

# Azole-resistant invasive aspergillosis in a patient with acute myeloid leukaemia in Germany

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**We report the first culture-proven case of invasive aspergillosis (IA) caused by azole-resistant *Aspergillus fumigatus* in a patient with acute myeloid leukaemia in Germany. IA presented as breakthrough infection under posaconazole prophylaxis. Analysis of the resistance mechanism revealed the TR/L98H mutation in the *cyp51A* gene, which indicates an environmental origin of the strain. This case underscores the need for monitoring azole resistance in *Aspergillus* spp. and for routine susceptibility testing of moulds.**

## Background

Invasive aspergillosis (IA) is the most frequent invasive fungal disease (IFD) in patients with haematological malignancies and in those undergoing allogeneic stem cell transplantation, with *A. fumigatus* being its prime causative agent. Azole antifungals are recommended as first-line treatment and prophylaxis of IA in high risk patients in most international guidelines. Azole resistance is observed in patients with long-term azole therapy, but also in azole-naïve patients, suggesting an environmental origin for the latter group. Resistance to azole antifungals in clinical *A. fumigatus* isolates was reported from the Netherlands, the United Kingdom, France, the United States and recently China and India and has been associated with a mortality of up to 88% [1-4]. In Europe, resistant clinical strains were mostly isolated from patients with chronic pulmonary disease and prior long-term antifungal therapy, e.g. patients with allergic bronchopulmonary aspergillosis, chronic pulmonary aspergillosis or cystic fibrosis [5, 6]. In patients with haematological malignancies, azole resistance has rarely been reported [7].

## Case report

A 35 year-old male patient was admitted to the University Hospital of Cologne with a first diagnosis of acute myeloid leukaemia (AML) in March 2012. He was

started on remission-induction chemotherapy on the same day (day 0) and he received oral posaconazole prophylaxis (200 mg three times a day) from day 5. The patient was included into the ongoing study on the epidemiology of invasive aspergillosis and resistance patterns of *Aspergillus* spp. (SEPIA study) for surveillance of patients with acute leukaemia. A baseline chest computed tomography scan (CT) showed no signs of IFD. A consecutive episode of febrile neutropenia (day 13) was treated with empirical broad-spectrum antibiotics, but fever persisted.

A PCR from a throat swab revealed respiratory syncytial virus (RSV) as the possible origin of fever, cough and dyspnoea. A chest CT scan showing bilateral patchy ground glass opacities was compatible with this diagnosis. However, a broncho-alveolar lavage (BAL) performed on day 25 after admission revealed an elevated galactomannan (GM) index of >2.5 from BAL fluid (norm: <0.9), which is indicative of IA. Serum levels of GM increased from 0.4 to 1.3 (norm: <0.5), even though posaconazole levels were adequate (2.01 mg/L; standard range not defined).

A novel PCR assay [8] performed from the same BAL fluid was positive for *A. fumigatus* and the TR/L98H mutation of *cyp51A*. In addition, *A. fumigatus sensu stricto* was isolated in culture, with elevated minimum inhibitory concentrations (MICs) for voriconazole (2 mg/L) and posaconazole (0.5 mg/L, Table 1). Treatment was switched to intravenous liposomal amphotericin B (LAmB) on day 35 at a dose of 3 mg/kg.

A follow-up chest CT scan on day 43, seven days after recovery from neutropenia, showed multiple larger lung nodules with air crescent sign in the right upper and left lower lobe and was strongly suggestive of pulmonary IA responding to LAmB. In addition, a new

**TABLE**

Minimum inhibitory concentrations of *Aspergillus fumigatus* isolate from a patient with acute myeloid leukaemia, and EUCAST clinical breakpoints [9], Germany 2012

Antifungal drug	Testing method	MIC [mg/L]	EUCAST breakpoint S/R
Voriconazole	EUCAST	2	-
Posaconazole	EUCAST	0.5	≤0.12/>0.25
Itraconazole	EUCAST	>16	≤1/>2
Amphotericin B	EUCAST	0.5	≤1/>2
Caspofungin	Etest	0.032	-

EUCAST: The European Committee on Antimicrobial Susceptibility Testing; MIC: minimum inhibitory concentration; R: resistant; S: susceptible.

splenic abscess was described. Splenectomy was performed on day 61. Culture of spleen tissue remained negative for bacteria and fungi, but the histological workup revealed abundant hyphae compatible with IA.

On day 140, the patient had recovered and was in complete remission from AML.

### Phenotypic identification, molecular identification and resistance testing

*A. fumigatus sensu stricto* was identified by morphological and molecular characteristics. DNA of the *A. fumigatus* strain was isolated using the QIAamp DNA mini kit (Qiagen, Hilden, Germany). Molecular identification was done by sequencing the internal transcribed spacer (ITS) and partial beta-tubulin gene of the isolate [10]. Susceptibility testing for azole antifungals and amphotericin B was performed by microdilution testing according to EUCAST [11]. Caspofungin MIC was determined by Etest (Biomérieux, Nürtingen, Germany) according to the manufacturer's recommendation. Presence of the L98H mutation and the 34 bp tandem repeat (TR) was evidenced by sequencing the complete *cyp51A* gene and its promotor region [12, 13]. Direct PCR from BAL fluid for the detection of *A. fumigatus* and azole-resistance genes was carried out as previously described [8].

### Discussion

*A. fumigatus* resistant to itraconazole and posaconazole bearing the TR/L98H mutation was identified in a patient with AML from Germany after only 14 days of azole treatment. This short duration of exposure as well as the TR/L98H mutation suggests an infection with a strain of environmental origin. The patient had not visited the neighbouring Netherlands within the last 12 months, where TR/L98H has frequently been described in azole-resistant *A. fumigatus* strains. Therefore, his

respiratory tract may have been colonised with a TR/L98H mutant strain from Germany prior to development of IA.

Azole resistance is usually mediated by different point mutations in the *cyp51A* gene, coding for 14 alpha-demethylase, a component of the ergosterol synthesis pathway and target of azole antifungals. The frequency of different resistance mechanisms varies between study locations, patient populations and countries. In the United Kingdom, several different mutations are commonly found, such as M220 or G54 [5]. In the Netherlands, the TR/L98H mutation prevails, which was detected in more than 90% of itraconazole resistant strains [4]. TR/L98H mutant strains are reported to be of environmental origin and as a consequence of the use of 14 alpha-demethylase inhibitors as fungicides in agriculture [14]. In these strains, a leucine to histidine substitution (L98H) is associated with a 34-bp TR in the promotor region [4]. According to recently published data, this resistance mechanism is spreading across Europe [15].

Considering that prophylaxis and first-line treatment of IA is usually based on azoles, further spread of resistant isolates could jeopardise the effectiveness of prophylactic strategies and targeted treatment.

Susceptibility testing of fungi, especially of moulds, is not carried out on a routine basis in most microbiology laboratories. In comparison to susceptibility testing of bacteria, testing of moulds is more laborious, it cannot be automated today and requires sound mycological knowledge. Breakpoints for antifungals in *Aspergillus* spp. were published only recently and for a limited number of species and drugs [9]. Detection of azole resistance can be challenging, as some isolates have only slightly elevated or normal MICs for posaconazole and voriconazole. Therefore, itraconazole should be routinely tested on all isolates from patients needing antifungal therapy, because *cyp51A* mutant strains usually show the highest MIC to this compound.

Furthermore, reliable detection of *A. fumigatus* and *cyp51A* mutations can be achieved directly from clinical samples by PCR assays with subsequent sequencing [8, 16], offering faster and culture independent recognition of resistance.

More data on the susceptibility of *Aspergillus* spp., both from environmental and clinical samples is needed to reliably describe the epidemiology of azole resistance in Europe. Susceptibility testing of *Aspergillus* spp. should be routinely carried out in clinical laboratories to detect resistant strains and prevent therapeutic failure..

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## Conflicts of Interest

A. Hamprecht has received a travel grant from Astellas. D. Buchheidt has received research grants from Gilead Sciences and Pfizer and served on the speakers' bureaux of Astellas, Gilead Sciences, Merck Sharp Dohme/ Merck and Pfizer. J.J. Vehreschild has received research grants from Astellas, Essex/Schering-Plough, Infectopharm, and Pfizer; and served on the speakers' bureau of Astellas, Essex/Schering-Plough, and Merck Sharp Dohme/ Merck. O.A. Cornely has received research grants from Astellas, Basilea, Bayer, Genzyme, Gilead, Merck/Schering, Merck/Serono, Optimer, and Pfizer, has been a consultant to Astellas, Basilea, F2G, Gilead, Merck/Schering, Optimer, and Pfizer. A. Steinbach has received a research grant from MSD and served on the speakers' bureau of Gilead Sciences. M.J.G.T. Vehreschild has served on the speakers' bureau of Schering-Plough/ Essex, Pfizer, MSD and Gilead Sciences. She has received a research grant from 3M.

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