDM posaconazole		No	2
		Sometimes*	3
		Always	3

BAL, Bronchoalveolar lavage; Resp, Respiratory; L-AmB, liposomal amphotericin-B; IFD, Invasive Fungal Diseases; PCR, Polymerase Chain Reaction; GM, Galactomannan; TDM, Therapeutic Drug Monitoring; +BAL possible/impossible: BAL sampling was possible/impossible; *Sometimes, when toxicity or therapeutic failure is suspected.

was higher than 2014 (7.2%) and 2015 (10.7%). In individual centres, resistance ranged from 9.5% to 20.5%.[6] Recently, a nationwide Dutch cohort study reported data from 144 patients with influenza pneumonia admitted to all 8 University Intensive Care Units. 23 patients (16%) were diagnosed with influenza-associated invasive aspergillosis and triazole resistance was reported in 29% of those with a positive *A. fumigatus* culture.[9] The clinical relevance of triazole resistance was also described in another recent study in which a multiplex real-time PCR test (AsperGenius© assay) was performed on BAL samples from 201 patients. This qPCR allows the simultaneous detection of *Aspergillus* species and identification of the most common mutations in the *A. fumigatus Cyp51A* conferring resistance by using melting curve analysis. In 11 of the 68 patients in which the resistance PCR could be successfully performed, the TR_{34}/L98H or TR_{46}/T289A/Y121F resistance pattern was documented. More importantly, the detection of resistance correlated with voriconazole treatment failure.[8]

4 | DISCUSSION

4.1 | Prophylaxis directed at *Candida*

The European Conference on Infections in Leukaemia (ECIL) 5 guidelines on antifungal prophylaxis recommends fluconazole (400 mg q24 h) when the mould infections are rare and a mould-directed diagnostic approach is in place (B-I).[10] The latter is the case in all centres that were surveyed but the dose of fluconazole varies among centres and is generally lower than was used in most randomised trials (400 mg q24 h).[11-15] Some studies suggest that lower doses may suffice.[16] Three centres use oral amphotericin B as primary prophylaxis and in others oral amphotericin B is given on top of fluconazole if surveillance cultures remain positive. In a pooled analysis of oral fluconazole vs amphotericin B no significant advantage of either of the two drugs was observed. Data on the efficacy of prophylactic amphotericin B are scarce.[17] According to the EBMT, fluconazole is the drug of choice for the prophylaxis of invasive candidiasis before engraftment in allo-HSCT recipients, and may be started at the beginning or after the end of the conditioning regimen (A-I).[18]

4.2 | Mould-active prophylaxis

The advantage of primary mould-active prophylaxis with posaconazole was shown in two randomised trials.[13,14] The Dutch guideline on antifungal management as well as the ECIL-5 guideline recommends posaconazole for primary prophylaxis (A-I) when the incidence of mould infections is high.[10,19] Firm criteria for what constitutes `high risk` are lacking but it has been proposed that subpopulations with >8%-10% fall into this category. Unfortunately, reliable data on the local prevalence of mould infections are often lacking.[20] One centre administers aerosolised L-AmB twice weekly for the prevention of IFD in AML patients undergoing intensive chemotherapy. Its efficacy and cost-effectiveness have been demonstrated in a single-centre randomised placebo-controlled trial and an observational study.[21,22] One centre uses itraconazole as antifungal prophylaxis. A major concern of itraconazole is its poor gastrointestinal tolerance and *CYP3A4* inhibitory properties. Both the ECIL-5 and the IDSA guidelines give moderate recommendations against its use.[10,23] All centres use a diagnostic protocol that includes a lung CT after three to 5 days of fever despite antibiotic

therapy and proceed to BAL sampling when infiltrates are documented. Indeed, a survival benefit of azole prophylaxis compared with a diagnostic-driven approach has not been convincingly shown and so both continue to be reasonable strategies.

4.3 | Mould-active prophylaxis in GVHD

Antifungal prophylaxis has been established as standard of care after allo-HSCT with grade II or higher GVHD, but issues concerning drug-drug interactions and factors compromising bioavailability have to be considered. Ullman et al. performed a randomised trial in which fluconazole and posaconazole oral solution were compared as fungal prophylaxis in patients with GVHD. Posaconazole prevented IA and resulted in lower numbers of deaths related to IFD although the overall mortality did not differ.[14] All centres administer azole prophylaxis (4 posaconazole, 2 voriconazole, 2 itraconazole) to patients with GVHD of grade II or higher in accordance with ECIL-5 recommendations in which an A-I recommendation is given for posaconazole and a B-I to itraconazole and voriconazole.[10]

4.4 | Diagnosis of IA

Pulmonary imaging with high-resolution CT (HRCT) was shown to accelerate and improve the diagnosis of IA.[23] The IDSA guideline advocates imaging with chest CT when a patient is suspected to have IA. IDSA guidelines also encourage BAL since signs, symptoms or imaging by itself are often aspecific. All centres use HRCT and BAL as the standard diagnostic procedure. Serum GM monitoring has a moderate sensitivity of ±70% but is insensitive in non-neutropenic patients and the specificity has varied between studies.[23,24] Only one centre routinely monitors serum GM in patients with prolonged neutropaenia. All centres measure BAL-GM and *Aspergillus* DNA PCR is performed in 3 centres on BAL fluid samples). The clinical implementation of PCR-based diagnosis was debated, though not recommended for routine clinical practice in the 2016 IDSA guidelines as few assays have been standardised and well validated.[23]

4.5 | Susceptibility testing

Azole-resistance was rare in The Netherlands before the year 2000 but its prevalence has continued to increase since then.[25] It is currently based on a limited number of resistance-associated mutations (RAMs) in the *cyp51A*-gene (TR_{34}/L98H, TR_{53}, and TR_{46}/Y121F/T289A) and is most likely caused by the environmental use of azole fungicides.[7,26,27] The TR_{34}/L98H and TR_{46}/Y121F/T289A accounted for 83% of resistance mutations in 2016.[6] IDSA guidelines do not recommend standard susceptibility testing but these guidelines cannot be applied to The Netherlands.[23,28] Case series indicate that IA caused by azole-resistant *Aspergillus*, is associated with a very high mortality.[5,8] The diagnostic tools used for the detection of azole-resistance vary from centre to centre. Most perform agar-based screening assays for resistance (VIPcheck™ testing). Phenotypic azole-resistance testing according to the EUCAST method is performed by the National mycology reference laboratory only (RefLab). Four centres directly send *Aspergillus* strains to the RefLab and three await the result of the screening assay.

Only very recently, the clinical validity and relevance of PCR-based susceptibility testing on BAL was demonstrated and may explain the limited uptake of resistance detection by PCR at the time of the survey. The AsperGenius® qPCR is a multiplex PCR and can detect the presence of *Aspergillus* DNA and in addition detect the 2 mutations described above.[8,29] In a recent study the diagnostic performance was evaluated on BAL-samples in 201 patients.[8,29] The *Aspergillus* BAL qPCR, had a sensitivity of 89% and a specificity of 89% and was able to detect *A. fumigatus* that carried resistance-associated mutations (RAM) in the majority of patients, even in culture-negative BAL. Furthermore, this study showed that response to voriconazole therapy, when given to patients infected with a resistant *A. fumigatus* was poor.[8]

4.6 | Treatment

The ECIL-6 guideline attributes an A-I recommendation to both voriconazole and isavuconazole for the treatment of IA.[30] Unfortunately, in 2016 surveillance data showed that triazole resistance was present in 101 of 784 (12.9%) patients with a positive *A. fumigatus* culture.[6] These data are based on clinical isolates and it is uncertain what fraction of these patients met EORTC/MSG criteria. However, the clinical relevance of azole-resistance in patients with an invasive *Aspergillus* infection was described in a recent multicenter study and small case series have reported a very high mortality in patients infected with a voriconazole resistant *A. fumigatus* that received initial therapy with voriconazole.[5,8] The management of IA in The Netherlands in the context of a progressively rising incidence of IA caused by azole-resistant *A. fumigatus* strains is challenging because evidence-based data on the most appropriate management of this emerging clinical problem are lacking. At the time of the survey, all centres start voriconazole when the patient is respiratory and clinically stable while awaiting culture and/or resistance PCR results. In a clinically unstable patient, five centres still start voriconazole, one centre starts posaconazole and another centre starts L-AmB. The feasibility to perform a BAL (and thus cultures) is the decisive factor for one centre. In 2015 an international consensus paper on the management of IA caused by azole-resistant *Aspergillus* isolates advised L-AmB or echinocandin-voriconazole combination as treatment of choice in regions with environmental triazole resistance rates of *Aspergillus* exceeding 10%.[25]

Therapeutic drug monitoring was systematically used in four centres for voriconazole, on indication or not at all in two centres each. Although some studies suggest a relation between voriconazole serum levels and the incidence of adverse events, randomised clinical trials that convincingly show the value of TDM are still lacking.[31]

Off-guideline management (as compared with the Dutch guideline on fungal infections) was observed in some of the centres.[19] One common reason for a delay in policy change after new convincing evidence was published and incorporated in guidelines is the absence

of a dedicated haematologist with special interest in infectious diseases and supportive haematological care who critically assesses the local practice on a regular basis. We asked the centres for the reasons of their off-guideline policies and the following answers were given: The continued use of itraconazole instead of posaconazole as anti-mould prophylaxis in 2 centres was driven by the higher costs of other azoles. Both centres recently moved to voriconazole after it became available as a generic drug. One centre preferred voriconazole over posaconazole and this was driven by the unpredictable absorption of the oral solution and the lack of an intravenous formulation of posaconazole when it first came on the market. Another centre used nebulised liposomal amphotericin-B as anti-mould prophylaxis and did this based on locally generated evidence that supports its (cost-)effectivity.[21,22] Finally, the continued use of oral amphotericin-B solution as anti-yeast prophylaxis (on top of fluconazole) was driven by the fact that it is a harmless intervention (as no systemic toxicity occurs with a non-absorbed drug) and because with this policy, the incidence of candidaemia had been very low with this policy for more than 15 years. Therefore, these centres were reluctant to change a safe policy that seems to be very efficacious.

4.7 | Protocol

Following this survey, a consensus meeting was organised with representatives of all 8 centres and led to the development of a standardised diagnostic and therapeutic protocol on the management of IFD in haematology patients (Figure 1). This protocol was developed in collaboration with the recently established Dutch-Belgian Mycosis Study Group (DB-MSG) and was implemented in all academic haematology centres in 2017 with the goal to gather evidence on the

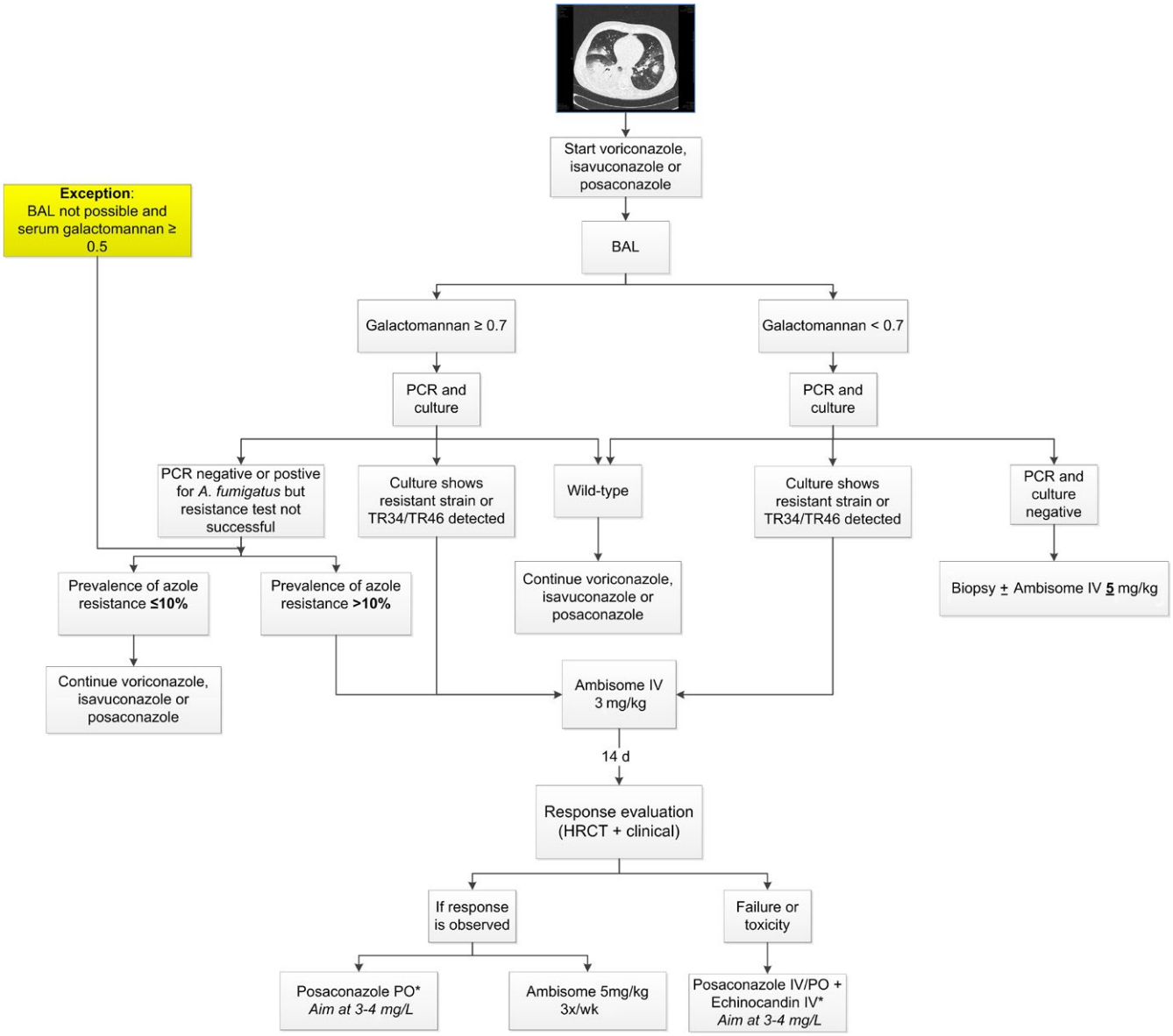

FIGURE 1 Treatment protocol for Azole-resistance Management-study. MIC, Minimal Inhibitory concentration; IV, Intravenously. *Posaconazole HD can only be considered as treatment option when the MIC (EUCAST) ≤1 g/dL. HRCT, High Resolution CT scan; PCR, polymerase chain reaction; PO, by mouth; BAL, Broncho-alveolar lavage

optimal approach towards IFD in the context of azole-resistance (The Azole-Resistance MANagement Study (AzoRMan) or DB-MSG 002 study, NCT03121235). The study aims to demonstrate that the use of resistance testing by PCR on BAL fluid from haematology patients with suspected IA will lead to an improved outcome by detecting resistance earlier and changing triazole therapy to L-AmB as soon as resistance is detected. Indeed, the majority of cases of IA remain culture negative and therefore, the use of resistance testing by PCR is considered crucial.[8,32] The AzoRMan-study is schematically depicted in Figure 1 and further information available at www.clinicaltrials.gov. In brief, treatment is based on the documentation of azole susceptibility or resistance and step-down treatment options for patients treated for documented or presumed azole-resistance are given.

Treatment with L-AmB is advised when azole-resistance is documented or when no susceptibility data are available and the local azole-resistance rate is >10%. This is supported by the fact that the *A. fumigatus* strains with the environmental TR_{34}/L98H or the TR_{46}/Y121F/T289A mutation pattern circulating in the Netherlands remain susceptible to L-AmB.[33] The activity of L-AmB was also confirmed in vivo in immunocompetent and immunosuppressive murine models of IA.[34] This approach may be less appropriate in different settings in which resistance mechanisms other than the environmental TR_{34}/L98H or the TR_{46}/Y121F/T289A mutation patterns are predominant.[35]

If a treatment response is observed during therapy with L-AmB 3 mg/kg/day, a switch to L-AmB 5 mg/kg/day three times a week or to oral posaconazole (when the posaconazole MIC is below 2 mg/L) is made with a posaconazole target trough serum level of 3-4 mg/L. The logical behind the posaconazole strategy is the observation that *Aspergillus* strains carrying RAMs often have a posaconazole minimum inhibitory concentrations (MIC) that is <2 mg/L.[36] The efficacy of posaconazole at high serum levels was demonstrated in a pharmacodynamic study in mice with invasive azole-resistant aspergillosis by Mavridou et al.[37] This study showed that posaconazole retains activity against an *A. fumigatus* strain that carried the TR_{34}/L98H mutation with a posaconazole MIC of 0.5 mg/L as long as serum drug levels are sufficiently high. No human data on the use of this treatment strategy have been published. However, in a phase 3 pharmacokinetics and safety study for posaconazole tablets the average serum concentration of posaconazole in quartile 4 of the 186 patients that received posaconazole tablets at 300 mg per day was 2.3-9.5 mg/L. It was not associated with a specific safety signal and therefore, a serum level between 3 and 4 mg/L is a realistic target.[38] Posaconazole with high serum trough levels is the only oral step-down treatment option for patients with azole-resistant IA. Although clinical evidence remains anecdotal, preclinical animal studies and experience in veterinary medicine provides proof op principle in its efficacy.[37,39]

5 | CONCLUSION

This survey shows the heterogeneous landscape in the prevention, diagnosis and treatment of IA in The Netherlands. In the context of the rapidly increasing prevalence of azole-resistance, the AzorMan study was implemented to evaluate a uniform diagnostic and therapeutic approach.

TRANSPARENCY DECLARATIONS

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