High-level panazole-resistant aspergillosis

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Abstract

High-level panazole-resistant *Aspergillus fumigatus* was recovered from four patients with chronic lung diseases. In one patient progressive resistance development followed long-term azole therapy and switching between antifungal azoles. The high-level panazole-resistant phenotypes were not associated with a specific cyp51A gene mutation. New strategies are needed that avoid progressive azole resistance development.
Azole resistance in *Aspergillus fumigatus* is an emerging problem, which is associated with treatment failure in patients with aspergillosis diseases (1). Resistance is commonly due to mutations in the *cyp51A* gene (1) that typically lead to high-level resistance (minimal inhibitory concentration (MIC) ≥8 mg/l) against one azole and low-level resistance (MICs close to the resistance breakpoint) against others (2,3).

We identified four *A. fumigatus* isolates with MIC ≥8 mg/l for all mold-active azoles, measured using EUCAST methodology (4). We labeled this unique phenotype high-level panazole resistance. We analyzed the *cyp51A* gene sequence of the isolates using previously published algorithms (5) and retrieved clinical data for these four patients (Table).

The first patient was a 22-year old male patient with Cystic Fibrosis. After being diagnosed with allergic bronchopulmonary aspergillosis (ABPA), he commenced itraconazole and steroid maintenance therapy. Fungal sputum cultures after 8 months of itraconazole therapy revealed *A. fumigatus* with the high-level panazole-resistant phenotype. The patient continued itraconazole maintenance therapy and the high-level panazole-resistant isolate was not recovered from repeat cultures.

The second patient was a 71-year-old male with a medical history of asthma, bronchiectasis and intermittent culture positivity with itraconazole and voriconazole susceptible *A. fumigatus*. The patient did not meet diagnostic criteria for ABPA and was never treated with azoles. The high-level panazole resistant *A. fumigatus* isolate was isolated once from a sputum sample; follow-up sputum cultures yielded azole-susceptible *A. fumigatus*.

The third patient was a 47-year-old female with severe pulmonary sarcoidosis, complicated by a pneumothorax with subsequent pleural empyema. From this empyema, an azole-susceptible *A. fumigatus* was cultured (itraconazole and voriconazole MICs 0.5 mg/l, posaconazole 0.063 mg/l). Treatment with itraconazole was started and later changed to voriconazole and...
ultimately posaconazole as a chronic suppressive therapy. After 18 months of azole therapy, the patient’s disease progressed and a sputum sample was ordered for fungal culture. This sample grew the high-level panazole-resistant isolate and induced a switch to liposomal amphotericin B therapy. Despite treatment, the patient’s condition deteriorated and she died of respiratory failure.

The fourth patient was a 39-year-old male diagnosed with chronic granulomatous disease and ABPA. The patient had been treated for multiple episodes of invasive pulmonary aspergillosis and he received secondary prophylaxis with itraconazole. He then presented with arthritis of the sternoclavicular joint, and itraconazole-resistant *A. fumigatus* was cultured from biopsies. Treatment with voriconazole was initiated, but visual disturbances forced a switch to liposomal amphotericin B and anidulafungin. The patient responded and was discharged on posaconazole maintenance therapy. After 11 months the patient presented with increasing dyspnea, dry cough and fever. A chest CT scan revealed a cavity and bronchiolitis in the right lower lobe. Broncho-alveolar lavage cultures were positive for *A. fumigatus*, which exhibited the high-level panazole-resistant phenotype and an M220R mutation in the *cyp51A* gene, absent in the patient’s previous isolates. Micafungin was added to posaconazole, based on susceptibility test results (Table). After 1 and 7 days of treatment, follow-up cultures remained positive with the high-level panazole-resistant strain. After 10 days of therapy, the patient died of pulmonary hemorrhage. Microsatellite typing (4) showed that the isolates from this patient were isogenic.

The isolates cultured from these four patients reveal a new and highly worrisome phenotype, characterized by high-level resistance to all mold-active azoles, including the new azole isavuconazole. Isavuconazole was shown to exhibit cross-resistance to voriconazole (6). All patients had chronic lung diseases, and in three patients a chronic aspergillus disease was
diagnosed. In patients 1 and 2 there was no clinical factor that might explain the single
recovery of the high-level panazole-resistant *A. fumigatus* isolate. The high-level panazole-
resistant isolates might have been acquired as such from the environment (7).
In patient 3 a persisting aspergillus infection was treated with various antifungal azoles.
Although the initial isolate was azole susceptible, during therapy the high-level panazole-
resistant isolate with an environmental resistance mechanism (TR46/Y121F/T289A) was
cultured. The high-level panazole-resistant phenotype may have developed during azole
therapy or was acquired as such from the environment. Clear evidence for progressive
resistance development was present in patient 4. As treatment was switched between the azole
compounds, *A. fumigatus* progressively developed resistance to each azole that was used for
treatment. Possibly the cavitary lesions or other difficult-to-reach sites with a high fungal
burden facilitated the development of resistance (8).
We were unable to detect genetic changes in the *cyp51A* gene that paralleled the development
of the high-level panazole-resistant phenotype (see Table). We did not find known azole
resistance mechanisms in the isolate of patient two, indicating that non-*cyp51A* mediated
resistance mechanisms may have emerged in these isolates. Two isolates (patients 1 and 3)
harbored *cyp51A* mutations that have been associated with environmental azole exposure,
TR34/L98H and TR46/Y121F/T289A (1,4). In the Netherlands, typical MICs of TR34/L98H
isolates are >16 mg/l for itraconazole, 4-8 mg/l for voriconazole and 1 mg/l for posaconazole,
and those of TR46/Y121F/T298A isolates are >16 mg/l for voriconazole, variable MICs for
itraconazole (1-16 mg/l) and 0.5–1 mg/l for posaconazole (2). The high-level panazole-
resistant phenotype, particularly the high posaconazole MIC, suggests that these isolates have
accumulated additional non-*cyp51A* resistance mechanisms. These could include the recently
described overexpression of *cyp51A*, *cyp51B* and the cdr1B efflux pump; for these three,
MICs of ≥8 mg/l for itraconazole, 1-8 mg/l for voriconazole and 0.125-2 mg/l for
posaconazole have been measured (9-11). Based on these reported phenotypes, particularly the low-level posaconazole resistance, these mechanisms are unlikely responsible for the high-level panazole-resistant phenotype observed in the four patients’ isolates. The M220R mutation (patient 4) has not been previously reported but M220K and M220T have been reported to lead to itraconazole MICs >8 mg/l but variable MICs to voriconazole (1-4 mg/l) and posaconazole (0.5 - >8 mg/l), so may equal the phenotype seen in patient 4 (12).

High-level panazole resistance has consequences for patient management. Dose escalation of voriconazole, (intravenous) posaconazole and azole-echinocandin combination therapy may be effective against low-level azole-resistant *A. fumigatus* (3,13). In high-level panazole-resistant infection dose escalation will be inadequate and the efficacy of combination therapy at best uncertain. In vitro, the activity of amphotericin B and the echinocandins remained unaffected. Liposomal amphotericin B has shown good efficacy in a nonneutropenic murine model of acute azole-resistant invasive aspergillosis (14).

Direct detection of resistance mutations in clinical samples is increasingly studied (15,16). These would have indicated a wild type *cyp51A* gene, M220R, a TR34/L98H or TR46/Y121F/T289A resistance mechanism but not the high-level panazole resistance in these four patients.

We report the emergence of high-level panazole-resistant *A. fumigatus* isolates. This phenotype might originate from the environment, but also may develop through switching between azole compounds in patients with chronic aspergillosis. There is a clear need to develop strategies in patients at risk for chronic aspergillosis that avoids high-level resistance development.
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References


Table. Patient characteristics, azole in vitro activity and cyp51A resistance mechanisms in four high-level azole-resistant *A. fumigatus* isolates.

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex/age</th>
<th>Underlying condition</th>
<th>Aspergillosis type</th>
<th>Prior azole exposure</th>
<th>Origin of isolate</th>
<th>MIC (mg/l)</th>
<th>cyp51A mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/22</td>
<td>Cystic Fibrosis</td>
<td>ABPA</td>
<td>ITC</td>
<td>Sputum</td>
<td>1</td>
<td>0.063 &gt;16 16 &gt;16 &gt;16 TR22/L98H</td>
</tr>
<tr>
<td>2</td>
<td>M/71</td>
<td>Bronchiectasis</td>
<td>none</td>
<td>No</td>
<td>Sputum</td>
<td>1</td>
<td>0.063 &gt;16 16 &gt;16 &gt;16 None detected</td>
</tr>
<tr>
<td>3</td>
<td>F/47</td>
<td>Sarcoidosis</td>
<td>empyema</td>
<td>ITC, VRC, POS</td>
<td>Sputum</td>
<td>0.5</td>
<td>0.016 &gt;16 &gt;16 &gt;16 &gt;16 TR37/V121F/T298A, M122L, G448S</td>
</tr>
<tr>
<td>4</td>
<td>M/59</td>
<td>Chronic granulomatous disease</td>
<td>chronic invasive</td>
<td>ITC, VRC, POS</td>
<td>BAL</td>
<td>0.5</td>
<td>0.063 &gt;16 8 &gt;16 16 M220R</td>
</tr>
</tbody>
</table>

ABPA, allergic bronchopulmonary aspergillosis; BAL, broncho-alveolar lavage; AmB, amphotericin B; AFG, anidulafungin; ITC, itraconazole; VRC, voriconazole; POS, posaconazole; ISA, isavuconazole. All MICs were determined using EUCAST methodology (2,4).