5th ADVANCES AGAINST ASPERGILLOSIS

Istanbul, Turkey

26 - 28 January 2012

Lufti Kirdar Convention & Exhibition Centre

www.AAA2012.org
Dear Advances Against Aspergillosis Colleague

We are excited to once again have assembled many of the leading clinicians and scientists from around the world to advance the scientific and medical research agenda in *Aspergillus* and aspergillosis for the 5th Advances Against Aspergillosis conference. There was an overwhelming feeling of success following the 1st Advances Against Aspergillosis meeting in 2004 in San Francisco where we had 364 attendees from 28 countries, the 2nd meeting in 2006 in Athens with 464 attendees from 44 countries, the 3rd meeting in 2008 in Miami with 351 attendees from 48 countries, and the 4th meeting in 2010 in Rome with 533 attendees from 49 countries. This conference has now clearly established itself as the premier forum for detailed and dedicated discussion of all aspects of *Aspergillus* infection and research, and previously published proceedings from the prior conferences have been very well-received.

The *Aspergillus* field is in a state of rapid advancement, including the publication of numerous post-genomic papers and substantial advances in translational and diagnostic research. Despite the incidence of invasive aspergillosis increasing and disease being the leading fungal cause of patient mortality, prior to the 1st Advances Against Aspergillosis meeting, there had been limited communication among experts in the area. Now we have another chance to gather the world’s aspergillosis experts in one venue. A fundamental tenet of this research colloquium continues to be to engender collaborative relationships amongst clinicians, scientists, and industry to further advance the field.

We thank the many corporate and foundation sponsors, listed in this program; without their support, this conference would not have been possible. We also thank the Scientific Committee for helping to assemble a truly international speaker list from the largest medical and scientific centers in the world, with a focus on contemporary topics in *Aspergillus* research. By our design, much of the newest published literature and hypotheses in the field have originated from the speakers of this conference. In the program, we have introduced many speakers who did not speak at the previous Advances Against Aspergillosis meetings, including some young scientists and clinicians - a pattern we would like to repeat in future years. This year we have also increased the number of oral presentations from submitted abstracts.

We also thank all the speakers and poster presenters, and every one of you, for contributing to the success of this effort. We hope you will enjoy the meeting, the conference hotel, and the exciting city of Istanbul. Please also join us at the welcome reception, the tour and dinner, and the poster sessions. An essential part of this conference is the new friendships we expect will result, and the support of young scientists entering the field.

The proceedings of this 5th meeting will once again be published in a special journal supplement, creating what we hope will be highlights of the newer insights from the many disciplines that encompass *Aspergillus* research and care. Our plan is to continue this conference every other year, and you will notice that there is a special open planning session for the next conference at the end of this meeting. We invite you to come and offer any constructive criticism of this meeting and suggestions for new sessions or topics or locations you would like to see in the future.

Yours sincerely,

William J. Steinbach  
Co-Chairman

David W. Denning  
Co-Chairman

David A. Stevens  
Co-Chairman
ACKNOWLEDGEMENTS

We would like to offer very special thanks to the following organizations for their generous educational grants. Their financial support makes this conference possible.

**Gold**

![Astellas](image1.png)  
GILEAD

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conference Chairmen, Scientific Committee</td>
<td>1</td>
</tr>
<tr>
<td>Faculty List</td>
<td>3</td>
</tr>
<tr>
<td>Faculty Disclosures</td>
<td>5</td>
</tr>
<tr>
<td>Poster Abstract Index</td>
<td>7</td>
</tr>
<tr>
<td>Final Programme</td>
<td></td>
</tr>
<tr>
<td>Wednesday 25 January (Pre-Meeting Workshop)</td>
<td>19</td>
</tr>
<tr>
<td>Thursday 26 January</td>
<td>20</td>
</tr>
<tr>
<td>Friday 27 January</td>
<td>22</td>
</tr>
<tr>
<td>Saturday 28 January</td>
<td>23</td>
</tr>
<tr>
<td>Abstracts</td>
<td></td>
</tr>
<tr>
<td>Invited Faculty</td>
<td>25</td>
</tr>
<tr>
<td>Poster Abstracts</td>
<td>63</td>
</tr>
<tr>
<td>Author Index</td>
<td>217</td>
</tr>
</tbody>
</table>
CONFERENCE CHAIRMEN AND
SCIENTIFIC COMMITTEE

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Massachusetts General Hospital, USA
### FACULTY DISCLOSURES

<table>
<thead>
<tr>
<th>NAME</th>
<th>Paid Speaking Engagements</th>
<th>Research Contracts</th>
<th>Consultancy</th>
<th>Travel and Accommodation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKOVA, Murat</td>
<td>Pfizer, MSD, Novartis, Gilead</td>
<td>Pfizer, MSD</td>
<td>Pfizer, MDS, Gilead</td>
<td>Pfizer, MSD, Gilead</td>
</tr>
<tr>
<td>ARIKAN-AKDAGLI, Sevtap</td>
<td>Astellas, Gilead, MSD, Pfizer</td>
<td>Pfizer</td>
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<td>MSD, Pfizer</td>
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<td>MSD, Astellas, Gilead, Pfizer</td>
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</tr>
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<td>Merck, Astellas, Novartis, Dainippon, Pfizer</td>
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<td>Sanofi Aventis, Merck, Wyeth, Bayer, Hikma, Abbott, Bristol-Meyers Squibb, The Liposome Company, Novartis, Astellas</td>
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<td>--------------------</td>
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<td>National Institutes of Health</td>
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</tr>
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<td>None</td>
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<tr>
<td>MARR, Keiren</td>
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</tr>
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<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>MOSS, Richard</td>
<td>None</td>
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<td>Novartis, Mpx, Vertex, Johnson &amp; Johnson, Gilead, 23 and Me, Locus Development</td>
<td>Novartis, Vertex</td>
</tr>
<tr>
<td>NETEA, Mihai</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
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<td>Astellas, Gilead, Pfizer, MSD, Novartis</td>
<td>Basilea, Astellas, Biomerieux, Fundación Mutua Madrileña, FIS</td>
<td>None</td>
<td>Astellas, Gilead, Pfizer, MSD</td>
</tr>
<tr>
<td>OSHEROV, Nir</td>
<td>None</td>
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<td>None</td>
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</tr>
<tr>
<td>PERLIN, David</td>
<td>Merck, Astellas, Novartis</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>Gilead, Cephalon, Pfizer, Wyeth, Schering, Merck, Aventis, Astellas, VIANEX</td>
<td>Pfizer, Gilead, Enzon, Schering, Wyeth</td>
<td>Gilead, Cephalon, Pfizer, Wyeth, Schering, Merck, Aventis, Astellas, VIANEX</td>
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<td>None</td>
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<td>None</td>
<td>None</td>
<td>None</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>None</td>
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<tr>
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<td>Enzon</td>
<td>Enzon, Pfizer, Astellas</td>
<td>Merck, Schering Plough, Gilead</td>
<td>Enzon, Merck, Schering Plough</td>
</tr>
<tr>
<td>TOPP, Max</td>
<td>None</td>
<td>None</td>
<td>None</td>
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<td>ULLMANN, Andrew</td>
<td>Basilea, Pfizer, MSD, Gilead</td>
<td>Basilea, Pfizer, MSD, Aicuris</td>
<td>Basilea, Pfizer, MSD, Gilead</td>
<td>None</td>
</tr>
<tr>
<td>VYAS, Jatin</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>POSTER #</td>
<td>ABSTRACT &amp; AUTHOR</td>
<td>PAGE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Antifungal and antioxidant potentials of <em>Piptostigma calophyllum</em>, <em>Uvariodendron calophyllum</em> and <em>Uvariodendron molundense</em> crude extracts</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EZ Menkem*, CT Mofor, FF Boyom</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Antioxydant and antifungal potentials of essential oils of <em>Ocimum gratissimum</em> (Lamiaceae) from Yaounde and Dschang (Cameroon)</td>
<td>66</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>JB Hzounda Fokou*, IF Kenfack Tsague, PM Jazet Dongmo, F Fekam Boyom, C Menut, PH Amvam Zollo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Synergistic action of honey and essential oils against <em>Aspergillus flavus</em> and <em>Aspergillus niger</em></td>
<td>67</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L Boukraâ*, F Abdellah, SM Hammoudi, HA Alzahrany, BA Bakhotmah</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>The place of the starch of ginger in the fight against <em>Aspergillus niger</em></td>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A Moussa, H Aaggad*, N Djebli, A Saad</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Antifungal effect of <em>lavandula, salvia, sumac, Glycyrrhiza</em> and <em>Althoca</em> extracts on <em>Aspergillus</em> species</td>
<td>69</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B Mousavi*, A Rashidi, B Davari, Q Zamini, MR Rahmani, MA Rezaei, SV Hosseini, Y Motaharinia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Anti-biofilm and anti-cancer activity of farnesol: a quorum sensing molecule</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DH Dusane*, S Dam, R Narr, A Beauvais, SS Zinjarde</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Azole resistance in <em>Aspergillus fumigatus</em> obtained from hospitals environments</td>
<td>71</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H Badali*, B Mousavi, B Ahmadi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>May avian farms constitute a source of azole-resistant <em>Aspergillus fumigatus</em> isolates?</td>
<td>72</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D Wang, M Gricourt, P Arnê*, D Séguin, R Chermette, W Huang, F Botterel, J Guillot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Management of aflatoxigenic fungi in groundnut (<em>arachis hypogaea L.</em>) varieties in Central Tigray through soil solarization and planting time</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MR Kahsay*, Dr Amare, Dr Dereje</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Antifungal susceptibility of <em>Aspergillus fumigatus</em> and relationship with azole exposure in Nagasaki, Japan</td>
<td>74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>First isolations in India of multiple-triazole resistant <em>Aspergillus fumigatus</em> strains, carrying the TR/L98H mutations in the <em>cypl51A</em> gene</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A Chowdhary*, S Kathuria, HS Randhawa, SN Gaur, CH Klaassen, JF Meis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Antifungal therapy affects the sensitivity of (1→3)-β-D-glucan, galactomannan, and lateral-flow device assays in serum but not in bronchoalveolar lavage fluids</td>
<td>76</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CR Thornton*, LK Najvar, R Bocanegra, WR Kirkpatrick, TF Patterson, NP Wiederhold</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Antifungal susceptibility of <em>Aspergillus sp.</em> under hypoxic growth conditions</td>
<td>77</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U Binder*, E Maurer, C Lass-Flörl</td>
<td></td>
<td></td>
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<tr>
<td>POSTER #</td>
<td>ABSTRACT &amp; AUTHOR</td>
<td></td>
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<tr>
<td>----------</td>
<td>-------------------</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>14</td>
<td>Aspergillus fumigatus biofilms releases extracellular DNA which plays a role in phase dependant antifungal resistance R Rajendran*, D Lappin, B Jones, C Williams, G Ramage</td>
<td></td>
<td></td>
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<td>NOTE: This abstract has also been selected for Oral Presentation</td>
<td></td>
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</tr>
<tr>
<td>16</td>
<td>Antifungal activity of imidazole, benzimidazole, triazole and benzo triazole derivatives against Aspergillus species K Pakshir*, K Zomorodian, Z Rezaei, S Khabnadideh, S Gholami</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>In vitro activity of extracts of Stevia ursicifolia (Asteraceae) against Aspergillus spp. LJ Nogueira, AM Nascimento, KN Machado, TFF Magalhães, CVB Martins, DL da Silva, FMR da Mata, MA Resende-Stoianoff*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>The effect of Anti-Aflatoxin essential oils of Cuminum cyminum, Ziziphora clinopodioiedes and Nigella sativa MH Minoeceanaghhighi*, AR Khosravi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>The effects of anti-Aspergillus essences of Cuminum cyminum, Ziziphora clinopodioiedes and Nigella sativa and the site of their activities MH Minoeceanaghhighi*, AR Khosravi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>The systems biology of azole resistance in Aspergillus fumigatus M Bromley, MF Fraæzek, M Kapushesky, I Gut, N Fedorova, W Nierman, DW Denning, P Bowyer*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Management of Aspergillus spp. and aflatoxin contamination in rice through fungicidal seed treatment CS Reddy*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Ochratoxin production and management of Aspergillus ochraceus contamination in rice CS Redddy*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Invasive aspergillosis of pituitary gland in an immunocompetent patient F Abbasi*, D Yadegarinia, M Haghighi, S Korooni</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Possible respiratory infection due to Aspergillus in workers from swineries and poultries R Sabino*, V Mateus, S Viegas, C Verissimo, C Viegas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Possible aflatoxin presence in Portuguese poultry units S Viegas, C Viegas, R Sabino*, C Verissimo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Invasive aspergillosis in Intensive Care Unit patients from Iran MT Hedayati*, S Khodavaisy, M Aliyali, S Mahdavi Omran, MR Habibi</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## POSTER # | ABSTRACT & AUTHOR | PAGE
--- | --- | ---
27 | Posaconazole plasma concentrations and invasive mould infections in patients with hematologic malignancies<br>M Hoenigl*, K Seeber, H Salzer, R Raggam, T Valentin, A Valentin, I Zollner-Schwetz, A Woelfler, R Krause | 91
28 | Risk factors associated with low posaconazole plasma concentrations in patients with hematologic malignancies<br>M Hoenigl*, H Salzer, T Valentin, A Valentin, K Seeber, A Woelfler, A Strohmaier, I Zollner Schwetz, R Raggam, R Krause | 93
29 | Impact of testing posaconazole plasma concentrations on epidemiology of antifungal prophylaxis and therapy in patients with hematologic malignancies<br>M Hoenigl*, H Salzer, R Raggam, T Valentin, A Valentin, A Woelfler, K Seeber, A Strohmaier, I Zollner Schwetz, H Sill, R Krause | 94
30 | Caffeine induced germination abnormality in *Aspergillus flavus*<br>S Debnath*, A. Tanti, I Phukan, B Barthakur | 96
31 | Recent developments in the pathogenesis of avian aspergillosis<br>L Van Waeyenberghe*, F Pasmans, F Haesebrouck, A Martel | 97
32 | Spectrum of infections associated with *Aspergillus* species in tertiary care hospital<br>I Xess*, A Gupta , Y Dabas | 98
33 | Aspergillus bronchitis in non-immunocompromised patients – case series, response to treatment and criteria for diagnosis<br>A Chrdle, S Mustakim, R Bright-Thomas, T Felton, CG Baxter, DW Denning*<br><em>NOTE: This abstract has also been selected for Oral Presentation</em> | 99
34 | Isolation and identification of *Aspergillus* spp from water in poultry farms in Khartoum State, Sudan<br>AG Wisal* | 100
35 | Ureteral obstruction due to *Aspergillus terreus* in a diabetic patient following ureteroscopic lithotripsy: A case report<br>N Najafi, T Shokohi, A Bassiri, M Parvin, D Yadegari, F Taghavi, MT Hedayati, R Abdii, B Mousavi* | 101
36 | Role of real time PCR and galactomannan in the classification of *Aspergillus* disease in cystic fibrosis<br>CG Baxter*, AK Webb, AM Jones, DW Denning | 102
37 | False positivity of *Aspergillus* galactomannan test in patients with hematological malignancies<br>Y Oz*, F Aksit, M Aslan, MO Akay, G Durmaz, N Kiraz | 103
<table>
<thead>
<tr>
<th>POSTER #</th>
<th>ABSTRACT &amp; AUTHOR</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>A case of chronic pulmonary aspergillosis due to itraconazole low-susceptible <em>Aspergillus fumigatus</em> of which resistance acquired during long-term administration of itraconazole</td>
<td>105</td>
</tr>
<tr>
<td>40</td>
<td>Evaluation on “real life” prescriptions of antifungal prophylaxis in high risk patients: preliminary results from a prospective survey</td>
<td>106</td>
</tr>
<tr>
<td>41</td>
<td>Granulocyte transfusions as adjunctive treatment of invasive fungal diseases in neutropenic patients</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>M Caira*, N Piccirillo, M Mancinelli, P Chiusolo, S Hohaus, L Laurenti, M Maresca, CG Valentini, G Leone, L Pagano</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>Prospective registry of invasive fungal diseases in acute myeloid leukemia: preliminary results on 142 cases</td>
<td>110</td>
</tr>
<tr>
<td>43</td>
<td>Pre-hospital risk factors for invasive fungal disease in patients with acute myeloid leukemia at diagnosis: preliminary results from the seifem 2010-study</td>
<td>111</td>
</tr>
<tr>
<td>44</td>
<td><em>Aspergillus tanneri</em> sp. nov, a new pathogenic <em>Aspergillus</em> that causes invasive disease refractory to antifungal therapy</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>JA Sugui*, SW Peterson, LP Clark, AM Zelazny, SM Holland, KJ Kwon-Chung</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>Impact of current antifungal therapy on PCR based investigation of bronchoalveolar lavage samples for diagnosing pulmonary aspergillosis in patients with hematologic malignancies</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>M Reinwald*, E Kovalevskaya, B Spiess, M Hummel, WK Hofmann, D Buchheidt</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>NOTE: This abstract has also been selected for Oral Presentation</strong></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>Antifungal management strategy for high risk neutropenic patients based on itraconazole levels</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>EL Giannatou*, JO Cleverley, CH Symeonidou, AR Prentice, CC Kibbler</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td><em>Aspergillus</em> &amp; <em>otomycosis</em> in Kermanshah Iran</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>A Mikaeli*, S Nazari</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>Testing for allergenicity of fungi and practices contributing to biotic pollution in air-conditioned indoor environments</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>AA Haleem Khan</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>A case of fungal rhinosinusitis presented with a maxillary mycetoma caused by <em>Aspergillus niger</em></td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>R Gumral*, Y Hidir, MA Saracli, E Ekberov, ST Yildiran</td>
<td></td>
</tr>
<tr>
<td>POSTER #</td>
<td>ABSTRACT &amp; AUTHOR</td>
<td>PAGE</td>
</tr>
<tr>
<td>----------</td>
<td>-------------------</td>
<td>------</td>
</tr>
<tr>
<td>50</td>
<td>Voriconazole and posaconazole improves asthma severity in allergic bronchopulmonary aspergillosis and severe asthma with fungal sensitization L Chishimba*, R Niven, J Cooley, DW Denning</td>
<td>118</td>
</tr>
<tr>
<td>51</td>
<td>Repeated courses of intravenous amphotericin B therapy including intermittent long-term treatment in patients with chronic pulmonary aspergillosis P Newton*, C Harris, DW Denning</td>
<td>119</td>
</tr>
<tr>
<td>52</td>
<td>Galactomannan assay performance in bronchoalveolar lavage for the diagnosis of invasive Aspergillosis in immunocompromised hosts MV Batista*, C Constâncio, CA Fonseca, SF Costa, CM Gamba, SV Campos, IL França, FL Dulley, GH Fonseca, MA Shikanai-Yasuda</td>
<td>120</td>
</tr>
<tr>
<td>53</td>
<td>Diagnosis and treatment of necrotizing malignant otitis caused by Aspergillus A Ghoubontini*, E Chaker, G Besbes, M Ferjaoui, T Ben Chaabane</td>
<td>121</td>
</tr>
<tr>
<td>54</td>
<td>Aspergillosis patient support meeting - actual and virtual MB Kirwan*, GL Powell, DL Kennedy, CV Harris, GT Atherton</td>
<td>122</td>
</tr>
<tr>
<td>55</td>
<td>Comparison of Aspergillus diagnostic yields from bronchial aspirate, bronchoalveolar lavage and sputa by real-time polymerase chain reaction compared to standard fungal culture MB Kirwan*, M Fraczek, J Martin, PV Barber, MD Richardson, CB Moore, DW Denning</td>
<td>123</td>
</tr>
<tr>
<td>56</td>
<td>Aspergillus-related respiratory diseases can impact pneumococcal antibody levels and response to both pneumococcal polysaccharide vaccines and pneumococcal conjugate vaccines GL Powell*, J Morris, R Borrow, DW Denning</td>
<td>124</td>
</tr>
<tr>
<td>57</td>
<td>RT-PCR is significantly more sensitive in detecting Aspergillus in respiratory samples than culture N Duddy*, CB Moore, P Kent, M Richardson, D Denning, R Rautema-Richardson</td>
<td>125</td>
</tr>
<tr>
<td>59</td>
<td>Chronic pulmonary aspergillosis severity and health status worsening; data from the National Aspergillosis Centre, Manchester, UK K Al-shair*, GT Atherton, S Whiteside, DW Denning</td>
<td>127</td>
</tr>
<tr>
<td>60</td>
<td>Aspergillus species isolated from clinical samples in Oman Abdullah Al-Hatmi*</td>
<td>128</td>
</tr>
<tr>
<td>61</td>
<td>Primary laryngeal aspergillosis related to oral sex? Y Ran*, Y Lu, L Cao, C Li, Y Dai, H Yang, X Ran, Y Liu, H Bai, C Zhang</td>
<td>129</td>
</tr>
<tr>
<td>62</td>
<td>Nosocomial and out-hospital invasive aspergillosis in haematological patients in Saint-Petersburg, Russia NN Klimko, SN Khostelidi*, YV Borzova, MO Popova, RM Chernopyatova, SM Ignatyeva, TS Bogomolova, RA Araviisky, NI Zubarovskaya, AS Kolbin, EG Boychenko, IS Zjuzgin, NV Vasilyeva</td>
<td>132</td>
</tr>
</tbody>
</table>
## POSTER # | ABSTRACT & AUTHOR | PAGE
--- | --- | ---
63 | *Aspergillus* species in our living environment  
IZ Ivica Zurak* | 134 |
64 | In vivo allergic immune responses of *Aspergillus* species isolated from Kathmandu Valley, Nepal  
US Khwakhali*, MS Hada, M Aryal, GR Shrestha | 135 |
65 | Detection and quantitation of Aflatoxin for the diagnosis of *Aspergillus flavus*  
G Shrestha*, A Mridha | 136 |
66 | *Aspergillus fumigatus* secretes factors that strongly activate human thrombocytes  
C Speth*, M Hagleitner, H Ott, C Lass-Flörl, K Pfüller, G Rambach | 137 |
67 | *Aspergillus fumigatus* secretes soluble factors that induce complement deposition on thrombocytes  
G Rambach*, A Toto, M Hagleitner, C Lass-Flörl, C Speth | 138 |
68 | Galactomannan detection in bronchoalveolar lavage fluid in Intensive Care Unit patients at risk for invasive aspergillosis  
S Khodavaisy*, MT Hedayati, M Alialy, MR Habibi | 139 |
69 | Role of the neutrophil and it’s interaction with the Th-17 pathway in the host response against *Aspergillus fumigatus*  
A Savers, M Parlato, M Brock, J-M Cavaillon, O Ibrahim-Granet* | 140 |
70 | Primary renal aspergillosis and associated cytokines in BALB/c mice in response to *Aspergillus flavus*  
R Anand*, J Shankar, AP Singh, BN Tiwary | 141 |
71 | Increased susceptibility to *Aspergillus fumigatus* in recipients with graft-versus-host disease after allogeneic bone marrow transplantation can be prevented by transfer of donor CD4+CD25+ regulatory T cells  
B Echtenacher*, K Doser, M Edinger, P Hoffmann | 142 |
72 | Vitamin D regulation of Th2 and Th17 immune response to *Aspergillus* in mice and humans: role of OX40L  
N Nguyen*, J Kreindler, C Steele, J Pilewski, A Ray, J Alcorn, S Zeigler, E Hartigan, M Kurs-Lasky, H Rockette, J Kolls | 143 |
73 | Anti-*Aspergillus* antibodies in haematological patients suspected for developing invasive aspergillosis  
V Arsic Arsenijevic*, A Barac, A Vidovic, N Suvajdžic Vukovic | 144 |
74 | T-cell responses to several *Aspergillus* antigens may be detected in patients with invasive aspergillosis and may be exploited for diagnostic and therapeutic purposes: a multicenter study  

*NOTE: This abstract has also been selected for Oral Presentation*
<table>
<thead>
<tr>
<th>POSTER #</th>
<th>ABSTRACT &amp; AUTHOR</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>Polymyxin B, in combination with voriconazole, modulates cytokine response of mammalian cells existing a potent fungicidal effect on <em>Aspergillus fumigatus</em></td>
<td>146</td>
</tr>
<tr>
<td></td>
<td>E Yesilyurt, A Kalkanci*, I Fidan, A Fouad</td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>Unique polysaccharide cell-wall composition responsible for dysregulated inflammation of <em>A. nidulans</em> infections in chronic granulomatous disease</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>S Henriët*, E Simonetti, AJMM Rijs, PE Verweij, PWM Hermans, A Warris</td>
<td></td>
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<tr>
<td><strong>NOTE:</strong> This abstract has also been selected for Oral Presentation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>Aspergillosis in cystic fibrosis: a multifactorial disease?</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>RG Iannitti*, G Giovannini, A DeLuca, A Carvalho, C Cunha, FM DeBenedictis, A Casagrande, L Romani</td>
<td></td>
</tr>
<tr>
<td>78</td>
<td>Regenerating islet-derived 3-gamma suppresses pulmonary Th17 immunity to <em>Aspergillus fumigatus</em></td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>JP McAleer*, N Nguyen*, DM Ricks, M Binnie, D Sheppard, LV Hooper, JK Kolls</td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>Identification of <em>A. fumigatus</em> secondary metabolites involved in neutrophil recruitment using a novel microfluidic platform</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>E Berthier*, DJ Beebe, NP Keller</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>β-glucan mediated autophagy induction regulates intracellular killing of <em>Aspergillus fumigatus</em> in human monocytes</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>E Kyrmizi, T Akoumianaki, MS Gresnigt, G Samonis, MG Netea, FL van de Veerdonk, DP Kontoyianniss, G Chamilos*</td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>Reduced gamma interferon (gIFN) production in chronic pulmonary aspergillosis (CPA)</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>R Dollfinger*, C Harris, S Lear, P Newton, H Alachkar, DS Kumarraratne, DW Denning</td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>The interaction of human platelets alone and in combination with anidulafungin against <em>Aspergillus fumigatus</em></td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>S Perkhofer*, B Striessnig, HW Ott, C Lass-Flörl</td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>Monocyte-derived dendritic cells pulsed with <em>Aspergillus fumigatus</em> derived β-3-glucan activate human natural killer cells and enhance their anti-<em>Aspergillus</em> effect</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>M Bouzani*, AL Schmitt, V Aimanianda, C Flechsig, SM Tan, GU Grigolet, JP Latge, H Einsele, J Loeffler</td>
<td></td>
</tr>
<tr>
<td>84</td>
<td>FIBCD1 binds to <em>Aspergillus fumigatus</em> chitin and modulates the cytokine production against fungal polysaccharides and proteases</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>LK Dubey*, A Schlosser, MA Hammond, JB Jensen, JB Moeller, GL Sorensen, U Holmskov</td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>DAMP signaling in aspergillosis: the S100B/RAGE hyperfunction is a risk factor for infection in stem cell transplant recipients</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>A Carvalho, C Cunha*, G Giovannini, G Sorci, R Donato, F Aversa, L Romani</td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>Immunogenetics of aspergillosis: from risk assessment to vaccinomics</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td>A Carvalho*, C Cunha, F Aversa, L Romani</td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>Immune response in immunocompetent rats with pulmonary <em>Aspergillus fumigatus</em> infection</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td>I Mirkov*, A Atia Mhfuod El-Muzghi, A Popov, J Glamoclija, D Miljkovic, S Belij, J Djokie, D Kataranovski, M Kataranovski</td>
<td></td>
</tr>
</tbody>
</table>
POSTER # | ABSTRACT & AUTHOR | PAGE
--- | --- | ---
88 | Immunoproteomic analysis of the antibody response to the human-pathogenic fungus *Aspergillus fumigatus*  
J Teutschbein, S Simon, N Koester-Eiserfunke*, J Loeffler, C Morten, J Springer,  
H Einsele, T Rogers, A Brakhage, O Kniemeyer | 159
89 | Modeling the effects of modern immunosuppressive therapies on *Aspergillus fumigatus* infection  
S Herbst*, I Teo, EM Bignell, S Shaunak, DPH Armstrong-James | 160
90 | Comparative virulence and susceptibility of *Aspergillus terreus* and *Aspergillus alabamensis*  
DA Stevens*, T Majumder, M Martinez, V Chen, D Alvarado, KV Clemons, SA Balajee,  
AM Tortorano, MA Viviani | 161
91 | Virulence of toxigenic and atoxigenic strains of *Aspergillus flavus*  
KV Clemons*, V Chen, M Martinez, B Campbell, P Cotty, DA Stevens | 162
92 | Molecular variability in aflatoxin producing strains of rice aspergilli  
C S Reddy* | 163
93 | A comparative analysis of polyketide synthases in *Aspergillus flavus*  
P Bietartiya*, A Bhaduri, Y Singh, T Madan, A Varma, P U Sarma | 164
94 | Comparative analysis of allergen produced by *Aspergillus* and *Penicillium* species  
isolated from Kathmandu, Nepal  
M Aryan*, US Khwakhali, MS Hada and GR Shrestha | 165
95 | The *ugm1* mutant of *Aspergillus fumigatus* activates human endothelial cells upon hyphae-cell contact  
GWP Neves*, JP Latgé, LM Lopes-Bezerra | 166
96 | Exposure of *Aspergillus fumigatus* to Caspofungin leads to leakage and *de novo* synthesis of gliotoxin  
A Eshwika*, K Kavanagh | 167
97 | Specific induction of cd203c expression in blood basophils discriminates between CF patients with *Aspergillus colonization* and those with CF-ABPA  
Y Gernez, C Everson, C Dunn, E Mitsunaga, Z Davis, LA Herzenberg, R Tirouvanziam, RB Moss* | 168
98 | Morphological and genotypic identification of environmental isolates of *Aspergillus* species based on sequencing of β-tubulin gene  
GH Shoohu*, H Mirhendi, P Kordbacheh, S Rezaei, A Rezaeimatekola, B Ahmadi, M Abastabar,  
N Jalali-Zand, M Nikaeen, K Makimura | 169
99 | The *Aspergillus* genome database: recent developments in comprehensive multispecies curation, comparative genomics and community resources  
G Sherlock*, MB Arnaud, GC Cerqueira, DO Inglis, MS Skrzypczek, J Binkley, MC Chibucos,  
J Crabtree, C Howarth, J Orvis, P Shah, F Wymore, G Binkley, SR Miyasato, M Simison,  
JR Wortman | 170
<table>
<thead>
<tr>
<th>POSTER #</th>
<th>ABSTRACT &amp; AUTHOR</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Aspergillus fumigatus supermaters: genome comparison and genetic recombination</td>
<td>171</td>
</tr>
<tr>
<td></td>
<td>NOTE: This abstract has also been selected for Oral Presentation</td>
<td></td>
</tr>
<tr>
<td>101</td>
<td>Identification of Aspergillus niger and Aspergillus tubingensis by PCR-RFLP method targeting the Beta-tubulin (β-TUB) gene</td>
<td>172</td>
</tr>
<tr>
<td></td>
<td>GH Shokhuhi*, H Mirhendi, P Kordbacheh, S Rezaei, A Rezaematekola, M Abastabar, N Jalali Zand, M Nikaein, K Makimura</td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>NCE102 homologue in Aspergillus fumigatus is required for normal sporulation, not hyphal growth or pathogenesis</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td>V Khalaj*, M Azizi, S Enayati, D Khorasanizadeh, E Mirabzadeh</td>
<td></td>
</tr>
<tr>
<td>103</td>
<td>Functional analysis of High Osmolarity Glycerol (HOG) pathway in Aspergillus fumigatus: a potential target for antifungal drug</td>
<td>174</td>
</tr>
<tr>
<td></td>
<td>D Hagiwara*, T Toyotome, K Kamei, T Gono, S Kawamoto</td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>Aspergillus fumigatus contamination monitoring in a French turkey hatchery using Multiple Locus VNTR Analysis (MLVA)</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>S Thierry, P Arné*, C Pourcel, F Botterel, R Chermette, J Guillot</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>Characterization of the veA gene in Aspergillus fumigatus</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>S Dhingra*, AM Calvo</td>
<td></td>
</tr>
<tr>
<td>106</td>
<td>Nail infection by Aspergillus candidus; the first report from Iran</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>B Ahmadi*, S Rezaei, SJ Hashemi, F Zaini, MR Shidfar, M Gerami-Shoar, R Daei, E Zibafar, S Ansari, L Hosseinpour</td>
<td></td>
</tr>
<tr>
<td>107</td>
<td>Onychomycosis due to Aspergillii species in 170 patients in Tehran, Iran</td>
<td>178</td>
</tr>
<tr>
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<td>B Ahmadi*, S Rezaei, SJ Hashemi, F Zaini, MR Shidfar, F Noorbakhsh, M Gerami-Shoar, R Daei, E Zibafar, L Hosseinpour</td>
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<tr>
<td>108</td>
<td>Classification and rapid identification of Aspergillus fumigatus and relative species</td>
<td>179</td>
</tr>
<tr>
<td></td>
<td>T Yaguchi*, T Matsuzawa, Y Imanishi, R Tanaka</td>
<td></td>
</tr>
<tr>
<td>109</td>
<td>Comparison of culture and nested-PCR in diagnosis of avian aspergillosis</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>A Nasrollahi, J Hashemi*, S Hajikhoodad, N Vaseghi, M Jafarpour</td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>MPS1 kinase is essential for the growth in Aspergillus fumigatus</td>
<td>181</td>
</tr>
<tr>
<td></td>
<td>T Umeyama*, S Yamagoe, K Tanabe, H Ohno, Y Miyazaki</td>
<td></td>
</tr>
<tr>
<td>111</td>
<td>Peptide aptamers as novel diagnostics, and for blocking PalH-mediated environmental sensing in A. fumigatus</td>
<td>182</td>
</tr>
<tr>
<td></td>
<td>CM Grice*, M Bertuzzi, E Bignell</td>
<td></td>
</tr>
<tr>
<td>112</td>
<td>Signalling requirements for, and cell wall dependency upon, PacC: A master regulator of A. fumigatus virulence factors</td>
<td>183</td>
</tr>
<tr>
<td></td>
<td>M Bertuzzi*, M Schrattl, T Cairns, N Fedorova, H Haas, W Nierman, EM Bignell</td>
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<td>PAGE</td>
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<td>------</td>
</tr>
<tr>
<td>113</td>
<td>Exploring the additive effects of multiple secondary metabolites during <em>Aspergillus fumigatus</em> virulence</td>
<td>184</td>
</tr>
<tr>
<td></td>
<td>L Alcazar-Fuoli*, T Cairns, N Fedorova, W Nierman, E Bignell</td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>Genetic diversity of <em>Aspergillus fumigatus</em> isolated from air at Manchester</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>FO Alshareef*, RD Geoffrey</td>
<td></td>
</tr>
<tr>
<td>115</td>
<td>High frequency transformation of <em>Aspergillus fumigatus</em> by <em>Agrobacterium</em> mediated transformation (AMT): the <em>Aspergillus</em> KU80 protein is required for random Ti plasmid transposon chromosomal integration</td>
<td>186</td>
</tr>
<tr>
<td></td>
<td>WC Nierman*, JA Sugui, L Losada, X Lin, J Kwon-Chung</td>
<td></td>
</tr>
<tr>
<td>116</td>
<td>Role of novel <em>Aspergillus fumigatus</em> gene clusters in host adaptation and virulence</td>
<td>187</td>
</tr>
<tr>
<td></td>
<td>A Ghosh*, L Alcazar-Fuoli, E Bignell</td>
<td></td>
</tr>
<tr>
<td>117</td>
<td>Survey the effect of licorice extract on <em>Aspergillus parasiticus</em> growth and aflatoxin production by MIC and HPLC technique</td>
<td>188</td>
</tr>
<tr>
<td></td>
<td>R Moliseni*, F Noorbakhsh, A Nasrollahi Omran, S Rezaie</td>
<td></td>
</tr>
<tr>
<td>118</td>
<td>Mutation and expression profiling of sequential patient isolates that developed azole resistance</td>
<td>189</td>
</tr>
<tr>
<td></td>
<td>ND Fedorova*, S Pakala, S Pakala, S Mounaud, DW Denning, P Bowyer, WC Nierman</td>
<td></td>
</tr>
<tr>
<td>119</td>
<td>Simple and highly discriminatory VNTR-based multiplex PCR for the typing of <em>Aspergillus flavus</em> isolates from different geographic origins and different hosts</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td>D Wang*, L Ziani, S Thierry, P Arné, R Chermette, C Pourcel, F Botterel, I Hadrich, A Ayadi, S Ranque, W Huang, J Guillot</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Identification of levels of <em>Aspergillus</em> spp conidia in different seasons and molecular pattern of two species <em>Aspergillus</em> (fumigatus and flavus) in the hospital</td>
<td>191</td>
</tr>
<tr>
<td></td>
<td>N Hosseini*, M Bayat, SH Roudbarmohammadi</td>
<td></td>
</tr>
<tr>
<td>121</td>
<td>Comparative analysis of metabolic pathways to identify and characterize the putative drug targets for <em>Aspergillus</em></td>
<td>192</td>
</tr>
<tr>
<td></td>
<td>VK Morya*, EK Kim</td>
<td></td>
</tr>
<tr>
<td>122</td>
<td>Fetuin A, a serum glycoprotein, promotes the growth and the biofilm formation of <em>Aspergillus fumigatus</em></td>
<td>193</td>
</tr>
<tr>
<td></td>
<td>T Toyotome*, A Watanabe, K Kamei</td>
<td></td>
</tr>
<tr>
<td>123</td>
<td>Interaction studies of <em>Aspergillus</em> and Human airway cells</td>
<td>194</td>
</tr>
<tr>
<td></td>
<td>M Safari*, T Keshavarz, M Clements</td>
<td></td>
</tr>
<tr>
<td>124</td>
<td>Microevolution of <em>Aspergillus fumigatus</em> in Aspergillomas</td>
<td>195</td>
</tr>
<tr>
<td></td>
<td>SJ Howard*, A Pasqualotto, M Anderson, H Leatherbarrow, A Al-Barrag, E Harrison, L Gregson, P Bowyer, DW Denning</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>Genetic diversity of <em>Aspergillus fumigatus</em> and <em>Aspergillus flavus</em> from poultry farms in Southern China, Guangxi Province</td>
<td>196</td>
</tr>
<tr>
<td></td>
<td>D Wang*, S Thierry, P Arné, R Chermette, W Huang, J Guillot</td>
<td></td>
</tr>
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<td>PAGE</td>
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<td>------</td>
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<tr>
<td>126</td>
<td>Molecular and cellular response of <em>Aspergillus fumigatus</em> to human platelets</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>S Perkhofer*, U Binder, I Wille, W Nussbaumer, C Lass-Flörl</td>
<td></td>
</tr>
<tr>
<td>127</td>
<td>Secondary soluble metabolites of <em>Aspergillus fumigatus</em> and its influence on inhibition of human WISH, CaCo and DU-145 cancer cell proliferation</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>A Barac, D Ivanovic*, M Pekmezovic, I Lukovic, V Arsic Arsenijevic</td>
<td></td>
</tr>
<tr>
<td>128</td>
<td>Genetics of heterokaryon incompatibility in <em>Aspergillus fumigatus</em></td>
<td>199</td>
</tr>
<tr>
<td></td>
<td>SR Weaver*, N Dunn-Coleman, MR Diaz-Torres, GD Robson</td>
<td></td>
</tr>
<tr>
<td>129</td>
<td>Characterization of a sialidase (KDNase) from the opportunistic fungal pathogen, <em>Aspergillus fumigatus</em></td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>JHF Yeung*, JC Telford, J Nesbitt, G Xu, J Chan, ML Warwas, A Bennet, GL Taylor, MM Moore</td>
<td></td>
</tr>
<tr>
<td>130</td>
<td>The importance of secreted siderophores in the diagnosis of invasive aspergillosis</td>
<td>201</td>
</tr>
<tr>
<td></td>
<td>CS Carroll*, LJS Pinto, MM Moore</td>
<td></td>
</tr>
<tr>
<td>131</td>
<td>Contribution of protein kinase C (pkaC) and albl genes involved in conidiation/pigmentation pathway in wild type (WT) and pigmentless, non-conidiating voriconazole-resistant (VCZ-R) <em>Aspergillus flavus</em></td>
<td>202</td>
</tr>
<tr>
<td></td>
<td>S Krishnan Natesan*, WJ Wu, PH Chandrasekar</td>
<td></td>
</tr>
<tr>
<td>132</td>
<td>Effect of pharmacological and genetic inhibition of the heat-shock protein 90 (Hsp90) in <em>Aspergillus fumigatus</em></td>
<td>203</td>
</tr>
<tr>
<td></td>
<td>F Lamoth*, PR Juvvadi, JR Fortwendel, WJ Steinbach</td>
<td></td>
</tr>
<tr>
<td>133</td>
<td>Phenotypic analysis of <em>Aspergillus fumigatus</em> strain lacking the sodium ATPase encoding gene <em>enaA</em></td>
<td>204</td>
</tr>
<tr>
<td></td>
<td>N Pinchai*, T Ponpinit, A Chaiprasert, S Foongladda</td>
<td></td>
</tr>
<tr>
<td>134</td>
<td>Diversity and comparison of short tandem repeats within <em>Aspergillus section Fumigati</em></td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>R Araujo*, A Amorim, L Gusmão</td>
<td></td>
</tr>
<tr>
<td>135</td>
<td>A rapid approach towards screening of <em>Aspergillus fumigatus</em> population structure using a single nucleotide polymorphism based method</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>R Caramalho*, L Gusmão, A Amorim, R Araujo</td>
<td></td>
</tr>
<tr>
<td>136</td>
<td>Genetic associations with plasminogen and related genes with chronic cavitary (CCPA) and allergic bronchopulmonary aspergillosis (ABPA) compared with healthy and asthmatic controls</td>
<td>207</td>
</tr>
<tr>
<td></td>
<td>NL Smith*, P Bowyer, DW Denning, A Simpson</td>
<td></td>
</tr>
<tr>
<td>137</td>
<td>Impaired switching of the normal IL1 response to <em>A. fumigatus</em> in chronic cavitary pulmonary aspergillosis (CCPA)</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>NL Smith*, A Simpson, DW Denning, P Bowyer</td>
<td></td>
</tr>
<tr>
<td>138</td>
<td>New diagnostic targets for <em>Aspergillus fumigatus</em> infections</td>
<td>209</td>
</tr>
<tr>
<td></td>
<td>E McCulloch, G Ramage, R Ranjendran, B Jones, C Williams*</td>
<td></td>
</tr>
<tr>
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<td>PAGE</td>
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<td>------</td>
</tr>
<tr>
<td>139</td>
<td><em>Aspergillus</em> proficiency testing - External quality assurance (EQA) for optimisation of diagnostic molecular testing</td>
<td>210</td>
</tr>
<tr>
<td>140</td>
<td>Modeling of <em>Aspergillus fumigatus</em> virulence: opportunities for novel therapeutic target discovery</td>
<td>211</td>
</tr>
<tr>
<td>141</td>
<td>Chemical genetic profiling and drug mechanism of action studies in <em>Aspergillus fumigatus</em></td>
<td>212</td>
</tr>
<tr>
<td>142</td>
<td>Isolation and identification of <em>Aspergillus</em> spp from airbourne of Sulaimani City</td>
<td>213</td>
</tr>
<tr>
<td>143</td>
<td>Comparison of antimicrobial potential of some <em>Aspergillus fumigatus</em> strains</td>
<td>214</td>
</tr>
<tr>
<td>144</td>
<td>Primary subcutaneous aspergillosis with sporotrichoid lesions</td>
<td>215</td>
</tr>
</tbody>
</table>
PROGRAMME

All General Sessions to be held in Room 2
Pre-Meeting Workshop (25 January) to be held in Room 3
Meet the Professor Sessions (26 + 28 January) to be held in Room 3

WEDNESDAY 25 JANUARY

Interactive Masterclass: Management of chronic and allergic pulmonary aspergillosis
Supported by an unrestricted educational grant from Gilead Sciences Europe Ltd

Faculty:
Professor David Denning, Professor of Medicine and Medical Mycology, University of Manchester,
Director, National Aspergillosis Centre
Dr Caroline Baxter, Clinical Research Fellow, National Aspergillosis Centre, University Hospital of South Manchester
Dr Timothy Felton, Clinical Research Fellow, National Aspergillosis Centre, University Hospital of South Manchester

08.30 - 08.45  Introduction and plan for the day
08.45 - 10.00  Clinical and radiological presentation, diagnosis
               David Denning
10.00 - 10.30  Clinical case quiz (what is the diagnosis?)
10.30 - 10.50  Break
10.50 - 12.00  Q & A
12.30 - 13.15 Lunch
13.15 - 14.30 Breakout session with real cases and management conundrums
               Group 1  David Denning
               Group 2  Caroline Baxter
               Group 3  Timothy Felton
14.30 - 15.00 Antifungal TDM and azole dose modifications
               Timothy Felton
15.00 - 15.20 Break
15.20 - 16.00 Dealing with antifungal toxicities
               Caroline Baxter and David Denning
16.00 - 16.45 Clinical failure and its management
               David Denning
16.45 - 17.15 EMQ questions
17.15 - 17.30 Q & A and EMQ answers
18.00  Close

Early Registration for 5th Advances Against Aspergillosis

17.00 - 19.00 Rumelli Hall Foyer
THURSDAY 26 JANUARY

08.00 - 08.45  Meet the Professor Session
               Controversies in immunology: Excessive inflammation in aspergillosis
               Luigina Romani, MD, PhD

09.00 - 09.10  Opening Remarks
               William J. Steinbach, MD

Session 1: Cutting Issues in the Pathogenesis of Aspergillosis
Chairs: William J. Steinbach, MD & Jean-Paul Latgé, PhD

09.15 - 09.40  Angiogenesis at the mold-host interface: a potential key to understanding and treating invasive aspergillosis
               Ronen Ben-Ami, MD

09.40 - 10.05  Aspergillus fumigatus survival in the lung environment
               Elaine Bignell, PhD

10.05 - 10.30  Visualising phagosomal killing of Aspergillus fumigatus
               Jatin M. Vyas, MD

10.30 - 10.55  Vitamin D and OX40L interaction
               Jay K. Kolls, MD

10.55 - 11.05  Oral Poster Presentation: Aspergillus fumigatus supermaters: genome comparison and genetic recombination
               Janyce Sugui, PhD

11.05 - 11.35  Coffee / Tea Break

Session 2: Emerging Clinical Associations with Aspergillosis
Chairs: David W. Denning, FMedSci & Omrum Uzun, MD

11.35 - 12.00  COPD and aspergillosis
               Jesús V. Guinea Ortega, PharmD, PhD

12.00 - 12.25  Aspergillosis in cystic fibrosis – insights from new and old diagnostics
               Caroline Baxter, MD

12.25 - 12.50  Invasive aspergillosis in ICU - are we moving forward?
               George Dimopoulos, MD

12.50 - 13.15  Immune regulation in idiopathic bronchiectasis
               Rosemary Boyton, PhD

13.15 - 14.45  Lunch
Session 3: Top Six Papers in Aspergillosis in 2011
Chairs: Sevtap Arikan-Akdagli, MD & Jay K. Kolls, MD

13.45 - 14.45 and concurrent with lunch
Murat Akova, MD and Nir Osherov, PhD

Session 4: Novel Immunological Insights into Aspergillosis
Chairs: Richard B. Moss, MD & Emmanuel Roilides, MD

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
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| 14.45 - 15.10 | Innate immunity to *Aspergillus*  
Mihai G. Netea, PhD |
| 15.10 - 15.35 | Neutrophil extracellular traps and *A. fumigatus*  
Matthias Gunzer, PhD |
| 15.35 - 15.45 | Control of neutrophil ROS responses to *A. fumigatus*  
Keith Boyle, MD |
| 15.45 - 15.55 | Oral Poster Presentation: T-cell responses to several *Aspergillus* antigens may be detected in patients with invasive aspergillosis and may be exploited for diagnostic and therapeutic purposes: a multicenter study  
Leonardo Potenza, MD, PhD |
| 15.55 - 16.25 | Coffee / Tea Break |

Session 5: Portraits of Non-Fumigatus *Aspergilli* - What is so Special About:
Chairs: Paul Bowyer, PhD & Karl Clemons, PhD

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
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| 16.25 - 16.50 | Specification of polarity sites during growth and development of *Aspergillus nidulans*  
Steven D. Harris, PhD |
| 16.50 - 17.15 | *Aspergillus niger* a perfect host for industrial biotechnology: systems biology approaches for the production of building block chemicals  
Peter J. Punt, PhD |
| 17.15 - 17.40 | Diversity of *Aspergillus flavus* on crops and in the environment can be exploited to reduce aflatoxin exposure and improve environmental health  
Peter J. Cotty, PhD |
| 17.40 - 17.50 | Oral Poster Presentation: Unique polysaccharide cell-wall comparison responsible for dysregulated inflammation of *A. nidulans* infections in chronic granulomatous disease  
Stefanie Henriet, MD |
| 17.50 - 19.50 | Welcome Reception, Drinks and Poster Session 1 |
FRIDAY 27 JANUARY

Session 6: Astellas Symposium
Chair: Katsuhiko Kamei, MD, PhD

08.00 - 09.00  Controversies in management: prophylaxis or diagnostics
Andrew Ullman, MD
Drosos E. Karageorgopoulos, MD

Session 7: Genomics and Proteomics
Chairs: Elaine Bignell, PhD & David S. Perlin, PhD

09.15 - 09.40  Aspergillus adhesins
Don Sheppard, MD

09.40 - 10.05  mRNAs in Aspergillus
Michelle Momany, PhD

10.05 - 10.30  The diverse applications of RNA-seq for functional genomics studies in Aspergillus fumitagus
Antonis Rokas, PhD

10.30 - 10.55  Alternative approaches to drug discovery in Aspergillus fumigatus
Michael Bromley, PhD

Markus Kalkum, PhD

11.20 - 11.50  Coffee / Tea Break

Session 8: Management of Aspergillosis
Chairs: John E. Bennett, MD & Volkan Korten, MD

11.50 - 12.15  Management of chronic pulmonary aspergillosis
Koichi Izumikawa, MD, PhD

12.15 - 12.40  Update on biologicals for ABPA and asthma
Richard B. Moss, MD

12.40 - 13.05  Paediatric aspergillosis
Andreas Groll, MD

13.05 - 13.15  Oral Poster Presentation: Aspergillus bronchitis in non-immunocompromised patients - case series, response to treatment and criteria for diagnosis
Timothy Felton, MD

13.15 - 14.15  Lunch

CONFERENCE SOCIAL EVENT: Tour followed by Dinner
SATURDAY 28 JANUARY

08.00 - 08.45 Meet the Professor Session
Stump the Professor - Interesting clinical dilemmas
John E. Bennett, MD
Souha Kanj, MD

Session 9: Diagnostics and Imaging
Chairs: Dimitrios P. Kontoyiannis, MD & Malcolm Richardson, PhD

09.15 - 09.40 Application of diagnostic markers for IA in children
Emmanuel Roilides, MD

09.40 - 10.05 Impact of prophylaxis on galactomannan, beta-D-glucan and PCR
J. Peter Donnelly, PhD

10.05 - 10.30 Which antigens are important and why?
Max S. Topp, MD

10.30 - 10.40 Oral Poster Presentation: Impact of current antifungal therapy on PCR based investigation of bronchoalveolar lavage samples for diagnosing pulmonary aspergillosis in patients with hematologic malignancies
Mark Reinwald, MD

10.40 - 11.10 Coffee / Tea Break

Session 10: Pfizer Symposium
The Treatment Options Strategies for IA
Chairman: J. Peter Donnelly, PhD

11.10 - 11.15 Welcome and introduction
J. Peter Donnelly, PhD

11.15 - 11.30 Stratification of risk for invasive aspergillosis in immunocompromised patients
Raoul Herbrecht, MD

11.30 - 11.45 Pre-emptive versus empirical anti-fungal therapy in neutropenic hematologic patients
Omrum Uzun, MD

11.45 - 12.00 Combination therapy: who benefits?
Keiren Marr, MD

12.00 - 12.10 Panel discussion

12.10 - 14.10 Lunch and Poster Session 2
## Session 11: Antifungal Resistance

Chairs: David A. Stevens, MD & Beyza Ener, MD

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Speaker(s)</th>
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<tbody>
<tr>
<td>14.10 - 14.35</td>
<td>Global status of azole resistance in Europe and Asia</td>
<td>Sevtap Arikan-Akdagli, MD</td>
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<td>14.35 - 15.00</td>
<td>Update on resistance mechanisms</td>
<td>David S. Perlin, PhD</td>
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<tr>
<td>15.00 - 15.10</td>
<td>Oral Poster Presentation: <em>Aspergillus fumigatus</em> biofilms releases extracellular DNA which plays a role in phase dependent antifungal resistance</td>
<td>Ranjith Rajendran, MSc</td>
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<tr>
<td>15.10 - 15.35</td>
<td>Current section and species complex concepts: recommendations for routine daily practice</td>
<td>Manuel Cuenca-Estrella, MD, PhD</td>
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<tr>
<td>15.35 - 16.00</td>
<td>Implications of resistance in <em>Aspergillus</em> treatment guidelines</td>
<td>Dimitrios P. Kontoyiannis, MD</td>
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<tr>
<td>16.00 - 16.10</td>
<td>Farewell</td>
<td>David A. Stevens, MD</td>
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<td>16.20</td>
<td>Farewell Drinks and Discussion about the next AAA Meeting</td>
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(to be held in Room 3)
ABSTRACTS OF INVITED FACULTY
CONTROVERSIES IN IMMUNOLOGY: EXCESSIVE INFLAMMATION IN ASPERGILLOSIS

Luigina Romani, MD, PhD

University of Perugia - Italy

THURSDAY 26 JANUARY 2012 (08.00 - 08.45)

Aspergillosis is a disease that predominantly affects patients with deregulated immunity. Compelling experimental evidence indicate that severe aspergillosis belongs to the spectrum of fungus-related immune reconstitution inflammatory syndrome. Some degree of inflammation is required for protection—particularly in mucosal tissues—during the transitional response occurring temporally between the rapid innate and slower adaptive response. However, progressive inflammation worsens disease and ultimately prevents pathogen eradication. The greatest challenge now is to pave the way from promising results in experimental models to the clinic. This demands for a better definition of mechanisms of resistance and immune tolerance to the fungus in the lung as well as of cellular and molecular pathways underlying pathogenic inflammation in fungal infection and allergy. The fact that virulence factors, traditionally viewed as fungal attributes, are contingent upon microbial adaptation to various environmental stresses encountered in the human host, implicates that the host and fungus are jointly responsible for pathogenicity. It is therefore very exciting that, in addition to the pathogen-derived ligands of pattern recognition receptors (PAMPs), primary metabolites associated with fungal growth under hypoxic conditions in the lung were found to contribute to fungal pathogenesis (1). Moreover, several damage-associated molecular patterns (DAMPs) have been shown to spatiotemporally regulate PAMP-induced, inflammation in experimental fungal pneumonia (2) and in humans where a genetically-determined hyperfunction of the DAMP signaling was associated with probable aspergillosis in HSCT patients (3). How these emerging themes in fungal pathogenesis better accommodate the susceptibility to fungal diseases in primary immunodeficiencies, such as Chronic Granulomatous Disease and Cystic Fibrosis, in the face of high-level inflammation, will be discussed. Thus, we are entering an exciting era where the control of inflammation leading to tolerance, the molecular bases of its regulation and rupture, and the way the host and the fungus on both sides contribute to fungal pathogenesis may pave the way to targeted anti-inflammatory strategies in difficult-to-treat patients (4).

References


Supported by the Specific Targeted Research Project ‘ALLFUN’ (FP7–HEALTH–2009 Contract number 260338) and the Italian Grant Application 2010 Fondazione per la Ricerca sulla Fibrosi Cistica (Research Project FFC#21/2010)
ANGIOGENESIS AT THE MOLD-HOST INTERFACE: A POTENTIAL KEY TO UNDERSTANDING AND TREATING INVASIVE ASPERGILLOSIS

Ronen Ben-Ami, MD

Tel Aviv Sourasky Medical Center - Israel

THURSDAY 26 JANUARY 2012 (09.15 - 09.40)

Angioinvasion and tissue necrosis are hallmarks of invasive aspergillosis in the neutropenic host. *Aspergillus* survival and virulence in this setting depend on maintenance of a hypoxic milieu in *Aspergillus*-infected tissue. Furthermore, tissue hypoxia represents a barrier to the antifungal actions of effector cells of the innate immune system and antifungal drugs. However, both tissue hypoxia and secretion of proinflammatory cytokines are powerful inducers of a compensatory angiogenic response, which can tip the balance in favor of the host by facilitating the influx of effector cells and antifungal drugs into the infected tissue. Recent work has shown that *Aspergillus fumigatus* inhibits angiogenesis during invasive growth, and this action is dependent upon secondary metabolism. More specifically, gliotoxin appears to be a key mediator of the anti-angiogenic effects of *A. fumigatus*. Modulation of angiogenesis is showing promise as an adjunct treatment to antifungal drugs in preclinical studies, and could represent a novel direction for future therapeutics.
Aspergillus fumigatus Survival in the Lung Environment

Elaine Bignell, PhD
Imperial College London - United Kingdom

Thursday 26 January 2012 (09.40 - 10.05)

Survival of Aspergillus fumigatus in the mammalian host is dependent upon the coordinated activities of multiple sensing and response systems. Many of these systems are fungus-specific, and possibly offer novel options for targeted antifungal therapies, but are not essential for viability in standard laboratory culture and have therefore been overlooked during screens for essential functions. Certain of these conditionally-essential sensing and response systems are required for tolerance to existing antifungal agents, and might offer novel options for targeted combination therapies.

Relative to the natural environment, the mammalian host represents an alkaline environment. In order to tolerate alkaline stress A. fumigatus utilises the conserved fungal transcription factor, PacC/Rim101. We found that A. fumigatus ∆pacC mutants are unable to grow at high pH and are attenuated for virulence in immunocompromised mice. Mutants lacking an upstream putative pH sensor, PalH, exhibited similar behaviour, both in vitro and in vivo, suggesting activity in the same signalling pathway. Examining the transcription profile of a ∆pacC mutant, relative to wild type, we identified cell wall biosynthesis as being significantly dysregulated during initiation of invasive infection. Concordant with this observation ∆pacC and ∆palH mutants are sensitive to cell wall-perturbing agents, including cell wall-active antifungal drugs.

To examine the molecular basis of pH sensing we exploited a yeast two hybrid screen to identify palH interactors in living fungal cells. This identified the fungal arrestin, PalF, as a direct interactor of PalH. We also discovered a novel self-interaction of PalH which suggests that dimerization of the 7TMD pH sensor is required during pH sensing. Exploiting extant epitope-tagged PalH alleles, the physiological relevance of this protein interaction was interrogated in A. nidulans demonstrating existence of PalH dimers, and mutational analyses were performed to find amino acid residues promoting dimerization. We hypothesise that PalH is a dimerising GPCR-like pH sensor, which is required for virulence and echinocandin tolerance suggesting utility as a combination therapy target. Future work will focus upon screening for inhibitory peptides, as well as applying this exploratory pipeline to other plasma membrane-based sensors.

One means of identifying functions required during growth in the host is to monitor fungal gene expression in mammalian models of infection. In recent years we have assayed global expression of A. fumigatus genes using a microarray approach [1]. The acquisition and comparative analysis of datasets obtained from various stages of murine infection and in vitro growth provides a powerful tool with which to dissect host-adaptive functions, and identifies novel virulence factors, including fungal gene clusters, required for virulence. We are currently curating all extant A. fumigatus transcriptomic data, and performing quantile normalisation, to provide a robust means of cross comparison between data originating from diverse sources. This will be made available to the research community as a new tool for A. fumigatus research.

Reference

Mammalian Toll-like receptors (TLRs) detect microbial products and initiate immune responses to infection. The different members of the TLR family recognize and signal to a broad range of microbial ligands, such as bacterial and fungal cell wall components, bacterial lipoproteins and bacterial and viral nucleic acids. The nucleic-acid sensing TLRs, TLR3, 7, 8 and 9, are localized to intracellular compartments whereas TLR1, 2, 4, 5 and 6 are expressed at the plasma membrane. TLR activation and signal transduction are regulated by subcellular compartmentalization of receptors and downstream signaling components and the intracellular localization of nucleic acid sensing TLRs appears to facilitate self-vs. non-self-discrimination. Evidence for the importance of innate immune mechanisms in fungal defense is mounting. The fungal β-glucan receptor Dectin-1 is essential in pulmonary defense against *A. fumigatus* and collaborates with TLR2 and TLR4 in providing critical immune signals. While most studies have focused on the importance of TLR2 and TLR4 in defense against *A. fumigatus*, increased susceptibility to ABPA is associated with a polymorphism in the TLR9 gene and intranasal CpG had a therapeutic effect during established murine fungal asthma. However, the cell biological processes underlying Toll-like receptor 9 (TLR9)-mediated *A. fumigatus* immune responses are still largely unresolved. TLR9 recognizes unmethylated CpG DNA and induces innate immune responses. TLR9 activation is a multistep process requiring proteolytic cleavage and trafficking to endolysosomal compartments for ligand-induced signaling. However, the rules that govern the dynamic subcellular trafficking for TLR9 after pathogen uptake have not been established. We demonstrate that uptake of *A. fumigatus* conidia induced drastic spatial redistribution of TLR9 to the membrane of *A. fumigatus*-containing phagosomes but not to bead-containing phagosomes in murine macrophages. Specific TLR9 recruitment to the fungal phagosome was consistent using *A. fumigatus* spores at different germination stages and selected mutants affecting the display of antigens on the fungal cell surface. Spatiotemporal regulation of TLR9 compartmentalization to the *A. fumigatus* phagosome was independent of TLR2, TLR4 and downstream TLR signaling. Our data demonstrate that the TLR9 N-terminal proteolytic cleavage domain was critical for successful intracellular trafficking and accumulation of TLR9 in CpG-containing compartments and *A. fumigatus*-phagosomal membranes. Our study provides evidence for a model in which *A. fumigatus* spore phagocytosis by macrophages specifically induces TLR9 recruitment to *A. fumigatus* phagosomes and may thereby mediate TLR9-induced antifungal innate immune responses.
VITAMIN D AND OX40L INTERACTION

Jay K. Kolls, MD

Rangos Research Centre - USA

THURSDAY 26 JANUARY 2012 (10.30 - 10.55)
COPD AND ASPERGILLOSIS

Jesús V. Guinea Ortega, PharmD, PhD

Hospital General Universitario Gregorio Marañón - Spain

THURSDAY 26 JANUARY 2012 (11.35 - 12.00)

Invasive pulmonary aspergillosis has traditionally affected patients with hematological cancer or patients receiving bone marrow or solid organ transplants. Antifungal prophylaxis and early initiation of preemptive therapy have helped decrease the incidence of invasive aspergillosis in these groups. Recent data indicate that the incidence of invasive aspergillosis in non-traditional hosts is increasing, and patients with chronic obstructive pulmonary disease (COPD) are one of the populations that have received most attention in the last few years [1]. Major changes in the lung architecture of patients with COPD may predispose to colonization by *Aspergillus*. Frequent administration of corticosteroids during exacerbations is thought to be responsible for the development of invasive aspergillosis. Exposure to high environmental loads of *Aspergillus* conidia can also play a role in the development of invasive aspergillosis. In COPD patients, the incidence of invasive aspergillosis has been estimated at 3 cases/1,000 admissions. Invasive aspergillosis in patients with COPD is characterized by non-specific respiratory signs and symptoms and presents as antibiotic-resistant pneumonia. The tools available for the diagnosis of invasive aspergillosis show several limitations when studied in patients with COPD. Detection of serum galactomannan has a sensitivity of 50% [2]. Characteristic radiologic findings in neutropenic patients, such as the halo sign, are frequently lacking. Other surrogate markers, such as β-1,3-d-glucan, have been poorly studied in this population, and their role for the diagnosis of invasive aspergillosis is still unknown. Procedures based on detection of *Aspergillus* DNA by PCR are encouraging but require more experience. The aforementioned limitations of the diagnostic procedures may explain the frequent and undesirable admission to the intensive care unit of patients with COPD and invasive aspergillosis, the >90% mortality, and the significantly higher hospital costs [3]. Suitable antifungal treatment of invasive aspergillosis in patients with COPD has not yet been defined, because very few patients with this disease have been included in clinical trials. Finally, the increasing number of azole-resistant *A. fumigatus* isolates is making patient management increasingly difficult [4]. My presentation reviews and updates current knowledge on the epidemiology of invasive aspergillosis in patients with COPD.

References

Aspergillus fumigatus causes significant morbidity in patients with cystic fibrosis (CF). It leads to a wide variety of diseases including life threatening invasive aspergillosis, chronic pulmonary aspergillosis, simple aspergilloma and allergic hypersensitivity. Hypersensitivity is the most common manifestation in CF and forms a spectrum from simple serological sensitisation (60%) to allergic bronchopulmonary aspergillosis (ABPA) (15%). It appears that patients with CF have an abnormal or heightened immunological response to A. fumigatus. It is unknown whether this relates to abnormalities in mucosal regulation and defence, genetic susceptibility, related treatments such as those for recurrent bacterial infections or expression of A. fumigatus virulence factors within CF lungs.

The diagnosis of ABPA in CF is notoriously difficult due to overlapping and concomitant bacterial infections. In 2003, defined criteria for diagnosis and monitoring of ABPA were introduced based on immunological tests, clinical parameters and radiology. Since this time a variety of serological tests have been evaluated for their role as superior diagnostic markers of ABPA including: thymus- and activation-regulated chemokine (TARC), IgG subclasses and cellular antigen stimulation test (CAST). Despite this, few serological markers have been identified that can reliably monitor treatment response.

Positive sputum culture has not been linked to the development of ABPA or sensitisation. However, the reported prevalence of A. fumigatus in CF sputum samples varies widely between studies, from 12-57%, indicating a need to improve and standardise methods of detection. Patients that do not meet criteria for ABPA are not treated and standards for monitoring are undefined. However, recent studies have shown that persistent A. fumigatus colonisation and simple immunological sensitisation are independent risk factors for a greater decline in lung function. Additionally, a separate clinical entity has recently been proposed - ‘aspergillus bronchitis’ - but diagnostic criteria and optimal treatment regimes are unknown. No serological markers have been found that can distinguish patients with simple colonisation from those sensitised or with ‘aspergillus bronchitis’.

Methods to categorize patients that do not meet criteria for ABPA are needed in order to evaluate whether these groups may also benefit from antifungal treatment. Furthermore, methods to allow monitoring of treatment response in these patients, and in those with ABPA, are also needed. Two new methods, sputum real time PCR and sputum galactomannan, have been studied in a cohort of 150 adult CF patients alongside traditional markers of infection and sensitisation. Real time PCR has been found to more accurately identify CF patients with Aspergillus in their sputum than standard culture. Both real time PCR and GM demonstrate good repeatability in CF sputum and may be useful to monitor treatment response to antifungals. Latent class analysis has demonstrated the strong statistical identification of 4 patient classes that can clinically be seen to represent those with ABPA, sensitisation, aspergillus bronchitis and normal. Using these methods to identify and monitor patients with Aspergillus disease in CF, a clinical trial of antifungal therapy is now required to determine treatment benefit.
Invasive pulmonary aspergillosis (IPA) generally occurs in hosts with impaired immune reaction. EORTC/MSG categorized IPA in proven, probable, and possible invasive fungal disease. A proven diagnosis requires histopathologic evidence of fungal invasion. A probable IPA requires the presence of host factors (neutropenia or treatment with immunosuppressive agents), radiologic features (dense, well circumscribed lesions, with or without a halo-sign, air crescent sign, or cavity) and positive mycology (cytology, direct microscopy, or culture) on any respiratory tract aspirate, or galactomannan antigen detection on Broncho-Alveolar lavage (BAL) fluid or serum. A diagnosis of possible IPA is made in the presence of host factors and clinical features, but in the absence of mycological criteria.

In mechanically ventilated intensive care unit (ICU) patients diagnosing IPA according to this strict classification is problematic because a) open lung biopsy might be contra-indicated because of coagulation disorders, b) IPA may develop in ICU patients without host factors, c) radiologic findings usually are non-specific and d) galactomannan antigen detection on serum is of little value in non-neutropenic patients as circulating neutrophils are capable of clearing the antigen.

The lack of specific criteria for diagnosing IPA in critically ill patients hampers timely initiation of appropriate antifungal therapy and may, as such, compromise the odds of survival. One of the black boxes in the diagnostic complex is the presence of *Aspergillus* species in endotracheal aspirate cultures, observed in up to 2% of mechanically ventilated ICU patients. According to EORTC/MSG a positive endotracheal aspirate culture could represent probable IPA in the presence of compatible signs, abnormal thoracic medical imaging and either host factors or BAL fluid positive for *Aspergillus* on direct microscopy and culture.

Efficacy has been shown for first-line therapy of invasive aspergillosis with voriconazole and liposomal amphotericin B. Posaconazole is recommended for prophylaxis in patients treated for acute myelogenous leukemia, myelodysplastic syndrome or patients with graft versus host disease after allogeneic transplantation. However gastrointestinal resorption for the azoles differ considerably in critically ill patients because of the frequently coexisted sepsis/septic shock.

References

IMMUNE REGULATION IN IDIOPATHIC BRONCHIECTASIS

Rosemary Boyton, MD, PhD

*Imperial College London - United Kingdom*

**THURSDAY 26 JANUARY 2012 (12.50 - 13.15)**

Bronchiectasis is a chronic, progressive lung disease with lung remodeling, mucus production, chronic airway inflammation, airflow obstruction and increased susceptibility to chronic bacterial infection. Allergic bronchopulmonary aspergillosis (ABPA) is an important cause of bronchiectasis and *aspergillus* related lung disease sometimes complicates established bronchiectasis. Bronchiectasis is a clinical diagnosis confirmed by high-resolution computed tomography (HRCT) of the thorax. The lung damage results from a vicious cycle of bacterial infection and chronic airway inflammation. There are two stages to the disease: an initial insult followed by a chronic inflammatory process with recurrent bacterial infection and progressive lung damage. Poor regulation of innate and adaptive immunity may predispose to bronchiectasis at both stages. Immunogenetic evidence suggests a link between the level of natural killer (NK) cell activation and disease susceptibility, implicating a predisposing role for innate immune mechanisms. Adaptive immune mechanisms are implicated by the genetic association of HLA-DR1, DQ5 with increased susceptibility to idiopathic bronchiectasis, compatible with an immune response gene effect on bacterial immunity and/or inflammatory damage. We have generated a transgenic model of the human disease; this model encompasses progressive lung remodeling, increased mucus production and chronic airway inflammation associated with increased resistance and reduced compliance in lung function studies and increased susceptibility to bacterial infection.
TOP SIX PAPERS IN ASPERGILLOSIS IN 2011

Murat Akova, MD  
Hacettepe University School of Medicine - Ankara, Turkey

Nir Osherov, PhD  
Sackler School of Medicine - Tel Aviv, Israel

THURSDAY 26 JANUARY 2012 (13.45 - 14.45)

Over 1,100 peer reviewed papers on aspergillosis were published in 2011. From these, we will attempt the impossible task of identifying and describing the top 6 papers.

Three papers selected by Dr Osherov will describe exciting recent advances in our understanding of Aspergillus fumigatus pathogenesis and the host response at the molecular level.

An additional three papers chosen by Dr Akova will outline novel developments in the diagnosis and treatment of invasive aspergillosis.
INNATE IMMUNITY TO *ASPERGILLUS*

Mihai G. Netea, PhD

*Radboud University Nijmegen Medical Center - The Netherlands*

**THURSDAY 26 JANUARY 2012 (14.45 - 15.10)**

The innate immune response was once considered to be a limited set of responses aimed to contain an infection by primitive ‘ingest and kill’ mechanisms, giving the host time to mount a specific humoral and cellular immune response. In the mid-1990s, however, the discovery of Toll-like receptors heralded a revolution in our understanding of how microorganisms are recognized by the innate immune system, and how this system is activated. Several major classes of pathogen-recognition receptors have now been described, each with specific abilities to recognize conserved bacterial structures. The challenge ahead is to understand the level of complexity that underlies the response that is triggered by pathogen recognition. A model of the recognition pathways through which the fungal pathogen *Aspergillus fumigatus* is recognized will be presented, as well as the initiation processes leading to the activation (and modulation) of the innate immune system.
NEUTROPHIL EXTRACELLULAR TRAPS AND A. FUMIGATUS

Matthias Gunzer, PhD

University Duisberg/Essen - Germany

THURSDAY 26 JANUARY 2012 (15.10 - 15.35)

Aspergillus fumigatus is a human pathogenic fungus that can cause life threatening pulmonary infections in individuals with a compromised immune system. While cells of the adaptive immune system can principally protect from an infection after successful priming via subclinical infections or vaccination, the immediate protection is offered exclusively by cells of the innate immune system, especially neutrophil granulocytes. We have investigated the physical interactions of neutrophils with diverse morphotypes of Aspergillus by life cell imaging in vitro and also in intact organs using 2-photon microscopy. Using this approach we have shown that the phagocytosis process is a highly dynamic cellular mechanism that is strongly dependent on the environmental cues. Despite phagocytosis neutrophils are also able to release nuclear DNA in an explosive process called NET-formation. We were able to demonstrate this not only in vitro but for the first time also in infected animal lungs and could prove that the de novo recruitment of neutrophils is essential for this process. Current projects in the lab aim at identifying the precise dynamics of neutrophil recruitment to infected organs as well as the release of NETs using novel reporter animals and advanced imaging. Together, these investigations have firmly established, that NET-formation by newly recruited neutrophils is a key aspect of immune responses against Aspergillus.
CONTROL OF NEUTROPHIL ROS RESPONSES TO A. FUMIGATUS

Keith Boyle, PhD

Babraham Institute - United Kingdom

THURSDAY 26 JANUARY 2012 (15.35 - 15.45)

Upon infection of the respiratory system with the fungus Aspergillus fumigatus various leukocytes, in particular neutrophils, are recruited to the lung to mount an immune response. Neutrophils respond by both phagocytosing conidia and mediating extracellular killing of germinated hyphae. Of paramount importance to an appropriate immune response is the neutrophil NADPH oxidase enzyme, which mediates the production of various reactive oxygen species (ROS). This is evidenced by the acute sensitivity of both oxidase-deficient humans and mice to invasive Aspergillosis (1). However, the signaling mechanisms regulating the generation of ROS in response to hyphae are poorly understood. Phosphoinositide 3-kinases (PI3Ks) are important regulators of numerous cellular processes, with much recent work describing unique roles for the four different Class I PI3K isoforms (Class IA α, β and δ isoforms and the Class IB γ isoform) (2). Class I PI3Ks are conventionally activated at the plasma membrane in response to either receptor tyrosine-kinase (Class IA) or G-protein coupled receptor (Class IB) activation. Here we show by live-cell imaging that murine neutrophils spread along the surface of hyphae and that the lipid products of Class I PI3Ks accumulate at the hyphal-bound neutrophil plasma membrane, as shown by use of a GFP-based reporter protein. By a combination of both pharmacological and genetic approaches we demonstrate essential, but overlapping, roles of PI3Kβ and PI3Kδ in both the ROS and spreading responses of neutrophils to hyphae (3). Dectin-1 is a cell surface receptor, deletion of which renders mice susceptible to A. fumigatus infection (4), and whose ligand, β-1,3-glucan, is present on hyphae. However, we found that neutrophils from Dectin-1 deficient mice had only a minor defect in hyphal-induced ROS production. On the other hand, deletion of β2-integrins, receptors with a variety of functions and known to recognize certain fungal-based ligands (5), elicited a substantial reduction in the ROS response. Nevertheless, addition of soluble algal glucans together with the abrogation of β2-integrin function was required to significantly inhibit activation of the PI3K-effector PKB, indicating that other unidentified receptors are utilised for PI3K, but not necessarily NADPH oxidase, activation. Genetic deletion of the non-receptor tyrosine kinase Syk completely abrogated the ROS response whilst, intriguingly, neither pharmacological inhibition of Syk nor deletion of both its conventional adaptor proteins FcRγ and DAP12 had any effect on ROS production pointing to an unconventional mode of Syk action. These results start to define the signaling network controlling neutrophil ROS responses to A. fumigatus hyphae.

References

SPECIFICATION OF POLARITY SITES DURING GROWTH AND DEVELOPMENT OF *ASPERGILLUS NIDULANS*

Steven D. Harris, PhD

*University of Nebraska - Lincoln, USA*

**THURSDAY 26 JANUARY 2012 (16.25 - 16.50)**

Because of its genetic tractability and amenability to post-genome analysis, *A. nidulans* is one of the best-established model filamentous fungi. A primary focus of research using *A. nidulans* has been to understand the mechanisms that direct cellular morphogenesis during hyphal growth and reproductive development. Although *A. nidulans* is capable of generating a variety of cell shapes, these variations likely reflect differences in the timing and location of polarized growth (Harris, 2010). Thus, elucidating the regulatory networks that determine where and when the morphogenetic machinery (i.e., the components of the cytoskeleton and vesicle trafficking complexes that enable polarized growth) is deployed should provide fundamental insight into cellular morphogenesis. Towards this end, we have focused our efforts on the Cdc42/Rac1 GTPase module, as well as its potential regulators and effectors. Our results implicate these GTPases in the control of polarity establishment during spore germination, and also show that Cdc42 regulates hyphal branching (Virag et al., 2007). More recently, our characterization of the effector PakB has revealed important roles for the Cdc42/Rac1 module in the control of conidiophore architecture. These roles include the control of vesicle formation, as well as the spatial regulation of “bud formation” from the vesicle and the transition to unicellular growth. Finally, we have initiated large-scale double screens that will permit the construction of genetic interaction networks focused on the Cdc42/Rac1 module. Ultimately, we envision that these networks will provide a cellular “roadmap” that can be exploited for the manipulation of morphogenetic mechanisms.

**References**

ASPERGILLUS NIGER A PERFECT HOST FOR INDUSTRIAL BIOTECHNOLOGY: SYSTEMS BIOLOGY APPROACHES FOR THE PRODUCTION OF BUILDING BLOCK CHEMICALS

Peter J. Punt, PhD

TNO Microbiology & Systems Biology/Institute for Biology Leiden University - The Netherlands

THURSDAY 26 JANUARY 2012 (16.50 - 17.15)

Among filamentous fungi Aspergillus niger is a well known production host for a wide variety of enzymes (amylase, cellulose, protease) and metabolites (organic acids). Based its performance in these more traditional fermentation processes A. niger is already used for the production of novel proteins (e.g. Punt et al., 2011). But even more recently this fungus is now also considered for the production of new so-called platform or building-block chemicals for the chemical industry. These chemicals, currently produced based on petrochemistry, are the starting point for the production of a wide variety of materials, such as resins, plastics, etc. Production of these compounds via biobased routes will be a major contribution towards a Biobased Economy.

For the production of these bulk compounds robust host organisms are required, suitable for using low cost lignocellulose-based feedstocks, resistant against adverse conditions due to inhibitory feedstock compounds and capable of coping with high product concentrations. A. niger was shown to fulfill most of these prerequisites (Rumbold et al., 2009-2010).

Based on the extended molecular genetic toolkit systems biology approaches were developed for A. niger and other fungi (e.g. Braaksma et al., 2010). These approaches may be followed to produce several of these platform chemicals in A. niger, as demonstrated by the example of itaconic acid (Li et al., 2011).

References
DIVERSITY OF *ASPERGILLUS FLAVUS* ON CROPS AND IN THE ENVIRONMENT CAN BE EXPLOITED TO REDUCE AFLATOXIN EXPOSURE AND IMPROVE ENVIRONMENTAL HEALTH

Peter J. Cotty, PhD

*University of Arizona - USA*

**THURSDAY 26 JANUARY 2012 (17.15 - 17.40)**

*Aspergillus flavus*, the primary causal agent of aflatoxin contamination of crops, is composed of many, highly diverse genetic groups called vegetative compatibility groups (VCGs). This diversity can be detected with simple sequence repeats (SSR, microsatellite markers), DNA sequence, and several physiological characters including aflatoxin-producing ability. SSR data suggests that *A. flavus* VCGs largely behave as clones in nature with certain VCGs exhibiting niche preference. *A. flavus* exists in the environment in highly complex communities composed of many VCGs varying in aflatoxin-producing ability from highly aflatoxigenic (do not produce aflatoxins) to atoxigenic. The success of individual VCGs within the environment is dictated by outcomes of competition among *A. flavus* genotypes during vegetative growth and reproduction under variable biotic (i.e. host) and abiotic (i.e. climate) conditions. The structure and average aflatoxin-producing potential of *A. flavus* populations influence incidences and severities of aflatoxin-contamination events. Human activities (i.e. farming, construction, composting, etc.) and environmental shifts cause changes to the structure of these communities. Thus by altering fungal communities, human activities change the prevalence of certain characteristics including aflatoxin-producing ability. In the United States, farmers have experienced severe losses as a result of aflatoxin contamination. For many of these farmers, a form of biocontrol in which atoxigenic isolates of *A. flavus* are used to competitively exclude aflatoxin producers has become a viable solution to aflatoxin contamination problems. Use of atoxigenic strains as biocontrol agents alters compositions of fungal communities associated with crops, reducing the prevalence of aflatoxin producers within the crop environment. Proper applications of atoxigenics typically provide over an 80% reduction in contamination with a single application of 10 kg/ha of formulated granular product. Atoxigenic strain applications can be made without increasing the overall quantity of *A. flavus* on the crop and throughout the environment and without increasing the proportion of the crop infected by *A. flavus*. Atoxigenics are naturally associated with crops, and application of biocontrol strains simply increases the frequency with which atoxigenics displace toxigenic *A. flavus* and reduce aflatoxin contamination. There are many atoxigenic VCGs of *A. flavus* and atoxigenics have been found in every target region examined to date. In many regions, there are sufficient endemic, well-adapted atoxigenic strains to permit rotation of strain mixtures between seasons and crops. Variation in the physiology of applied atoxigenic VCGs allows for divergence in adaptation to hosts, climate, and soil characteristics. These divergences influence the extent to which individual atoxigenics compete across landscapes and through crop rotations. The future of atoxigenic strain technology may depend on designing formulations that contain mixtures of atoxigenics with the greatest potential for long-term residence in the target region.
CONTROVERSIES IN MANAGEMENT: PROPHYLAXIS OR DIAGNOSTICS

Andrew Ullman, MD

Klinikum der Johannes-Gutenberg-Universitat - Germany

FRIDAY 27 JANUARY 2012 (08.00 - 09.00)

This presentation will touch on a few important issues of prophylaxis: Invasive aspergillosis remains a challenge in the care of patients with hematological malignancies. Despite low incidences rates and improved survival data, mortality rates remain a concern. Diagnostic tools still are unsatisfactory to prove or rule out disease. Various therapeutic options are available starting with prophylaxis, empiric/preemptive, through targeted treatment. Prophylaxis is expensive because of its wide use in patients at risk but most of the approved agents demonstrate cost effectiveness. Not all agents though were able to demonstrate superiority in prophylaxis. On the other hand antifungal prophylaxis hampers the diagnostic sensitivity of biomarkers challenging the issue of breakthrough infections. Resistance development is a point of concern but possibly not a real clinical issue as of yet. And another question will be addressed whether other alternative agents can be recommended in the case of azole exposure problems, intolerance, or toxicity?
ASPERGILLUS ADHESINS

Don Sheppard, MD

McGill University - Canada

FRIDAY 27 JANUARY 2012 (09.15 - 09.40)

During infection, Aspergillus fumigatus adheres to and interacts with a number of host cells including airway and alveolar epithelial cells, leukocytes and endothelial cells. Surprisingly, however, the molecular mechanisms underlying the adherence of A. fumigatus to host constituents remain largely unknown. To date only disruption of the conidial hydrophobin gene rodA has been shown to impact adherence of A. fumigatus, and only to collagen but not other host macromolecules.

To identify A. fumigatus adhesins, we screened a collection of regulatory mutants for adherence to epithelial and endothelial cells as well as the ability to form adherent biofilms. Mutants deficient in either of two developmental regulatory genes, medA and stuA, were markedly impaired in host cell adherence and did not form adherent biofilms when grown on polystyrene. Electron microscopy of these strains demonstrated abnormalities in the cell wall, suggesting that alterations in cell wall composition might mediate the impairment in adherence. Comparative transcriptome analysis of these two mutant strains identified a cluster of genes that were markedly downregulated in both strains and contained genes predicted to be involved in polygalactosamine synthesis, including uge3, encoding a putative UDP-4-epimerase. Consistent with this observation, analysis of the cell walls of the medA and stuA mutant demonstrated a near absence of galactosamine, and galactosaminogalactan (GAG), a novel glycan, was absent from culture supernatants of these mutants. Further, the addition of exogeneous GAG isolated from wild-type hyphae restored the adherence of the ΔmedA and ΔstuA mutant in a dose dependent manner. Collectively these results suggested that GAG mediates A. fumigatus adherence.

Since MedA and StuA govern the expression of many genes, we constructed a mutant deficient in uge3 alone. Cell walls of the Δuge3 null mutant contained no detectable galactosamine, and no GAG was recovered from the culture supernatants of this mutant. Consistent with the hypothesis that GAG mediates adherence, the Δuge3 mutant was completely non-adherent to epithelial and endothelial cells and was unable to form adherent biofilms. Addition of wild-type GAG, but not zymosan increased adherence of the Δuge3 mutant to epithelial cells. The Δuge3 mutant induced markedly less damage of epithelial cells in vitro, and was attenuated in virulence in a corticosteroid treated mouse model of invasive aspergillosis.

Thus, GAG functions as a major adhesin of A. fumigatus in vitro and in vivo. Future work will define the host receptors bound by this novel carbohydrate, and the biochemical pathways governing its synthesis.

References
When dormant conidia of *Aspergillus fumigatus* come in contact with a carbon source they synchronously break dormancy and begin nuclear division and morphological development. Conidia expand isotropically for a short while, after which they establish an axis of polarity and the primary germ tube emerges. This primary germ tube continues to extend by tip growth and is soon partitioned by the formation of the first septum near the basal end of the germ tube. Only the polarly extending tip cell remains mitotically active. Polar growth allows the fungus to explore new territory and, in the case of invasive aspergillosis, to invade host tissue. We took advantage of the synchronous early development of *A. fumigatus* to analyze temporal and spatial gene expression patterns.

To investigate temporal patterns of gene expression, we isolated RNA from several developmental stages during early growth and analyzed changes in gene expression using hybridization to microarrays. Surprisingly, microarray experiments did not show an increase in the transcription of polarity-related genes during the switch from isotropic to polar growth. The largest changes in the expression of polarity-related and other genes were seen during early isotropic expansion.

To investigate spatial patterns of mRNA localization, we used laser microdissection with pressure catapulting (LMPC) to harvest conidium, base and tip regions from pre-septation hyphae. RNA isolated from these hyphal samples was then deep sequenced using 454. Statistical analysis showed that many RNAs are asymmetrically localized in hyphae. Surprisingly, we could detect no correlation of spatial patterns of RNA localization with functional categories.
THE DIVERSE APPLICATIONS OF RNA-SEQ FOR FUNCTIONAL GENOMICS
STUDIES IN ASPERGILLUS FUMIGATUS

Antonis Rokas, PhD

Vanderbilt University - Nashville, USA

FRIDAY 27 JANUARY 2012 (10.05 - 10.30)

Next-generation DNA sequencing technologies (NGSTs) have revolutionized the study of genomics in non-model organisms [1, 2]. Aside from the higher throughput and lower sequencing cost, two key features of NGSTs are that they are by design aimed at sequencing samples that contain a mixture of different DNA pieces and that this sequencing simultaneously provides qualitative and quantitative information about each DNA piece analyzed. This has given rise to a variety of novel genome-wide sequencing-based functional assays that enable very accurate and precise determination of the genome architecture of any organism [3].

One of the most successful and widely implemented NGST applications is RNA-Seq, the deep sequencing of the mRNA population of a sample [e.g., Ref. 4]. RNA-Seq applications include the cataloging and quantification of transcriptome variation, precise annotation of transcriptome structure, and transcriptome profiling. We recently used RNA-Seq to study the transcriptome of *Aspergillus fumigatus* ATCC 46645, a very common and deadly human fungal pathogen, in two different growth conditions, biofilm growth and liquid planktonic growth [5]. We have used this data to highlight and demonstrate the potential of RNA-Seq for functional genomics studies in *A. fumigatus* and other fungal human pathogens. Specifically, we will report the results of using RNA-Seq to characterize transcriptome variation between strains, identify previously unannotated and novel genes, identify and quantify the impact of alternative splicing on the transcriptome, as well as compare the transcriptome-wide expression profile during in vitro biofilm and planktonic growth conditions.

References

Fungal pathogens remain a major cause of morbidity and mortality. The introduction of a number of new drugs since the late 1990’s has improved matters for several disease subgroups however in most cases mortality rates remain unacceptably high. Given their obvious importance it is perhaps surprising that only four classes of drugs are available to treat systemic fungal infections, polyenes (namely amphotericin B), flucytosine, triazoles and the echinocandins. Resistance to flucytosine and the triazoles, particularly itraconazole, is common while amphotericin B has significant toxicity issues and the echinocandins are limited to intravenous use.

We are exploring a potential solution to the scarcity of known antifungal agents through the validation of completely novel drug targets. We are in the process of develop a drug mechanism-of action screen, we term CG-FABS (chemical genetic-fitness analysis by sequencing), in the model pathogenic fungus *Aspergillus fumigatus* to allow the identification of ‘druggable’ targets. A similar process has been successfully used to reveal the mechanism of action of a number of promising compounds in *Candida albicans* and *Saccharomyces cerevisiae* but the technology to recreate this system in filamentous fungi has proved difficult. The creation of a comprehensive heterozygous knockout collection in *A. fumigatus* has so far been hampered by a slow and laborious transformation protocol, inefficient gene knockout methodologies and the lack of suitable diploid *A. fumigatus* strains. Here we present an optimization of the common transformation protocol for *A. fumigatus* which permits high-throughput gene disruption. In addition we have been able to demonstrate that chemical genetic haploinsufficiency studies are possible in filamentous fungi. This offers new possibilities for antifungal research, enables high-throughput methods for surveying the genome of *A. fumigatus* for new drug targets and supports unveiling the mechanisms of action of antifungal drugs.
TARGETS FOR BROAD SPECTRUM ANTIFUNGAL VACCINES? QUANTITATIVE PROTEOMIC COMPARISONS OF ASPERGILLUS & CO

Markus Kalkum, PhD

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FRIDAY 27 JANUARY 2012 (10.55 - 11.20)

Several protein antigens from distinct fungal pathogens show cross reactive immunogenicity, suggesting that structural similarities exists, which may be exploited for the generation of broad-spectrum antifungal vaccines. For example, a whole cell *Saccharomyces cerevisiae* vaccine has shown protection against *Aspergillus fumigatus* and *Coccidioides* (1). We have quantitatively analyzed and compared the hyphal proteome of *C. posadasii* with that of *A. fumigatus* and other fungi. Our proteomic analysis focused on intracellular proteins as well as cell wall-associated and secreted proteins. Protein samples were reduced, alkylated and digested with trypsin. A novel mass spectrometric method, named MSE (2), was carried out in combination with two-dimensional Ultra Performance Liquid Chromatography to analyze the complex mixtures of digest peptides. Rabbit glycogen phosphorylase peptides were added for label-free MSE protein quantification (2). The data was analyzed with Protein Lynx Global Server and Scaffold software for protein identification and quantification. In total, 514 *A. fumigatus* proteins were identified and quantified. In hyphal extracts alone, 331 proteins from *C. posadasii* and 331 proteins from *A. fumigatus* could be identified and simultaneously quantified. Seven of the 20 most abundant hyphal proteins of each species had more than 50% inter-species sequence identity. E.g., the known vaccine candidates Pmp1 from *C. posadasii* and Asp f3 (3, 4) from *A. fumigatus* were among the five most abundant proteins, respectively, and share 69% identical sequence. Other highly homologous proteins were GAPDH, enolase, EF-1α, HSP70, EF-2, and SSC1. *A. fumigatus* cell wall proteins had high degrees of sequence homologies with those of several other fungi. Crf-1 was detected as the third most abundant cell wall protein in *A. fumigatus*. Parts of its sequence were highly homologous to that of *Candida albicans*, *Cryptococcus*, *Saccharomyces*, *Acremonium*, *Mucor*, *Rhizopus*, *Penicillium*, *Fusarium*, and *Coccidioides* species; and a cross reactive T cell epitope of Crf-1 is reportedly involved in vaccine-induced cross-protection against both *A. fumigatus* and *C. albicans* infections (5). Our study has revealed a number of promising protein targets for the development of broad-spectrum antifungal vaccines.

References

Chronic pulmonary aspergillosis (CPA) is a relatively rare, slowly progressive pulmonary syndrome due to Aspergillus spp. which requires specific knowledge in terms of disease entity, diagnosis, management and azole-resistance. One of the major unsolved issues regarding this disease is the scarcity of clinical evidence for management. It is difficult to establish a simple disease entity for this disease due to the complex backgrounds of CPA patients, such as existence of chronic pulmonary underlying diseases (e.g., tuberculosis sequelae, bronchiectasis, and chronic obstructive pulmonary disease) with mild immunosuppression (e.g., low-dose steroid administration, diabetes, collagen diseases, or alcohol) as well as co-infection with other microorganisms. Thus, it is also difficult to conduct large-scale randomized clinical trials for CPA cases.

The latest IDSA guidelines for the treatment of CPA recommend oral VRCZ or ITCZ for CNPA and CCPA as primary treatment. Surgical resection is recommended for simple aspergilloma cases. Since only few case series reports are currently available, the evidence for these recommendations is insufficient. Additionally, the following clinical factors have not been standardized or even described in IDSA guidelines: timing of the initiation of treatment, duration of treatment and timing of discontinuation of treatment. From the early 1990s to date, the reported efficacy of oral ITCZ is somewhat variable, with a range of 30-82.1%, a duration of administration of approximately 4-12 months and adverse effects being seen in 16-33%. In the last decade, the reported efficacy of oral VRCZ in terms of response rate ranged from 53-65% with several months’ administration, and the frequency of adverse effects which resulting in discontinuation of therapy ranged from 9-27%. Posaconazole is a new triazole drug with a wide spectrum of activity including zygomycoses and its data for CPA is extremely limited.

On the other hand, azole-resistant A. fumigatus is strikingly increasing and becoming one of major clinical concerns in the treatment of Aspergillus infection. We recently found the existence of azole-resistant A. fumigatus at certain level in Japan (data will be presented at poster session). Since relatively prolonged administration of azoles is the mainstay of CPA treatment, drug-resistant to azoles may be a great potential threat for the management of CPA. Furthermore, prolonged oral azole administration itself might produce furtherazole resistant of A. fumigatus in CPA patients. We actually experienced a case that A. fumigatus probably acquired azole resistance by long term administration of ITCZ (the case will be presented at poster session).

It is the time that we should consider alternative ways of management of CPA due to these issues. One of options is using intravenous candins and I will present the result of the first large scale prospective study comparing intravenous micafungin (MCFG) and intravenous voriconazole (VRCZ) in CPA. However, the utility of intravenous antifungal drugs has not been well evaluated and still controversial, as they are expensive and need hospital admission of patients. I will overview the general management of CPA and focus on current problems in this session.
CURRENT UNDERSTANDING OF ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA) IS BUILT ON THE HYPOTHESIS THAT IT IS BASICALLY A TH2-DRIVEN PROCESS, OPENING UP THE POSSIBILITY OF TARGETED BIOLOGICAL THERAPIES ALONG THE TH2 DISEASE PATHWAY. ABPA CAN BE VIEWED, IN THIS REGARD, AS AN EXTREME, FUNGAL-INDUCED MANIFESTATION OF THE TH2 ASTHMA ENDOTYPE [1,2].

The first identified target molecule amenable to clinical intervention with a biological was IgE. The production of polyclonal (total) and allergen-specific IgE are both essential features of ABPA. Double-blind, placebo-controlled, randomized trials in allergic asthma have clearly established the clinical efficacy and safety of omalizumab, a recombinant humanized IgG1 monoclonal antibody (Mab) that binds to circulating but not cell-bound IgE, targeting the same epitope on the IgE Fc region that initiates inflammatory signalling upon binding to FcεR1 [3]. Omalizumab is approved in many countries for treatment of severe allergic asthma. Published omalizumab use in ABPA has to date been limited to uncontrolled case reports and series, but with strongly positive clinical benefits reported [4-5]. Recently other Th2 down-regulating biologicals with promise in various animal models have entered clinical trials in asthma. With crucial proper selection for the Th2-high asthma endotype, trials of Mabs directed against the Th2 cytokine mediators IL-5 and IL-13 have begun showing clinical benefit, while Mabs directed against TNF-α, IL-4, IL-4Ra and several other targets have so far been ineffective or equivocal [6-8]. Finally, therapeutic small molecule-directed immune deviation is also plausible with biologicals, as demonstrated by reduced Th2 responses to Aspergillus in cystic fibrosis-ABPA upon vitamin D repletion [9].

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PEDIATRIC ASPERGILLOSIS

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FRIDAY 27 JANUARY 2012 (12.40 - 13.05)

Invasive aspergillosis is an important cause of infectious morbidity and mortality in children with innate or acquired deficiencies in phagocytic host defenses and anatomical barriers. Similar to adults, it most commonly affects the lung, and remains difficult to diagnose. Prognosis depends on early recognition, prompt institution of appropriate treatment and restoration of host defenses. Pediatric patients represent a distinct population in regard to clinical presentation and epidemiology, and in particular, the utility of diagnostic tools such as the serum galactomannan ELISA and high resolution CT scan imaging. The disposition of antifungal agents with clinical efficacy against invasive aspergillosis is different, resulting in different dosages across age groups and differential therapeutic algorithms due to the lack of either published clinical experience or a pediatric dosage as in the setting of antifungal prophylaxis. This presentation reviews the epidemiology of invasive aspergillosis in pediatric patients, discusses the value of currents diagnostics and outlines the options for treatment and prevention with a focus on unmet needs for further research.
APPLICATION OF DIAGNOSTIC MARKERS FOR IA IN CHILDREN

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SATURDAY 28 JANUARY 2012 (09.15 - 09.40)

Serological and radiological markers have been evaluated and applied in adults at risk for IA. These markers have been less predictive in children. Galactomannan (GM) is released by *Aspergillus* spp. Based on studies in adults, GM positivity in serum, BAL and CSF are included as a mycological criterion in the revised EORTC/MSG definitions. Retrospective analysis in 59 immunocompromised children (9 with proven/probable IPA) suggests that BAL GM is a valuable adjunctive diagnostic tool. GM testing in the CNS is supported by small retrospective case reports and case series. While there are some studies evaluating serum GM in hematological children (7 prospective), no formal recommendation for GM testing has been made for pediatric populations. Prospective monitoring every 3-4 days in children at high risk for IFD is reasonable for early diagnosis of invasive aspergillosis (serum GM threshold 0.5). Limited published data support the value of GM in the diagnosis of pulmonary aspergillosis (BAL GM 1) and CNS aspergillosis (CSF GM 0.5) in children. Mould-active prophylaxis may decrease the performance of the test.

β-D-Glucan (BG) can be detected in infections due to many fungi as well as bacteria. In healthy individuals BG is absent as well as in cryptococcosis and zygomycosis. Antibiotics may cause positive BG levels. Similar to GM, BG is included as mycological criterion in the revised EORTC/MSG definitions. Although BG testing has been shown to be useful in diagnosing IFD in adults, data are too limited to make any recommendations in children.

Limited data on imaging studies in children with underlying malignancies and persistent febrile neutropenia exist. In contrast to adult patients, typical signs of IFD (halo sign, air crescent sign, and cavities) are not seen in the majority of children. Radiographic findings are often unspecific, in particular in the younger age group (<5 years): multiple nodules or fluffy masses and infiltrate-like mass lesions were the two basic types of involvement. In high-risk children with persistent febrile neutropenia that persists beyond 96h or with focal clinical findings, imaging studies (lung CT-scan or adequate imaging of the symptomatic region) should be performed. In chest X-ray and/or CT scan, typical signs of invasive pulmonary fungal disease are often missing, in particular in the younger age group. In contrast, even atypical pulmonary infiltrates (e.g., fluffy masses) may support the diagnosis of invasive pulmonary fungal disease in patients at high risk. Further diagnostic work-up (BAL, biopsy) should be considered.

References

IMPACT OF PROPHYLAXIS ON GALACTOMANNAN, BETA-D-GLUCAN AND PCR

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SATURDAY 28 JANUARY 2012 (09.40 - 10.05)

It has become generally accepted that antifungal prophylaxis will have a marked effect on tests for galactomannan, beta-d-glucan and Aspergillus DNA. This is partly the result of intuition and partly drawn from the literature. For instance, exposure to itraconazole given for therapy resulted in a lower sensitivity of the ELISA test for galactomannan raising the question about whether or not antifungal prophylaxis would do the same. In a recent systematic review of galactomannan testing, Leeflang et al concluded that antifungal prophylaxis was associated with a significantly lower specificity but a higher than average sensitivity and that antifungal therapy increased both sensitivity and specificity. Whilst most studies reported using a formulation of amphotericin B for therapy none provided details of the agent used for prophylaxis. Most likely the agent was an azole perhaps fluconazole in some cases and a mould-active azole in other cases. Never the less it seems clear that exposure to antifungals affects performance of the galactomannan test. There has been no systematic review of the effect of these agents on the beta-d-glucan test or on PCR to detect Aspergillus. Whether or not used for treatment or prophylaxis Yet all the azoles appear equally active in vitro against Aspergillus species with MICs within an acceptable range. The biology of Aspergillus suggests that galactomannan and beta-D-glucan are released from the hyphae during growth and, that that DNA is also released. This has been shown in animal models of infection in which the levels of DNA, beta-D-glucan and galactomannan can be used to estimate the burden of disease in lung tissue. This is exactly what has been shown to occur in vitro. Exposing animals to antifungals after infection leads to a decline in all these fungal products. This is also true in patients so much so that it has been advocated that a steady decline in galactomannan levels corresponds to a satisfactory response to therapy. However not all antifungal drugs appear to have the same impact on these tests nor would one expect them too given their modes of action. Exposing mice infected with Aspergillus fumigatus to posaconazole or caspofungin has been shown reduce the sensitivity of a PCR test but not that of galactomannan. By contrast amphotericin B had no effect on either fungal product.

However, the question is would these findings translate to the setting of antifungal prophylaxis given to prevent either a patient from becoming infected with Aspergillus spores or infection from advancing to disease? Clinical studies suggest that prophylaxis with posaconazole may reduce the sensitivity of the galactomannan test since there were fewer cases of probable IA among those given the azole than was found among those being treated for acute leukaemia and that this difference was determined by the presence or absence of galactomannan. This suggests the effect of antifungal prophylaxis in this case is that it interrupts infection at a stage before the host responds enough for disease to become manifest. Since similar doses of an antifungal drug are used as for therapy and prophylaxis it is not unreasonable to suppose that the exposure will be the same and that therefore the impact of prophylaxis on galactomannan, beta-d-glucan and PCR will be similar as that found for therapy. This has implications for diagnostics in the setting of mould-active prophylaxis as these tests will be less sensitive and may also be less specific bring their utility into question.
References


WHICH ANTIGENS ARE IMPORTANT AND WHY?

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SATURDAY 28 JANUARY 2012 (10.05 - 10.30)
Most frequent underlying conditions predisposing to invasive aspergillosis have been well identified. According to the two largest randomized clinical trials assessing primary antifungal therapy, over 90% of the patients included had a hematological malignancy or were hematopoietic stem cell transplant recipients. Remission induction chemotherapy and graft versus host disease in allogeneic stem transplantation recipients certainly are the two groups of patient at highest risk for invasive aspergillosis. However in real life, approximately than half of the of patients with invasive aspergillosis are not belonging to any of these two groups: patients with uncontrolled leukemia, lymphoma or myeloma, even in the absence of high-dose chemotherapy administration; solid cancer or intensive care patients with comorbidities such as chronic lung disease especially if this respiratory disease is treated with systemic or inhaled steroids or concomitant diabetes; or patients receiving T-cell suppressants (e.g. fludarabine, alemtuzumab, anti-TNF agents) for chronic lymphoproliferative diseases or for non-hematological diseases. In addition, the impact of innate immunity may be critical in the risk to develop an invasive aspergillosis. Very few clinical studies have so far been done to assess the role of innate immunity and routine assessment of the gene polymorphism of innate immunity protein is not yet a standard practice. Finally, colonization is a pre-requisite to develop the invasive disease. So far, no test allows identification of patients colonized for *Aspergillus* but it is obvious that environmental factors also play a critical role in the occurrence or not of an invasive aspergillosis. 

When combining all these factors together, categorization of the patients according to their real risk of invasive aspergillosis is not easy. We can try to sort them into a high risk group or into a low (lower) risk group. High risk group patients would include the allogeneic stem cell transplant recipients and the AML patient undergoing remission induction chemotherapy. Patients with an ALL receiving induction chemotherapy or with a highly pre-treated chronic lymphoproliferative diseases, patients receiving steroids or T-cell immunosuppressant belong to the lower risk group except if they combined another factor able to transform a low risk into a high risk. Examples of such factors are prior respiratory disease, history of prior *Aspergillus* infection; known colonization by *Aspergillus*, high environmental exposure to *Aspergillus* spores, refractory hematological malignancy. 

This risk stratification will then allows deciding whether the patient should receive anti-mold prophylaxis or require, in the absence of anti-mold prophylaxis, a close monitoring for detection of early clinical, radiological or biological signs of invasive infection and receive preemptive (diagnostic driven) antifungal therapy.
PRE-EMPTIVE VERSUS EMPIRICAL ANTI-FUNGAL THERAPY IN NEUTROPENIC HEMATOLOGIC PATIENTS

Omrum Uzun, MD

Hacettepe University School of Medicine - Turkey

SATURDAY 28 JANUARY 2012 (11.30 - 11.45)
COMBINATION THERAPY: WHO BENEFITS?

Keiren Marr, MD

Johns Hopkins University School of Medicine - USA

SATURDAY 28 JANUARY 2012 (11.45 - 12.00)
GLOBAL STATUS OF AZOLE RESISTANCE IN EUROPE AND ASIA

Sevtap Arikan-Akdagli, MD

Hacettepe University School of Medicine - Turkey

SATURDAY 28 JANUARY 2012 (14.10 - 14.35)

Azole resistance in genus *Aspergillus* has initially drawn attention following the report of itraconazole-resistant *Aspergillus fumigatus* strains in 1997. Over the years, resistant strains have been detected in international, nationwide and single-center studies in European and Asian countries, USA and Canada. In the prospective international surveillance study (SCARE), strains from 23 participating centers in 20 countries were included. The prevalence of azole resistance in *Aspergillus* section *fumigati* was found to be 0-4.2% per center and resistant strains were detected in isolates from Austria, Australia, Belgium, Denmark, France, Italy, Netherlands, Spain, Sweden, Switzerland, and UK. In ARTEMIS global surveillance study, on the other hand, isolates from 62 medical centers were tested. Among the 2008-2009 *A. fumigatus* isolates, 5.8% generated elevated MICs to one or more triazoles. Most (82.3%) of these resistant strains were from Hangzhou, China and the rest were from centers at Czech Republic, Portugal, Brazil, and USA. The prospective nationwide multicenter study from the Netherlands (June 2007-January 2009) showed an itraconazole resistance prevalence of 5.3% (range: 0.8-9.5%) for *A. fumigatus*, suggesting a widespread multi-azole resistance in the country. In Manchester, UK, the frequency of itraconazole resistance in clinical *A. fumigatus* strains was reported as 5%. Importantly, 65 and 74% of these isolates were cross-resistant to voriconazole and posaconazole, respectively. Itraconazole MICs of > 2 µg/ml were detected at a rate of 4.6% in *A. fumigatus* strains isolated from cystic fibrosis patients at Cochin University Hospital, France. Among the clinical *A. fumigatus* strains isolated in Nagasaki University Hospital, Nagasaki, Japan, the percentages of non-wild type isolates were found as 7.1, 2.6, and 4.1 for itraconazole, posaconazole, and voriconazole, respectively. Multi-azole resistant *A. fumigatus* strains have been reported from India as well (two of 103 clinical strains isolated at University of Delhi). In summary, azole resistance in *Aspergillus* strains remains remarkable and tends to emerge in various centers and countries with varying frequencies. In general, exposure of *Aspergillus* to antifungal agents via medical or environmental (agricultural) use of these compounds appears to have the possible major impact on acquisition of resistance of this genus to triazoles. Based on the limited rates of isolation of *Aspergillus* from clinical samples in routine practice and the limited number of the studies carried out so far for screening of azole resistance, the true prevalence of triazole resistance remains to be further and continuously elucidated by individual centers.

References

Triazole resistance in *Aspergillus fumigatus* is an emerging and highly significant problem for patients with acute and chronic forms of invasive disease requiring aggressive antifungal therapy\(^1,2\). Intrinsic and acquired azole resistance in *Aspergillus fumigatus* results primarily from amino acid modifications in the drug target site for azoles, lanosterol 14α-demethylase, which catalyzes a central step in the biosynthetic pathway of the critical membrane sterol ergosterol. Mutations in *CYP51A*, encoding the demethylase, result in structural alterations to the enzyme, which alter the apparent tight binding of drugs. Prominent amino acid substitutions in Cyp51a conferring acquired resistance include Gly54, Met220, Leu98, Gly138, Gly448. In some cases, these amino acid substitutions confer resistance to the entire class of drugs while other substitutions induce resistance to only some drugs\(^3\). In the Netherlands, highly triazole resistant isolates are selected from environment as a consequence of azole use in the agricultural world, and arise from a common mechanism involving tandem mutations in the promoter region of Cyp51A and the codon for Leu 98 (TR/L98H)\(^4\). This dominant mechanism appears to be spreading throughout Europe and beyond\(^5\), although it has not been observed in patients that acquire resistance during therapy. Overexpression of ABC and MFS drug transporters has also been described, as they confer resistance to itraconazole, voriconazole and posaconazole. Finally, in a small percentage of isolates, the mechanism of triazole resistance is unclear and may be novel.

References

CURRENT SECTION AND SPECIES COMPLEX CONCEPTS: RECOMMENDATIONS FOR ROUTINE DAILY PRACTICE

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SATURDAY 28 JANUARY 2012 (15.10 - 15.35)

Prospective surveillance on species distribution and antifungal resistance in moulds has rarely been conducted in different geographical areas. To our knowledge, quite a few prospective studies have been done so far. However, there have been several articles reporting an increasing incidence of species and isolates showing resistance to antifungal drugs even among species regarded as susceptible such as *Aspergillus*. Invasive aspergillosis is mainly caused by *Aspergillus fumigatus*, although other species, such as *Aspergillus terreus*, *Aspergillus niger*, and *Aspergillus flavus* can also cause invasive infections. These fungi are really species complex made up of several taxons which are very difficult to classify using phenotypic techniques and subsequently molecular methods of identification have been developed to identify different *Aspergillus* spp. In addition recent studies have reported cases of aspergillosis caused by other *Aspergillus* species that belong to *Aspergillus* section *Fumigati* and some of those have been described as resistant to antifungal agents. *Aspergillus* section *Fumigati* has been reclassified based on molecular techniques of identification and currently contains 25 different species, with 8 anamorphs. In the section *Fumigati*, besides *A. fumigatus*, other species, such as *Neorsartorya fischeri*, *Neorsartorya pseudofischeri*, *Neorsartorya hiratsukae*, *Aspergillus viridinutans*, *Aspergillus fumigatiaffinis*, *Aspergillus fumisynnematus*, and *A. lentulus* have been reported to be human pathogens. These non-*fumigatus* species can exhibit resistance in vitro to antifungal agents, particularly *A. lentulus* that seems to be resistant in vitro to amphotericin B and azole compounds. Resistant species belonging to other *Aspergillus* sections have been also isolated in human infections. Epidemiological studies have to be done in order to know the real prevalence of these new pathogens. The identification of these new pathogens is based on molecular methods of classification and correct characterization of these species can be significant at therapeutic level in view of their distinct antifungal susceptibility profile. Molecular method of identification could be unpractical for clinical laboratories being more practical to perform susceptibility testing of clinical isolates to recommend the most appropriate treatment. Direct detection and identification of fungal species from clinical samples using PCR-based procedures could be useful for the clinical laboratories but further studies are needed.

References

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Evidence-based recommendations have put voriconazole as the preferred treatment for documented aspergillosis. However, recent reports coming from some, but not all western European countries, alert to the possibility of environmental *Aspergillus* isolates that carry a dominant mutation conferring multi-azole resistance. These isolates retain their fitness in experimental models and are associated with high crude mortality in immunosuppressed hosts where the majority of cases of multi-azole resistant aspergillosis have been reported. Agricultural use of azole fungicidals partially explains some emergence of azole resistance. Alarmingly, such isolates have been also described lately from Asia in isolated case reports. These recent cases complement sporadic secondary azole resistance seen in patients with chronic cavitary aspergillosis syndromes following prolonged use of itraconazole. Finally, cryptic *Aspergillus* species having innate isolate resistance to azoles have also been described. Importantly, the frequency of triazole resistance might be underestimated based on conventional culturing systems as it was found to be much more common by PCR in non-culturable *Aspergillus* fumigatus from lungs of patients with chronic lung disease.

Several questions remain regarding the epidemiology, risk factors and the natural history of azole-resistant aspergillosis. There is little doubt that azole resistance could contribute to treatment failure for invasive aspergillosis treated initially with a broad-spectrum triazole. However, it is still unclear whether this is becoming a growing problem that has reached a “threshold” to modify treatment guidelines. The apparent low background rates of azole resistance in *Aspergillus* create a perception among many clinicians that risks surrounding antifungal resistance are still greatly outweighed by the benefits and clinical advantages of using triazoles for the treatment of aspergillosis. However, it is possible that azole resistance in *Aspergillus* might become a greater challenge in the next decade and it would be critical that molecular diagnostics and treatment tools to manage azole-resistant *Aspergillus* species be developed now. Prudent use of azoles as in agriculture, careful multinational surveys and potentially antifungal stewardship programs would be needed, so we are better equipped to deal with the challenges on the horizon.

**References**

POSTER ABSTRACTS
ANTIFUNGAL AND ANTIOXIDANT POTENTIALS OF PIPTOSTIGMA CALOPHYLLUM, UVARIODENDRON CALOPHYLLUM AND UVARIODENDRON MOLUNDENSE CRUDE EXTRACTS

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Purpose:
Aspergillosis is a large spectrum of fungal diseases, which primarily affect the lungs and are caused by members of the genus Aspergillus. Aspergillus fumigatus, followed by Aspergillus flavus, and Aspergillus niger seems to be the most frequent species. The transmission of fungal spores to the human host is via inhalation. The clinical manifestation depend upon the immunological state of the patient, and range from hypersensitivity reactions (allergic bronchopulmonary aspergillosis (ABPA) to noninvasive colonization of previously damaged tissue (pulmonary aspergilloma) to acute or chronic limited invasive disease to rapidly progressive invasive disease (invasive aspergillosis). This infection is related with the production of free radicals, which in excess create an unbalance state exposing the body to oxidative stress related diseases. The present study describes the in vitro anti-mould and antioxidant activity of the ethanolic extracts of the leaves and twigs of Uvariodendron molundense, Uvariodendron calophyllum, and Piptostigma calophyllum (Annonaceae).

Methods:
The anti-mould activity was evaluated on moulds (Aspergillus fumigatus, Aspergillus flavus, and Aspergillus niger) using agar dilution method. DPPH free radical scavenging and the metal chelating activity methods served for antioxidant activity. Plant extracts undergo phytochemical screening.

Results:
These plant extracts presented different potencies with highest MIC value range from 22mg/ml to 36mg/ml and A. flavus was the most sensitive to Uvariodendron calophyllum twig extract with an MIC of 22 mg/mL. The MFC/MIC ratio showed that most extracts are fungicidal except for Uvariodendron molundense leaf extract on mould strains. The strongest scavenging activity obtained for P. calophyllum leaf extracts with SC50 of 0.201 mg/mL and the weakest was that of Uvariodendron molundense twig extracts with an SC50 > 4mg/mL. The plant extracts proved to be potent iron chelators with U. Calophyllum twig extract being the most active with IC50 of 3.07mg/mL. Other plant extracts showed IC50 > 4mg/mL. The phytochemical analyses revealed the presence of tannins, phenols, and glycosides being common among the crude extracts.

Conclusions:
These results suggested that the studied plants could be a potential source of antifungal and antioxidants.
Antioxydant and Antifungal Potentials of Essential Oils of Ocimum Gratissimum (Lamiaceae) from Yaoundé and Dschang (Cameroon)

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Purpose:
Due to the increasing number of immunocompromised individuals, mycoses have increased in the last two decades, affecting millions people worldwide. In order to control these fungal infections and oxidative stress, our aim was to evaluate the anti-oxidant and antifungal properties of the essential oils of Ocimum gratissimum L. from Cameroon.

Methods:
The fresh leaves of Ocimum gratissimum harvested from Dschang and Yaoundé and essential oils were extracted by hydrodistillation. The chemical composition was analysed using GC and GC/MS. In addition, the antioxidant properties were evaluated using the free radical scavenging activity of DPPH and the β-carotene bleaching methods. Moreover the sensitivity on Aspergillus flavus, Aspergillus fumigatus, and Aspergillus niger were evaluated using agar dilution method. Then that on Candida albicans, Candida parapsilosis and Cryptococcus neoformans was done using the paper disc, broth microdilution and spectrophotometric methods.

Results:
The extraction yields were 0.15% and 0.61% of the essential oils for Ocimum gratissimum from Dschang and Yaoundé respectively. The major compounds found in Dschang extract were thymol (40.7%), γ-terpinène (24.5%), and that of Yaoundé were eugenol (46.2%), thymol (20.6%). Furthermore the essential oil of Dschang with PA = 5,88x10^-4±9,95x10^-5 mol/g and IC50 = 0.28 ± 0.03 µg /ml possess more antiradical and antioxidant activities than the one from Yaoundé with PA = 3,37x10^-4±1,86x10^-6 mol/g and IC50 3.28 ± 0 µg/ml (p <0.05).

Aspergillus flavus (MIC= 283,5±0,06 µg/l and 334,8±0,01µg/l) were the more susceptible to our essential oils and Aspergillus niger (329,5±0,01µg/L and 422,4±0,01µg/l) were the more resistant. The more susceptible yeast strain was Candida albicans (CMI= 0,32 µg/mL and 2,5µg/mL) and the most resistant was Cryptococcus neoformans (CMI=2,57µg/mL and 4,68µg/mL) of the essential oils from Yaoundé and Dschang respectively.

Conclusions:
This study highlight in one hand the antioxidant property of the essentials oils of Dschang and Yaoundé and in other hand the in vitro antifungal activity against human pathogens causing mycosis. But it also opens the door to in vivo anti fungal investigation.
SYNERGISTIC ACTION OF HONEY AND ESSENTIAL OILS AGAINST
ASPERGILLUS FLAVUS AND ASPERGILLUS NIGER

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Purpose:
To evaluate the synergistic action of essential oils (EO) on the antifungal activity of honey, a comparative method of adding honey with and without EO to culture media was used.

Methods:
Aspergillus niger and Aspergillus flavus were used to determine the minimum inhibitory concentration (MIC) of five varieties of EO. In the second step, lower concentrations of honey than the MIC were mixed with a set of sub-MIC of EO and then added to media to determine the minimum synergistic inhibitory concentration (MSIC).

Results:
The MIC of honey without EO was 47% (v/v) against A. niger and 50% (v/v) against A. flavus. When EO were mixed with honey and then added to media, a MIC drop was noticed with each variety of EO. Isobolographic representation shows a synergistic action between honey and EO against the tested species.

Conclusions:
The current prevalence of antifungal-resistant species has led to a re-evaluation of the therapeutic use of ancient remedies, including honey and EO. Further research studies are needed to elucidate and optimize the effective combination of these natural products in clinical practice.
THE PLACE OF THE STARCH OF GINGER IN THE FIGHT AGAINST ASPERGILLUS NIGER

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Purpose:
The additive effect of the starch of ginger on the capacity antifungal of four honey samples of Algerian origin was evaluated in vitro on Aspergillus niger.

Methods:
The Minimum Additive Inhibitory Concentration (MAIC). For the four honey samples added with starch concentrations, were determined by the method of dilution in gélose Sabouraud.

Results:
The starch of ginger showed a remarkable additive potential on the Minimum Inhibitory Concentration (MIC) of honey, against yeast tested; whereas no activity was noted when the starch paste of ginger is employed only. The CMI for honey alone were of 53% (v/v) with 57% (v/v) where as the MAIC for the four honey samples tested opposite Aspergillus niger was located between starch and 47% honey (v/v), with 2% starch and starch and 51% honey (v/v), with 2% starch.

Conclusions:
The use of the starch paste of ginger would allow a honey benefit and would constitute a valid alternative against Aspergillus niger.
ANTIFUNGAL EFFECT OF LAVANDULA, SALVIA, SUMAC, GLYCYRRHIZA AND ALTHOCA EXTRACTS ON ASPERGILLUS SPECIES

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Purpose:
Many studies have shown that plants extracts have antibacterial, antifungal, antiviral and antiparasitic activity. Furthermore some plant extracts such as Lavandula, salvia, sumac, glycyrrhiza and Althoca have been described to have medicinal usages in some fungal infections like Aspergillosis. Our aim is to study the inhibitory effects of alcoholic extract of plants on Aspergillus spp.

Methods:
Strains including Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger were used from clinical samples. We prepared serial dilution of the extract including 500, 200, 100, 50, 25 and 12.5 in DMSO solvent. The effect of anti-fungal extracts was assessed separately using broth macrodilution on these strains in vitro. Finally, the minimum inhibitory concentration (MIC) and minimum fungicidal concentrations (MFC) extracts were determined.

Results:
This study showed that, lavandula, salvia, sumac, glycyrrhiza and Althoca extracts have anti-fungal effects. sumac, glycyrrhiza, lavandula extracts significantly were different than salvia extract so that MIC of sumac extract was in the range 25-50 and lavandula extract with MIC in the range 12.5-100 and lycyrrhiza extract with MFC in the range 25-100 is stronger and more significant (p≤0.05).

Conclusions:
The result of this study showed that lavandula, salvia, sumac, glycyrrhiza and Althoca extracts can inhibit growth of Aspergillus niger, Aspergillus fumigatus and Aspergillus flavus that were isolated clinically from patients. Among these fungi, the most sensitive strain was Aspergillus flavus and the less sensitive was Aspergillus fumigatus to antifungal effects of the extracts (p≤0.05). Therefore, these extracts can be used in medicine and as a replacement instead of chemical preservatives to the introduced food industry. Further work is necessary to elucidate the extent and mechanism of these changes.
ANTI-BIOFILM AND ANTI-CANCER ACTIVITY OF FARNESOL: A QUORUM SENSING MOLECULE

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Purpose:
Farnesol, a quorum sensing molecule is a sesquiterpene alcohol found in Candida albicans. Its role as a quorum sensing molecule and as a virulence factor of C. albicans has been well described. The application of farnesol in the treatment of infectious diseases and cancer has received scanty attention.

Methods:
The present study investigates the ability of farnesol to inhibit Aspergillus fumigatus biofilms and display anticancer activity. The fungus, Aspergillus fumigatus is a major causative agent of aspergillosis, cystic fibrosis and allergic bronchopulmonary diseases, associated with high mortality rates ranging from 30% to 90%. Farnesol was shown to inhibit growth of A. fumigatus and mycelium formation. Farnesol inhibited growth of A. fumigatus biofilms during co-incubation however; it was ineffective against pre-established biofilms. Anti-cancer activity of farnesol was observed against HeLa cells by MTT assay.

Results:
The results suggest potential application of a quorum sensing molecule, farnesol in the treatment of detrimental biofilm related infections and cancer.

Conclusions:
This research paves new avenue in the application of quorum sensing molecules for the eradication of infections.
AZOLE RESISTANCE IN *ASPERGILLUS FUMIGATUS* OBTAINED FROM HOSPITALS ENVIRONMENTS

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**Purpose:**

*Aspergillus fumigatus* is a saprotroph widespread in nature which typically found in soil and decaying organic matter which it cause severe infection like invasive aspergillosis especially in immunocompromised host. Environmental study of azole-resistant *Aspergillus fumigates* and other *Aspergillus* species which are surrounded at the high risk host would be essential study to explore the prevalence of azole-resistant *Aspergillus*.

**Methods:**

In the present study we obtained all samples from the soil and air in different hospitals of Tehran and Sari, Iran. The obtained soils were plated on Sabouraud’s dextrose agar (SDA) and SDA supplemented with chloramphenicol and in each of the multidish plate containing RPMI 1640–2% glucose agar supplemented with itraconazole (4 mg/liter), voriconazole (1 mg/liter), and incubated at 37°C and checked for azole resistance *Aspergillus* species. *Aspergillus* species were diagnosed by conventional and molecular tools. Those *Aspergillus fumigatus* species which grew on multidish azole agar were tested for in vitro antifungal susceptibility based on E-test method according to the manufactures’ instructions, and then PCR amplification and DNA sequencing of *cyp51A* gene were performed to identify azole-resistant *A. fumigatus* isolates.

**Results:**

*Aspergillus fumigatus* was isolated in 3 out of 70 samples from Tehran’s Hospital. The majority of *A. fumigatus* isolates obtained from the hospital soil samples rather than environment. In vitro susceptibility results have shown that MIC for itraconazole >8 mg/L and for voriconazole 4 mg/L. Results of sequenced *cyp51A* gene from 3 strains compared with susceptible wild type of *A. fumigatus* and revealed a single mutation at codon 98, which was substituting the amino acid leucine to histidine (L98H).

**Conclusions:**

Due to multi-azole-resistant *Aspergillus fumigatus* has increased in hospital area which patients are in the high risk. Thus understanding of the prevalence of azole-resistant organisms which they are in the first line to cause severs disorder with a potential efficacy is significantly recommend. With present results we suggest that in vitro antifungal susceptibility testing of clinical samples is crucially important and have to be done routinely at all hospital labs before start the treatment.
MAY AVIAN FARMS CONSTITUTE A SOURCE OF AZOLE-RESISTANT ASPERGILLUS FUMIGATUS ISOLATES?

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Purpose:
Aspergillus fumigatus is frequently isolated in avian farms. The fungus can therefore be exposed to antifungal drugs regularly used for the prevention of avian mycoses (parconazole to prevent infection due to Candida albicans and enilconazole to prevent infection due to A. fumigatus). Since limited data exist on the risk represented by the use of azoles in avian farms, we undertook a drug susceptibility study including a large number of A. fumigatus isolates from birds and their environment in France and in Southern China.

Methods:
Identification was based on the macro- and micromorphology. Determination of partial DNA sequences of the beta-tubulin gene was performed to confirm the species. Azole resistance was screened using itraconazole E-test strips. The promoter and entire coding sequence of the cyp51A gene were further sequenced for all the isolates.

Results:
Aspergillus fumigatus was recovered in 108 out of 292 samples (36.7%). MIC values were comprised between 0.19 to 3 microg/mL and similar for clinical and environmental isolates. Only 3 isolates with high itraconazole MIC (≥ 2 microg/mL) were detected. All the isolates with elevated azole MICs harbored mutations of cyp 51A but these mutations were different from that (TR/L98H mutation) usually reported in resistant A. fumigatus isolates collected in hospitals. A few isolates (n=5) with low azole MICs also harbored mutations of cyp 51A gene. The prevalence of azole-resistant isolates was not higher in farms where azole derivatives were used to prevent avian mycosis.

Conclusions:
Taken together, our results demonstrated that A. fumigatus isolates with high MIC values are circulating in avian farms in France and China. However, the use of azole derivatives was not proved to have a significant impact in the emergence of resistance and additional investigations are still required.
MANAGEMENT OF AFLATOXIGENIC FUNGI IN GROUNDNUT (ARACHIS HYPOGAEA L.) VARIETIES IN CENTRAL TIGRAY THROUGH SOIL SOLARIZATION AND PLANTING TIME

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Purpose:
Field experiments were conducted in Northern Ethiopia, Central Zone of Tigray Administrative Region at two locations to determine the effect of soil solarization on Aspergillus spp. inoculum in the soil and to evaluate the effect of soil solarization and time of planting on Aspergillus spp. seed invasion and yield of groundnut varieties.

Methods:
Soil samples were taken in three rounds and analysed for aflatoxigenic population.

Results:
Soil solarization reduced fungal inoculum and increased groundnut yields. Individual fungi and total cfu g^-1 of soil was determined before, after and at harvest. Four Aspergillus spp. namely, A. flavus, A. parasiticus A. niger and A. terrues were identified and their densities were significantly (p< 0.05) reduced after solarization. In the solarized plots, A. flavus and A. parasiticus were found reduced by 53.8 and 45% cfu g^-1 at Ramma and 36.4 and 44% cfu g^-1 at 5 and 10 cm soil depths at Mayweyni respectively, after soil solarization in the solarized plots than the nonsolarized plots. At harvest, Fusarium spp., A. flavus and A. terrues were detected. Pod yields were found increased by 263 kg ha^-1 and 182.22 kg ha^-1 on solarized plots at Mayweyni and Ramma respectively. Increased in yield related parameters were found from early planting dates as compared to later planting time at Mayweyni.

Conclusions:
Generally, yield varied across locations, mean pod yield in Mayweyni was 360.9 kg ha^-1 higher than the yield in Ramma. Three Aspergillus spp. namely, A.flavus, A. niger and A. parasiticus were isolated from seed samples plated on Czapek Dox Agar medium. Early planting of the varieties showed the lowest seed infection by A. flavus.
ANTIFUNGAL SUSCEPTIBILITY OF *ASPERGILLUS FUMIGATUS* AND RELATIONSHIP WITH AZOLE EXPOSURE IN NAGASAKI, JAPAN

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**Purpose:**

During recent years the appearance of azole-resistant *Aspergillus fumigatus* comes under scrutiny in several countries. Those origins are important problem to develop strategies for prevent an outbreak of aspergillosis caused by resistant isolates. Despite the existence of case reports about development of azole resistance during azole therapy, there is as yet few information of possible azole amount used for patients to develop azole resistance. We evaluated drug susceptibility of clinical *A. fumigatus* isolates and cumulative amount of used azoles for patients as at isolation date of each isolates.

**Methods:**

We investigated the triazole, amphotericin B, and micafungin susceptibilities of 196 *A. fumigatus* clinical isolates obtained in Nagasaki, Japan. Analysis of *cyp51A* gene was also conducted. For 154 isolates of those 196 isolates, we analyzed a relationship between previous exposure of triazoles and their MICs. Detailed analysis included microsatellite genotyping had been performed for isolates from patients infected azole-resistant *A. fumigatus*. ECVs used in this study were as follows: itraconazole, 1 μg/ml; posaconazole, 0.5 μg/ml; voriconazole, 1 μg/ml as previously suggested.

**Results:**

In this study, using the ECVs, the percentages of non-wild-type (non-WT) isolates for itraconazole, posaconazole, and voriconazole were 7.1%, 2.6%, and 4.1%, respectively. No consistent trend of increased proportion of non-WT isolates was observed. Amphotericin B MICs of ≥2 μg/ml were recorded for 1.0% of the isolates (2/196); micafungin MECs of ≥16 μg/ml were recorded for 1.0% of the isolates (2/196). G54 mutation in *cyp51A* was detected in 64.2% (9/14 isolates) and 100% (5/5 isolates) of itraconazole and posaconazole non-WT isolates, respectively. The amount and term of itraconazole exposure caused increase of MICs and showed positive correlation (r = 0.5700, p <0.0001). The number of isolates, which had never been exposed itraconazole, was furiously less according to increase of itraconazole MIC, especially over 2 μg/ml (0.5 μg/ml vs. 2μg/ml, p = 0.03). For other azoles, positive correlative relationship existed between itraconazole exposed period and posaconazole MIC (r = 0.5237, p <0.0001). The number of isolates, which had never been exposed itraconazole, was also less according to increase of posaconazole MIC, especially over 0.5 μg/ml (0.25 μg/ml vs. 0.5 μg/ml, p = 0.04). On the other hand, correlation coefficient obtained from scattergram of itraconazole usage and voriconazole MIC was small (r = -0.2627, p =0.001). No significant difference was observed in percentages of isolates never been exposed itraconazole about each individual MIC of voriconazole. There was no tendency to change susceptibility of three triazole agents as change period of voriconazole exposure. We confirmed existence of acquired resistance to itraconazole and posaconazole in a patient with chronic pulmonary aspergillosis after oral itraconazole therapy.

**Conclusions:**

No consistent trend of increased proportion of non-WT isolates was observed during investigated period. It might be better to consider the possibility of acquired azole-resistant in *A. fumigatus*, during itraconazole usage.
FIRST ISOLATIONS IN INDIA OF MULTIPLE-TRIAZOLE RESISTANT ASPERGILLUS FUMIGATUS STRAINS, CARRYING THE TR/L98H MUTATIONS IN THE CYP51A GENE

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Purpose:
Aspergillus fumigatus is the commonest etiologic agent of invasive and chronic pulmonary aspergillosis. Azole resistance in Aspergillus fumigatus isolates impacts the management of aspergillosis since the azoles are primary agents used for prophylaxis and therapy. We report the emergence of resistance to triazoles in two A. fumigatus isolates from patients in Delhi, India.

Methods:
One hundred and three A. fumigatus isolates, collected from 85 patients suspected of broncho-pulmonary aspergillosis, admitted to the Clinical Research Centre of V. P. Chest Institute (VPCI), Delhi, India, during 2005-2010 were investigated for susceptibility to itraconazole, voriconazole, posaconazole and isavuconazole using CLSI M38-A2 broth microdilution method. Identification of the resistant isolates was confirmed by internal transcribed spacer (ITS) sequencing. We undertook mixed-format real-time PCR assay for detection of mutations leading to triazole resistance in A. fumigatus. Genotyping was performed with a panel of nine short tandem repeats. For phylogenetic analysis, 25 Dutch clinical and environmental isolates of A. fumigatus containing the TR/L98H genotype were included along with the Indian isolates.

Results:
Of the 103 A. fumigatus isolates tested, only two had high MIC values of itraconazole (>16 mg/L), voriconazole (2 mg/L), posaconazole (2 mg/L) and isavuconazole (8 mg/L). The resistant A. fumigatus isolates exhibited the TR/L98H genotype and showed identical patterns by microsatellite typing but were different from 25 Dutch TR/L98H isolates. Isolate VPCI 1042/09 originated from sputum of a 55-year-old, male outpatient of VPCI, diagnosed with chronic obstructive pulmonary disease with bilateral bronchiectasis and cor pulmonale. The second multiple-triazole resistant A. fumigatus isolate, VPCI 942/09, originated from sputum of a 22-year-old male labourer who presented to the outpatient department of VPCI with complaints of productive cough since two years and fever on and off since one year. His diagnosis included pulmonary tuberculosis and allergic bronchopulmonary aspergillosis.

Conclusions:
To our knowledge, this is the first report from India on the occurrence of multiple-triazole resistant A. fumigatus isolates, carrying the TR/L98H genotype, in patients with chronic respiratory diseases. The TR/L98H mutation associated with pan-azole resistance in A. fumigatus has been reported so far only from Europe and recently from China. In the present study, 1.9% (2/103) of the A. fumigatus clinical isolates were multiple-triazole resistant, whereas in Dutch hospitals, the corresponding figure is 6-12.8%. As both of our triazole-resistant A. fumigatus isolates were epidemiologically unrelated but showed identical STR genotypes, and were obtained from patients without history of exposure to azoles or of travel to Europe, it is highly likely that the resistance was acquired from the environment in India. Keeping in mind the emergence of azole resistance in environmental strains, continued surveillance of resistance in clinical A. fumigatus strains is desirable for successful therapy of aspergillosis.
ANTIFUNGAL THERAPY AFFECTS THE SENSITIVITY OF (1→3)-β-D-GLUCAN, GALACTOMANNAN, AND LATERAL-FLOW DEVICE ASSAYS IN SERUM BUT NOT IN BRONCHOALVEOLAR LAVAGE FLUIDS

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Purpose:
Reduced sensitivity of diagnostic markers for invasive pulmonary aspergillosis during antifungal exposure is of concern as many centres use these assays to monitor for breakthrough infections in those receiving antifungal prophylaxis or empiric therapy (Marr et al. Clin Infect Dis 2005; Senn et al. Clin Infect Dis 2008). Previous work by our group has also demonstrated decreased sensitivity of the (1→3)-β-D-glucan and galactomannan assays in the serum of guinea pigs administered antifungal agents (Wiederhold et al. ICAAC 2009). However, it is unknown if diagnostic markers are affected in the bronchoalveolar lavage (BAL) fluid. Our objective was to compare the sensitivities of clinically available (1→3)-β-D-glucan and galactomannan assays as well as an investigational lateral-flow device using serum and BAL fluids from guinea pigs with invasive pulmonary aspergillosis receiving antifungal treatment.

Methods:
Immunosuppressed guinea pigs were inoculated with *A. fumigatus* isolate AF293 using an aerosol chamber. For treatment (N = 8 per group), posaconazole (20 mg/kg PO BID), voriconazole (20 mg/kg PO BID), liposomal amphotericin B (10 mg/kg/day IP), or caspofungin (2 mg/kg/day IP) were given on days 1 - 8, with serial blood samples collected on days 5, 7, and 11. Terminal BAL fluid was collected from moribund animals (days 6 – 11), and the lungs were harvested to measure colony-forming units. (1→3)-β-D-glucan was assayed using the Fungitell assay (threshold for positivity 80 pg/mL) and galactomannan by the Platelia *Aspergillus* assay (galactomannan index serum threshold 0.5, & BAL threshold 1.0). For the lateral-flow assay, serum was pre-treated with EDTA and heat, while BAL was applied untreated.

Results:
In untreated controls, each diagnostic marker became positive within the serum by day 7. In guinea pigs treated with antifungals, the majority of serum samples were negative for each marker at each time point. In contrast, each marker was positive in the majority of terminal BAL samples, including those from animals treated with antifungals. The BAL results are in agreement with the colony-forming unit data, which remained elevated in each antifungal group and did not differ significantly from untreated controls.

Conclusions:
Antifungal exposure reduced the serum sensitivity of the (1→3)-β-D-glucan, galactomannan, and lateral-flow device assays. However, each diagnostic marker remained elevated within the BAL fluid even with antifungal treatment. These results suggest that serum markers of infection may be of limited value in the setting of antifungal therapy. Further work is needed to assess the utility of BAL samples in this setting.
13 ANTIFUNGAL SUSCEPTIBILITY OF \textit{ASPERGILLUS} SP. UNDER HYPOXIC GROWTH CONDITIONS

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\textbf{Purpose:}
Invasive aspergillosis is a major life-threatening disease in immunocompromised patients, with mortality rates ranging from 40 \% up to 90 \% in high-risk populations. The most common species causing aspergillosis is \textit{Aspergillus (A.) fumigatus}, accounting for approximately 90\% of \textit{Aspergillus} infections. Depending on regional distinctions \textit{A. flavus} and \textit{A. terreus} are frequently reported as well, and there is evidence that these non-fumigatus pathogens are increasingly common etiologic agents.

During infection, fungal pathogens must adapt to microenvironmental stresses, including hypoxia as well as high CO2 levels. Such oxystress conditions are usually not taken into account in current models of infection and assessment of antifungal sensitivities.

\textbf{Methods:}
Using Etests as antifungal test system, we compared the in vitro activity of amphotericin B, various azoles and echinocandines in hypoxic conditions (1 \% O2, 5 \% CO2) to their activity in normoxic conditions against 47 isolates of \textit{Aspergillus} sp. belonging to three different species. The test group comprises 9 strains of the \textit{A. flavus} complex, 16 strains belonging to \textit{A. terreus} complex and 22 isolates of the \textit{A. fumigatus} complex.

\textbf{Results:}
We found that in hypoxic conditions similar to those that might occur in \textit{aspergillus}-infected tissue, a reduction in the in vitro MIC of amphotericin B for species of all three groups occurred. For some of the azoles this could be shown as well, mainly for \textit{A. flavus} species, while for echinocandines differences where less significant and the phenomenon of trailing was also persistent in hypoxic conditions, which makes determination of MIC rather difficult. Most interestingly, some strains of the \textit{A. terreus} complex exhibited amphotericin B susceptibility in hypoxic conditions, using a breakpoint of > 2 \(\mu\)g/ml. It is important to note, that for none of the tested strains the MIC increased in hypoxia, while all tested isolates showed severe morphological changes resulting in a fluffy appearance. Additionally, conidiation was inhibited in the \textit{A. terreus} and \textit{A. flavus} complex in hypoxic conditions, but not for the \textit{A. fumigatus} isolates tested.

\textbf{Conclusions:}
Further tests are currently in progress to find out if similar results can be obtained with microbroth dilution assays, where not only gas concentrations are regulated to mimic host environments, but also other parameters such as pH, iron limitation or the provision of host cell components can be manipulated.
**ASPERGILLUS FUMIGATUS BIOFILMS RELEASES EXTRACELLULAR DNA WHICH PLAYS A ROLE IN PHASE DEPENDANT ANTIFUNGAL RESISTANCE**

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**Purpose:**
The biofilm formation by *Aspergillus fumigatus* is one of several significant virulence factors associated with life threatening pulmonary infections in immunocompromised individuals. In our previous studies, we demonstrated phase dependant antifungal activity against *A. fumigatus* biofilms and association of efflux pumps in early stage biofilm resistance. In this study, we investigated the presence and role of extracellular DNA (eDNA) in *A. fumigatus* biofilms.

**Methods:**
Different phases of *A. fumigatus* (n=5) biofilms were grown for 8, 12, 24 and 48h in 96 or 24 well polystyrene plates in RPMI media. Extracellular material were extracted from biofilms by EDTA-treatment and eDNA was assessed by microplate fluorescence assay (MFA) using the DNA-binding dye SYBR Green I. The MIC of different classes of antifungals was assessed in the presence and absence of DNase by the broth microdilution method. Biofilm disruption by DNase was evaluated by fluorescent microscopy and biomass assessed by crystal violet assay.

**Results:**
The fluorescent micrographs of propidium iodide stained biofilms showed the presence of eDNA in *A. fumigatus* biofilms. The MFA demonstrated that eDNA release was highest in the 48h populations, followed by 24 and 12h, respectively. Digestion of eDNA by DNase significantly reduces biofilm formation and also disrupts preformed biofilms in a dose-dependant manner. The biofilm susceptibility to Amphotericin B was increased in the presence of DNase, but no significant change in the MIC of other antifungals were observed. DNase did not appear to alter 8h biofilm susceptibility, but with 12h, 24h and 48h biofilms it increased the sensitivity of 1-2 fold, 8 fold and 8-16 fold reductions in MIC, respectively.

**Conclusions:**
In this study we established the presence of eDNA in *A. fumigatus* biofilms. The biofilm biomass decreased with DNAses treatment, indicating the association of eDNA in biofilm stability. The MIC value of different phases of *A. fumigatus* growth decreased significantly in the presence of DNase, indicating that decreased susceptibility to antifungals in the *A. fumigatus* is mediated in part by eDNA. Our previous studies have shown that biofilms are more resistant to antifungals than immature germlings, and analysis of their eDNA in this study showed that this may potentiate resistance. Overall these results suggest the presence of eDNA in *A. fumigatus* contributes to the biofilm architecture and is partly associated with antifungal resistance, particularly when within complex hyperfilamentous biofilm populations.

**NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION**
AMPHOTERICIN B: ANALYSES OF THE MODE OF ACTION/CAUSATION OF RESISTANCE IN ASPERGILLUS TERREUS

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Purpose:
Invasive aspergillosis (IA) has emerged worldwide as an important cause of infections among patients undergoing cancer chemotherapy, hematopoietic stem-cell transplantation, or solid organ transplantation. Aspergilli are ubiquitous fungi and the major pathogenic agents are *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus terreus*. Among the different species *A. terreus* takes an exceptional position: almost all isolates are in vitro and in vivo resistant to Amphotericin B (AMB), which is one of the broadest antifungal drugs and widely used for life threatening fungal infections; the reason for AMB resistance is unknown yet. This pathogen is a common cause of IA in some geographically unrelated institutions, such as The University of Texas M. D. Anderson Cancer Center (MDACC) in Houston, TX, and The University Hospital of Innsbruck (UHI), Austria. At the UHI, a tertiary-care hospital with 2000 beds, infections due to *A. terreus* have been noted since 1994.

Methods:
The objective of this study was to identify differences in the mode of AMB action of AMB resistant *A. terreus* (ATR; MIC ≥8 µg/ml) compared to AMB susceptible *A. terreus* (ATS; MIC ≤0.5 µg/ml) in response to sub-lethal (0.1 µg/ml) and lethal (10 µg/ml) AMB concentrations.

Results:
Whereas ergosterol contents of ATR (5.45 ± 0.7 µg/mg) and ATS (5.26 ± 0.9 µg/mg) were similar, intracellular AMB uptake was higher (up to 80%) in ATS. Amino acid disposal and protein release were higher in ATR; potassium release after AMB treatment was similar in both groups (ATR and ATS). Basic catalase activity was significant higher in ATR (P < 0.05) compared to ATS. AMB treatment resulted in stronger inhibition of intracellular GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) activity in ATS.

Conclusions:
The presented data demonstrate that ergosterol content, amino acid- and protein release do not play a crucial role in AMB resistance; higher AMB uptake and stronger GAPDH activity inhibition in ATS indicate that AMB impact/resistance is connected with cell membrane passage of AMB molecules and thus possible inhibit intracellular enzymes/processes.
ANTIFUNGAL ACTIVITY OF IMIDAZOLE, BENZIMIDAZOLE, TRIAZOLE AND BENZOTRIAZOLE DERIVATIVES AGAINST *ASPERGILLUS* SPECIES

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**Purpose:**
Nowadays antifungal drugs especially azole group did use widely against opportunistic mycoses and there are many reports about resistance to this antifungal group. The aim of this study was to synthesized of many azole derivatives and evaluation of antifungal activity of these compounds against *Aspergillus* species.

**Methods:**
Sixteen azole derivatives did synthesized and their antifungal activities were evaluated against *Aspergillus fumigatus* and *Aspergillus flavus* standard strains, using microdilution method by CLSI protocol.

**Results:**
Two compounds had good and strong activity against *Aspergillus* species. Eight compounds had variable activities and six compounds had no antifungal activities against the fungi.

**Conclusions:**
This study reveals that some of these compounds have a potential for used as antifungal agents and further studies needed to confirm these results.
IN VITRO ACTIVITY OF EXTRACTS OF STEVIA URTICIFOLIA (ASTERACEAE) AGAINST ASPERGILLUS SPP

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Purpose:
The species of Aspergillus have been increasingly recognized as a cause of invasive disease and mortality from invasive pulmonary aspergillosis (IPA) remains above 50% to 95% in certain situations. Aspergillus fumigatus is the species responsible for most cases of mortality (85% to 90%) infections in IPA, followed by A. flavus, A. terreus, A. nidulans and A. niger. Patients suffering from haematological disorders and patients who underwent transplantation therefore have a particularly high risk of IPA. In the study for the development of new chemotherapeutic agents against aspergillosis, we can highlight the advancement of research of new herbal medicines as an alternative route in the therapy and the search for drugs with low side effects (toxicity). Studies have shown that extracts of plants belonging to the Asteraceae family have potential trypanocidal activity. The description of biological activity of the species Stevia urticifolia is unprecedented in the literature, and the investigation of in vitro antifungal activity of these extracts is promising. This paper presents the results of the study of antifungal activity of three species of Stevia urticifolia extracts: ethylacetate extract (EA), ethanol extract (EE), hexane extract (HE) against Aspergillus spp.

Methods:
The in vitro antifungal activity of three extracts of Stevia urticifolia was evaluated against Aspergillus clavatus, A. fumigatus, A. niger and A. tamarii, according to the protocol of CLSI (M38-A2), with some modifications. The minimum inhibitory concentration (MIC) were determined by microdilution test, and used the amphotericin B (AMB), as positive control.

Results:
All extracts showed activity against the species tested. The EH and EA extracts showed similar results with MIC values of 64 µg/mL against A. clavatus and 128 µg/mL against A. fumigatus, A. niger and A. tamarii. EE extract proved to be the most active among the three, with MIC values of 64 µg/mL against A. clavatus and A. tamarii, and 128 µg/mL against A. fumigatus and A. niger. The MIC values for AMB ranged from 1.0 to 0.03 µg/mL.

Conclusions:
There are a large number of citations involving the family Asteraceae to a wide variety of chemical constituents and therapeutic applications. Among the substances chemically and biologically isolated from species of the family Asteraceae is worth noting the sesquiterpene lactones, they are considered chemotaxinomic markers of the family. In research with yeast fungi, extracts of Stevia urticifolia species showed antifungal activity against Candida albicans, C. krusei, C. parapsilosis and C. tropicalis. Additional studies are being conducted to verify the toxicity of the active and research.
18 THE EFFECT OF ANTI-AFLATOXIN ESSENTIAL OILS OF CUMINUM CYMINUM, ZIZIPHORA CLINOPODIOIDEES AND NIGELLA SATIVA

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Purpose:
Aflatoxins are the large groups of Mycotoxins are produced by certain species of Aspergillus, including Aspergillus flavus, A. parasiticus and A. numius. This group of fungal toxins is considered as a leader of all Mycotoxins. Therefore, performed many efforts to elimination or neutralization of these compositions in food and feed stuffs. The aim of this study is to survey the effect of Anti-aflatoxin essential oils of Cuminum cyminum, Ziziphora clinopodioiedes and Nigella sativa growth in Khorasan Razavi (northeastern Iran).

Methods:
In this experimental study, herbal substances which include Cuminum cyminum, Ziziphora clinopodioiedes and Nigella sativa were selected and collected on the basis of traditional medicine and their growing region, during the year 2009. Then, their essential oils (essences) were extracted by hydro-distillation procedure. The concentration of Aspergillus parasiticus aflatoxins (B1, B2, G1, G2, Total) was detected by Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) technique based on ng/ml (ppb) and three times repetition. Also, the final concentration of Aflatoxin after the impact of Cuminum cyminum, Ziziphora clinopodioiedes and Nigella sativa Essences were measured in concentrations of 0.25, 0.25 and 1.5 mg/ml, respectively.

Results:
The results from these measures were analyzed by the SPSS-16 software and statistical tests Kruskal–Wallis and Mann-Whitney. (P ≤ 0.05). The ability of above-mentioned essential oils to prohibit producing of any Aflatoxins is essences of Cuminum cyminum and Nigella sativa and Ziziphora clinopodioiedes, respectively. There was a significant and positive correlation between fungus dry weight and amount of Total Aflatoxin. (P = 0.0005, r = 0.896).

Conclusions:
The findings of this study showed that Cuminum cyminum, Nigella sativa and Ziziphora clinopodioiedes have the ability to inhibit Aflatoxin production by Aspergillus parasiticus and there is the possibility of using it for medical affairs, pharmaceutical, veterinary, food industry, Cosmetics and elements as anti-fungal drugs and anti-toxin. This study also confirmed the traditional use of these plants against microbial infections. Therefore, they can be used as anti-fungal and anti-toxin elements.
THE EFFECTS OF ANTI-ASPERGILLUS ESSENCES OF CUMINUM CYMINUM, ZIZIPHORA CLINOPODOIOEDES AND NIGELLA SATIVA AND THE SITE OF THEIR ACTIVITIES

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Purpose:
Considering the clinical importance due to fungal infections and increasing drug resistance fungi, essences may be a promising and suitable proposal. The aim of the study is to survey the effects of anti Aspergillus essences of Cuminum cyminum, Ziziphora clinopodioiedes and Nigella sativa and the site of their effects.

Methods:
In this experimental study, herbal substances which include Cuminum cyminum, Ziziphora clinopodioiedes and Nigella sativa were selected and collected during the year 2009 from Northeast of Iran. Then, their essential oils were extracted by hydro-distillation procedure. By applying Broth Macro Dilution and Broth Micro Dilution technique and four times of repetition, the mean of Minimum Inhibitory Concentration (MIC90) and Minimum Fungicidal Concentration (MFC) of the above-mentioned essences were certified against Aspergillus fumigatus and Aspergillus parasiticus in concentrations of 3, 2.5, 2, 1.5, 1, 0.5 and 0.25 mg/ml in Broth Macro Dilution and 8, 6, 5, 4, 3, 2.5, 2, 1.5, 1.25, 1, 0.75 and 0.5 mg/ml in Broth Micro Dilution technique. The testing fungi were observed from growth level point of view after being cultured in 28 degrees centigrade in Yeast Extract Sucrose (YES) broth and in RPMI 1640 culture medium in Broth Macro Dilution and Broth Micro Dilution techniques respectively, for 48, 72 and 96 hours in order. Then, MIC and MFC of them were determined by being subcultured in Potato Dextrose Agar (PDA). Analysis of chemical composition of the essential oils were performed by using Gc/Ms. The results were analyzed by the SPSS-16 software. Conidial and mycelial samples exposed to 0.25, 0.5, 1, 1.5 and 2 mg essential oils/ml for 5 days in 2% yeast extracted granulated plus 15% Saccharose media were processed for Transmission Electron Microscopy (TEM) based on broth dilution methods.

Results:
In general, after repeating the test 4 times, the mean of MIC90 and MFC of the above-mentioned essences against both types of fungi was determined from a low of 0.25 to a high of 1.75 and a low of 0.5 to a high of 2.5 mg/ml, respectively. According to the results of this study, the essential oils of Cuminum cyminum and Ziziphora clinopodioiedes showed the best and most tonic activity against both types of fungi (0.25 ≤ MIC90 ≤ 0.43) while Nigella sativa showed a fairly moderate activity against them (1.25 ≤ MIC90 ≤ 1.75). Overall Broth Macro Dilution method showed clearer results, with less standard deviation, lower MIC90 and MFC in compare with Broth Micro Dilution method. The main changes observed by TEM in the cell wall, plasma membrane and membranous organelles; in particular, the nuclei and mitochondria. Aspergillus fumigatus and A. parasiticus growth inhibition induced by these oils were found to be wellcorrelated with subsequent morphological changes of the fungi exposed to different fungistatic concentrations of the oils.

Conclusions:
The findings of this study showed that Cuminum cyminum, Ziziphora clinopodioiedes and Nigella sativa have fungicidal and anti-fungal functions against Aspergillus fumigatus and Aspergillus parasiticus. Therefore, they can be used in medical pharmacology, veterinary, food industries, aromathrapy and health affairs as drugs and anti-fungal elements.
THE SYSTEMS BIOLOGY OF AZOLE RESISTANCE IN *ASPERGILLUS FUMIGATUS*

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**Purpose:**
Treatment for fungal disease is limited to a small number of drug classes including azoles, candins, and polyenes. Long term azole therapy for invasive, allergic and chronic pulmonary aspergillosis is the norm, and increasingly used. Antifungal drug resistance complicates therapy with worse outcomes. The mortality of patients with multi-azole resistance aspergillosis was 88% compared with 30-50% who were infected with azole sensitive strains. *Aspergillus fumigatus* azole resistance in several centres has recently increased in frequency, in Manchester 10% to 50% of resistant isolates carry non-CYP51A mutations, which remain unidentified. These isolates represent both a diagnostic challenge and an opportunity to study the underlying non-target basis of azole resistance in *A. fumigatus*.

**Methods:**
To identify the biological systems that play a role in acquired drug resistance, we used a multi-omics approach involving Illumina GAII genome sequencing of resistant clinical isolates, RNAseq of strains in the presence and absence of azoles, saturation transposon mutagenesis to discover genes involved in azole resistance and sensitivity and high throughput gene knockout to identify genes and pathways involved in azole resistance and sensitivity.

**Results:**
Currently >25 strains resistant to Itraconazole, Voriconazole, Posaconazole or to multiple azoles, some from the same patient, others from diverse sources, have been sequenced. RNAseq of sensitive strains in the presence or absence of azole reveals 156 up-regulated and 201 down-regulated transcripts including efflux transporters, transcription factors, gliotoxin biosynthetic genes and lipid biosynthetic genes. Insertional mutagenesis reveals a number of transcriptional networks involved in azole resistance including 4 key transcription factors. Finally, analysis of drug resistant strains coupled with RT-PCR analysis of transporter gene expression has allowed us to identify two efflux transporters involved in azole resistance as well as evidence that up-regulation of CYP51B may play a role in resistance.

**Conclusions:**
The combination of different -omics techniques has allowed us to analyse drug resistance systems in *A. fumigatus* with identification of 9 novel genes or pathways involved in resistance. Azole resistance is a complex phenomenon in *A. fumigatus*, as might be expected from its remarkable versatility.
21 MANAGEMENT OF *ASPERGILLUS* SPP. AND AFLATOXIN CONTAMINATION IN RICE THROUGH FUNGICIDAL SEED TREATMENT

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**Purpose:**
Rice, the principal food of majority of population is also the residence for several seed borne fungi. The structure and chemical composition of this cereal grass grain has made it an ideal substrate for the establishment and growth of fungal species, especially toxigenic Aspergilli that secrete mycotoxins including the aflatoxins. *Aspergillus* contamination (*A. flavus*, *A. parasiticus*, *A. ochraceus* and *A. niger*) was found on paddy seed samples collected from different rice growing areas in India. As it is of paramount importance to check the seed contamination and infection, a few of the commercially available fungicides were evaluated against the growth of these Aspergilli on the seed and subsequent toxin contamination.

**Methods:**
Naturally contaminated rice seeds with Aspergilli were treated with the test fungicides, and plated on the standard media. Observations were recorded on the inhibition of mycelia growth of Aspergilli *vis a vis* the untreated control seeds. The contaminated seed samples were extracted and assayed by following the procedure for the detection of Aflatoxin B1 (AFB1) by ELISA both in case of treated and untreated seeds.

**Results:**
Of the fungicides evaluated, carbendazim 50 WP, hexaconazole 5 EC, tebuconazole 250 EC, propiconazole 25 EC and carbendazim-mancozeb 75 WP combination (@ 1 g or ml/Kg seed) showed high efficacy in checking the growth of all *Aspergillus* spp., followed by carproamid 30 SC, tricyclazole 75 WP (@ 4 g/Kg seed) and bitertanol 25 WP (@ 2 g/Kg seed) on *A. niger*. However, propineb 70 WP (@ 3 g/Kg seed) and bitertanol 25 WP (@ 4 g/Kg seed) completely inhibited the growth of *A. parasiticus*.

All the test fungicides were found effective in checking the AFB1 production with increasing concentration, though carbendazim, hexaconazole, tebuconazole, propiconazole and carbendazim-mancozeb combination inhibited the Aflatoxin B1 @ 1 g or ml/Kg seed itself, followed by tricyclazole @ 4 g/Kg seed.

**Conclusions:**
Fungi are known to contaminate the seed and produce toxic substances, which pose real threat to human health. Seed treatment of *Aspergillus* contaminated rice with the fungicides like carbendazim, hexaconazole, tebuconazole, propiconazole, carbendazim-mancozeb combination, propineb and bitertanol protect the seed from toxigenic fungal infection and subsequent Aflatoxin production.
OCHRATOXIN PRODUCTION AND MANAGEMENT OF ASPERGILLUS OCHRACEUS CONTAMINATION IN RICE

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Purpose:
Contamination of rice grains by Aspergillus spp. is known to occur widely. During their growth, they produce various toxic metabolites including Ochratoxins. Ochratoxin A (OTA), a mycotoxin contaminating foods and beverages, has been classified as a human carcinogen. Consumption of such contaminated grains and food is hazardous to humans. However, detailed information on OTA contamination in rice is not available. Therefore, an attempt was made to isolate OTA producing mycoflora in the seed samples collected from different rice growing areas of India and evaluate plant extracts, biocontrol agents, fungicides and chemicals to limit the growth of A. ochraceus.

Methods:
Five hundred and five seed samples were collected from 36 locations in 21 states in the country. Using agar plate method, presence of A. ochraceus was observed and OTA producing mycoflora were isolated. These isolates were grown on Pusa basmati rice grains for 7 days at room temperature, extracted the OTA and identified on TLC plate along with standard in different solvent systems. Plant extracts viz., Pongamia, onion, custard apple, garlic, Eucalyptus, neem leaf and kernel, biocontrol agents viz., Trichoderma viride, T. harzianum, T. virens, T. reseii, T. koningi and Pseudomonas fluorescens, fungicides viz., propineb 70 WP, bitertanol 25 WP, carbendazim 50 WP and tricyclazole 75 WP and chemicals viz., acetic acid, benzoic acid, propionic acid, vanillin and sodium chloride were tested for their efficacy on the growth of A. ochraceus.

Results:
Natural contamination of Aspergillus ochraceus was detected in forty six rice samples collected from areas exposed to rain or flood or stored in storage bins or rice mills. The OTA isolated in this study and standard showed the same Rf values in different solvent systems. The OTA was confirmed by changing the colour from greenish blue to blue after spraying 6% NaOH in 20% ethanol on TLC plate. Garlic bulb extract proved significantly more effective with complete inhibition of A. ochraceus. Pongamia glaberima kernel extract showed 71% inhibition on mycelia growth of A. ochraceus. Among biocontrol agents, Trichoderma virens completely inhibited the growth of A. ochraceus while Pseudomonas fluorescens had effectively inhibited (98%) the mycelia growth of A. ochraceus. Among the fungicides tested, carbendazim was effective even at 100 ppm in inhibiting A. ochraceus. Propineb completely reduced the growth at 750 ppm. Tricyclazole and bitertinol had shown the efficacy in the range of 53 to 100% on A. ochraceus at the highest concentration tested. Fungicidal effect was more pronounced in all the test fungicides with any increase in concentration. Among the chemicals tested benzoic acid completely inhibited the mycelia growth at 0.1% concentration.

Conclusions:
The study in general, had shown that plant extract like garlic bulb, biological agents Trichoderma virens and Pseudomonas fluorescens, fungicides carbendazim and propineb, and chemical like benzoic acid prevent or drastically reduce the incidence of A. ochraceus on rice during storage. Therefore, further studies are warranted to deploy them usefully as seed treatment to avoid the occurrence of OTA contamination in rice.
23 INVASIVE ASPERGILLOSIS OF PITUITARY GLAND IN AN IMMUNOCOMPETENT PATIENT

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Purpose:
Invasive aspergillosis is a major cause of morbidity and mortality in immunosuppressed patients. This infection is caused by *Aspergillus*, a hyaline mould, the etiologic agent of many different manifestations. Although infection in apparently normal hosts can occur, aspergillosis is extremely uncommon in immunocompetent hosts.

Case Description:
A 63 year old woman, housewife, living in north of Iran, presented with the history of right eye ptosis after a mild head trauma since about 3 weeks ago. She had positive history of on and off headache, with good response to analgesic several weeks before ptosis. The patient was a known case of poorly controlled insulin dependent diabetes mellitus (DM) since about 20 years ago. The patient was HIV negative and no risk factor for immune deficiency was detected.

The patient’s left eye was blind due to cataract. During this period she had negative history of focal neurologic signs, left and right extremities weakness, inability to walk and sensory loss.

On physical examination, the patient was not febrile, other vital signs were normal. Mild ptosis of right eye was detected. There was no change in right eye vision during this period. Right eye pupil reflex was normal, extraocular movements of both eyes were normal. Cranial nerves were normal. There was no neck rigidity. Kernig’s and Brudzinski’s signs were negative. Other neurologic examinations were normal. Physical examinations of lung, heart, abdomen, extremities were normal.

Brain MRI was done that shows a pituitary mass measuring about 4 cm. Differential diagnoses in this stage were:

1. Pituitary adenoma
2. Granulomatous diseases (tuberculosis, fungal infection, etc.)
3. Vascular aneurysm

CT-angiography was done to rule out vascular aneurysm. The result was negative. Trans sphenoidal surgery was done and the mass was excised. Pathological study showed hyphae of aspergillosis (Figure 1, 2). Positive culture of aspergillus confirmed the diagnosis. Amphotericin B was started for her and then was changed to oral itraconazole. After surgery, in the hospital course ptosis gradually improved and headache disappeared. Evaluation of sinuses, chest, vertebrae and other sites shows no evidence of aspergillosis. The patient discharged with oral itraconazole 400 mg daily as maintenance therapy and out pateint follow up. She was followed for one year and no complication or relapse occurred and the patient’s condition is now good.

Conclusions:
Aspergillosis should be considered in differential diagnosis of pituitary gland mass. Itraconazole can be used as maintenance therapy for invasive aspergillosis.
POSSIBLE RESPIRATORY INFECTION DUE TO *ASPERGILLUS* IN WORKERS FROM SWINERIES AND POUltRIES

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**Introduction:**
*Aspergillus* is among a growing list of allergens that can aggravate asthmatic responses. Significant pulmonary pathology is associated with *Aspergillus*-induced allergic and asthmatic lung disease. Environments with high levels of exposure to fungi are found in animal production facilities like swineries and poultry which farmers are at increased risk of occupational respiratory diseases.

**Purpose:**
Seven Portuguese poultries and seven swineries were analyzed in order to estimate the prevalence, amount and distribution of *Aspergillus* species, as well as to evaluate the existence of clinical symptoms associated with asthma and other allergy diseases in these highly contaminated settings.

**Method:**
A total of 83 air samples (through impaction method), 66 surface samples (swab sampling) and 28 samples of different material covering their floor were analyzed.

**Results:**
The collected fungal isolates (699) were distributed by 40 different genus, *Aspergillus* presented a prevalence of 17.6% (average) and regarding poultries only, its prevalence showed a higher value (22.7%). The most frequently isolated *Aspergillus* species were *A. versicolor*, *A. flavus* and *A. fumigatus*. In poultries, *A. flavus* presented the highest level of airborne spores (>2000 ufc/m3) whereas in swineries was *A. versicolor*, with a prevalence four times higher than the other referred species.

Eighty workers of these settings were analyzed, with ages ranging from 17 to 93 years old. Seven of these workers were medical diagnosed with asthma and four of them reported the first attack after the age of 40 years old, which possibly could be related with their occupational exposure. Brain MRI was done that shows a pituitary mass measuring about 4 cm. Differential diagnoses in this stage were:

A high prevalence of respiratory symptoms in professionals without asthma was observed, namely wheezing associated with dyspnea (23.8%) and dyspnea after strenuous activities (12.3%), suggesting an under diagnosed respiratory problem. Moreover, 32.5% of all the inquired workers refer an improvement of their respiratory ability during the resting days and holiday.

**Conclusions:**
Considering the strong association between fungal sensitization and severity of asthma and that *Aspergillus* species are one of the most important fungal agents causing sensitization of the individual, this study contributes to the knowledge of *Aspergillus* prevalence and distribution in Portuguese poultries and swineries. These data, together with data regarding determinations of specific IgE levels and monitorization of mycotoxin exposure using biomarkers will also help to understand how and which *Aspergillus* species can affect workers of these settings.
POSSIBLE AFLATOXIN PRESENCE IN PORTUGUESE POULTRY UNITS

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Introduction:
Aflatoxins are known to be human carcinogens based on sufficient evidence of carcinogenicity in humans (hepatocellular carcinoma, or primary liver-cell cancer). Aflatoxin B1 is one of the most deeply studied mycotoxins, known for a long time as belonging to the group of toxins produced by the genus Aspergillus (A. flavus, A. parasiticus, A. niger). The presence in food stuffs depends on their geographical origin and production methods. Occupational exposure to aflatoxins can occur by inhalation of dust generated during the handling and processing of contaminated crops and feeds. Therefore, farmers and other agricultural workers have one of the greatest risks of occupational exposure to these mycotoxins.

Purpose:
To characterize A. flavus prevalence in seven poultry units, with emphasis to the possible presence of aflatoxin in the air.

Method:
A descriptive study was developed to monitor air fungal contamination in seven poultry units. Nineteen interior air samples of 25 litres were collected through impaction method.

Results:
From the seven poultry units analyzed, A. flavus was found in three of them. From all fungal genus identified in the referred units, A. flavus was the third species most frequently found in air samples (7.23%). Moreover, in those units, and from the Aspergillus genus, A. flavus was the most frequently isolated species in air samples (74.5%).

Conclusions:
Regarding the observed results and considering the high number of units contaminated by fungi known as possible aflatoxin producers, we have to believe that exposition can occur by inhalation (workers) and ingestion (consumers). This situation might represent a public health problem considering that aflatoxin is a known cancer agent.
INVASIVE ASPERGILLOSIS IN INTENSIVE CARE UNIT PATIENTS FROM IRAN

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Purpose:
In the present study we assessed the intensive care unit (ICU) patients for invasive aspergillosis (IA) with culture and non-culture based diagnostic methods from Iran.

Method:
Thirty six ICU patients with underlying predisposing conditions for IA were enrolled in the study. Sixty eight Bronchoalveolar lavage (BAL) samples were collected by bronchoscope twice a weekly. BAL samples were analyzed by direct microscopic examination using CalcoFlour White staining, fungal culture and GM detection. The Platelia Aspergillus GM EIA was used to quantify GM indices as per the manufacturer’s instructions. Samples with a BAL GM index ≥1 were considered as positive for GM. Patients were classified as having probable or possible IA, based on the European Organization for the Research and Treatment of Cancer/ Mycoses Study Group (EORTC/MSG) case definitions.

Results:
Out of 36 suspected patients to IA, 13 (36.1%) cases showed IA. According to criteria presented by EORTC/MSG, they were categorized as: 4 cases (30.8%) of possible IA and 9 (69.2%) of probable IA. A total of 21 BAL samples from patients with IA were analyzed by microscopic and culture methods; 18 (85.7%) and 11 (52.4%) of these samples were positive for septate hyphae and Aspergillus growth, respectively. A. flavus and A. fumigatus were equally isolated from positive samples for Aspergillus growth. Of these BAL collected samples, 16(76.2%) were positive for GM. From 13 patients with IA, 11(52.4%) had at least one positive BAL GM index. Of these patients, 9(81.8%) showed probable IA. The main underlying predisposing conditions were neutropenia (53.8%), COPD (30.8%) and hematologic malignancy (15.4%).

Conclusions:
The results of our study showed that IA is a life threatening infectious disease in ICU. Our study has also indicated that non classic risk factors especially COPD must be considered as a main predisposing condition for occurrence of aspergillosis in ICU patients. Our data have also revealed that GM detection in BAL samples play a significant role to IA diagnosis.
POSACONAZOLE PLASMA CONCENTRATIONS AND INVASIVE MOULD INFECTIONS IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Purpose:
Posaconazole is a new triazole antifungal agent that has broad activity against pathogenic fungi and is increasingly used for prophylaxis and treatment of invasive mould infections (IMI). Posaconazole is available only as oral formulation with varying absorption from the gastro-intestinal tract. Reports correlating posaconazole plasma concentrations (PPCs) with breakthrough IMI, however, are rare. The study objective was to analyze posaconazole serum levels in patients with hematologic malignancies and posaconazol prophylaxis.

Method:
We analyzed posaconazole plasma concentrations (PPCs) in patients with hematological malignancies and evaluated the impact on antifungal prophylaxis, therapy and outcome by conducting a prospective observational single-centre study for seven months in 2010.

Results:
A total of 109 PPCs were measured in 34 patients receiving posaconazole prophylaxis (n=31) or treatment (n=3). Insufficient levels were detected in 70% (24/34) of patients; in 15 of these patients concentrations were found under the limit of detection (<0.20 µg/ml). Insufficient PPCs yielded either way in a modification of intake procedures, discontinuation of PPIs or switch of antifungal therapy. In 12 of these 24 cases with insufficient PPC modification of intake, i.e. with a high fat meal, led to sufficient PPCs. As discontinuation of PPIs had benefit in only 1/24 cases, antifungal therapy had to be switched due to insufficient PPCs in 5 cases. In three patients with insufficient PPCs antifungal therapy had to be changed from posaconazole prophylaxis to echinocandin empiric treatment due to development of febrile neutropenia. In these patients no fungal pathogen was detected. Three patients on posaconazole prophylaxis met the criteria of probable or proven breakthrough IMI. Prior to development of invasive fungal infection (IFI), however, PPCs were insufficient in all three patients (0.28, <0.20, and 0.31 µg/ml respectively).

Table: IMI under Posaconazole prophylaxis

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Patient’s age years/sex</th>
<th>Specimen of fungal detection</th>
<th>Days of posaconazole prophylaxis before breakthrough infection</th>
<th>Last posaconazole plasma level before diagnosis of IMI (days before diagnosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>58/f</td>
<td>BAL</td>
<td>8</td>
<td>0.28 µg/ml (1)</td>
</tr>
<tr>
<td><em>Aspergillus spp.</em></td>
<td>20/m</td>
<td>BAL / Serum</td>
<td>23</td>
<td>0.20 µg/ml (4)</td>
</tr>
<tr>
<td><em>Geosmithia argillacea</em></td>
<td>52/m</td>
<td>BAL / Blood culture</td>
<td>&gt;60</td>
<td>0.31 µg/ml (7)</td>
</tr>
</tbody>
</table>

Abbreviation: f, female; m, male; BAL, Bronchoalveolar-lavage fluid
Conclusions:
Our data suggest that insufficient PPCs may lead to development of IMI which may be misinterpreted as breakthrough infection, as three patients developed insufficient PPC associated probable or proven IMI while on posaconazole prophylaxis. Previously four cases of possible IFI and one proven IFI under posaconazole prophylaxis all associated with insufficient PPCs have been reported. Considering that we found insufficient posaconazole plasma levels in 70% of patients testing of PPCs on a regular basis seems to be meaningful. Considering that we found insufficient posaconazole plasma levels in 70% of patients, introduction of PPC measurement on a regular basis and, if necessary, consecutive modification of intake procedures or switch of antifungal therapy may lead to a decrease in rates of invasive mould infection.
RISK FACTORS ASSOCIATED WITH LOW POSACONAZOLE PLASMA CONCENTRATIONS IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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3AGES PharmMed/Austrian Agency for Health and Food Safety, Vienna, Austria.

Purpose:
Posaconazole has broad activity against pathogenic fungi and is an attractive option for prophylaxis and treatment of invasive fungal infections. Recent data have shown the benefit of therapeutic drug monitoring (TDM) in patients receiving posaconazole. Various factors had been reported to influence posaconazole absorption, including age, food (specifically fat), gastric pH and the use of proton pump inhibitors (PPIs), body mass index (BMI), or gastrointestinal disorders. Whether TDM is indicated in all immunosuppressed patients receiving posaconazole or only in subgroups (e.g. patients with gastrointestinal disorders) is still a point of discussion. We analyzed risk factors associated with low posaconazole plasma concentrations (PPCs) in patients with hematologic malignancies.

Method:
We analyzed posaconazole plasma concentrations (PPCs) in patients with hematological malignancies by conducting a prospective observational single-centre study for seven months in 2010. PPCs were measured by using High Performance Liquid Chromatography and indicated as sufficient if above 0.50 µg/ml (limit of detection 0.20 µg/ml). Factors previously described as being associated with PPC were assessed in patients receiving posaconazole medication. Statistical analysis was performed using SPSS, version 17 (SPSS Inc., Chicago, IL, USA). A P-value of less than 0.05 was considered statistically significant.

Results:
PPCs were significantly associated with type of chemotherapy (P= 0.001) and found highest in patients receiving low dose/palliative chemotherapy (median 0.90 µg/ml; [IQR] 0.55-1.29 µg/ml), and lowest in the high dose or induction chemotherapy receiving group of patients (median 0.29 µg/ml; [IQR] <0.20-0.59 µg/ml, Kruskal-Wallis test). PPI treatment was associated with higher PPCs compared to no PPI treatment (P= 0.043). Correlation between PPI treatment and type of chemotherapy was ruled out (Spearmans rho 0.016; p=0.712). Linear regression analysis failed to identify a significant relationship between PPCs and patient age (R2= 0.004), body mass index (R2= 0.002), or weight (R2= 0.004). Fishers exact test identified low PPC being more prevalent among women than men (65% versus 43%; P= 0.033) and tending to be associated with therapy rather than prophylaxis (P=0.062). No association was found for other parameters such as gastrointestinal symptoms (emesis and/or diarrhea), age, BMI, weight, or GVHD (data not shown).

Conclusions:
Interestingly, there was a significant positive correlation between PPI treatment and high PPCs. These findings are in contrast to previous findings reporting decreased PPCs in case of concomitant use of PPIs. Other studies, however, found no correlation between insufficient PPCs and PPI treatment. Due to low patient numbers further studies are needed to investigate this issue. For the first time this study reports an association of PPCs with chemotherapeutic approach used for treatment of underlying hematological malignancy and development of IFI. Other than some previous reports we found no association between PPCs and BMI, weight, age, diarrhea (3 patients) and/or emesis (3 patients).

Given that previously reported risk factors for insufficient PPCs vary between studies, we suggest that posaconazole TDM should be performed in all patients with hematological malignancies receiving posaconazole prophylaxis/treatment independent of potential risk factors.
IMPACT OF TESTING POSACONAZOLE PLASMA CONCENTRATIONS ON EPIDEMIOLOGY OF ANTIFUNGAL PROPHYLAXIS AND THERAPY IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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⁴AGES PharmMed/Austrian Agency for Health and Food Safety, Vienna, Austria

Purpose:
Posaconazole is a new triazole antifungal agent that has broad activity against pathogenic fungi and increasingly used for prophylaxis and treatment of invasive mould infections (IMI). Posaconazole is available as oral formulation only with varying absorption from the gastro-intestinal tract. We analyzed posaconazole plasma concentrations (PPCs) in patients with hematologic malignancies and evaluated the impact on antifungal prophylaxis and therapy.

Method:
Applied antifungal prophylaxis and therapy were assessed in patients with underlying hematologic diseases by conducting a prospective observational single-centre study at the Division of Hematology, Medical University of Graz, Austria, for seven months in 2010. To analyze the impact of PPC testing on antifungal prophylaxis and therapy results obtained were compared to a representative collective of patients assessed by the same investigators at the same institution over seven months in 2007 before testing of posaconazole plasma levels has been introduced.

Results:
In 2010, 129/729 (18%) of cases with hematologic malignancies received systemic antifungal prophylaxis and therapy. Of those, fifty-seven percent of cases received prophylactic, 44% empiric, 30% preemptive and 6% directed antifungal therapy. Main reasons for prophylaxis were neutropenia in AML patients (40/74; 54%), followed by GVHD (18/74; 24%) and allogeneic HSCT (16/74; 22%). Eleven out of 39 (28%) cases receiving preemptive therapy had clinical/radiological and microbiological evidence of IFI, 26/39 (67%) had clinical/radiological and 2/39 (5%) only microbiological evidence of IFI. In 2010 posaconazole was the most commonly administered antifungal agent followed by caspofungin which had been the leading antifungal agent at the study site in 2007. Posaconazole usage increased significantly after introduction of posaconazole TDM (P <0.05). Concerning prescription rates of antifungal agents other than posaconazole no significant difference was found. In both study collectives (2007 and 2010) posaconazole was the primary antifungal agent used for prophylaxis, while itraconazole was used mainly in allogeneic HSCT. Demographic data, chemotherapeutic approach and antifungal modalities for cases receiving antifungal therapy in 2007 and 2010, respectively, are depicted in table 1.
Table 1: Demographic data, and antifungal therapy among patients with hematologic malignancies

<table>
<thead>
<tr>
<th></th>
<th>Antifungal therapy 2007 (b)</th>
<th>Antifungal therapy 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>117/690 (17%)</td>
<td>129/729 (18%)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>70/117 (60%)</td>
<td>85/129 (66%)</td>
</tr>
<tr>
<td>female</td>
<td>47/117 (40%)</td>
<td>44/129 (34%)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median age</td>
<td>51,2</td>
<td>53,5</td>
</tr>
<tr>
<td><strong>Chemotherapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>high dose (a)</td>
<td>55/117 (47%)</td>
<td>81/129 (63%)</td>
</tr>
<tr>
<td>palliative (a)</td>
<td>39/117 (33%)</td>
<td>17/129 (13%)</td>
</tr>
<tr>
<td><strong>Rational for antifungal therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>directed</td>
<td>4/117 (4%)</td>
<td>8/129 (6%)</td>
</tr>
<tr>
<td>preemptive (a)</td>
<td>50/117 (43%)</td>
<td>39/129 (30%)</td>
</tr>
<tr>
<td>empiric (a)</td>
<td>31/117 (36%)</td>
<td>57/129 (44%)</td>
</tr>
<tr>
<td>prophylactic</td>
<td>52/117 (44%)</td>
<td>74/129 (57%)</td>
</tr>
<tr>
<td><strong>Antifungal agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>posaconazole (a)</td>
<td>38/117 (32%)</td>
<td>81/129 (63%)</td>
</tr>
<tr>
<td>caspofungin</td>
<td>72/117 (62%)</td>
<td>65/129 (50%)</td>
</tr>
<tr>
<td>lip amph B</td>
<td>7/117 (6%)</td>
<td>10/129 (8%)</td>
</tr>
<tr>
<td>voriconazole</td>
<td>14/117 (12%)</td>
<td>18/129 (14%)</td>
</tr>
<tr>
<td>itraconazole</td>
<td>15/117 (13%)</td>
<td>19/129 (15%)</td>
</tr>
<tr>
<td>fluconazole</td>
<td>7/117 (6%)</td>
<td>7/129 (5%)</td>
</tr>
<tr>
<td>anidulafungin</td>
<td>0</td>
<td>1/129 (1%)</td>
</tr>
</tbody>
</table>

**Note:**

\(a\) Significant difference between 2007 and 2010 in patient collective with antifungal therapy \(P <0.05\)

\(b\) Data from 2007 previously published (11)

**Conclusions:**

We found a significant increase of posaconazole usage after introduction of posaconazole therapeutic drug monitoring (TDM). As TDM was available for posaconazole only, the feasibility of monitoring plasma concentrations may have influenced the selection of this antifungal agent in clinical routine. Prescribing regimens did not change significantly and study collectives were comparable concerning prescription rates of antifungal agents other than posaconazole. We did, however, observe a significant increase in the empiric and decrease in the preemptive treatment approach in 2010 compared to 2007. This shift was based on the recent findings by Cordonnier et al. suggesting that preemptive treatment increases the incidence of invasive fungal disease and that empirical treatment may provide better survival rates for patients receiving induction chemotherapy.
CAFFEINE INDUCED GERMINATION ABNORMALITY IN *ASPERGILLUS FLAVUS*

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**Purpose:**
Caffeine, a 3,7 methylxanthine is one of the quality components of tea and occur in black tea ranging from 2-3.5 percent depending on the type of tea cultivars and methods of processing. Inhibitory influence of caffeine on mold and yeasts are well known and interaction of caffeine known to interfere in the biosynthetic pathway of aflatoxin formation by toxigenic species of *A. flavus*. The present study was initiated to measure the extent of growth inhibition and germination abnormality in an isolate of *A. flavus*(icft-1234)caused by different concentration of caffeine.

**Methods:**
Water agar (2%) mixed with measured quantity of caffeine was used to get desired concentration of 1,10,100,1000 and 10000 ppm for germination test as per (APS-1934). The fungus *A. flavus* was raised on Czapek medium mixed with above quanty of caffeine and incubated for recording mycelial dry weight.

**Results:**
It was observed that caffeine reduced biomass production of *A. flavus* which increased with increasing concentration of caffeine. Caffeine exerted considerable inhibitory influence on conidial germination of *A. flavus* accompanied by swelling of germ tubes and repeated curling of germ tube hyphae. At 1000 ppm caffeine caused increased growth abnormality of reproductive structures (vesicles and conidiophores) and significant reduction of spore production.

**Conclusions:**
The antifungal properties of caffeine was discussed in relation to risk reduction in mold and mycotoxin problem in tea processing.
RECENT DEVELOPMENTS IN THE PATHOGENESIS OF AVIAN ASPERGILLOSIS

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Purpose:
Aspergillosis is an important cause of morbidity and mortality in birds. Aspergillosis in birds is mainly caused by *Aspergillus fumigatus*, a ubiquitous and opportunistic saprophyte. Although avian aspergillosis is a frequently occurring disease, the pathogenesis in birds is poorly known.

Methods:
In a first study, we examined whether aspergillosis in birds is caused by avian adapted strains. For this purpose, microsatellite length polymorphism was used to compare environmental, human and avian isolates of *A. fumigatus*. Short tandem repeat (STR) typing, based on a panel of 9 STR markers, was used to analyze 65 clinical avian isolates and 23 environmental isolates of *A. fumigatus*. Typing data were imported into BioNumerics and analyzed using the categorical multistate similarity coefficient with UPGMA clustering. The 78 genotypes obtained were compared to a database containing genotypes of approximately 2500 strains isolated from human clinical samples and from the environment. No specific association between the observed genotypes and the origin of the isolates (environment, human or bird) was found.

Secondly, we examined whether host susceptibility varies between avian species. For this purpose, we compared the course of an experimental infection with *A. fumigatus* in infected falcons (supposedly highly susceptible) and pigeons (supposedly low susceptible). We inoculated hybrid falcons and pigeons with different dosages of *A. fumigatus* conidia (10^7, 10^5 and 10^3) intratracheally. The animals were weighed daily and observed at least twice daily. The presence of ruffled feathers, dyspnea, sneezing and stridor were scored daily. Animals with severe dyspnea (open beak breathing) or extreme weight loss were euthanized. At 28 days post inoculation all animals were euthanized. At necropsy, macroscopic lesions were described. Samples of the trachea, lungs, air sacs, heart, pericardium, liver, kidney, brain, pectoral muscle, and abdominal fluid were inoculated on Sabouraud dextrose agar plates and incubated at 37°C at aerobic conditions to isolate *A. fumigatus* for 72 h. Samples of the lungs, air sacs, liver, spleen and kidney were fixed in phosphate buffered formaldehyde solution, sectioned and stained with Hematoxylin and Eosin (HE) and Periodic acid Schiff reagents (PAS) for visualization of fungal elements.

Results:
Clinical signs including loss of appetite, vomiting, discolouration of urates and dyspnoea were observed in 4 out of 5 falcons and 2 out of 5 pigeons inoculated with 10^7 *A. fumigatus* conidia. In the lower dosage groups, no clinical signs were noticed except in one falcon inoculated with 10^5 *A. fumigatus* conidia. Necropsy revealed the presence of granulomas in the air sacs in 4 out of 5 falcons and 3 out of 5 pigeons in the high dosage group. *A. fumigatus* could be isolated from these granulomas in 3 falcons and 2 pigeons. The presence of fungal hyphae was detected with PAS staining in 3 out of 5 falcons and 3 out of 5 pigeons in the high dosage group. In the others dosage groups, no granulomas, no positive cultures or fungal hyphae were present. It was not possible to demonstrate respiratory macrophages in healthy lungs of falcons and pigeons with a concanavalin A staining. A lot of macrophages were observed in and around fungal granulomas of diseased lungs and airsacs demonstrating its role in the defense against avian aspergillosis. In conclusion, falcons were not more susceptible to aspergillosis than pigeons under experimental conditions.

Finally, we studied the early interaction between the avian respiratory macrophage and *A. fumigatus* spores. The phagocytic and killing capacity of avian respiratory macrophages was evaluated using pigeon respiratory macrophages that were inoculated with *A. fumigatus* conidia. On average, 25% of the conidia were phagocytised after one hour and 16.4% of the conidia were killed after 5 hours in pigeon respiratory macrophages. In 2% of the pigeon respiratory macrophages, intracellular germination of *A. fumigatus* conidia occurred after 6 hours. Intracellularly germinating conidia were located free in the cytoplasm, as shown using transmission electron microscopy.

Conclusions:
The results showed that avian respiratory macrophages may prevent early establishment of infection unless overwhelming amounts of *A. fumigatus* conidia exceed their killing capacity, leading to intracellular germination and colonization of the respiratory tract.
SPECTRUM OF INFECTIONS ASSOCIATED WITH *ASPERGILLUS* SPECIES IN TERTIARY CARE HOSPITAL

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**Purpose:**

*Aspergillus* species cause a variety of diseases including allergic syndrome, colonizing sinuses or cavities, acute or subacute and chronic invasive diseases. We have reviewed spectrum of infections associated with *Aspergillus* spp., in the clinical samples received during Jan 2010- May 2011

**Methods:**

All the samples from the suspected cases of Aspergillosis received during these periods were processed by standard laboratory procedure like direct microscopy and culture

**Results:**

A total of 59 clinical samples were positive by both direct microscopy & culture. Of these, 44 (74.6%) were having fungal sinusitis/nasal mass, 5 (8.4%) had respiratory tract involvement, 3 (5%) had brain tissue involvement, 2 (3.4%) each were of infective endocarditis & dermatologic involvement & 1 (1.7%) each were from dental eschar, superficial involvement of exposed heart in ectopic cordis & chest wall mass. Additionally, 2 cases were of disseminated aspergillosis in a renal transplant patient & in a HIV positive patient, wherein, bone marrow aspirate & blood culture grew *Aspergillus* spp. Furthermore, we also identified 22 cases which were culture negative but, showed hyaline, thin, septate hyphae on direct microscopy. Of these, 12 (54.5%) were of fungal sinusitis/ nasal mass, 6 (27.3%) had respiratory tract involvement, 3 (13.6%) were having cutaneous involvement & 1(4.5%) was from lung biopsy in a patient of Acute Myeloid Leukemia having lung mass. Of the 61 culture positive cases *Aspergillus flavus* was grown in 50 (82%), *A. fumigatus* in 9 (14.7%) & *A. niger* & *A. nidulans* in 1 (1.6%) case each

**Conclusions:**

*Aspergillus* is still a major problem causing spectrum of infections in our country. In an attempt to recognition pathogen in various sample and its distribution of the species helps the clinician for better management of the patients.
**Purpose:**

Few patients have been described previously with *Aspergillus* (aspergillary) bronchitis, unless immunocompromised. We reviewed records of patients referred who fulfilled proposed criteria for *Aspergillus* bronchitis.

**Method:**

From >400 patients referred to the National Aspergillosis Centre we conducted a retrospective chart review of possible *Aspergillus* bronchitis. Patients with persistent chest symptoms or bronchial obstruction who did not fulfill criteria for allergic, chronic or invasive pulmonary aspergillosis were analysed. Patients with an elevated *Aspergillus* IgG or precipitins and a positive culture or *Aspergillus* real-time PCR, were reviewed.

**Results:**

28 patients’ notes were examined; 17 fulfilled the criteria selected for review. 14 were women and the mean age was 57 years (range 39-76). 4 were overtly immunocompromised, 2 were not immunocompromised at all, and 11 had subtle immunocompromising factors. 16 had a productive cough and 8 high volume tenacious sputum. 8 had MRC dyspnoea scores of 4-5 (1 = normal, 5 is breathless getting dressed or talking). 7 had recurrent chest infections, and 4 significant fatigue. 3 had lost weight and 2 prior haemoptysis. 12 of 14 (86%) patients had bronchiectasis on CT scan. Bacterial co-infection was common, but un-responsive to antibiotics. 13 grew *A. fumigatus*, 3. *A. niger* and 1 *A. terreus*. 12 of 17 had elevated *Aspergillus* IgG (47-137mg/L, mean 89.2) (Phadia) and 5 had elevated *Aspergillus* precipitins, 4 with a normal *Aspergillus* IgG. 7/15 (47%) had a major response to oral antifungal therapy the first course given for 1-52 weeks (median 20 weeks) and 5/8 (63%) who discontinued therapy relapsed. 8 of 15 (53%) had adverse reactions to itraconazole, 3 of 4 (75%) with voriconazole.

**Conclusions:**

Patients with underlying structural lung disease usually with immunological compromise of varying severity may present with *Aspergillus* (aspergillary) bronchitis. We suggest that the combination of relapsing chronic bronchitis symptoms with microbiological (culture or PCR) and immunological (*Aspergillus* IgG) evidence of *Aspergillus* infection are characteristic of *Aspergillus* bronchitis. This entity needs to be distinguished from asymptomatic fungal colonisation as well as invasive, allergic and chronic pulmonary aspergillosis. *Aspergillus* bronchitis responds to antifungal therapy.

**NOTE:** THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION
ISOLATION AND IDENTIFICATION OF *ASPERGILLUS SPP* AND OTHER MOULD FROM POULTRY FARMS IN KHARTOUM STATE

W Abdalla*

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**Purpose:**
To determine the source of the cases of aflatoxins in poultry farm to know if the feed the only source of infection.

**Methods:**
Twenty water samples were collected from ten different poultry farms in Khartoum state, from tap water, tanks and drinkers in sterile sample bottles (400ml). Fungi were isolated by using plating method, 500 μl of samples were placed on agar plates. Each colony from the primary plate was sub-cultured onto fresh Sabouraud’s dextrose agar. This was further incubated at room temperature for 7 days. Fungal colonies were isolated upon formation, stained with lactophenol cotton blue and observed under the microscope Fungi so observed were identified using appropriate taxonomic guides.

**Results:**
Eleven species of mould belonging to nine genera were isolated. These included: *Aspergillus nigar* (20.9%), *A. flavus* (19.4%), *A. terreus* (17.9%), *Penicillium, Mucor, Curvularia, Cladosporium, Rhizomucor, Aureobasidium, Alternaria* and *Exerophilum.*

**Conclusions:**
Water can be source of aflatoxins cases in poultry farms.
URETERAL OBSTRUCTION DUE TO \textit{ASPERGILLUS TERREUS} IN A DIABETIC PATIENT FOLLOWING URETEROSCOPIC LITHOTRIPTSY: A CASE REPORT

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\textsuperscript{2}Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
\textsuperscript{3}Urology and Nephrology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
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\textsuperscript{5}Department of Infectious Diseases, Shahid Beheshti University of Medical Sciences Tehran, Iran
\textsuperscript{6}Private Clinic, Ghemshahr, Iran
\textsuperscript{7}Department of Radiology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

\textbf{Purpose:}
Renoureteral aspergillosis following ureteroscopic lithotripsy and insertion of ureteral stent is a rare entity. Due to inadequate sterilization of the instrument during the ureterscopy and insertion of stent, a localized \textit{aspergillus} infection may occur which may cause pyouria, ureteral obstruction, perinephric abscess and hydronephrosis.

\textbf{Case presentation:}
We describe a case of unilateral ureteral obstruction caused by \textit{Aspergillus terreus} following ureteroscopic lithotripsy in a 45 years old diabetic man. He admitted with complain of passing whitish soft mass along with urine and hydronephrosis in left kidney. Three months prior to hospital admission, he had undergone ureteroscopic lithotripsy for removal of stone and a ureteral stent (DJ catheter) had been placed for three weeks. However, due to abnormal discharge and persistent obstruction the stent was removed and prescribed antibiotics for while. As he did not respond to antibiotic treatment, the retrograde pylography was done and removed the stent and placed the new one. In ureteral washing and urine a whitish soft mass were visible and a mesh of fungal hyphae were seen in direct microscopy exam and culture in SDA yielded growth of \textit{Aspergillus terreus}. He was treated initially with oral itraconazole 400mg/day for one week and then the dosage is diminished to 200mg/ day due gastric intolerance. However it was stopped after 25 days due to persistent whitish mass in urine. The isolate was susceptible to caspofungin (0.038 μg/mL), posaconazole (0.032 μg/mL), itraconazole (0.032 μg/mL) and voriconazole (0.023 μg/mL) in Etest antifungal susceptibility test. The endoscopic removal of fungal mass was done and antifungal was changed to oral voriconazole (400mg/day). Sonography and CT scan showed a complete relief, the patient’s renal function completely recovered; ceased passing whitish mass in urine, and urine culture was negative for fungi after 4 month of treatment.

\textbf{Conclusions:}
Obstructive uropathy related to \textit{Aspergillus} mass may be suspected in diabetic patient with history of manipulation, impaired renal function and persistent passage of a soft mass in urine. Direct microscopy and culture of multiple urine and ureteral washing is necessary for diagnosis. Treatment with voriconazole and endoscopic removal mass are effective.
ROLE OF REAL TIME PCR AND GALACTOMANNAN IN THE CLASSIFICATION OF ASPERGILLUS DISEASE IN CYSTIC FIBROSIS

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²School of Translational Medicine, University of Manchester, Manchester, UK
³Manchester Adult Cystic Fibrosis Centre, University Hospital of South Manchester, Manchester, UK

Purpose:
Cystic fibrosis (CF) patients with allergic bronchopulmonary aspergillosis (ABPA) are routinely treated with azole antifungals. However, it is not known whether Aspergillus colonised or sensitised patients would similarly benefit from antifungal treatment. To aid these treatment decisions and monitor treatment response, accurate methods to detect Aspergillus in sputum are first needed. This study aimed to validate and then integrate real time PCR and galactomannan antigen detection, from CF sputum, with serological analysis to identify patients groups that may benefit from antifungal therapy.

Methods:
146 adult CF patients provided a sputum sample and a blood sample. 30 of these patients provided a second sputum sample within 6 months. Serological tests included total IgE (tIgE), specific A.fumigatus IgE (sIgE) and specific A.fumigatus IgG (sIgG) performed by Phadia ImmunoCAP® assay. Sputum samples were homogenised with sputasol (Oxoid, UK) and sonication for 120 seconds (Sonics® VC505). 10µL was cultured on sabouraud agar with chloramphenicol (Oxoid, UK) for 72 hours at 37°C. 300µL was used in the Platelia™ Aspergillus EIA kit (Bio-Rad, Marrnes-La-Cocquette, France) to measure GM antigen, the cut-off optical index of ≥ 0.5 being used to define positivity. The remaining sputum sample had fungal DNA extracted using the MycXtra™ DNA extraction kit (Myconostica, Manchester, UK) and Aspergillus DNA was detected using the real time PCR kit MycAssay™ Aspergillus (Myconostica, Manchester, UK). Latent class analysis, using Mplus version 6.1 software, defined patient groups.

Results:
37% (39) of the 146 sputum samples were positive for Aspergillus species by standard culture whereas 74% (108) were positive for Aspergillus species using real time PCR. Repeatability over 6 months was performed in 30 patients. All PCR positive patients remained positive and 5 negative patients became positive. PCR repeatability within a sputum sample was performed for 10 samples. Repeatability was 100% with Ct values varying by <1 cycle. 47% (68) of the 146 samples were GM positive. Over 6 months all positive patients remained positive and 5 negative patients became positive - the same 5 patients that became PCR positive. GM inter-assay coefficient of variation (CV) over 5 days was 12% and intra-assay CV was 5%. Latent class analysis identified 4 classes based on serological results, PCR and GM. The average latent class probabilities were: class 1: 0.981, class 2: 0.999, class 3: 0.921, class 4: 0.992. Table 1 demonstrates the serological, PCR and GM characteristics of each class.

Conclusions:
Real time PCR can more accurately identify CF patients with Aspergillus in their sputum than standard culture. Both real time PCR and GM demonstrate good repeatability in CF sputum and may be used to monitor treatment response. Latent class statistical analysis suggests the presence of 4 distinct groups of patients based on serological, PCR and GM data. These 4 classes can be clinically interpreted as: Class 1 = normal, Class 2 = ABPA, Class 3 = sensitised and Class 4 = Aspergillus bronchitis. A randomised trial of antifungal therapy is required to determine if there is clinical benefit in treating class 3 or 4 patients.
FALSE POSITIVITY OF ASPERGILLUS GALACTOMANNAN TEST IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES PATIENTS

Y Oz¹, F Aksit¹, M Aslan¹, MO Akay², G Durmaz¹, N Kiraz³

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²Department of Hematology, Eskisehir Osmangazi University Faculty of Medicine, Eskisehir, Turkey
³Department of Microbiology, Istanbul University Cerrahpasa Medical Faculty, Istanbul, Turkey

Purpose:
Galactomannan (GM) antigen test monitoring is an approved noninvasive tool for early detection of invasive aspergillosis (IA) that has been validated in adult patients with hematologic malignancies and undergoing bone marrow transplantation. However, the observation of false-positive results of GM test is a major disadvantage of this technique and it caused to unnecessary invasive intervention or antifungal therapy. We aimed to investigate the rate and reasons of false positive GM test results in our institute.

Methods:
We evaluated the patients with hematological malignancies in our hospital from January 2008 to May 2011 retrospectively. During hospitalization, serum galactomannan levels were measured twice a week using the Platelia Aspergillus EIA test kit. Positivity criterion of GM antigenemia test was defined as two consecutive GM test results with an optical density index above 0.5. Patients were evaluated according to criteria of the European Organization for Research and Treatment of Cancer.

Results:
During the analysis period, 5624 serum samples were obtained from 2127 hospitalization periods of 945 patients and at least one serum specimen was positive for 150 (15.8%) of these patients for Aspergillus GM antigen test. Twenty-six patients had at least two consecutive positive test results. These patients were diagnosed as acute leukemia (n=15), malignant lenfoma (n=5), multiple myeloma (n=4), and aplastic anemia (n=2). Fifteen of 26 patients had clinical features of IA and were diagnosed as probable IA. Remaining 11 patients with positive antigenemia test had no adequate evidences for diagnosis of proven or probable IA and their episodes were considered as false-positive. While, 6 of 11 patients were treated with a beta lactam antibiotic (piperacillin-tazobactam or cepharerazone-sulbactam), 5 patients did not receive any antibiotherapy and 4 of them had the diagnosis of multiple myeloma.

Conclusions:
The Aspergillus galactomannan test is a valuable tool in the diagnosis of invasive aspergillosis and it was introduced as microbiological evidence in the European Organization for Research and Treatment of Cancer (EORTC) criteria for opportunistic invasive fungal infection. However, one of the major limitations of this test is false positivity. We reported false-positive results in patients with multiple myeloma and receiving beta lactam antibiotics. In addition to beta lactam antibiotic treatment, multiple myeloma may also be an important cause of false GM positivity.
BRONCHIAL ASPERGILLOSIS DUE TO ASPERGILLUS UDAGAWAE: A RARE CASE REPORT TITLE

S Ide1*, K Izumikawa1, H Gyotoku1, A Minematsu1, K Hirano1, N Hosogaya1, Y Nagayoshi1, M Tashiro1, T Mihara1, Y Morinaga2, S Nakamura1, Y Imamura1, T Miyazaki2, H Ikeda1, H Kakeya1, Y Yamamoto1, K Yanagihara2, T Kanda1, T Tashiro1, S Kohno1

1Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
2Department of Laboratory Medicine, Nagasaki University Hospital, Nagasaki, Japan
3Department of Respiratory Medicine, Goto Chuoh Hospital, Nagasaki, Japan

Purpose:
Aspergillus udagawae and A. fumigatus share similar morphological features but are genetically different. This is an important clinical distinction because A. udagawae is less sensitive to amphotericin B compared to A. fumigatus. We present a rare case of bronchial infection with A. udagawae that was successfully treated with voriconazole with the results of the profile of the strain isolated in this case acquired by several in vitro studies.

Methods:
A case report.

Results:
An 82-year-old female patient with diabetes mellitus complained bloody sputum and admitted. Bronchoscopy revealed white plugged region at the origin of right B4. Cytology found a clot of filamentous fungi and Aspergillus spp. was detected by culture. Molecular identification revealed that causative agent was A. udagawae. MICs of itraconazole, voriconazole and amphotericin B were 0.5, 0.5 and 2 μg/ml, respectively and MEC of micafungin was ≤ 0.015μg/ml.

The patient was treated with voriconazole for a month and switched to itraconazole due to drug-induced hepatitis. Her symptoms were improved within several days, and treatment was discontinued after three months. The infiltrates in chest X-ray films were completely diminished, and no recurrence has been confirmed to date.

The pathogenesis of this strain was evaluated using immunosuppressed mice model; A. udagawae is statistically less virulent compared to A. fumigatus B-5233, reference strain. Effect of hydrogen peroxide on these strains were evaluated by XTT assay and there was significant reduction in the metabolic activity of A. udagawae compared to that of A. fumigatus.

Conclusions:
The A. udagawae isolated in this case was 1) less sensitive to amphotericin B, 2) less virulent in immunosuppressed mice and 3) more sensitive to hydrogen peroxide, features that are almost identical to those of previously reported A. udagawae strains. We should be aware that new Aspergillus strains might pose a clinical threat.
A CASE OF CHRONIC PULMONARY ASPERGILLOSIS DUE TO ITRACONAZOLE LOW-SUSCEPTIBLE ASPERGILLUS FUMIGATUS OF WHICH RESISTANCE ACQUIRED DURING LONG-TERM ADMINISTRATION OF ITRACONAZOLE

K Hirano1*, K Izumikawa1, M Tashiro1, S Ide1, N Iwanaga1, A Minematsu1, Y Morinaga1, S Kurigihara1, S Nakamura1, Y Imamura1, T Miyazaki1, M Tsukamoto1, H Kakeya1, Y Yamamoto1, K Yanagihara2, T Tashiro1, S Kohno1

1Department of Molecular Microbiology and Immunology, Nagasaki University, Graduate School of Biomedical Sciences, Nagasaki, Japan
2Department of Laboratory Medicine, Nagasaki University Hospital, Nagasaki, Japan

Introduction:
The tolerance to azole antifungal drug has become a serious concern recently. We previously found that the frequency of itraconazole (ITCZ) resistant *Aspergillus fumigatus* strains isolated in Nagasaki, Japan was 7.1%. Retrospective study of azole low-susceptible strain isolated cases revealed that there was a case of chronic pulmonary aspergillosis due to ITCZ low-susceptible *A. fumigatus* of which resistance acquired during long-term administration of ITCZ.

Case:
Fifty nine years-old male with history of pleuritis and operation of pneumothorax of both lungs, complained hemosputum and fever. He was diagnosed as chronic pulmonary aspergillosis with the fungus ball among cystic lesions in chest CT and positive serum *aspergillus* antibody test.

ITCZ (200mg/day, orally) was given for 189 days continuously followed by 150mg/day of intravenous micafungin (MCFG) for 111 days due to unsatisfactory efficacy of ITCZ treatment. Since his symptom was improved after MCFG treatment, oral ITCZ (400-800mg/day) was continued and stopped after 394 days. Five months after discontinuation of ITCZ, hemosputum occurred again and 200-300mg/day of intravenous voriconazole (VRCZ) was started. The status was improved after 82 days of VRCZ infusion and discontinued. *A. fumigatus* had been isolated from his sputum continuously before the medication of VRCZ and disappeared after VRCZ treatment. The MIC (Minimum Inhibitory Concentration) of ITCZ, VRCZ, and posaconazole (POSA) of three *A. fumigatus* strains isolated within 189 days of cumulative period of ITCZ medication were 0.25-0.5 μg/ml, 0.25-0.5 μg/ml, and 0.06 μg/ml, respectively. Those MICs against six *A. fumigatus* strains isolated after 507 days of cumulative period of azole medication were 2- >8 μg/ml, 0.125-0.5 μg/ml, 0.5-2 μg/ml, respectively. A total of 9 *A. fumigatus* strains were confirmed as genetically identical by the microsatellite analysis. The genetic variation of cyp51A was found in 5 strains among 6 ITCZ low-susceptible *A. fumigatus* strains, and major genetic variation of G54E was identified.

Conclusions:
We experienced a chronic pulmonary aspergillosis case with ITCZ low-susceptible *A. fumigatus*, possibly the resistance acquired by long-term administration of azoles. Although the mechanism of acquiring resistance to azoles has not been revealed clearly, our case suggested the resistance may be induced by long-term exposure to azoles. Further investigation is warranted for understanding of azole resistance in *Aspergillus*. 
EVALUATION ON “REAL LIFE” PRESCRIPTIONS OF ANTIFUNGAL PROPHYLAXIS IN HIGH RISK PATIENTS: PRELIMINARY RESULTS FROM A PROSPECTIVE SURVEY

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1Hematology, Catholic University, Rome, Italy; 2Le Molinette Hospital, Turin; 3S.Giovanni Rotondo Hospital; 4Hematology, University of Udine; 5University of Parma; 6University of Bari; 7University of Firenze; 8Brescia Hospital; 9University of Cagliari; 10Lece Hospital; 11Federico II University, Napoli; 12University of Palermo; 13Hematology, San Matteo Hospital, Pavia; 14Catholic University, Campobasso; 15Internal Medicine, San Matteo Hospital, Pavia; 16Pescara Hospital; 17Reggio Calabria Hospital; 18San Camillo Hospital, Rome; 19San Giovanni Addolorata Hospital, Rome; 20Tor Vergata University, Rome; 21University of Bologna; 22University of Verona; 23University of Modena; 24Niguarda Hospital, Milan; 25University of Perugia

Purpose:
To describe the current use of antifungal (AF) prophylaxis in consecutive, unselected adult patient with acute myeloid leukemia patients (AMLs) at first induction of remission and to analyze the efficacy of prophylaxis with posaconazole (POS) when compared to old azoles in a “real life” setting.

Methods:
From January 2010 to March 2011, all newly diagnosed AMLs have been consecutively registered and prospectively monitored in 31 Italian participating centers. Only adult cases that received conventional chemotherapy were included in the present study. Principal demographic and clinical data, as well as antifungal treatments were collected. In particular we analyzed data about systemic AF prophylaxis: the drug of choice, the duration of treatment, and its efficacy were thus evaluated. To determine prophylaxis efficacy, incidence of IFDs was assessed at 30th day from the end of chemotherapy. IFDs outcome was also evaluated.

Results:
During the study period 498 pts received conventional chemotherapy as first induction for AML. Median age was 60 (range 18-81), with a male/female ratio of 1.6/1. The most part of them (448, 90%) received systemic antifungal prophylaxis. POS was the most frequently employed drug (224/448, 50%), followed by fluconazole (128, 29%) and itraconazole (86, 19%). When comparing the POS group (224 pts) to those receiving itraconazole or fluconazole (214 pts) (FLU/ITRA) no significant differences emerged in terms of the main risk factors for IFDs (table). In particular the 2 groups resulted to be comparable in terms of age, sex, frequency and duration of deep neutropenia, days of prophylaxis. On the contrary, there were significant differences in breakthrough IFDs (6.2% in POS vs 11.7% in FLU/ITRA, p-value 0.04). Except for one case of fusariosis, all mould infections were invasive aspergillosis. Yeast infections also were more frequent in the FLU/ITRA group. Caspofungin and amphotericin B compounds were the most frequently employed drugs, as empirical/pre-emptive treatments. There were no significant differences in the response rate, nor in the IFDs attributable mortality rate.
Table 1: comparison between POS and FLU/ITRA groups

<table>
<thead>
<tr>
<th></th>
<th>FLU/ITRA</th>
<th>POS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>57.7</td>
<td>53.5</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- male</td>
<td>112 (52%)</td>
<td>101 (45%)</td>
</tr>
<tr>
<td>- female</td>
<td>102 (48%)</td>
<td>123 (55%)</td>
</tr>
<tr>
<td>Deep neutropenia</td>
<td>210 (98%)</td>
<td>213 (95%)</td>
</tr>
<tr>
<td>Central venous catheter</td>
<td>119 (56%)</td>
<td>143 (64%)</td>
</tr>
<tr>
<td>Mean duration of deep neutropenia (days)</td>
<td>19.5</td>
<td>21.5</td>
</tr>
<tr>
<td>Median AF prophylaxis duration (days)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Empirical/pre-emptive therapies</td>
<td>59 (28%)</td>
<td>71 (32%)</td>
</tr>
<tr>
<td>Most used drugs as empirical/pre-emptive therapy</td>
<td>- L-AmB 18 (30%)</td>
<td>- L-AmB 19 (55%)</td>
</tr>
<tr>
<td></td>
<td>- Caspo 17 (29%)</td>
<td>- Caspo 12 (17%)</td>
</tr>
<tr>
<td></td>
<td>- Vorico 8 (14%)</td>
<td>- Vorico 6 (8%)</td>
</tr>
<tr>
<td></td>
<td>- CL-AmB 8 (14%)</td>
<td>- CL-AmB 2 (3%)</td>
</tr>
<tr>
<td></td>
<td>- Others 8 (14%)</td>
<td>- Others 12 (17%)</td>
</tr>
<tr>
<td>Proven/probable IFDs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Moulds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Aspergillus spp</td>
<td>25 (11.7%)</td>
<td>14 (6.2%)</td>
</tr>
<tr>
<td>- Other moulds</td>
<td>17 (7.9%)</td>
<td>11 (4.9%)</td>
</tr>
<tr>
<td>- Yeasts</td>
<td>8 (3.7%)</td>
<td>3 (1.3%)**</td>
</tr>
<tr>
<td>Moulds/yeasts ratio</td>
<td>2.1/1</td>
<td>3.7/1</td>
</tr>
<tr>
<td>Favourable responses (RR)</td>
<td>14/25 (56%)</td>
<td>11/14 (79%)</td>
</tr>
<tr>
<td>Nº deaths (AMR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- moulds</td>
<td>7/25 (28%)</td>
<td>2/14 (14%)</td>
</tr>
<tr>
<td>- Aspergillus spp</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>- yeasts</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>


* 1 Fusarium sporotrichoides
** 1 Trichosporon spp included

Conclusions:
During the last few years the use of POS prophylaxis in high risk pts has significantly increased. Although not randomized, our study demonstrates in a “real life” setting the increased use and the higher efficacy of POS prophylaxis, when compared to FLU/ITRA. Only 14 patients developed a breakthrough IFDs. Surprisingly, POS superiority emerged for both moulds and yeasts infections. Previous AF prophylaxis does not seem to impact IFDs outcome.
GRANULOCYTE TRANSFUSIONS AS ADJUNCTIVE TREATMENT OF INVASIVE Fungal DISEASES IN NEUTROPenic PATIENTS

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¹Hematology Catholic University, Rome, Italy

Purpose:
The degree and duration of neutropenia are crucial prognostic factors in hematological patients (pts) with invasive infections. Since the introduction of granulocyte colony stimulating factor (G-CSF), there has been a renewal of interest in granulocyte transfusions (GTX). Aim of the study was to evaluate feasibility, efficacy and safety of GTX as adjunctive treatment of infections in neutropenic pts unresponsive to antimicrobial therapy.

Methods:
Retrospective analysis on adult patients with hematological malignancies (HM) and fever during neutropenia (ANC<500 x 10⁶/l and anticipated duration >7 days) who received GTX after no clinical response to antimicrobial therapy. Volunteer donors received G-CSF 12h before the first of 2 consecutive collection procedures (5μg/kg). All of them had signed an informed consent for G-CSF administration and leukapheresis.

Results:
During a 7 years period (2004-10) 46 courses of GTX were administered. Patients were suffering from acute leukemia (30 myeloid and 5 lymphoid), lymphoma (9), multiple myeloma (2). Overall, 209 GTX were administered, with a median of 4 GTX per episode of infection (range 1-20). Infections causing fever were identified in 41 episodes: the majority of them (24/41, 59%) were IFDs (including 1 case of mixed bacterial/fungal sepsis), while 17 cases were bacterial sepsis (17 cases). Remaining 5 episodes were classified as fever of unknown origin (FUO) (5 cases). IFDs included 16 cases of pulmonary aspergillosis (proven/probable), 5 candidemia, 1 invasive zygomycosis, 1 invasive fusariosis and 1 infection due to Blastospizomices capitatus.

Donors’ mean white blood cell (WBC) count at first leukapheresis was 27 x 10⁹/l (range 13-45); at second procedure WBC count was lower (15 x 10⁹/l, range 8-33), as expected. The mean yield was 25.6 x 10⁹ PMN (range 3.5-75.8) at first procedure and 11.1 x 10⁹ PMN at the second one (range 0.6-42.4). Mean transfused dose was 3.7 x 10⁹/kg at first day (range 0.6-9.6) and 1.4 x 10⁹/kg at second day (range 0.1-4.7).

The combination of antimicrobial therapy with GTX led to a favourable clinical response in 33 of 46 valuable pts (72%); the acute infection-attributable mortality rate at 30th day after the last GTX was 22% for IFDs, 29% for bacterial sepsis, and 40% for FUO.

Table: Univariate analysis of main risk factors for IFD

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>Nº pts</th>
<th>Nº cases</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
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</tr>
<tr>
<td>males</td>
<td>232</td>
<td>28 (12%)</td>
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</tr>
<tr>
<td>females</td>
<td>215</td>
<td>15 (7%)</td>
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<tr>
<td>Performance status</td>
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<tr>
<td>0-1</td>
<td>342</td>
<td>22 (6%)</td>
<td>&lt;0.001</td>
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<tr>
<td>2-3</td>
<td>97</td>
<td>18 (19%)</td>
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</tr>
<tr>
<td>4</td>
<td>8</td>
<td>3 (38%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
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<tr>
<td>No</td>
<td>409</td>
<td>36 (9%)</td>
<td>0.054</td>
</tr>
<tr>
<td>Yes</td>
<td>38</td>
<td>7 (18%)</td>
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## Chronic kidney failure

<table>
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<tr>
<td>No</td>
<td>434</td>
<td>13</td>
<td>0.09</td>
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<tr>
<td>Yes</td>
<td>40 (9%)</td>
<td>3 (23%)</td>
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## COPD

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<tr>
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<th>No</th>
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<tr>
<td>No</td>
<td>424</td>
<td>23</td>
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<tr>
<td>Yes</td>
<td>38 (9%)</td>
<td>5 (21%)</td>
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## Liver disease

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<tr>
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<th>No</th>
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<tbody>
<tr>
<td>No</td>
<td>428</td>
<td>19</td>
<td>0.08</td>
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<tr>
<td>Yes</td>
<td>39 (9%)</td>
<td>4 (21%)</td>
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## Cigarette smoking

<table>
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<th></th>
<th>No</th>
<th>Yes</th>
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<tr>
<td>No</td>
<td>327</td>
<td>120</td>
<td>0.8</td>
</tr>
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<td>Yes</td>
<td>31 (9%)</td>
<td>12 (10%)</td>
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## Mucosal damage*

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<td>0</td>
<td>207</td>
<td>240</td>
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<tr>
<td>&gt; 0</td>
<td>17 (8%)</td>
<td>26 (11%)</td>
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## CVC

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<td>112</td>
<td>335</td>
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<td>Yes</td>
<td>9 (8%)</td>
<td>34 (10%)</td>
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## Urinary catheter

<table>
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<td>No</td>
<td>369</td>
<td>78</td>
<td>&lt;0.001</td>
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<td>24 (7%)</td>
<td>19 (24%)</td>
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## Neutropenia§

<table>
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<td>No</td>
<td>37</td>
<td>410</td>
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<tr>
<td>Yes</td>
<td>0</td>
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## Type of house

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<thead>
<tr>
<th></th>
<th>Flat</th>
<th>House with garden</th>
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<tbody>
<tr>
<td>Flat</td>
<td>248</td>
<td>199</td>
<td>0.058</td>
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<tr>
<td>House with garden</td>
<td>18 (7%)</td>
<td>25 (13%)</td>
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## Home restructuring^†

<table>
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<tr>
<th></th>
<th>No</th>
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<td>49</td>
<td>0.24</td>
</tr>
<tr>
<td>Yes</td>
<td>36 (9%)</td>
<td>7 (14%)</td>
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## Construction sites near home°

<table>
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<tr>
<th></th>
<th>No</th>
<th>Yes</th>
<th>Χ² (p)</th>
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<tbody>
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<td>310</td>
<td>137</td>
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<tr>
<td>Yes</td>
<td>26 (8%)</td>
<td>17 (12%)</td>
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</table>

### Legend:

CVC: central venous catheter; COPD: chronic obstructive pulmonary disease

*: it comprehend oral mucositis, esophagitis, vomiting, diarrhea. Zero means none of them.

§: Neutrophils <500/µl, duration > 7 days.

^: In the last 6 months.

°: in the last 3 months, within 500 meters.

### Conclusions:

At preliminary analysis GTX may be safe and efficacious in HM with severe infection to bridge the period of deep neutropenia, when antimicrobial therapy has failed. Controlled studies are needed to confirm this datum, and to define the proper role of this procedure and the optimal schedule for HM.
PROSPECTIVE REGISTRY OF INVASIVE FUNGAL DISEASES IN ACUTE MYELOID LEUKEMIA: PRELIMINARY RESULTS ON 142 CASES

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Purpose:
To evaluate epidemiological characteristics, treatments and outcome of invasive fungal diseases (IFDs) in acute myeloid leukemia patients (AMLs).

Methods:
From January 2010 to March 2011, 31 Italian participating centers registered all consecutive cases of IFDs in adult AMLs at first induction (until 30th day from the end of chemotherapy). The parameters we analyzed were: age, sex, severity and duration of neutropenia, antifungal prophylaxis, certainty of IFD diagnosis, empirical/pre-emptive therapy, target therapy, etiologic agent, outcome. Response rate to antifungal therapy and mortality rate were thus analyzed.

Results:
Over a 15 month period, 142 IFDs were collected in 593 newly diagnosed AMLs (incidence 23.6%). Median age was 60 (range 18-81), with a male/female ratio of 1.6/1. The most part of IFDs (128, 90%) occurred in pts who had received conventional chemotherapy (128/498, incidence 25.7%). As expected, IFDs incidence was lower in those receiving either supportive or low dose therapy (14/95, 14.7%). Probable and proven IFDs were 37 and 14, respectively; remaining cases were classified as possible IFDs (91, 64%). A deep neutropenia (PMN count <500/μl) lasting for at least 7 days occurred in 129 of them (91%). Antifungal approaches are reported in the table. Most of pts had received systemic antifungal prophylaxis (120/142, 85%), more frequently with posaconazole. Liposomal AmB and caspofungin were the most frequently employed drugs, as empirical/pre-emptive therapies. Of 51 proven/probable IFDs, the majority were mold infections (36, 69%), with a mold/yeast ratio of 2.4/1. Among molds, aspergillosis (IA) were predominant (27, 75%). Four cases of rare fungal agents were identified (1 Fusarium, 1 Blastoschizomices, 1 Geotrichum and 1 Trichosporon). At 30th day, 104 pts had achieved a favourable response; the overall response rate was 73%. IFD-attributable mortality rate (AMR) was 11.3%, ranging from 5.5% for possible to 21.6% for proven/probable cases.

Conclusions:
IFDs continue to be a challenging complication in high risk patients. Our results confirm the recently reported trend in reduction of IFD-AMR. On the contrary, cases with unidentified origin continue to be the most frequent. This datum makes it necessary to improve our diagnostic work-up to better target treatment and preventive strategies, and to reduce the risk of overtreatment.
PRE-HOSPITAL RISK FACTORS FOR INVASIVE FUNGAL DISEASE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AT DIAGNOSIS: PRELIMINARY RESULTS FROM THE SEIFEM 2010-STUDY

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Purpose:
To investigate the potential relationship between pre-hospital exposures to fungal sources and the development of invasive fungal diseases (IFDs) in adult acute myeloid leukemia patients (AMLs).

Methods:
From January 2010 to March 2011, in 31 Italian participating centers, all consecutive patients (pts) with newly diagnosed AMLs were registered. Information about personal habits and possible environmental exposures were investigated. In particular we collected data about: comorbidities, job, hygienic habits, work and living environment, voluptuary habits (i.e. smoking, alcohol, illicit substances abuse), hobbies, pets. We also included data on other well-known risk factors, such as age, neutropenia, mucosal damages, etc. In order to make our study population very homogeneous, we focuses on pts treated with conventional chemotherapy only. All cases of proven/probable IFDs occurred until the 30th day from the end of first induction were recorded.

Results:
593 pts were enrolled in the study; of them, 447 were included in the present analysis and 43 developed a proven/probable IFDs (30 molds and 13 yeasts) (incidence 9.6%). Median age was 61 (range 18-81). Main variables included in the risk analysis have been reported in the table. In particular, at preliminary analysis a significant association with IFDs development was found for performance status (p <0.001), chronic obstructive pulmonary diseases (p 0.04), urinary catheter (p <0.001), neutropenia (<500 neutrophils/µl, > 7 days) (p 0.03).

A not significant trend was noted for incidence by gender (males 12% vs females 7%), for diabetes (yes 18%, no 9%), construction sites in the last 3 months to less than 500 meters from home (yes 12%, no 8%), home restructuring in the last 6 months (yes 14%, no 9%). We did not find any association for weight, occupational exposure, geographical origin.

For mold infections only, those patients living in a flat resulted to be at higher risk when compared to those living in house with garden (p 0.03). Other variables showing a correlation with the onset of invasive yeast diseases were chronic kidney failure (p 0.006) and liver diseases (p <0.001).

Conclusions:
Several hospital-independent fungal sources emerged at univariate analysis to potentially influence IFDs onset. Investigation of these factors at time of admission may be helpful in defining patient’ risk category and in better targeting prophylactic strategies.
ASPERGILLUS TANNERI SP. NOV, A NEW PATHOGENIC ASPERGILLUS THAT CAUSES INVASIVE DISEASE REFRACTORY TO ANTIFUNGAL THERAPY

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Purpose:
Invasive aspergillosis (IA) is most commonly caused by Aspergillus fumigatus, which is generally sensitive to amphotericine B, triazoles and caspofungin. Refractory IA has been documented with several sister species such as Aspergillus udagawae and Aspergillus lentulus. We describe here a new Aspergillus species genetically unrelated to A. fumigatus but related to members of Aspergillus Section Circumdati that caused fatal IA refractory to antifungals in two patients with Chronic Granulomatous Disease (CGD). The species is described as Aspergillus tanneri sp. nov.

Methods:
Isolates were inoculated on Czapek-Dox, malt extract and corn meal agar media and growth was evaluated at 25, 30, 37 and 42°C. DNA sequences of ITS, Mcm7, RPB2 and Tsr1 were aligned with datasets using CLUSTALW. PAUP* 4.0b10 was used for parsimony analysis and construction of phylogenetic trees. Virulence of the fungus was compared with a virulent strain of A. fumigatus in moth (Galleria mellonella) as well as mice (BALB/c mice immunosuppressed with hydrocortisone and CGD mice). Drug susceptibility was evaluated using E test (Biomérieux).

Results:
The two CGD patients, ages 16 and 18, developed pulmonary infection caused by a “white mold” which failed to sporulate when first grown from biopsy specimen or from bronchial washing on routine agar media used in clinical laboratories. Despite treatment with an array of currently available antifungal drugs (polyenes, azoles and echinocandins), the infection progressed and the patients succumbed to the disseminated disease. The “white mold” grew well on malt extract and Czapek-Dox media, but poorly on corn meal agar. Conidial structures typical of Aspergillus species were initially observed only on cornmeal agar. However, once sporulation was induced on corn meal agar, subcultures on malt extract maintained sporulation. The fungus grew faster at 30°C than at 37°C and very slowly at 25°C. No growth was observed at 42°C. Morphological characteristics of the fungus did not show similarity to any known Aspergillus species. ITS sequences of both isolates did not show identity above 95% with any other fungal species (BLASTX/GenBank). The phylogenetic tree based on combined sequencing data of Mcm7, RPB2 and Tsr1 indicated that the two strains represent a new Aspergillus species phylogenetically close to the members of Aspergillus Section Circumdati. The species was described as Aspergillus tanneri sp. nov. Virulence studies indicated that A. tanneri is less virulent in CGD mice, equally virulent in BALB/c hydrocortisone treated mice, but more virulent in moths compared to the virulent A. fumigatus, B-5233. E-test showed A. tanneri to be more resistant to voriconazole, posaconazole and itraconazole than B-5233, confirming its resistance to antifungal drugs.

Conclusions:
This is the first report documenting fatal invasive aspergillosis caused by a new pathogenic Aspergillus species that is inherently resistant to antifungal drugs. Phenotypic characteristics of A. tanneri combined with the molecular approach enabled diagnosis of this new pathogen. This study underscores the importance of fungal diagnosis at the species level in order for appropriate patient management.
IMPACT OF CURRENT ANTIFUNGAL THERAPY ON PCR BASED INVESTIGATION OF BRONCHOALVEOLAR LAVAGE SAMPLES FOR DIAGNOSING PULMONARY ASPERGILLOSIS IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Purpose:
Invasive pulmonary aspergillosis (IPA) remains a major cause for morbidity and mortality in patients (pts) with hematologic malignancies. As culture-based methods only yield results in a minority of patients, using non-culture-based methods for detection of aspergillosis in clinical specimens becomes increasingly important. Analyzing bronchoalveolar lavage (BAL) samples with polymerase chain reaction (PCR) is promising, however, the influence of current antifungal drugs on the performance of this diagnostic tool remains controversial.

Methods:
Results of a validated nested Aspergillus PCR assay testing BAL samples from 221 hematological pts at high risk of IPA were retrospectively analyzed with regard to the influence of antifungal treatment on the performance of the PCR assay. According to EORTC/MSG 2008 Criteria 7 patients could be identified as having proven IPA, 46 patients were classified as probable, 115 patients were classified as possible while 53 pts did not fulfill the EORTC criteria for at least possible IPA.

Antifungal treatment in probable or proven IPA pts consisted of amphotericin B formulations, voriconazole, itraconazole, posaconazole and caspofungin. Median number of administered antifungal regimes were 1 (range 0-4) prior to BAL sampling.

Results:
Of 221 pts, 141 (64%) had received antifungals prior to BAL sampling, 92 pts were treated with one, 38 pts had received 2 and 12 pts had received 3 or more antifungals.

Sensitivity (sens), specificity (spec), positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odds ratio (OR) for BAL of proven and probable pts receiving one or no antifungal (n=37) were 0.57, 0.84, 3.5, 0.5 and 6.9, whereas the values decreased for patients receiving more than one antifungal (n=16) prior to BAL sampling to 0.25, 0.84, 1.6, 0.9, 1.7 (sens, spec, PLR, NLR, OR), respectively.

Statistical analysis showed sensitivity of BAL PCR in probable/proven IA patients to be significantly lower when receiving more than one antifungal prior to BAL sampling (Mann-Whitney-U-test, p ≤ 0.04).

Conclusions:
In terms of sensitivity a statistically significant reduced performance of BAL PCR testing in patients receiving more than one novel antifungal drug prior to BAL sampling was observed whereas specificity and NPV remained high.

This underlines the crucial need to investigate BAL samples as early as possible in case of suspected IPA, especially in the current clinical setting of mold active prophylaxis and treatment with novel antifungals.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION
ANTIFUNGAL MANAGEMENT STRATEGY FOR HIGH RISK NEUTROPENIC PATIENTS BASED ON ITRACONAZOLE LEVELS

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Objectives:
This study assessed a pilot antifungal prevention strategy based on itraconazole levels in consecutive high risk patients with the aim of reducing empirical treatment. The patients underwent chemotherapy or allogeneic stem cell transplantation for haematological malignancies and received itraconazole suspension prophylaxis.

Methods:
103 febrile neutropenic episodes (FNEs) in 81 adult patients (26 allogeneic haematopoetic stem cell transplants, 48 with acute myelogenic leukemia, 7 with acute lymphocytic leukemia) were studied. All patients had serum galactomannan (gal) assays (EIA) twice a week and trough itraconazole levels measured on day one of fever. If they were still febrile after 4 days of broad spectrum antibiotics initiated on day one, HRCT scan chest was performed and action taken was based on initial radiology report and the itraconazole level (itra). Antifungal treatment was required to be given only when well defined clinical, microbiological and radiological criteria were present. Invasive Fungal Disease (IFD) is defined according to EORTC/MSG criteria 2008. For the purposes of IFD incidence calculation all HRCTs were subsequently assessed by 2 independent radiologists blind to clinical information.

Results (Fig 1):
A. 30 out of 103 FNEs had responded to antibiotics by day 4. In 21 of these itra levels were normal (>500ng/L) and no action was taken (1 death: non-IFD). In the rest, 9 were subtherapeutic (<500ng/L) and oral itra suspension was switched to iv itra (all alive). B. 73 out of 103 FNEs were refractory to 4 days of antibiotics. In 14 out of these 73 (initial HRCT suggestive of fungal infection and or gal: positive) targeted antifungal treatment was given. 6 of these 14 had possible IFD (1 died), 1 probable IFD, and the remaining 7 had non-specific CT findings (1 died). 12 had normal itra levels and in one probable IFD and one airway disease case levels were subtherapeutic. In the remaining 59 out of 73 HRCT findings were not suggestive of fungal infection. C. The 59 FNEs with negative HRCTs are divided into 2 groups: 1) 43 with normal itra levels. 12 of these 43 were treated on physician suspicion (1 death: non-IFD) while no action was taken for the remaining 31. 2) 16 with subtherapeutic itra levels. Twelve of these 16 were treated on physician suspicion (2 deaths: non-IFD) while the other 4 were switched to iv itra (all alive). The incidence of proven or probable IFD in the 103 FNEs was 1.9% and of possible IFD 10.6% (overall incidence 12.6%). Antifungal treatment was given to 38 of the 73 patients (52%) with persistent FNE.

Conclusions:
Patients given liquid itraconazole prophylaxis can achieve adequate (>500ng/L) levels at the time of febrile neutropenia in the majority of cases. The incidence of documented IFD is low in this setting and breakthrough infection occurred in patients with low itra levels. The measurement of day 0 itra levels, combined with HRCT and gal screening, reduced antifungal therapy by 48% in comparison with an empirical strategy.
ASPERGILLUS & OTOMYCOSIS IN KERMANSHAH IRAN

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Purpose:
Otomycosis is a common external ear canal disorder resulting from the invasion of mould species, yeast or dermatophyte and gives rise to some diverse clinical presentations. The purpose of the present study was to isolate and identify the causative fungi of otomycosis in the Kermanshah Iran.

Methods:
Samples were examined both by direct microscopical observation of fungal elements in KOH preparations and in culture for the identification of the causative agent.

All samples were inoculated on (i) Sabouraud dextrose agar (SDA, Merck), (ii) SDA with 5% chloramphenicol and cycloheximide in duplicate for dermatophyte and (iii) SDA with 5% chloramphenicol in triplicate for mould isolation. The criteria for the diagnosis of otomycosis were based on microscopical observation of fungal elements and growth of the same mould in all triplicate culture.

A mycological study was undertaken in 100 patients suspected of otomycosis in Kermanshah, a western province of Iran, to gain more insight into the prevalence and aetiology of this infection.

Results:
Direct microscopy and culture of the sample positive in 13(0%13) cases and Aspergillus niger with 8(0%08) cases had most frequent agents of otomycosis and A. flavius 3(0%03) A. fumigatus 2(0%02), were other agents of otomycosis in kermanshah.

Conclusions:
Aspergillus niger was the most prevalent species as same as other studies. The frequency of A. niger in otomycosis is noticeable.
TESTING FOR ALLERGENICITY OF FUNGI AND PRACTICES CONTRIBUTING TO BIOTIC POLLUTION IN AIR-CONDITIONED INDOOR ENVIRONMENTS

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Purpose:
Indoor environments play important roles in human health. The health hazards posed by polluted indoor environments include allergy, infections and toxicity. The term ‘sick building syndrome’ (SBS) describes causes of building occupants experiencing adverse health effects that appear to be linked to the time spent in a building. In the present study investigations were done by using questionnaire-based data collected from the people who live or work in air-conditioned (A/C) rooms, and responses of the occupants were analyzed to understand the practices that may contribute in biotic pollution of air-conditioned indoor environments. The fungi from air-conditioned environments were isolated, their allergic extracts prepared and the fungi causing allergies are identified by skin prick test.

Methods:
Survey on practices of A/C users: A questionnaire was prepared and data were collected from people who live or work in air-conditioned rooms. Responses of the participants were analyzed to understand the practices that may contribute to the health of indoor environments.

Isolation and identification of fungi: Potato dextrose agar plates in triplicates containing 40 mg l-1 Rose Bengal were exposed to air from A/C units for 20 min. Sampling interval was once a fortnight for 3 months or weekly once for 2 months. The fungi were identified based on their macroscopic and microscopic features.

Extraction of fungal allergic extracts: Fungal allergenic extract of 11 indoor fungal isolates and standard fungal isolates were made by Creative Drug Industries, Vashi, Mumbai, India.

Skin prick test for indoor fungal allergenic extract: Seventy-five patients were tested over a period of 3 months. All the subjects were suffering from atopic allergy viz. sneezing, rhinorea, nasal blockage, urticaria and asthma. These patients were referred by ENT specialists, chest physicians, and dermatologists for allergy testing. The preferred suitable area for skin prick test was on the back of the patient. Skin prick tests were done in the clinic of Dr. Sunaina Waghray, Kamineni Hospital, Hyderabad, India.

Results:
Twelve fungi were isolated and identified from air-conditioned rooms. Of the 12, 7 were species of Aspergillus, viz. A. niger, A. oryzae, A. fumigatus, A. terreus, A. nidulans, A. versicolor and A. parasiticus. Other fungi were Penicillium citrinum, Fusarium oxysporum, Trichoderma viride, Neurospora crassa and Alternaria alternata. A. niger was present in 80% of the locations. Some of the fungi isolated in this study could be opportunistic fungal pathogens, like A. fumigatus, A. niger and Penicillium citrinum, and were found to be allergenic.

Conclusions:
Results of this study indicate that air-conditioned rooms could be reservoirs of fungi and may cause allergic problems or infections in healthy or immunocompromised individuals living in these environments. Responses of the occupants were analyzed to understand the practices contributing to biotic pollution of indoor air-conditioned environments. Our survey revealed some interesting facts about the users of A/C rooms and their practices that may be contributing to the indoor air quality. Cleaning practices may reduce the complaints.
Purpose:
Fungal infections of the paranasal sinuses have recently been blamed for causing most cases of chronic rhinosinusitis. The most common pathogens are from *Aspergillus* and Mucor species. Whereas invasive fungal rhinosinusitis usually occurs in diabetic and immunocompromised patients, non-invasive form can be seen immunocompetent patients. Infection is usually suspected upon reviewing the CT scan results. The most common pathogens associated with fungal sinusitis are *Aspergillus fumigatus* and *Aspergillus flavus*.

Methods:
We report here a case having chronic rhinosinusitis symptoms lasting for 3 months in a 39-year-old patient.

Results:
She was was not immunocompromised and presented with mycetoma in the right maxillary sinus on computed tomographic scan. The patient did not respond to antibiotics. The sinus aspirate yielded the growth of a mold identified as *Aspergillus niger*. Surgical treatment was planned for removal of the mycetoma from the maxillary sinus.

Conclusions:
We emphasize that clinicians should consider fungal pathogens in the differential diagnoses of all chronic sinusitis that did not respond to antibiotics.
VORICONAZOLE AND POSACONAZOLE IMPROVES ASTHMA SEVERITY IN ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS AND SEVERE ASTHMA WITH FUNGAL SENSITIZATION

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²The National Aspergillosis Centre, North West Lung Centre, University Hospital of South Manchester, Manchester, UK
³Respiratory Research, School of Translational Medicine, University of Manchester, Manchester, UK

Background:
Allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitization (SAFS) are progressive allergic fungal lung diseases. Current treatment with itraconazole is associated with a 40% failure rate and adverse events (AEs).

Purpose:
The purpose of the study was to assess the effect of voriconazole or posaconazole as second and third line therapy.

Methods:
We conducted a retrospective review of adult asthmatic patients with either ABPA or SAFS receiving voriconazole or posaconazole. Clinical, radiological and immunological evaluation was used to assess response.

Results:
There were 26 patients, ABPA (n = 21) or SAFS (n = 5), 11 males, median age = 59 yrs. All patients had failed itraconazole (n=14) or developed adverse events (AEs) (n=12). There were 34 courses of therapy analyzed, 25 with voriconazole and 9 with posaconazole. Clinical response to voriconazole was observed in 17/25 (68%) at 3 months, 15/20 (75%) at 6 months and 12/16 (75%) at 12 months, compared to 7/9 (78%) at 3, 6 and 12 months for posaconazole. Eighteen of 24 (75%) discontinued oral corticosteroids, 12 of them within 3 months of starting antifungal therapy. Six of 23 (26%) patients on voriconazole had AEs requiring discontinuation before 6 months compared to none on posaconazole (p=0.15). Four relapsed (57%), one at 3 months and 3 at 12 months after discontinuation.

Conclusions:
Both voriconazole and posaconazole are safe and effective alternative treatment options for SAFS and ABPA, although there is a substantial AE rate with voriconazole. Larger prospective studies are required.
REPEATED COURSES OF INTRAVENOUS AMPHOTERICIN B THERAPY INCLUDING INTERMITTENT LONG-TERM TREATMENT IN PATIENTS WITH CHRONIC PULMONARY ASPERGILLOSIS

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Purpose:
Intravenous (IV) amphotericin B therapy is a recognised treatment for patients with chronic pulmonary aspergillosis (CPA). There are limited data available on the use of IV amphotericin B therapy in this patient group and none on repeated courses. This audit was therefore undertaken to ascertain whether CPA patients derive clinical benefit from IV amphotericin B therapy.

Methods:
CPA patients who received two or more courses of IV liposomal amphotericin B therapy (Gilead Sciences), either as repeated short courses or a short course(s) followed by intermittent long-term therapy, at our clinical centre were identified. A retrospective review of patient case-notes was performed using a standardised proforma. Data collected included patient demographics, the indication for treatment, the dose and duration of treatment and the subsequent clinical response.

Results:
9 CPA patients (5 females, 4 males) aged between 47 and 74 years (median 61 years) at the time of their first dose of IV amphotericin B were identified. 4 patients had repeated short courses of treatment (2 courses, n=3; 3 courses, n=1) whilst 5 patients had at least 1 short course of treatment prior to commencing long-term intermittent IV amphotericin B therapy. The dose of IV amphotericin B given for short courses and intermittent long-term treatment ranged between 2.53 - 4.62 (mean 3.03) mg/Kg daily and 2.53 - 6.7 (mean 4) mg/Kg/dose three times a week respectively. The duration of treatment ranged between 3 - 36 days (mean 20.3) and 4 - 12 (mean 11.2) months respectively for short versus intermittent long-term courses of treatment. Indications for repeated short courses of IV amphotericin B were primarily deterioration in respiratory symptoms (62%) and / or constitutional symptoms (56%). Intermittent long-term therapy was generally given to patients who had no other oral options available either due to drug intolerance and / or the development of azole drug resistance. Intermittent long-term therapy was delivered via a Portacath IV line.

Improvements in respiratory and constitutional symptoms were observed in 5 out of 9 (56%) and 4 out of 9 (44%) patients receiving repeated short courses of IV amphotericin B respectively. All patients (n=5) on intermittent long-term therapy noticed an improvement in their symptoms (respiratory, n=1; constitutional, n=4). An improvement in the immunological markers of Aspergillus infection were noted in 3 out of 6 (50%) and 1 out of 3 (33%) patients with adequate immunological data available in the short course versus intermittent long-term IV amphotericin B groups respectively. Of the 5 patients who had long-term intermittent IV amphotericin B therapy, 1 patient stopped treatment following a pneumonectomy and the other 4 patients subsequently failed this treatment.

Conclusions:
Around 44 - 56% of CPA patients receiving repeated short courses of IV amphotericin B therapy noted an improvement in their respiratory and / or constitutional symptoms. Intermittent long-term therapy was reserved for patients with limited treatment options and all patients in this treatment group gained clinical benefit from this treatment.
GALACTOMANAN ASSAY PERFORMANCE IN BRONCHOALVEOLAR LAVAGE FOR THE DIAGNOSIS OF INVASIVE ASPERGILLOSIS IN IMMUNOCOMPROMISED HOSTS

MV Batista\textsuperscript{1,2,3}, C Constância\textsuperscript{3}, CA Fonseca\textsuperscript{1}, SF Costa\textsuperscript{1,2}, CM Gamba\textsuperscript{1,2}, SV Campos\textsuperscript{1}, IL França\textsuperscript{1,2}, FL Dulley\textsuperscript{4}, GH Fonseca\textsuperscript{4}, MA Shikanai-Yasuda\textsuperscript{1,2,3}

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\textsuperscript{2}Department of Infectious and Parasitic Diseases, FMUSP, São Paulo, Brazil
\textsuperscript{3}Laboratory of Immunology (LIM 48), FMUSP, São Paulo, Brazil
\textsuperscript{4}Hematology and Stem Cell Transplantation Clinics, FMUSP, São Paulo, Brazil

\textbf{Purpose:}
Invasive aspergillosis (IA) is a major cause of morbidity and mortality in immunocompromised patients, particularly those with hematological malignancies in the setting of profound neutropenia and/or hematopoietic stem cell transplantation. The diagnosis of IA in immunocompromised patients is a challenge. Recently, research has shown that galactomannan (GM) assays are useful in diagnosis, especially when performed in bronchoalveolar lavage (BAL) where there is a greater sensitivity and earlier kinetics compared with serum.

\textbf{Methods:}
We performed a prospective study from December 2008 to June 2011 to determine the clinical features and diagnosis of invasive aspergillosis in 47 immunocompromised patients of the Hospital das Clínicas da Faculdade de Medicina da USP, classified according to the EORTC criteria as proven, probable or possible IA along with 11 controls with other diseases such as tuberculosis, herpes, sepsis, febrile neutropenia, lymphoma and lung cancer. We evaluated the effectiveness of BAL GM assay in diagnosing IA in these populations.

\textbf{Results:}
Four samples were associated with proven, 15 with probable, and 28 with possible IA and 11 were associated with no IA. Using BAL GM $\geq 0.8$ (cutoff for serum GM) and $\geq 1.00$, the sensitivity in diagnosing proven or probable IPA was 58\% (11/19) and 58\% (11/19), respectively, and specificity was 90\% (10/11) and 100\% (11/11). At these cutoffs, positive and negative predictive values were 91\% (11/12) and 55\% (10/18), and 100\% (11/11) and 58\% (11/19), respectively. In this study 98\% (56/57) of patients were already receiving antifungal agents for at least 72 hours at time of BAL.

\textbf{Conclusions:}
The BAL GM assay appears promising for the diagnosis of IA. However, treatment with certain antifungals may interfere with the results of the BAL GM assay.
DIAGNOSIS AND TREATMENT OF NECROTIZING MALIGNANT OTITIS CAUSED BY *ASPERGILLUS*

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Purpose:
Necrotizing malignant otitis is caused by bacterial or fungal agents. Aspergillosis invasive otitis is associated to a diabetes millitus. Infection extends towards cranial bones, facial deeps spaces and neurologic and vascular structures. We report cases of invasive otitis caused by *Aspergillus* and efficacy of voriconazole.

Methods:
Patients are selected after clinical examination, serology of aspergillosis, rate of galactomannan in blood and medical imaging of ear by CT scan or MRI. Patients are then treated by daily dose of 400 mg of voriconazole on average during six months.

Results:
Between 2007 and 2010, twelve have an invasive otitis caused by *Aspergillus*. *Aspergillus flavus* is the main species isolated at the tunisian patients. Serology and galactomannan antigen are positive respectively in 90% and 60% of patients.

Scanning and MRI was pathologic in all of cases. Temporomandibular arthritis is present at six patients (50%). Two patients had a facial pralysis and a patient has an extension to the brain with an archnoiditis. Among eight patient how completed their treatment photosensitivity is the most frequent and most annoying side effect.

Conclusions:
Diagnosis of invasive otitis caused by *Aspergillus* requires the combination of several methods: mycology and medical imaging allow to confirm infection, in our patients *Aspergillus flavus* is the most frequent species. Because it’s well tolerated and effective, Voriconazole has to be the treatment of choice.
Purpose:
The UK National Aspergillosis Centre (NAC) was commissioned in 2009 to provide highly-specialised quality care for Chronic Pulmonary Aspergillosis patients. The official launch incorporated the first Aspergillosis Patient Meeting, positively evaluated by those patients and carers in attendance. Monthly meetings have continued with developments, offering a combination of interactive educational talks and peer group support. Subjects are patient requested and focussed and include nutrition, anti-fungal drugs, physiotherapy, environmental exposure risks and a virtual clinic “visit” role-play.

The aims of the meetings are to provide ongoing support to Aspergillosis patients and carers; to empower them to improve their quality of life; promote self-management skills; encourage concordance with anti-fungal treatment; develop strategies to improve physical functioning; reduce risk of environmental exposure; feedback on patient engagement in research and a platform for the patients’ voices to be heard. These meetings are a valuable tool for empowering patients.

Methods:
A suitable meeting room to seat people comfortably with nearby toilet access is required. Disabled access is a pre-requisite for debilitated oxygen-dependant, wheelchair-assisted patients and any in-patients transferred directly from the ward.

The meeting room requires audio-visual projection and flip-chart for interactive discussions and explanations. Expert speakers are from the NAC team, the wider hospital Trust and external experts.

The virtual aspect of the meeting is achieved by streaming live onto the Internet and is recorded for future presentation and reference. Internet chat software enables remote, virtually attending patients the facility to post real-time questions. A two-way conversation is maintained throughout the duration of the live meeting.

Publicity is through the Aspergillosis Patient Website, email circulars and newsletter distribution during out-patient clinics. Talks and typed meeting-notes are uploaded onto the patient’s website for future reference. Refreshments are provided by the Fungal Research Trust. For health and safety purposes, an Aspergillosis Nurse Specialist is present to intervene should any patient develop a clinical need.

Results:
The benefits of the meetings are the promotion and encouragement of patients to become experts in their own health, through empowerment, by empathetically tailored education, based on their own patient stories and experiences, delivered by their own expert clinical, scientific and research focussed staff. The meetings are continually positively evaluated by the patients and carers as helping them understand more about their diagnosis and treatment plans.

A testament to the meetings success is the continued high attendance and increasing number of online attendees. The meeting gives an opportunity to meet and speak, often for the first time, to fellow Aspergillosis sufferers. It gives them a chance to share their stories in a friendly, supportive arena. A direct response to the meetings is the patients’ initiatives to raise funds for the Fungal Research Trust.

Conclusions:
The Aspergillosis Patient Meeting is an innovative and creative approach to providing patient education and support. An integral component of the meeting delivery is the role of the Clinical Information Architect. Simultaneous actual and virtual meetings reach a much wider vulnerable audience, immediately and later. Importantly, those who are too ill or debilitated to travel still benefit from virtual attendance. Healthcare professionals can improve the quality of life of their patients with long-term condition by effectively providing patient education and support; in-reach in the hospital setting and more importantly, out-reach into the patients’ homes.
COMPARISON OF *ASPERGILLUS* DIAGNOSTIC YIELDS FROM BRONCHIAL ASPIRATE, BRONCHOALVEOLAR LAVAGE AND SPUTA BY REAL-TIME POLYMERASE CHAIN REACTION COMPARED TO STANDARD FUNGAL CULTURE

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**Purpose:**
The primary objective of this small study was to compare *Aspergillus* species diagnostic yield from Bronchoalveolar lavage (BAL), bronchial aspirate and post bronchoscopy sputum samples by quantitative detection using Real Time - Polymerase Chain Reaction (RT-PCR) compared to standard fungal culture. *Aspergillus* is a major contributor to the morbidity and mortality of immunocompromised patients and those with primary diagnoses of asthma, bronchiectasis, cystic fibrosis and chronic pulmonary Aspergillosis. Despite impacting significantly on the disease burden of these patients, positive culture remain problematic from standard bronchoscopic and sputum sampling methods. Often presumptive clinical diagnosis is required in the absence of standard culturable results.

**Methods:**
We prospectively recruited five patients from the National Aspergillosis Centre, Manchester UK who were scheduled for diagnostic bronchoscopy for suspected *Aspergillus* bronchitis. Patients were recruited under the auspices of the large Fungal Exposure and Colonisation in Respiratory Disease; Research Ethics Committee (REC) reference: 07/Q1403/70. Four patients had a primary diagnosis of bronchiectasis and all five a presumed clinical diagnosis of *Aspergillus* bronchitis. A sputum sample was obtained pre-bronchoscopy (n=4:5). During bronchoscopy after initial entry in to the main bronchi trap specimens were taken (n=4:5) and an initial wash of 5-20ml 0.9% Normal-Saline performed in n=4:5 patients. Based on chest radiograph, the bronchi with a consolidation or shadowing was wedged and between 10-120ml 0.9% Normal Saline instilled for the BAL. Volume instilled was discretionary by Bronchoscopist based on clinical condition and oxygen-saturations. A second sputum was obtained post-bronchoscopy (n=5:5). All specimens were transferred to the Mycology Reference Centre Manchester (MRCM) (http://www.mycologymanchester.org) for processing. Each sample was split into 3; one sent to the Clinical Sciences Laboratory University Hospital South Manchester for standard microscopy and fungal culture; one processed for DNA extraction and RT-PCR; one sample underwent microscopy, fungal culture and sensitivities within the MRCM.

**Results:**
Pre-bronchoscopy sputa samples were obtained in 4 of 5 patients (n=4:5). 75% (n=3:4) positive standard culture rate compared to 100% (4:4) by RT-PCR. Bronchoscopic trap specimens were obtained in 4 of 5 patients (n=4:5). 50% (n=2:4) positive standard fungal culture rate compared to 100% (4:4) by RT-PCR. Initial wash samples were obtained in 4 of 5 patients (n=4:5). 25% (n=1:4) positive standard fungal culture rate compared to 100% (4:4) by RT-PCR. In standard practice this initial wash is usually discarded. BAL samples obtained on 5 of 5 patients (n=5:5) yielding a 20% (n=1:5) positive standard fungal culture rate compared to 100% (n=5:5) by RT-PCR. Post -bronchoscopy sputa samples were obtained in 5 of 5 patients (n=5:5) yielding 40% (n=2:5) by standard culture compared to 100% (4:4) by RT-PCR. One patient underwent bilateral BAL and 4 separate specimens obtained. Only 1 specimen proved positive on standard culture compared to 100% by RT-PCR.

**Conclusions:**
These results demonstrate an important comparison between the diagnostic yields rates between standard fungal culture testing and RT-PCR using a commercial real-time assay for *Aspergillus*. This small single centre study suggests that improved respiratory Aspergillosis diagnostics can be achieved through RT-PCR.
**ASPERGILLUS-RELATED RESPIRATORY DISEASES CAN IMPACT PNEUMOCOCCAL ANTIBODY LEVELS AND RESPONSE TO BOTH PNEUMOCOCCAL POLYSACCHARIDE VACCINES AND PNEUMOCOCCAL CONJUGATE VACCINES**

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**Purpose:**
Streptococcus pneumoniae is a leading cause of infection worldwide. The 23 valent polysaccharide vaccine (PPV-23) was introduced in 2003 in the UK for all people aged 65yrs and over, the 7-valent pneumococcal conjugate vaccine (PCV-7) was introduced into the infant immunisation programme in 2006, replaced by PCV-13 in April 2010. Our objective was to establish if patients with chronic respiratory disease who have not achieved adequate pneumococcal antibody response following PPV-23, can achieve adequate response to the PCV-7 or PCV-13.

**Methods:**
Patients attending specialist respiratory clinics routinely had pneumococcal antibody levels tested. Antibody levels to 12 serotypes were reported for patients with chronic pulmonary aspergillosis (CPA), allergic bronchopulmonary aspergillosis (ABPA), bronchiectasis and asthma/severe asthma with fungal sensitisation (SAFS). A level of >0.35µg/ml signified adequate response and patients with low levels to >6/12 serotypes were given PPV-23. Those who continued to have low levels went on to receive 1 or 2 PCV doses, either PCV-7 or PCV-13 (only 1 patient received PCV-7 followed by PCV-13).

**Results:**
All patients had received PPV-23 within the previous 5 years and had not achieved adequate antibody levels. They were, therefore, eligible for repeat vaccine with PCV rather than PPV. Pre and post-vaccination serotype-specific IgG levels were available for 20 patients – CPA n=13 (3 of whom received PCV 13), ABPA n=3, bronchiectasis n=1 and asthma/SAFS n=3. 17 patients received PCV-7 and 3 received PCV-13. For those who received 1st dose of PCV-7 the best responses were achieved for serotypes 4 (41% increase); 6B (36% increase); 18C (47% increase); 19F (24% increase); 23F (59% increase) and 7F (29%). The poorest serotype levels were for serotypes 1 & 5 (not contained in PCV-7). Following a single dose of PCV-13, 2 out of 3 patients (all CPA) had an overall adequate response. 12/20 patients required a 2nd dose of PCV 7 or 13, due to poor response. To date only 3/12 had antibody levels measured post 2nd vaccine dose. 2 CPA patients had a 2nd dose of PCV-7 with neither responding. The other patient received PCV-13 following PCV-7 with adequate response.

**Conclusion**
Although the numbers of patients are small and comparison between the disease groups was not possible we were able to show that PCV is an effective 2nd line vaccine (post PPV-23) for certain serotypes, however most patients (60%) do need to have a 2nd PCV dose. The efficacy of PCV-13 in adults over 65yrs is being evaluated via a randomised controlled trial. Efficacy of PCV-13 following PPV-23 in our aspergillosis patients, also needs further investigation, as the numbers reported here are small.
RT-PCR IS SIGNIFICANTLY MORE SENSITIVE IN DETECTING ASPERGILLUS IN RESPIRATORY SAMPLES THAN CULTURE

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Purpose:
The National Aspergillosis Centre (NAC) is a specialist referral centre that has been providing long-term care for patients with chronic pulmonary aspergillosis in the UK since 2009. The Mycology Reference Centre, Manchester is the referral laboratory and provides a specialist mycology service for the NAC including detection, identification and susceptibility testing of yeasts and moulds. Since January 2010, culture, PCR and serology have been employed to detect Aspergillus in respiratory samples. MycXtra DNA extraction and MycAssay Aspergillus RT-PCR kits (Myconostica, Cambridge, UK) are used according to the manufacturer’s instructions. This system detects DNA from Penicillium spp. in addition to that of Aspergillus. The BSOP57 method, ‘Investigation of Bronchoalveolar lavage, Sputum and Associated Specimens’ is used for culture. The correlation between the performance of PCR and culture in the detection of Aspergillus in respiratory samples from chronic and allergic pulmonary aspergillosis patients has not been analysed before.

This retrospective audit aims to analyse the concordance between two methods (culture and PCR) for the detection of Aspergillus of the first 100 NAC patient respiratory samples.

Methods:
A database search of the laboratory reporting system was used to collect the data. The first 100 consecutive respiratory samples of NAC patients where PCR and fungal culture had been performed were included.

Results:
The 100 samples included in the study had been collected between January and April 2010 (80 days) from 83 patients that attended the NAC. A total of 42 samples were found positive for Aspergillus with either method. Of the patient samples, 40% were positive by PCR and 18% by culture. Four cases were negative in PCR but positive in culture. There was a 66% concordance between the two methods. In two cases the positive PCR was due to Penicillium sp. as detected by culture. In addition, two cases of Penicillium was detected by culture but the PCR remained negative. Of the 40 cases positive in PCR, 50% gave a strong signal (CT≤36) including the two false positive cases, and 50% gave a weak signal (36<CT≤38). In this patient group and sample type the sensitivity of the PCR was 90% and that of the culture was 33% (P=0.0156). The specificity of the PCR system was 98%.

Conclusions:
PCR was found to be significantly more sensitive in detecting Aspergillus in respiratory samples than culture.
ASPERGILLUS FUMIGATUS DURING STABLE STATE AND EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE


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Purpose:
Chronic obstructive pulmonary disease (COPD) is associated with significant morbidity and mortality. Airways of patients with chronic lung disease including COPD are often colonised with potential pathogenic microorganisms which give rise to increased airway inflammation. Bacteria and viruses have been implicated in exacerbations of COPD and bacteria are often isolated in stable state, whereas the potential role of fungal colonisation and infection in the pathogenesis of COPD is poorly understood. The objectives of this study were to determine the frequency of filamentous fungal culture and IgE sensitisation to Aspergillus fumigatus in COPD and define their relationship with clinical outcomes.

Methods:
Subjects with COPD were recruited from a single centre into a 1 year observational study. Assessments of lung function, allergen testing, and sputum analysis for inflammation, bacterial and fungal cultures were undertaken in COPD subjects and in controls. For the mycology, sputum culture was focused towards optimised isolation of A. fumigatus. A. fumigatus was identified from macroscopic and microscopic features; other fungi were identified by DNA sequencing.

Results:
Fungal culture and baseline demographic data was obtained in 128 patients with COPD. A fungus was isolated that was predominantly Aspergillus and Penicillium species in 49% of COPD patients. A. fumigatus was cultured in 37%; whilst identification of any Aspergillus species was present in 42% of patients. Pathogenic bacteria and A. fumigatus co-culture was found in 14% of patients at study entry. A fungus was cultured in 3/22 controls (2 were A. fumigatus) which was significantly lower than observed in COPD (p=0.002). The total sputum cell count, sputum neutrophil % and inhaled corticosteroid dosage were significantly increased in COPD patients with a positive fungal culture at baseline (p<0.05), but the within subject repeatability of fungal culture between stable visits (examined in 70 subjects at two stable visits three months apart) was low (κ=-0.04). Sensitization to A. fumigatus was present in 13% of COPD subjects and was associated with worse lung function (FEV1 % predicted 39% versus 51%; p=0.01), but not related to fungal culture. Positive fungal cultures were present in 42/110 exacerbations and were not associated with bacterial culture or severity of exacerbation.

Conclusions:
Positive fungal culture is a common feature of COPD. A. fumigatus is commonly detected in COPD subjects during stable state and exacerbations. Neutrophilic airway inflammation in COPD is related to A. fumigatus culture whilst sensitisation is associated with lower lung function. The clinical significance of this remains uncertain.
CHRONIC PULMONARY ASPERGILLOSIS SEVERITY AND HEALTH STATUS WORSENING; DATA FROM THE NATIONAL ASPERGILLOSIS CENTRE, MANCHESTER, UK

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Purpose:
Chronic pulmonary aspergillosis (CPA) is a leading cause of illness with a high mortality rate. We aimed to investigate the association of CPA severity with health status deterioration or improvement in patients with CPA. We also compared the discriminative ability of a respiratory disease-specific instrument (the St. George’s Respiratory Questionnaire (SGRQ)) to a widely used generic instrument the health survey SF-36.

Method:
To rate disease severity, we used our National Aspergillosis Centre criteria for banding CPA patients (band 1 = mild), (band 2 = moderate), (band 3 = severe). Health status was assessed using the respiratory specific scale St. George’s Respiratory Questionnaire (SGRQ) (1-100, 100 poor) and a generic scale the health survey SF-36 which its two summary scores, a physical component summary (PCS) and mental component summary (MCS) score were calculate. Differences in means for continuous variables between CPA severity grades were examined using analysis of variance (ANOVA) and the non-parametric equivalent (Kruskal-Wallis). Differences in means for continuous variables between CPA grades 1 versus 3 were examined using independent sample T-Test and Mann-Whitney test. Binary logistic regression was also used.

Results:
We examined 128 patients where 35 (27%), 72 (56%) and 21 (17%) had mild, moderate and severe CPA respectively. Using total SGRQ score and its activity and impact domains only, worse health status was consistently associated with increasing severity of CPA where patients with severe CPA has worse health status than patients with mild or moderate disease (P = 0.02, 0.049 and 0.03 respectively). A stronger correlation was observed comparing only mild CPA versus severe CPA; we found patients with severe CPA had worse health status as measured by the total SGRQ, SGRQ activity and impact domains p=0.015, 0.02 and 0.017 respectively.

We did not find statistically significant difference in the mean score of SF-36 PCS and SF-36 MCS between different stages of CPA. However, patients with severe CPA had higher scores of SF-36 PCS only comparing to patients with mild disease (p= 0.055). No statistically significant difference was seen between patients for SGRQ symptoms domain and SF-36 MCS.

Binary logistic regression analysis using mild versus moderate/severe CPA showed that patients with disease severity suffered more health deterioration represented by total SGRQ score and its activity domain, and SF-36 PCS only after controlling for age, FEV1% and FVC% (OR 1.06, 95% CI 1 – 1.03, p = 0.04; OR 1.07, 95% CI 1.01 – 1.2, p= 0.02 & OR 0.89, 95% CI 0.89 – 0.98, p= 0.02 respectively). The impact domain showed a trend but it did not reach a statistical significance (p= 0.07).

Conclusions:
The SGRQ showed better discriminating validity among different levels of CPA severity than generic health instrument. This suggests that SGRQ may provide CPA studies with better statistical power than SF-36 summary scores to identify meaningful differences in daily clinical practice.
Purpose:
To identify the most common Aspergillus species which cause fungal infections in human samples by applying conventional and molecular techniques and compare both results in Oman.

Methods:
18 strains of Aspergillus were isolated from clinical samples in Oman and identified by conventional methods such as culture and microscopy then, DNA was isolated from pure cultures of fungi by simple and rapid chemical based DNA extraction protocol. After the PCR products were sequenced, GenBank Blast searches were performed, and the sequence-based and conventional identifications were compared. Also all the isolates of Aspergillus species were further identified by using ITS-RFLP.

PCR:
The PCR assay was performed in a 50 μl-volume reaction as described by Vilgalys et al, (1990). 5 μl of 10x PCR buffer (10 mM Tris-HCL, [pH 8.3], 50 mM KCl, 1.5 mM MgCl2, 0.001 % w/v gelatine) (Applied Biosystems, Foster City, CA), 5 μl of 2 mM each dATP, dGTP, dCTP, and dTTP (Roche Diagnostics), 3 μl of 50mM MgCl2 solution (Roche Diagnostics), 5 μl of 10ng/μl primer SR6R (5’ AAGTATAAGTCGTAACAAGG 3’) and 5 μl of 10ng/μl primer LR1 (5’GGTTGGTTTCTTTTTCCT 3’) (Sigma-Genosys, Australia), 0.5 μl of 5 U of Taq DNA polymerase (Bioline), 10 μl of DNA, 16.5 μl of dH2O. Amplification was performed on a thermal cycler 480 (model, Watson Perkin Elmer). After the initial denaturation of the DNA at 97°C for 3 minutes, 35 cycles where performed. Each cycle consisted of a denaturation step at 97°C for 35 seconds, an annealing step at 50°C for 55 seconds, an extension step at 72°C for 45 seconds. A final extension step was at 72°C for 6 minutes following the last cycle.

ITS-RFLP:
First 25.8 μl PCR products of the ITS was added to the tube. Then, 4.2 μl of the master mix was added to the same tube. One drop of mineral oil was added to prevent evaporation. The tubes were closed and placed in the thermo-block. Digestions were incubated at 37°C for 3 hours. The PCR products of ITS was cut with Hha1 and after the digestion RFLP fragments were stored at 4°C.

Results:
This study shows that 13 strains were identified up to the species level and 7 up to the genus level by using conventional methods. The most common were Aspergillus niger (61.2%) followed by Aspergillus flavus (22.2%) and Aspergillus terreus (5.5%). By contrast 18 Aspergillus species were all identified up to species level by using ITS-sequencing and ITS-RFLP. All Aspergillus species were identified by PCR-sequencing as definitive identification test and showed that (100%) identified to species level. Also PCR-RFLP showed (100%) similarity to the identification by ITS sequencing and (72.2%) with culture results. There was 100% agreement between the PCR-RFLP analyses and ITS-sequencing analyses for the identifications obtained of the studied Aspergillus species.

Conclusions:
The conventional methods have mainly relied on culture isolation and subsequent observations of morphological features. Molecular techniques such as PCR followed by sequencing were found sensitive and accurate but, quite expensive. Analysis of the RFLP is a valuable tool for rapid and reliable identification of fungi in medical laboratories.
Primary laryngeal aspergillosis is an extremely rare opportunistic infection, especially in an immunocompetent host. So far, the disease process is not explicit. We report here a case of primary laryngeal aspergillosis in an immunocompetent female with the history of oral sex, which may be an independent aetiological factor.

**Case Report:**
A 23-year-old female undergraduate student who had been experiencing hoarseness in the throat for 15 days presented to our clinic. Initially, she began to experience hoarseness, severe paroxysmal coughing and tachypnea without significant causes. Due to obvious wheezing and crackles, she was diagnosed with asthma in a local hospital. Subsequently, white plaques were found on her vocal cords and laryngeal ventricle by laryngoscopy. After 10 days of anti-inflammatory inpatient treatment, her cough and tachypnea disappeared; however the hoarseness became worse than before. We carefully interviewed the patient; she was healthy in the past and denied history of asthma and long-term use of antibiotics or corticosteroids. However, she revealed a history of oral sex (fellatio), the latest occurrence being 1 week before she became ill. A laryngoscopy was performed again and revealed obvious white plaques on the swollen vocal cords and laryngeal ventricle (Fig. 1a). Samples were taken from the vocal cords. Numerous hyphae branching at 45° angles were detected by microscopy (Fig. 2a), electron microscopy (Fig. 2b) and pathology (Fig. 2c). A velvety and powdery colony was developed on Sabouraud Dextrose Agar (SDA) (Fig. 2d), at 28 °C. Microscopic examination of a slide culture (Fig. 2e) was consistent with the features of *Aspergillus fumigatus*. DNA sequencing was also carried out to confirm the identification as previously described (1). Genomic DNA was extracted using a DNA kit (Omega bio-tek, USA) and amplification of the intergenic transcribed spacer (ITS) regions flanking the 5.8 S region of the rDNA was performed by PCR, employing the ITS-1 (5’-TCC GTA GGT GAA CCT GCG G) and ITS-4 (5’-TCC TCC GCT TAT TGA TAT GC) primers provided by Shanghai Invitrogen Biotech. The samples were subsequently sent to Invitrogen Life Technologies for DNA purification and bidirectional sequencing. The sequences were aligned using Clustal X software. A BLAST search in GenBank using the ITS sequence showed 100% homology to *A. fumigatus* (435/435) with the accession number JF958125. Moreover, routine laboratory tests showed her hepatic function, kidney function and immunity were normal, serum HIV-antibody was negative, and computerized axial tomography showed a normal image of her chest.

The patient was diagnosed with primary laryngeal aspergillosis and treated with oral itraconazole solution at 200 mg (20ml) twice a day (keep the solution in mouth for a moment, then swallow slowly) for 5 days, followed by itraconazole capsules at the same dose for another 25 days (Sporanox, XIAN-JANSSEN Pharmaceutical). On the fifth day of treatment her hoarseness was relieved, and laryngoscopy showed lighter swelling of the vocal cords with white plaques markedly reduced, with negative results for both microscopy and fungal culture. After 4 weeks of therapy of itraconazole, the patient was cured and gradually recovered her normal voice; laryngoscopy showed that vocal cords were smooth without any white plaques (Fig. 1b). We followed up on the patient for half a year and there was no recurrence.
Table SI. Features of 18 cases of primary laryngeal aspergillosis in immunocompetent patients

<table>
<thead>
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<th>Case No. (ref)</th>
<th>Published Year</th>
<th>Sex</th>
<th>Age (years)</th>
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<tbody>
<tr>
<td>1. Rao PB. (3)</td>
<td>1969</td>
<td>M</td>
<td>48</td>
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<td>2. Ferlito A. (4)</td>
<td>1974</td>
<td>M</td>
<td>76</td>
</tr>
<tr>
<td>5. Nong et al (7)</td>
<td>1997</td>
<td>F and 4 M</td>
<td>30-40</td>
</tr>
<tr>
<td>6. Dean et al (8)</td>
<td>2001</td>
<td>F</td>
<td>21</td>
</tr>
<tr>
<td>7. Wittkopf et al (9)</td>
<td>2006</td>
<td>F</td>
<td>62</td>
</tr>
<tr>
<td>8. Ran et al (1)</td>
<td>2008</td>
<td>F</td>
<td>36</td>
</tr>
<tr>
<td>9. Liu et al (10)</td>
<td>2010</td>
<td>2 F</td>
<td>30/32</td>
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<tr>
<td>10. Ran et al (11)</td>
<td>2010</td>
<td>F</td>
<td>30</td>
</tr>
</tbody>
</table>

Fig. 1. (a) The initial laryngoscopy showed that both the vocal cords and the ventricle of larynx were swollen with hyperplasia and white plaques. (b) After 4 weeks of antifungal treatment, the vocal cords were smooth without any white plaques and almost recovered the normal function.

Fig. 2. (a) Microscopy (×400) showed numerous hyphae, some branching at 45° angles. (b) Histopathological examination showed many hyphae which had septate branches at 45° angles, suggested Aspergillus infection. The tissue was infiltrated with neutrophils (H&E, ×400). (c) SEM showed crowded hyphae branches at 45° angles covering and passing throughout the damaged tissue. (d) The culture of the isolate on SDA, showed the green velvety and powdery colony. (e) The slide culture of the isolate. (methylene blue, ×400).
Conclusions:
Primary laryngeal aspergillosis is extremely rare. So far as we know, since 1969 fewer than 50 cases have been reported in the English language literature. Advanced age, diabetes, long-term steroid therapy, chronic obstructive pulmonary disease, decline of CD4 lymphocyte cells, leukemia, lymphoma, HIV infection and other causes leading to immunocompromise are generally the aetiological factors (1). Still, for immunocompetent patients, there are no noticeable events to explain the development of the rare infection. For our patient, fellatio may be an important cause, although it may not be the direct cause as in the transmission of condyloma acuminata, HIV, herpes, and gonorrhea by oral-genital contact (2). By repeated friction, fellatio may cause physical changes such as local mucosal injury and edema, which may greatly contribute to the colonization of *Aspergillus* spores, followed by the occurrence of disease, and the relative serious symptom of this patient.

To review the related English literature, the age distribution and gender proportion of patients with laryngeal aspergillosis have changed dramatically over the years (Table 1). Noticeably, the number of reported cases has increased rapidly in the past twenty years. Females between the ages of 20-40 years (who are sexually active) have a much higher incidence than males, who were the primary patients until 1997, especially in the past decade. The demographic shift from old to young and male to female may be explained by the remarkably increased practice of oral sex (2). However, dates and other details are still needed, and unfortunately, for privacy of the patient and overlook of the doctor, many case reports lack such details.

Because of its extremely low incidence and lack of pathognomonic features, primary laryngeal aspergillosis is often misdiagnosed. Hoarseness may be the only symptom in most cases. For those who have received anti-inflammatory treatment without relief, laryngoscopy is needed. Hyphae can be found by microscopy, pathological examination and SEM. Culture and non-culture identification should be done to identify the isolate, and an antifungal susceptibility test is recommended. Itraconazole and amphotericin B are first-line agents to treat primary laryngeal aspergillosis; excision (9, 10) is also effective for patients with drug contraindication. For our patient, keep the oral itraconazole solution in mouth seems very good tolerance, then gently swallow allow the solution cover the mucous membrane of vocal cords and laryngeal ventricle, with a higher concentration of the itraconazole in the focus, to kill the invaded *A. fumigatus*, resulted quick relief of patient symptoms.
Objective:
To define demographic parameters, underlying diseases, etiology, clinical features and survival rate of haematological patients with nosocomial invasive aspergillosis (IA) in St. Petersburg, Russia.

Methods:
The prospective study was conducted during the period of 1998-2010 y.y. The diagnosis of IA was established with criteria EORTC / MSG 2008. Cases of nosocomial IA were considered on the basis of WHO criteria: "nosocomial infections (hospital-acquired infections), are infections acquired during hospital care which are not present or incubating at admission, if the disease occurred more than 48 hours after admission are usually to the hospital”.

Results:
During the study period 274 haematological with IA were observed. Proven and probable IA we diagnosed in 50% of patients (n=138). In 138 patients: nosocomial IA – 75% (n=103), out-hospital - 25% (35).

Among patients with nosocomial IA: male - 56%, female - 44%. Age of patients - from 2.5 to 68 years (median 31 ± 18 years). Adults were 78 (76%, median - 39 ± 15 years), children - 25 (24%, median - 10 ± 5 years). In patients with out-hospital IA: male - 63%, female - 37%. The median age: 40 ± 19 years (from 3 to 76 years). Adults - 31 (89%, median - 44 ± 18 years), children - 4 (11%, median - 16 ± 6 years).

In patients with IA the most frequent haematological malignancies were acute myeloid leukemia, acute lymphoblastic leukemia, non-Hodgkin’s lymphoma, and Hodgkin’s disease. We diagnosed nosocomial IA in more patients with acute myeloid and lymphoblastic leukemia than out-hospital (68% vs. 40%, p = 0.004).

Risk factors of nosocomial and out-hospital IA were: cytostatic chemotherapy (98%vs. 89%, p=0.017), neutropenia (91% vs. 71%, p=0.0036), lymphocytopenia (80% vs. 67%), corticosteroids (65% vs. 60%), and allo-BMT (36% vs. 31%).

Etiology of nosocomial IA: *A. fumigatus* (42%), *A. niger* (42%), *A. flavus* (16). In other group - *A. fumigatus* (39%), *A. niger* (35%), *A. flavus* (17%), *A. ochraceus* (4%), *A. versicolor* (4%), >2 *Aspergillus* spp. – 13%.

Lungs were involved in 95% vs. 97% patients, paranasal sinuses - 13% vs. 6%, CNS - 4% vs. 6%, eyes - 1% vs. 0, two or more organs - 12% vs. 9.

CT scan signs of nosocomial and out-hospital IA were: infiltrative lesions - 76% vs. 85%, focuses - 76% vs. 56%, “crescent” - 14% vs. 18%, “halo” - 3% vs. 9%.

GM in serum was positive in 81% vs. 88% of cases. Direct microscopic examination of BAL, sputum, CSF and sinuses aspirate was positive in 35% vs. 41% of cases and *Aspergillus* spp. was isolated from 35% vs. 62% (p=0.015).

Probable and proven IA we diagnosed in 94% and 6% of patients in both group.
Two or more antifungals received 85% vs. 83%, combined antifungal therapy - 18% vs. 3%. The most frequent for initial therapy was amphotericin B 52% vs. 29% of patients, voriconazole - 24% vs. 28%, caspofungin – 14% vs. 14%, itraconazole - 7% vs. 23%. Duration of treatment 2 to 277 days (median - 49 ± 23) vs. 2 to 420 days (median - 70 ± 35) (p = 0.004).

12 week overall survival was 78% vs. 82%, 12 months - 51% vs. 50%. 12 months overall survival was improved with use of voriconazole as initial therapy (55% vs 34%, p = 0.0099), surgical treatment (85% vs 40%, p = 0.0067) and secondary antifungal prophylaxis (70% vs 27%, p <0.0001).

Conclusions:
In hematological patients IA was nosocomial 75% cases. The main risk factors of nosocomial IA - chemotherapy, neutropenia, lymphocytopenia, and allo-BMT. Main pathogens of nosocomial IA are *A.fumigatus, A.niger* and *A.flavus*. 12 months overall survival of patients with nosocomial IA was 51% and improved with voriconazole use, surgical treatment and secondary antifungal prophylaxis.
**ASPERGILLUS SPECIES IN OUR LIVING ENVIRONMENT**

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**Introduction:**
The *Aspergillus* species are fungal organisms with a wide distribution in nature, found worldwide in the environment. The *Aspergillus* species are the most common fungal species, able to cause disease and allergic reactions in humans and animals. They produce mycotoxins in food and feedstuff. Numerous food products, spices, seasonings and other plant species contain spores of *Aspergillus* species. The aim of this study was to determine the presence of *Aspergillus* species in fresh vegetables and in herbal teas.

**Method:**
*Aspergillus* species grow well on standard media and can be easily isolated in laboratories. For the isolation of the *Aspergillus* species we used the Brain Heart Infusion Broth, Sabouraud Dextrose agar and Emmons Agar without adding antibiotics (as an antibiotic replacement, a selective supplement was used, containing: mucin, potassium metabisulfite and cupric sulfate). For the samples, we used fresh vegetables and herbal teas from the market. The samples were directly applied to the broth and to the surface of the agar, incubated at 24°C for 24h, and longer. After the incubation period, the broths were subculture on appropriate agars for the isolation of the *Aspergillus* species.

**Results:**
The results showed that, among other fungal species, the following *Aspergillus* species were isolated from the vegetables and teas: *A. niger* 38.5%, *A. fumigatus* 27.9%, *A. flavus* 15.7%, *A. terreus* 9.7%, and rest 8.2% is: *A. clavatus, A. glaucus, A. candidus* and *A. nidulans*.

**Conclusions:**
Although the spores of the Aspergillum species are found all around us in the air, the majority of people do not develop diseases because their immune systems are well developed. The spores are transmitted by air and this is the most common route of disease, which is why the Aspergillum species have emerged as an important cause of life-threatening infections among people with a weakened immune system, immunocompromised patients. The spores are most common during autumn and winter periods. Among the isolated *Aspergillus* species, only three cause infections in humans: *A. niger, A. fumigatus* and *A. flavus*. Eliminating any type of fresh or dried plant species, fruits and vegetables from the household will reduce the accumulation of *Aspergillus* species spores. The occurrence of multiple drug resistance of certain bacteria (especially the Pseudomonas and *E.coli* species) is the reason for the occurrence and increasing number of bacteria among fungal species on primary cultures. The fungi, whose growth is slower, can be suppressed by the bacteria, especially when the fungi are smaller in number and surrounded by numerous bacteria. The selective combination added to the Emmons agar acidifies the medium, thus eliminating the persistent bacterial contaminants. Microorganisms cannot develop resistance to chemicals, which is why such salts can successfully serve as a substitute for antibiotics in selective media for the isolation of fungi from specimens contaminated by various bacteria.
IN VIVO ALLERGIC IMMUNE RESPONSES OF *ASPERGILLUS* SPECIES ISOLATED FROM KATHMANDU VALLEY, NEPAL

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**Purpose:**
The species of *Aspergillus*, a major risk factor for both invasive and allergic aspergillosis, is an important cause of morbidity and mortality. Exposure to *Aspergillus* allergen, a strong carcinogen and toxin, causes an IgE response to *Aspergillus* antigen and hypersensitivity pneumonitis. At higher concentration, allergen directly affects the specific organs and tissues. *Aspergillus* species including *A. niger*, *A. flavus*, *A. fumigates* and *A. sulphurious* have been reported as allergenic sources. In vivo allergic immune responses of these species have been studied as these are prevalent in core areas of Kathmandu Valley.

**Methods:**
Screening of fungi and isolation of *Aspergillus* species was carried out by gravity plate method in core areas of Kathmandu Valley in different seasons between the year 2010-2011 AD and isolates of *A. niger*, *A. flavus*, *A. fumigates* and *A. sulphurious* were studied for comparing their allergic components. The allergens were extracted (Tilak, 1989), characterized by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE) and in vivo allergic immune responses were evaluated by intracutaneous inoculation in animal model.

**Results:**
More than 27 different spore types belonging to 21 genera were identified from different areas of Kathmandu Valley in different seasons. Species of *Aspergillus* (7.4%), *Penicillium* (19.7%), *Cladosporium* (29.7%) and *Fusarium* (2.8%) were the most prevalent. On SDS-PAGE allergic bands of 30 to 120 kDa were common in all the species of *Aspergillus*. The most allergenic bands of 20-35 kDa were prominent in *A. fumigatus* followed by *A. flavus*. In the in-vivo immune responses of allergens, *A. fumigatus* was the most allergenic than other species.

**Conclusions:**
The fungal spores were the predominant air contaminant in Kathmandu Valley that distributed uniformly. The extracts of *A. fumigatus* is more allergic than *A. flavus*, *A. niger* and *A. sulphurious* as characterized by the protein analysis and observed in the in-vivo allergic immune responses of *Aspergillus* species.
DETECTION AND QUANTITATION OF AFLATOXIN FOR THE DIAGNOSIS OF ASPERGILLUS FLAVUS

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Purpose:
Mycotoxins have been known to cause acute kidney failure (ochratoxin), damage of central nervous system (tremorgenic mycotoxin) and damage of the upper respiratory tract. Aflatoxins produced by Aspergillus flavus is a known carcinogenic hepatotoxin. The immunological assays for the detection and quantitation of toxin provide a technique for the diagnosis of disease. Aflatoxin from A. flavus was screened for its detection and quantitation by immunoassay.

Methods:
A. flavus producing mycotoxin were screened on Aflatoxin Producing Medium (APM) as described Donald et al (1981). Extraction of Aflatoxins were carried out (Abarca et al, 1988 and Chu, 1987) and detection of Aflatoxin B1 was done by TLC using pre-coated silica gel plates (Merck) and further confirmed by AOAC (Association of Official Analytical Chemists) method (Horwitz, 1975). Quantitation of Aflatoxin was done by ELISA (Chu, 1987). The data were analyzed by SPSS 16.0.

Results:
The highest amounts of Aflatoxin B1 were reported in synthetic medium, YES (68.56ng/ml and 200ng/ml) with 2% sucrose, pH 5.5 after 14 days of inoculation at 28°C (p-value 0.05), and in natural medium, groundnuts (121.20ng/g and 500ng/kg) by ELISA and TLC methods respectively.

Conclusions:
Aflatoxins produced by Aspergillus species could be used as a marker for the diagnosis of disease. The spores of A. flavus under the favourable environmental conditions produce sufficient amount of Aflatoxins B1 that was detected and quantified by ELISA and TLC. Detection and quantification of these adducts by immunoassays have been suggested to be an alternative methods to detect human exposure to aflatoxins in blood samples.
ASPERGILLUS FUMIGATUS SECRETES FACTORS THAT STRONGLY ACTIVATE HUMAN THROMBOCYTES

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Purpose:
Platelets were recently discovered to be part of the innate immunity and thus might participate in the immune defense during invasive Aspergillose; on the other hand, they might also contribute to some hallmarks of this disease such as inflammation and thrombosis. We studied the hypothesis that *A. fumigatus* secretes soluble factors that modify activity and functionality of thrombocytes.

Methods:
*A. fumigatus* was grown for 2 days in medium. The supernatant was harvested and different volumes were added to freshly isolated human platelets. After the incubation activation of thrombocytes was quantified by FACS analysis of the marker CD62P, annexin binding and phagocytic capacity. Furthermore platelet aggregation was studied by aggregometry.

Results:
Even minimal volumes of the fungal culture supernatant were capable to potently stimulate the platelets, inducing high expression of CD62P on the surface. Furthermore the secreted fungal factors increased annexin binding of the platelets and induced significant thrombocyte aggregation, even after few minutes of incubation. Experiments using different signal transduction inhibitors indicated that calcium release, PI-3 kinase and the protein tyrosine kinase Syk participated in the signal transduction pathways leading to the stimulation of thrombocytes. Two active components in the fungal culture supernatant could be identified. First, the role of a fungal serine protease was confirmed by use of serine protease inhibitors, which partly eliminated the thrombocyte-stimulating capacity of the *A. fumigatus* supernatant. Second, the mycotoxin gliotoxin seems to play a role, since an *A. fumigatus* mutant unable to synthesize this mycotoxin does not stimulate the thrombocytes to an large extent. Furthermore, the effect of the fungal supernatant could be mimicked by purified gliotoxin. Preliminary experiments with glutathione, a reducing compound that inactivates gliotoxin, suggest the possibility to counteract the action of the mycotoxin and thus to reduce the danger of excessive platelet activation during invasive aspergillosis.

Conclusions:
Secreted fungal factors such as proteases and mycotoxins might participate in thrombocyte activation during invasive aspergillosis. Putative consequences could be a platelet-driven antimicrobial response but also, on the other hand, thrombosis and thrombocytopenia.
**ASPERGILLUS FUMIGATUS SECRETES SOLUBLE FACTORS THAT INDUCE COMPLEMENT DEPOSITION ON THROMBOCYTES**

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**Purpose:**
As the mechanisms of pathogenesis and immune defense in invasive aspergillosis are still insufficiently clarified, further studies are an urgent need that may help to develop new therapeutic approaches. The complement system is considered as a pivotal weapon of innate immunity against fungal infections. Similarly, thrombocytes are progressively accepted as antimicrobial actors. Various proteins of the complement system interact specifically with thrombocytes to activate them and vice versa. In our studies we aimed to examine the influence of soluble factors secreted by *A. fumigatus* on the deposition of complement proteins on platelets.

**Methods:**
*A. fumigatus* was grown in RPMI medium; culture supernatant was harvested after 2 days. Platelets were incubated with different concentrations of the supernatant and subsequently opsonized with serum. Fluorescent antibodies were used to label deposited complement proteins and the platelet activation marker CD62P (P-selectin). Complement deposition and platelet activation were analyzed by flow cytometry.

**Results:**
*Aspergillus fumigatus*, when grown in cell culture medium, secretes factors that are able to activate human thrombocytes, as shown by appearance of the activation marker CD62P on the platelet surface. As CD62P has been described to serve as a receptor for complement factors, we investigated the deposition of complement on thrombocytes as a consequence of incubation with the fungal supernatant. Our FACS analysis showed a strong opsonization of the thrombocytes with complement factor C3. Incubation times of only 30 minutes were sufficient to increase the appearance of C3 on the platelet surface. In addition, also other complement factors such as C1q and C5 could be detected on the thrombocytes. Kinetic studies showed a perfect correlation between the fungal factor-induced membrane exposure of CD62P and the amount of deposited complement proteins. Furthermore, we could show that even very low volumes of the *A. fumigatus* culture supernatant were sufficient to elicit the effects of thrombocytes activation and complement deposition.

**Conclusions:**
The secretion of soluble factors by *A. fumigatus* induces platelet activation and the deposition of complement factors on the platelet surface; these processes may lead on to various possible consequences that need to be further clarified. On one hand, the spreading of the fungus in the host may be facilitated by consumption of complement proteins and probable ingestion of opsonized thrombocytes by phagocytes. On the other hand, the concurrent activation of platelets and the complement system might induce increased cytokine production and attract immune cells to the focus of infection, which could help to limit the fungal dissemination.
GALACTOMANNAN DETECTION IN BRONCHOALVEOLAR LAVAGE FLUID IN INTENSIVE CARE UNIT PATIENTS AT RISK FOR INVASIVE ASPERGILLOSION

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Purpose:
Invasive aspergillosis (IA) is a serious and often fatal infection among immunocompromised patients in the intensive care unit (ICU). Delayed diagnosis and therapy may lead to poor outcomes. Diagnosis may be facilitated by a test for galactomannan (GM) antigen detection using an enzyme immunoassay. In the present study we assessed GM testing of bronchoalveolar lavage (BAL) fluid as a tool for early diagnosis of IA among ICU patients who were at risk for developing IA.

Methods:
The prospectively performed study from August 2009 through September 2010 in ICU patients with underlying predisposing conditions for IA. BAL samples for direct microscopic examination, culture, and GM detection were obtained once or twice weekly. GM in BAL levels were measured using the Platellia Aspergillus EIA test kit. According to modified EORTC/MSG criteria, patients identified were classified.

Results:
A total of 36 patients fulfilling the inclusion criteria were enrolled, 15 patients IA were classified as having 4 cases (30.8%) possible and 9 cases (69.2%) probable IA. From patients IA, 11(52.4%) had at least one positive BAL GM index. The sensitivity, specificity, positive and negative predictive values for BAL GM at a cut-off ≥0.5 were 100%, 85.7%, 65.7% and 96%, and cut-off ≥1.0 were 73%, 92.7%, 89% and 87.3%, respectively. In 6 cases, BAL culture or direct microscopic examination remained negative, whereas GM in BAL was positive.

Conclusions:
Our data have revealed that the sensitivity of BAL GM testing was better than that of conventional tests such as clinical signs, radiological diagnoses, BAL cytology and culture. The use of GM in BAL as a means of establishing or excluding early diagnosis and monitoring of the course of IA in ICU patients.
ROLE OF THE NEUTROPHIL AND IT’S INTERACTION WITH THE TH-17 PATHWAY IN THE HOST RESPONSE AGAINST ASPERGILLUS FUMIGATUS

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Purpose:
Innate immunity plays an important role in host defense against Aspergillus fumigatus. The effectiveness of this defense system is mainly based on the phagocytic and antimicrobial activity of different cells that predominantly consist of macrophages and neutrophils and their ability to produce pro-inflammatory mediators. The alteration of this defense system results in the development of infection. By knocking down various cell populations in murine models of invasive aspergillosis the essential role of neutrophils in the immunocompromised host defense has been demonstrated by using a bioluminescent strain of A. fumigatus.

Recent studies have focused on the role of Th17 (LTh17) lymphocyte subpopulation in infection and inflammation. The LTh17 seems to play a role in chronic inflammatory and autoimmune diseases. When activated, Th17 cells rapidly initiate an inflammatory response that is dominated by neutrophils. However, the role of Th-17 response in the host defense against A. fumigatus is controversial. In addition, the impact of the Th-17 pathway on neutrophil recruitment during onset and progression of aspergillosis has not been studied yet.

Methods:
We use flow cytometry and cytokines analysis to investigate the different immunological mechanisms implemented by the immnocompetent host effector cells in the defense against A. fumigatus by comparing wild-type (WT) mice to CXCR2 knock out (KO) mice, in which neutrophil recruitment is impaired and are, thus, susceptible for aspergillosis. The progression of invasive aspergillosis was monitored using the bioluminescent optimised-codon strain of A. fumigatus.

Results:
Our data show a decrease of LTh17 cells in wild-type mice, whereas this response is reversed in CXCR2 knock out mice. In addition, the impairment of neutrophils results in a high inflammatory response as inferred from the cytokine and chemokine pattern from lungs homogenates of susceptible mice. At day 6 after infection, the high level of IL-17, but not IFN gamma, indicates that the implemented adaptative immune response is of type LTh-17.

To further evaluate the involvement of Th-17 lymphocytes in the recruitment of neutrophils in the host response, cell populations were compared following the infection of WT type and RAG knock out mice, which are devoid of T, B and NKT cells. Although both WT and RAG KO mice survived the infection, the number of recruited neutrophils was decreased in the KO mice.

Conclusions:
In immunocompetent mice, T cells are not essential for the survival. However, in the situation of neutrophils deficiency, the increase of the Th-17 cells suggest that they are involved in the recruitment of neutrophils that remain the major cells required for an efficient immune response against A. fumigatus.
PRIMARY RENAL ASPERGILLOSIS AND ASSOCIATED CYTOKINES IN BALB/C MICE IN RESPONSE TO ASPERGILLUS FLAVUS

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Purpose:
Although Aspergillus flavus has been known to be more virulent than almost all other Aspergillus sp., there is no report on the immune responses elicited in the murine model of systemic infection. Thus we wanted to determine the immune response in one of the primarily infected organ (Kidneys) of mice challenged with A. flavus.

Methods:
The immunocompetent male BALB/c mice were infected intravenously with 3.3×10⁵ conidia of A. flavus. Cytokines TNF-α, IFN-γ, IL-6, IL-12/IL-23p40, IL-4 and IL-10 were determined by the murine specific Cytokine enzyme linked immunosorbent assay (ELISA) in the infected or control mice at different time points from 6 hours post infection (h PI) to 96 h PI during the progression of infection. Further, the extent of infection was also evaluated by histopathology and by analysis of colony forming unit (CFU) in kidneys homogenates.

Results:
During the initial phase of infection up to 12 h PI, the rate of clearance of A. fumigatus was high, presumably by the recruited neutrophils and the resident macrophages with concurrent significant release of pro-inflammatory cytokines (TNF-α, IFN-γ, IL-12/IL-23p40, IL-6) in comparison to control mice. However at later phase of infection, there was a rise in the number of A. flavus leading to renal aspergillosis as determined by histopathology and CFU counts. The fungal growth was evident in the histological sections at 72 and 96 h PI and we got some clue about the production of blastoconidia of A. flavus after three days post infection in the sections of kidneys. Concommitantly, at later time-points, a significant and steady decrease in the production of pro-inflammatory cytokines except IL-6 and IL-12/IL-23p40 in A. flavus infected group of mice was observed. The cytokine IL-6 showed significant increase at 72 and 96 h PI, however, IL-12/IL23p40 demonstrated a significant rise at 96 h PI in comparison to respective controls. There were no any significant change in the Th2 cytokines IL-4 and IL-10 during the present investigations.

Conclusions:
(1) In the early phase of infection the protective Th1 pathways was active as evident by the significant increase in Th1 cytokines TNF-α, IFN-γ, IL-12/IL-23p40. The increased antifungal activity as demonstrated by a high rate of decrease of CFU during the early hours further supports the Th1 type response during the early hours.

(2) The later phase of infection demonstrated a decrease in the Th1 cytokines like TNF-α and IFN-γ and a significant increase in the cytokines IL-6 and IL-12/IL-23p40 (Both IL-6 and IL-23 promotes the activation of Th17 subsets of T cells) suggesting the operation of Th17 response.

(3) The ureter and pelvis were the primary renal tissues, which demonstrated the fungal growth.
INCREASED SUSCEPTIBILITY TO \textit{ASPERGILLUS FUMIGATUS} IN RECIPIENTS WITH GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION CAN BE PREVENTED BY TRANSFER OF DONOR CD4+CD25+ REGULATORY T CELLS

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\textbf{Purpose:}
Graft-versus-host disease (GvHD) is a frequent and life-threatening complication after allogeneic bone marrow transplantation (alloBMT). It is initiated by interaction of host antigen-presenting cells with mature alloreactive donor T cells and leads to dysregulated pro-inflammatory cytokine secretion and target organ destruction. Patients after alloBMT are severely immunocompromised and thus particularly prone to opportunistic bacterial and fungal infections. We showed before that co-transplantation of donor CD4+CD25+ Treg cells protects mice from lethal GvHD. We here tested the impact of GVHD as well as of co-transplanted donor Treg cells on course and severity of an opportunistic fungal infection after alloBMT.

\textbf{Methods:}
We employed a completely MHC-mismatched murine C57BL/6 into BALB/c alloBMT model. Recipients with or without GVHD were infected with the clinically relevant pathogen \textit{Aspergillus fumigatus} on day 28 after BMT when the animals had recovered from irradiation-induced neutropenia and symptoms of GvHD had developed. Part of the animals received donor CD4+CD25+ Treg cells for GVHD prophylaxis.

\textbf{Results:}
After infection with \textit{Aspergillus fumigatus} all animals with GVHD died within 10 d after infection, whereas 60% of the animals without GVHD survived for more than 35 d. Survival of recipients protected from GVHD after co-transplantation of donor CD4+CD25+ Treg cells was significantly better than that of unprotected recipients with GVHD. Interestingly, clearance of the fungus from the lung after i.t. infection, or from spleen, liver, lung, kidney and brain after i.v. infection, was rapid and comparable in mice with and without GvHD and no live fungus was detectable in moribund animals. However, lymphocytes isolated from spleen and liver of infected animals with GVHD and restimulated in vitro with germinating conidia secreted significantly more pro-inflammatory TNF, IFN-\textgamma and IL-6 than those from control mice. Co-transplanted donor Treg cells did not interfere with pathogen clearance, but normalized the dysregulated cytokine secretion.

\textbf{Conclusions:}
Our data show that co-transplantation of donor CD4+CD25+ Treg cells protects mice not only from lethal GvHD but also from infection-related co-mortality. Furthermore, they support the hypothesis that an uncontrolled inflammatory immune response contributes to the high morbidity and mortality of opportunistic infections in GVHD.
VITAMIN D REGULATION OF TH2 AND TH17 IMMUNE RESPONSE TO ASPERGILLUS IN MICE AND HUMANS: ROLE OF OX40L

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Purpose:
4-15% of cystic fibrosis (CF) patients develop Allergic Bronchopulmonary Aspergillosis (ABPA) characterized by Th2 responses to Aspergillus. Colonization rates with Aspergillus in CF can approach 40-50% suggesting immune tolerance may also prevent ABPA. We have previously shown that CD11c dendritic cells (DCs), OX40 Ligand, and Vitamin D play a role in sensitization versus tolerance to Aspergillus. We have set up an animal model to further explore the roles of CD11c DCs, OX40L, and Vitamin D response to Aspergillus.

Methods:
In the clinical arm of the study, patients with ABPA (defined by the NACF Consensus Statement) and patients with Aspergillus colonization alone were enrolled. A group of ABPA patients were placed on supplemental Vitamin D. CD11c+ DCs were cultured with (control DCs) and without TSLP (TSLP DCs), and pulsed with Aspergillus extract (Af). CD4+ T-cells were added and cultured for 4 days.

For the mouse model, Vitamin D deficient mice (by nutritional diet) and Vitamin D normal mice where sensitized and challenged with Aspergillus and with/without anti-OX40 ligand. Lung, lymph nodes (LN), and bronchoalveolar lavage (BAL) fluid where analyzed for Th2 and Th17 cytokine production. In vitro, Mouse CD11c DCs from Vitamin D deficient and Vitamin D normal mice where cultured with naïve CD4+ T cells and pulsed with Aspergillus extract. Outcome measures included IL-4, IL-5, IL-13, IL-10, and FASC analysis for CD4, CD25, FoxP3, IL-10 and TGFbeta in both human and mouse experiments.

Results:
Th2 responses were significantly attenuated in ABPA patients by pre-incubation with anti-OX40L antibody. In contrast, Af colonized patients had significant increases in CD4, CD25+ FoxP3+ cells and higher surface TGFb on these cells. ABPA patients had no differences in BMI, Vitamin A or E levels but were significantly Vitamin D deficient compared to the Aspergillus colonized cohort. Addition of active Vitamin D in vitro decreased the Af Th2 response and increased TGFb+ Treg cells. Similar results were seen in ABPA CF patients given supplemental Vitamin D.

In vitro, cD11c DCs from Vitamin D deficient mice elicit significantly greater Th2 and Th17 responses compared to Vitamin D normal mice. Addition of 1,25 OH Vitamin D3 to cell culture significantly inhibits Th2 and Th17 production from Vitamin D deficient mice. In vivo, Vitamin D deficient mice have increased Th2 and OX40L response to Aspergillus in lung, BAL, and LN. In addition, treating Vitamin D deficient mice with anti-OX40L prior to Aspergillus infection decreases Th2 response suggesting a critical role of OX40L in Vitamin D mediated Th2 suppression.

Conclusions:
These results support therapeutic use of Vitamin D to treat ABPA in CF and suggest Vitamin D plays a role in immune tolerance to Af.
ANTI-ASPERGILLUS ANTIBODIES IN HAEMATOLOGICAL PATIENTS SUSPECTED FOR DEVELOPING INVASIVE ASPERGILLOSIS

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Purpose:
Invasive fungal infections continue to cause considerable morbidity and mortality in patients with haematological malignancies, especially invasive mould infections caused by Aspergillus spp. Survival of patients with invasive aspergillosis (IA) depends on early diagnosis, but clinical manifestations of IA are not specific, also standard laboratory methods are not enough sensitive. Therefore, detection of early laboratory fungal biomarker glactomannan (GM) nowadays is essential for timely diagnosis. Despite the fact that GM is approved by Food and Drug Administration, the shortcomings are in the existence of false negative and false positive findings. Therefore, using two or more tests can improve diagnosis.

Methods:
The aim of this study was to investigate cumulative diagnostic potential of screening for GM and anti-Aspergillus antibodies in our adult hematological patients during two years period (2007-2009). A total of 371 serum samples from 150 patients were collected and analyzed.

Results:
Both GM and anti-Aspergillus antibodies were negative in 79/150 (52.66%). In positive patients (n=71/150) GM was detected in 20/71, anti-Aspergillus antibodies were detected in 33/71, while both GM and anti-Aspergillus antibodies were detected in 18/71 patients. Our results indicate that monitoring of GM as the only laboratory biomarker reduces the number of suspected patients (25%). If GM and anti-Aspergillus antibodies are monitored simultaneously the number of suspected patients is higher (47%).

Conclusions:
Screening for GM and anti-Aspergillus antibodies significantly increases the number of patients suspected for developing IA. This finding is important because it can indicate early diagnosis of IA in GM negative haematological patients.
T-CELL RESPONSES TO SEVERAL ASPERGILLUS ANTIGENS MAY BE DETECTED IN PATIENTS WITH INVASIVE ASPERGILLOSIS AND MAY BE EXPLOITED FOR DIAGNOSTIC AND THERAPEUTIC PURPOSES: A MULTICENTER STUDY

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Purpose:
We have investigated whether: 1. the identification of Aspergillus-specific T cells may be effective in the diagnosis of invasive aspergillosis (IA) in high risk patients; 2. Aspergillus antigens, most frequently targeted by protective immune responses, could be identified in patients with proven IA; 3. specific-protective T cells could be expanded from the peripheral blood (PB) of proven patients, during IA.

Methods:
180 patients have been enrolled, including 18 proven, 35 probable, 17 possible IA cases and 110 controls. Specific immune responses producing interleukin-10 (IL-10), interferon-gamma (IFN-γ), IL-4 and IL-17A were detected and characterized by enzyme-linked immunospot (ELISpot) assay and cytokine secretion assay (CSA). The antigens used were Aspergillus conidia and the recombinant GEL1p, CRF1p, PEP1p, SOD1p, α1–3glucan, β1–3glucan, and galactomannan. Cytotoxicity has been investigated with the XTT assay. Aspergillus-specific T cells were obtained by culturing PB mononuclear cells with PEP1p, GEL1p, α1-3 glucan and β1-3 glucan. The infection course were divided into 4 phases, defined from t1 to t4, each of fifteen days interval, starting from the radiological diagnosis of IA.

Results:
The sensitivity and specificity of ELISPOT for the diagnosis of IA resulted 94.4% and 98.2%, respectively. The PPV and the NPV of the test were 89.5% and 99.1%. The efficiency was 97.6%. The positive likelihood ratio resulted 51.89, the negative was 0.06. In proven IA patients, Aspergillus-specific T cells producing either IL-10 or IFN-γ were detected to all the antigens, but galactomannan. The number of antigens targeted by IFN-γ producing specific T cells progressively increased along the course of IA, from 3 at t1 to 6 at t4. At t1, IFN-γ producing specific T cells were only detected to GEL1p, α1-3 glucan and β1-3 glucan. GEL1p and α1-3 glucan resulted the antigens most constantly targeted by IFN-γ producing specific T cells, persisting the responses to these antigens in all the phases of IA. Aspergillus-specific T cells producing IL-17A were detected in only one patients, and targeted CRF1p. Specific T cells to galactomannan and producing IL-4 to all the antigens could be shown only by CSA, suggesting that they are present only at very low frequencies during the infection. After 13-day cultures, Aspergillus-specific T cells were expanded from five out of five patients. They included a median of 95.8% CD3+ cells, either CD4+ or CD8+ T cells, and showed a median lytic activity of 9.45% either at 3/1 or at 5/1 effector/target cells ratios.

Conclusions:
Our findings demonstrate the potential of ELISPOT in diagnosing IA, and, because of a high PPV, suggest that it might be well combined with the other non-coltural diagnostic methods. Moreover, in patients with IA, at the onset of IA, protective immune responses target antigens involved in the cell wall biosynthesis of Aspergillus. Such T cells may be expanded, even in the course of IA and are able to directly kill fungal hyphae. The above mentioned antigens and the corresponding protective T cells may be exploited for therapeutic strategies of either vaccine or autologous cytotoxic cell infusions in patients at high risk for IA.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION
POLYMIXIN B, IN COMBINATION WITH VORICONAZOLE, MODULATES CYTOKINE RESPONSE OF MAMMALIAN CELLS EXISTING A POTENT FUNGICIDAL EFFECT ON *ASPERGILLUS FUMIGATUS*

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**Purpose:**
There is an urgent need for new antifungal therapies due to the limited number of clinically available antifungals and the fact that many antifungals either lack potency or are toxic to the host. The purpose of this study was to evaluate antifungal and the immunomodulatory effects of voriconazole and polymyxin B, in combination, on human peripheral blood mononuclear cells (PBMC) stimulated with *Aspergillus fumigatus*.

**Methods:**
The antifungal effect of cationic peptide antibiotic polymyxin B and its synergistic interactions with voriconazole were tested against *Aspergillus fumigatus* using microdilution and disc diffusion techniques. The toxicity of the drug combination of polymyxin B and voriconazole was compared with that of each drug alone in PBMC cultures. Cell proliferation was assayed by colorimetric method using MTT -(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide). Levels of cytokines, such as IFN-gamma, IL-2, IL-6, IL-10, and TGF-beta, in the cell culture supernatants were determined by specific Enzyme-Linked Immunosorbent Assay (ELISA) technique.

**Results:**
We found that polymyxin B possesses anti-*Aspergillus* activity at relatively high concentrations. However, because of its synergistic interactions with voriconazole, polymyxin B at much lower concentrations exerts a potent fungicidal effect against *A. fumigatus*. The drug combination displayed no additional toxicity compared with polymyxin B alone when tested in cell culture, moreover the combination lead to proliferation of PBMC. In the presence of voriconazole and polymyxin B, the levels of IL-2, IFN-gamma, and IL-6 increased remarkably in PBMC stimulated by *A. fumigatus*, while TGF-beta and IL-10 were not increased.

**Conclusions:**
The combination of polymyxin B and voriconazole has the potential to be used in the clinic to treat systemic aspergillosis.
Introduction:
Invasive aspergillosis is a major threat to patients suffering from chronic granulomatous disease (CGD). While *A. fumigatus* is the most commonly encountered species, *A. nidulans* is unique in its interaction with CGD patients. Previously we showed that *A. nidulans* is killed independently by the presence of an active NADPH-oxidase and that CGD phagocytic cells can cope with *A. nidulans* as efficiently as healthy cells do. In this study, we questioned whether a difference in cytokine profile could be responsible for the observed *A. nidulans* pathogenesis in CGD patients and whether this could be related to differences in melanin or polysaccharide cell-wall composition between *A. nidulans* and *A. fumigatus*.

Materials & Methods:
Blood was drawn from healthy volunteers (n=6) and two X-CGD patients (gp91phox deficient) after informed consent. PBMC were isolated by density gradient centrifugation. PBMC (5x 10⁶ cells/ml) were inoculated in duplicate and stimulated for 24h at 37°C at 5% CO₂. As stimuli were used: LPS (10ng/ml), 1x10⁶/ml live *A. nidulans* conidia (V44-46) and *A. fumigatus* (V45-07), live albino *A. nidulans* (A191) and *A. fumigatus* (RGD12), isolated cell-wall fragments of *A. nidulans* (FGSC strain A28) and *A. fumigatus* (A237) (kindly provided by Prof. Momany, University of Georgia, USA). IL-1β and TNFα (Sanquin) were assessed by ELISA. *Aspergillus* galactomannan antigen (GM) was detected by PlateliaTM *Aspergillus* EIA. Fungal damage was assessed by XTT. Inter-group comparisons were made using the non-parametric Mann-Whitney method. A p-value of <0.05 was considered statistically significant.

Results:
*A. nidulans* conidia induces significantly more pro-inflammatory cytokines (IL-1β and TNF-α) compared to *A. fumigatus*. In the absence of a functional NADPH-oxidase, the pro-inflammatory phenotype of *A. nidulans* is more boosted compared to the cytokine production induced by *A. fumigatus*. It has been shown that *A. fumigatus* conidial melanin has immunomodulatory capacity’s. Indeed *A. fumigatus* conidia devoid of melanin induce significantly more IL-1β and TNF-α compared to wild type. Interestingly, no difference in cytokine production could be observed between albino *A. nidulans* and the corresponding wild type conidia, suggesting a major role of the cell-wall composition in the pathogenesis of *A. nidulans* in the CGD host. GM is one of the major structural components of fungi. However, no differences were found in analyzing the amount of GM released by *A. fumigatus* compared to *A. nidulans*. In contrast, stimulation of PBMC by isolated cell-wall fragments of *A. nidulans* compared to *A. fumigatus* confirmed the significant higher IL-1β production induced by *A. nidulans* compared to *A. fumigatus*. Finally, fungal damage to albino *A. fumigatus* was significantly higher compared to wild type. In contrast, absence of *A. nidulans* melanin did not result in increased fungal damage.

Conclusions:
*A. nidulans* induces a more pro-inflammatory phenotype (IL-1β and TNF-α) compared to *A. fumigatus*. Melanin of *A. nidulans* does not play a major role as virulence factor in the host-pathogen interaction. Dysregulated inflammation is a crucial factor in the pathogenesis of *A. nidulans* in CGD an further analysis of the cell-wall composition of *A. nidulans* is urgently needed to understand and to manage this life-threatening infections.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION
Purpose:
In cystic fibrosis (CF), progressive pulmonary diseases and complications are the most dominant clinical features, characterized by chronic airway infection and inflammation. *Aspergillus fumigatus* is commonly found in the respiratory secretions of patients with CF. Although allergic bronchopulmonary aspergillosis (ABPA) is associated with deterioration of lung function, the relative contribution of *A. fumigatus* colonization versus host factors in lung function is not clear.

Methods:
To evaluate susceptibility to pulmonary aspergillosis in CF, we used Cftr tm1Unc (Cftr-/-) mice, reported to mimic, to some extent, human CF. Infection was carried out through intranasal instillation of a suspension of *A. fumigatus* resting conidia. After 1 week, we evaluated the mice for parameters of airway inflammation.

Results:
We found that, despite visible signs of inflammatory pathology in the lung parenchyma, only a moderate fungal growth in the lungs of Cftr-/- mice was observed, eventually resulting in a chronic infection. Neutrophil recruitment and infiltration in the lung parenchyma was observed in Cftr-/- mice, as well as high levels of inflammatory cytokines such as IL-17A and TNF-α. In the face of the heightened inflammatory response, defective fungal clearance, due to impaired activity of local effector cells, was paradoxically observed in Cftr-/-mice. These findings suggest that dysregulated host mechanisms, in addition to or by influencing fungal colonization, may contribute to the pathogenesis of inflammation in infection in CF.

Conclusions:
We know that inflammation results from recognition of pathogen–associated molecular patterns (PAMPs) and from reaction to tissue damage–associated molecular patterns (DAMPs). Thus, host-derived metabolic and stress signals may play a non secondary role in the pathogenesis of the infection in CF. Combined murine and human studies are underway to define the host metabolic signaling pathways contributing to CF patient’s susceptibility to infection by *Aspergillus* spp.
Purpose:
The ubiquitous fungus *Aspergillus fumigatus* can lead to severe pulmonary disease in immunocompromised patients, such as fungal pneumonia or allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis patients. The anti-microbial regenerating islet-derived 3 (Reg3) family of lectins have been shown to play a role in intestinal immunity; however, their role during fungal infections in the lung has not been characterized. Here, we investigated the impact of Reg3gamma (Reg3g) on pulmonary *A. fumigatus* infection.

Methods:
Expression of the Reg3 family was analyzed by PCR and ELISA from lung tissues of B6 mice infected with *A. fumigatus*. To determine if Reg3 proteins adhere to *A. fumigatus*, recombinant mouse Reg3g-human IgG1 (Reg3g-Fc) and Reg3beta-Fc fusion proteins were expressed in 293T cells, purified with protein G and used to stain *A. fumigatus* organisms by flow cytometry. To assess the immune response to *A. fumigatus*, B6 and Reg3g-/- mice were infected by oropharyngeal aspiration, and the expression of IL-17, IL-22 and IL-23 were analyzed by PCR, ELISA and flow cytometry. IL-17 production by small intestinal lamina propria lymphocytes was analyzed by flow cytometry.

Results:
Pulmonary infection with *A. fumigatus* increased the expression of Reg3b, Reg3d and Reg3g in lung tissue. Recombinant Reg3 proteins were capable of binding to *A. fumigatus* organisms, indicating a potential role in host defense. Reg3g-/- mice were not defective in fungal clearance, and produced increased IL-17 following infection. Further, naïve Reg3g-/- mice were found to have increased numbers of CD4 T cells producing IL-17 (Th17 cells) in small intestinal lamina propria, suggesting a role for commensal bacteria in the gut. In support, antibiotic treatment of Reg3g-/- mice suppressed their pulmonary ovalbumin-specific Th17 response.

Conclusions:
This data suggests that Reg3g may be involved in anti-fungal immunity by binding to inhaled *A. fumigatus* organisms and perhaps decreasing its virulence. More importantly, however, Reg3g suppresses pulmonary Th17 responses by altering the gut microbiome. This data has implications for allergic bronchopulmonary aspergillosis, and suggests that Reg3g-sensitive commensal bacteria contribute to pulmonary Th17-driven inflammation.
IDENTIFICATION OF A. FUMIGATUS SECONDARY METABOLITES INVOLVED IN NEUTROPHIL RECRUITMENT USING A NOVEL MICROFLUIDIC PLATFORM

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Purpose:
The innate immune system – and neutrophils in particular – play a central role in host-defense against Aspergillus infections. An important parameter in host defense is neutrophil recruitment to Aspergillus spores and hyphae. While the ability of neutrophils to home to the site of the infection is essential, relatively few compounds have been identified enabling neutrophil recruitment, and even fewer platforms exist to assess the chemotaxis potential of putative compounds in a high-throughput manner.

Methods:
We developed a microfluidic platform allowing the screening of neutrophil recruitment to a large number of samples of interest. The migration of human primary neutrophils to supernatant of mutants of A. fumigatus and A. nidulans in liquid shake cultures, spore suspensions, germinating spores, and phagocytosed spores of was recorded. High-throughput image analysis software was developed to allow processing of the microscopy data collected from hundreds of migration experiments.

Results:
Using a novel microfluidic platform for screening neutrophil recruitment to natural products, we have found that the supernatant from Aspergillus spp. grown in liquid shake conditions acts as a neutrophil chemoattractant. We show that extracts from ppoA deletants unable to generate specific lipid derived secondary metabolites, result in loss of neutrophil recruitment ability.

Conclusions:
Fungal hyphae secretes secondary metabolites which directly induces an innate immune response. The panel of secondary metabolites secreted by A. fumigatus is wide and varies considerably based on the growth conditions and the specific fungal strains, making the identification of these metabolites challenging. The establishment of novel microfluidic platforms have the ability to significantly increase the scope of in-vitro experiments that can be made and allow the identification of metabolites inducing an immune response from HPLC fractionation.
B-GLUCAN MEDIATED AUTOPHAGY INDUCTION REGULATES INTRACELLULAR KILLING OF ASPERGILLUS FUMIGATUS IN HUMAN MONOCYTES

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Purpose:
Aspergillus fumigatus is a leading cause of fungal infections in an expanding population of immunocompromised patients. In immunocompetent individuals, phagocytic cells efficiently eliminate A. fumigatus spores, thus preventing germination and invasive fungal growth. However, little is known on the molecular mechanisms regulating intracellular killing of A. fumigatus by human phagocytes.

Methods:
We evaluated intracellular trafficking and the induction of autophagy pathway during A. fumigatus infection of human monocytes/macrophages by confocal imaging microscopy, FACS and western blot analysis. Primary human monocytes or THP-1 differentiated macrophages were stimulated with GFP-expressing or fluorochrome-labeled A. fumigatus spores, purified β-glucan particles and latex beads coated with different TLR ligands. Conditional inactivation of autophagy related genes and transfection of monocytes with a GFP-LC3 plasmid was performed using the Amaxa electroporation system.

Results:
We found that fungal cell wall swelling during A. fumigatus infection within human monocytes triggered an early and selective induction of the autophagy pathway. The formation of autophagosomes containing A. fumigatus germinating spores was tightly regulated by β-glucan surface exposure as it was completely abolished following β-glucan enzymatic digestion or competitive inhibition by non-immunostimulatory β-glucans. Induction of the autophagy response by A. fumigatus germinating spores required phosphorylation of syk kinase and downstream activation of PI3K III and ERK signaling. Conditional inactivation of autophagy-related genes in human THP-1 macrophages resulted in significant impairment in phagolysosomal fusion and killing of A. fumigatus. Importantly, robust induction of the autophagy pathway by the Aspergillus pigmentless mutant PKSP, which has increased β-glucan surface exposure, was associated with increased susceptibility to killing by human macrophages.

Conclusions:
Overall, our studies identify autophagy as a novel pathway in innate immunity against A. fumigatus.
REDUCED GAMMA INTERFERON (GIFN) PRODUCTION IN CHRONIC PULMONARY ASPERGILLOSIS (CPA)

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Purpose:
TH1 responses are crucial for effective defence against Aspergillus spp. Two patients with poor lymphocyte proliferation and gifn production and CPA have been described. CPA is a slowly progressive destructive disease, usually of the upper lobes, which is characterised by chronic inflammation and a failure to halt the intra-cavitary growth of A. fumigatus (usually)

Methods:
We analysed gifn, TNFalpha, IL-6, IL-12 and IL-10 production after in vitro stimulation of Whole Blood culture to a variety of defined stimuli including LPS, beta-glucan, PHA, IFN gamma, IL-12 and IL-18 to interrogate the IL-12 and gifn dependant pathways. Blood was collected from CPA patients and a healthy control in Manchester attending the NAC and sent by courier to Cambridge for analysis. Cells were processed the same day in Cambridge. Cytokines were measured by ELISA or multiplexed particle based flow cytometry.

Results:
Results from 30 CPA patients were available. Only 7 (23%) of the patients showed normal in vitro gifn production. 9 (30%) had a reduced (2-5 fold) and 14 (47%) a very low (> 5 fold) gifn production when compared to healthy controls.

Different patterns can be observed among the gifn deficient responses with more than half of them (54%) being deficient in their response to all stimuli. Approx one third (35%) did have a normal response to PHA. A smaller group (11%) showed a weak response to PHA only. In comparison, there was a normal response to gifn and a normal production of and response to IL-12, a major physiological inducer of gifn. IL-12 had a normal capacity to synergistically up-regulate the production of gifn.

More than two thirds of the patients showed an increased production of TNF-alpha and IL-6. Three patients were treated with gifn with clinical improvement (2 with community acquired acute Aspergillus pneumonia which became CPA). This was paralleled by improved gifn secretion in vitro, suggestive of the positive feedback loop being inducible with treatment.

Conclusions:
The majority of patients with CPA appear to have low gifn production, but a normal response pathway. As treatment with gifn is usually reasonably well-tolerated, supplementation should be considered. Additional work is required to ascertain if the low gifn production remains permanent or is remediable with treatment. Increased TNF and IL6 production is of interest
THE INTERACTION OF HUMAN PLATELETS ALONE AND IN COMBINATION WITH ANIDULAFUNGIN AGAINST *ASPERGILLUS FUMIGATUS*

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Purpose:
The incidence of opportunistic systemic diseases, such as invasive aspergillosis, continues to increase and is still associated with a high mortality rate. Of interest, these typical risk group patients with chemotherapy-induced neutropenia and recipients of hematopoietic stem cell transplants very often have concurrent thrombocytopenia in addition to neutropenia. Furthermore, liver transplant recipient patients with thrombocytopenia exert a considerably higher incidence of fungal infection than those patients without thrombocytopenia. Recently, we observed that human platelets have the capacity to attenuate the virulence of *Aspergillus* spp. and Zygomycetes spp. in vitro. Human platelets are known to show synergetic effects in combination with antimycotics against aspergilli. Still, there are no data showing the potential synergetic antifungal effects of human platelets in combination with anidulafungin.

Methods:
Therefore, we investigated whether human platelets and anidulafungin used alone and in combination have a potential synergetic effect on inhibition of germination, hyphal elongation and hyphal damage of two clinical *Aspergillus fumigatus* isolates. Furthermore, fungal fks gene expression analysis was performed.

Results:
The combination of platelets plus anidulafungin at 0.03 µg/ml and 0.0078 µg/ml significantly (P < 0.05) reduced fungal germination rate and hyphal elongation and induced greater hyphal damage compared to either anidulafungin or platelets alone. Furthermore, platelets decreased the expression of the fungal fks gene, which plays an important role in cell wall synthesis.

Conclusions:
These data indicate that human platelets act beneficially with antimycotic substances by inhibiting fungal germination and hyphal elongation, which are both essential mechanisms in the development of invasive fungal disease. Collectively, these data contribute to analysis of the potential synergetic effects of human immune defense cells with the antimycotic substance anidulafungin against *A. fumigatus*.
MONOCYTE-DERIVED DENDRITIC CELLS PULSED WITH ASPERGILLUS FUMIGATUS DERIVED B-3-GLUCAN ACTIVATE HUMAN NATURAL KILLER CELLS AND ENHANCE THEIR ANTI-ASPERGILLUS EFFECT

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Purpose:
Dendritic cells (DCs) are recognized for their immunoregulatory ability towards adaptive and innate immune cells. Moreover, it has been shown that Natural Killer (NK) cells activated by DCs demonstrate increased IFN-γ release, proliferation and cytotoxicity against malignant cells. Recently, we demonstrated an anti-Aspergillus fumigatus effect of NK cells, mainly mediated by the release of IFN-γ, which showed direct fungicidal properties. In our study, we investigate the potential effect of in vitro generated monocyte-derived DCs (moDCs), previously pulsed with fungal proteins and polysaccharides, on NK cells and on their cytotoxicity against A. fumigatus.

Methods:
For the generation of moDCs, we used CD14+ cells isolated from peripheral blood of healthy donors by positive selection with magnetic microbeads. Cells were cultured for 5 days in serum-free GMP grade medium supplemented with GM-CSF and IL-4. On the second day of culture, immature DCs were pulsed either with the polysaccharide β-(1,3)-glucan extracted from fungal mycelia, inactivated germ tubes or with the recombinant catalase Cat1 and transglycosidase Crf1. Three hours later, DCs were matured with TNF-α. Before being used, DC profile was characterized by quantifying the expression of CD1a, CD14, CD40, CD80, CD83, CD86, HLA-I, HLA-DR, CCR7, Dectin-1, TLR-2, TLR-4, IL12p35, IL15, and IFN-γ. Autologous NK cells were obtained by magnetic negative selection of peripheral mononuclear cells and were kept frozen in human serum supplemented with 8% DMSO at -80°C. DC/ NK cell co-cultures were set in unconditioned GMP grade medium on different ratios (1:1, 1:5, 1:10). To evaluate proliferation, NK cells were stained with CFSE. Proliferation was measured at the 7th day of DC / NK co-culture. Activation markers on NK cells were measured by FACS analysis after 48 h of DC / NK co-culture. Finally, the antifungal impact on A. fumigatus germ tubes was evaluated by 2,3-bis[2-methoxy-4-nitro-5-sulpho-phenyl]2H-tetrazolium-5-carboxanilide (XTT) assays.

Results:
DCs pulsed with the different fungal stimuli demonstrated a ‘mature’ surface profile with negative CD1a & CD14 expression and high positivity of CD40, CD80, CD83, CD86, HLA-I, HLA-DR & CCR7. All DCs produced IL12p35 and IL15, but no IFN-γ. The surface expression of Dectin-1 was down-regulated by β-(1,3)-glucan and germlings and it was unaffected after Cat1 and Crf1 stimulation. This observation suggests a major involvement of Dectin-1 and its ligand, β-(1,3)-glucan in the interaction of moDCs with A. fumigatus. In contrast, TLR-2 and TLR-4 were not found to be regulated on DCs. Furthermore, DCs induced the proliferation of NK cells, higher DC / NK ratios elicited higher NK cell expansion. Among the stimuli, β-(1,3)-glucan provoked a significantly higher proliferation of NK cells, enhanced the expression of activating markers like CD69 on NK cells and mediated higher fungal damage.

Conclusions:
To summarize, our study shows for the first time that moDCs pulsed with β-(1,3)-glucan induce NK cell proliferation and activation and potentiate cytotoxicity against A. fumigatus. These results provide evidence for a role of moDCs and β-(1,3)-glucan as activators of NK cells towards an immune response against IA.
84 FIBCD1 BINDS TO ASPERGILLUS FUMIGATUS CHITIN AND MODULATES THE CYTOKINE PRODUCTION AGAINST FUNGAL POLYSACCHARIDES AND PROTEASES

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Purpose:
To investigate the role of FIBCD1 in fungal chitin recognition and modulation of immune response induced by Aspergillus fumigatus polysaccharides and proteases in vitro.

Methods:
We used fluorescence microscopy, flow cytometry (FACS) and pull down experiments to study the putative cell wall ligand interacting with FIBCD1. We further used A549 lung epithelial cell line stably transfected with fibcd1 gene or sham gene for cell stimulation experiments. Aspergillus fumigatus cell wall polysaccharides and one-week grown culture filtrate (1WCF) were used as stimulus for cellular experiments. A time and dose dependent studies were performed to evaluate IL-8 expression at mRNA and protein level.

Results:
FIBCD1 is a type 2 transmembrane protein having a fibrinogen related domain (FReD). In this study for the first time, we demonstrate that the FReD of FIBCD1 binds to Aspergillus fumigatus cell wall chitin. Fluorescence microscopy revealed that FIBCD1 recognizes chitin rich zones like the septum, budding region, and bud scar in fungal cell wall and colocalize with WGA (a chitin binding lectin). We further confirmed our finding by isolating the chitin rich fungal cell wall fragments and showed binding to FIBCD1 using pull down, FACS and colocalization experiments. Inhibition of chitin synthesis using nikkomycin-Z or by destroying the cell wall chitin using chemical or chitinases treatment abolishes recognition by FIBCD1. We next stably transfected the A549 lung epithelial cell line with sham gene or full length FIBCD1 and confirm the transfection and expression of gene by making western blot of cell lysate and by FACS analysis. We used the isolated Afu cell wall polysaccharides (rich in β-glucan and chitin) and 1WCF for stimulation of A549 cells. A549 cell stimulation resulted in significant release of IL-8 from epithelial cells in a time and dose dependent manner. When tested for the role of FIBCD1 we found that FIBCD1 overexpressing A549 lung epithelial cell line down regulated IL-8 production after stimulation with β-glucan-chitin complex and with 1WCF reflecting a possible FIBCD1 mediated effect. Sham transfected and FIBCD1 transfected A549 cells stimulated with live conidia and 1WCF showed a marked increase in IL-8 secretion seen after 6 hour compared to unstimulated cells however sham transfected cells have higher IL-8 production compared to FIBCD1 transfected cells against 1WCF and live conidia indicating FIBCD1 might be involved in regulating IL-8 synthesis during fungal infection and inflammation.

Conclusions:
FIBCD1 recognizes chitin rich zones in Aspergillus fumigatus and modulate IL-8 secretion in A549 epithelial cells stimulated with fungal polysaccharides and proteases. We will now analyze the role of FIBCD1 in modulation of allergic immune responses induced by polysaccharides and secretory proteases (culture filtrate) in vivo by establishing an Aspergillus fumigatus allergic and infectious model in wild type and fibcd1 deficient mice. The identification of mechanisms involved in chitin induced modulation of innate and adaptive immune responses may provide novel insight into prevention of allergic airway inflammation.
DAMP SIGNALING IN ASPERGILLOSIS: THE S100B/RAGE HYPERFUNCTION IS A RISK FACTOR FOR INFECTION IN STEM CELL TRANSPLANT RECIPIENTS

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Purpose:
The most important risk factor for invasive aspergillosis (IA) in allogeneic hematopoietic stem cell transplantation (HSCT) has historically been neutropenia. However, and although displaying apparently similar “immunocompromised” phenotypes, not all patients develop IA suggesting that genetically-determined immune defects may also play a role in defining susceptibility to this disease. Pathogen sensing through pathogen-associated molecular patterns (PAMPs) combined with host damage perception via damage-associated molecular patterns (DAMPs) is critical in promoting inflammation and its resolution in infection. DAMPs, such as S100B proteins, are known to activate inflammation through interaction with the multiligand receptor for advanced glycation end products (RAGE).

Methods:
To assess the PAMP/DAMP system interaction in aspergillosis, we analyzed a cohort of 223 consecutive patients undergoing HSCT for the association of selected genetic variants in the S100B/RAGE axis and susceptibility to IA. Furthermore, studies addressing the functional consequences of these variants were performed in preclinical settings as well as in a mouse model of pulmonary aspergillosis.

Results:
We found significant associations between two distinct polymorphisms in RAGE (-374T/A) and S100B (+427C/T) genes and susceptibility to IA in patients undergoing HSCT. Functional assays demonstrated a gain-of-function phenotype of both variants, as shown by the enhanced expression of inflammatory cytokines in RAGE polymorphic cells and increased S100B secretion in the presence of the S100B polymorphism, suggesting that the susceptibility phenotype is correlated with skewing towards a proinflammatory signaling by means of a positive feedback loop occurring between increased RAGE expression in conditions of S100B accumulation. Furthermore, a tight control of S100B is critical to restrain inflammation in experimental aspergillosis, through an evolving braking circuit whereby an endogenous danger protects the host against pathogen–induced inflammation and a nucleic acid–sensing mechanism terminates danger–induced inflammation.

Conclusions:
This study indicates how the S100B/RAGE axis, in sensing danger, plays a critical and unanticipated role as a fine modulator of inflammation during infection and represents a proof-of-principle demonstration that a defective danger sensing may underlie individual differences in the clinical course of invasive aspergillosis and the inherent patient’s susceptibility to infection.

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IMMUNOGENETICS OF ASPERGILLOSIS: FROM RISK ASSESSMENT TO VACCINOMICS

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Purpose:
Innate control of fungal infection requires the recognition of fungal structures by immune receptors that initiate inflammatory and subsequent adaptive responses. Therefore, genetic variants affecting Toll-like receptors (TLR), dectin-1 or danger signaling pathways have been reported as predictive factors for aspergillosis in immunocompromised patients. TLR signaling has been regarded as potential adjuvant candidates for vaccination strategies. However, the role of TLR3 in this scenario is far from being completely understood.

Methods:
We evaluated the potential contribution of genetic variants of TLR3 to susceptibility to aspergillosis in a cohort of 223 hematological patients undergoing allogeneic stem cell transplantation (HSCT). In addition, the impact of TLR3 deficiency in antifungal responses was addressed in preclinical models of aspergillosis and in vitro with human cells.

Results:
We have identified a genetic variant in donor TLR3 that increased the risk for invasive aspergillosis following stem cell transplantation. This variant was found to result in a loss-of-function phenotype of human dendritic cells and concomitant failure to activate antifungal CD8+ T cells. Studies in preclinical models of infection have confirmed that TLR3 deficiency ablates the activation of class I MHC-restricted protective memory CD8+ T cell responses to *A. fumigatus* through impaired sensing of fungal RNA by cross-presenting dendritic cells.

Conclusions:
These findings point to important implications of human genetic variability and support the use of vaccinomic approaches in the design and application of vaccine candidates against *Aspergillus*. Moreover, they attest the prognostic value of polymorphisms in immune gene to predict susceptibility to aspergillosis and emphasize the utility of the “two-way road” translational approach: bench to bedside and back.

This study was supported by the Targeted Research Project “ALLFUN” (FP7-HEALTH-2010, contract number 260338).
IMMUNE RESPONSE IN IMMUNOCOMPETENT RATS WITH PULMONARY ASPERGILLUS FUMIGATUS INFECTION

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Purpose:
Studies of mechanisms of immune response to Aspergillus fumigatus, the most prevalent air-borne fungal pathogen, are mainly performed in mice treated with immunosuppressive agents. A role of Th cytokines in resistance (interferon-γ) or susceptibility (interleukin-4) to A. fumigatus infection in these mice was shown. In contrast, there are no coherent data concerning IL-17, as both protective as well as detrimental effects of this cytokine was shown in Aspergillus infection in immunosuppressed mice. Studies of immune response to A. fumigatus in immunocompetent (nonsuppressed) animals are less numerous, and there are virtually no data concerning immune response to A. fumigatus in other animal species. Considering all the above quoted, we investigated pulmonary immune response in immunocompetent rats following A. fumigatus infection by comparative investigation of individuals of a Th1 prone Dark Agouti (DA) rats and a Th2 prone Albino Oxford (AO) rats.

Methods:
Dynamic of fungal elimination from lungs, determination of IFN-γ (Th1 cytokine), IL-17 (Th17) and IL-4 (Th2) content in lung tissue, as well as, activity of thoracic lymph nodes (cellularity, proliferation and cytokine production) were investigate in immunocompetent individuals of two rat strains at days 1, 3, 7 and 15 after intratracheal (i.t.) infection with nonlethal inoculum (1×10^7 conidia/per lung) of Aspergillus fumigatus.

Results:
Significantly lower fungal burden in lungs, as well as faster elimination of A. fumigatus from lungs in immunocompetent DA rats compared to AO rats were noted. Determination of cytokine content in lung homogenates revealed increase in IFN-γ and IL-17 content in individuals of both rat strains, but significantly higher in DA rats. No changes in lung IL-4 content were noted in both strains. Activity of draining lymph nodes (where the lymphocytes and their differentiation into effector cells occurs) was investigated further. Increased lymphocyte numbers and cell proliferation were noted in individuals of both rat strains (from day 1 after infection in DA rats and from day 3 in AO rats). In response to conidia stimulation IFN-γ (in both rat strains from day 1) and IL-17 response (earlier and significantly higher in DA rats) occurs. Down-regulation of IL-4 production was noted in DA rats as well.

Conclusions:
Pulmonary A. fumigatus infection tipped the balance towards pro-inflammatory antifungal response by increasing IFN-γ and IL-17 production in both rat strains without changing the IL-4 production in AO rats, in contrast to down-regulating IL-4 production in DA rats. Earlier and higher IL-17 production in DA rats coincided with faster removal of A. fumigatus from lungs of this rat strain and may indicate the higher relevance of Th17 and IL-17 in resistance to pulmonary aspergillosis in immunocompetent rats.
Purpose:
One major risk of patients with a strongly immunosuppressed health status, is to develop invasive mycoses, particularly Invasive Aspergillosis (IA). IA is mainly caused by the prevalent opportunistic human pathogenic fungus *Aspergillus fumigatus*, whose spores, when inhaled by immunosuppressed humans, can germinate and form hyphae that are able to penetrate lung tissue and to disperse via the bloodstream resulting in a life-threatening invasive infection. For the success rate of antifungal therapy of IA-patients, early diagnosis of IA is crucial and a better prognosis for the chance to develop IA and the course of an IA-infection would be helpful. Therefore, our research focus lays on first, the detection of *Aspergillus* proteins within patient sera and the identification of immunologically reactive proteins to improve diagnosis of IA and second, the correlation of a patient’s immune response to *Aspergillus fumigatus* proteins as a prognostic marker.

Methods:
Proteome analysis was performed by using *Aspergillus fumigatus* mycelial protein extracts and extracts generated from culture filtrates which were separated by 2D-gel electrophoresis. To identify *A. fumigatus* antigens that are specifically recognized by natural human anti-*A. fumigatus* antibodies, immunoblotting was followed, using several patients sera and HRP-conjugated goat anti-human IgG antibody. Protein spots that were bound by antibodies within a patient serum, were identified by MALDI-TOF/TOF. In a bioinformatic approach, we used a supervised machine learning method based on decision tree induction, to analyse the immunoproteome data.

Results:
We identified several proteins that were recognized either exclusively by antibodies in sera of patients likely suffering of IA or in control sera or in both. By bioinformatic analysis we could in part detect a correlation between the pattern of *A. fumigatus* proteins that immunoreacted with a particular patient serum and the likelihood of an IA-infection, based on revised EORTC definitions. In parallel, this approach helped us to identify *Aspergillus* proteins that might be useful targets for the generation of reagents to detect an *Aspergillus* infection.

Conclusions:
We conclude that with our immunoproteomic approach, we could give first insights in the interrelation of a patient’s anti-*Aspergillus* antibody repertoire and the occurrence of IA. In addition, we identified promising protein targets that could be used for the establishment of diagnostic reagents like monoclonal antibodies.
MODELING THE EFFECTS OF MODERN IMMUNOSUPPRESSIVE THERAPIES ON ASPERGILLUS FUMIGATUS INFECTION

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Background:
Tacrolimus (FK-506), corticosteroids and mycophenolate mofetil (MMF) have become core components of maintenance transplant immuno-suppressive therapy. Invasive fungal infections (IFI) are a major cause of mortality in this patient group with Aspergillus spp. accounting for 20% of all IFIs. Relatively few studies have investigated the effects of modern immunosuppressive regimes on the host immunity to IFIs. To address this issue we have developed a murine transplant immuno-suppression model of invasive aspergillosis.

Methods:
Male Balb/c mice were immunosuppressed with FK-506, MMF and hydrocortisone and infected with 5 x 10^6 A. fumigatus conidia. Survival was monitored for 9 days or mice were culled at 20% weight loss. FK-506 and MMF levels were determined by blood LC-MS. Lung fungal burden was measured by PCR and lung cytokine levels were analysed by Luminex. Infiltrating cell types were analysed by Quick-Diff staining of BAL cytospins. For histopathological analysis lungs were fixed in formaldehyde and sections were stained with Periodic acid-Schiff stain.

Results:
Fk-506 doses of 1-5 mg/kg/day and MMF doses of 50 to 100 mg/kg/day reached therapeutic levels. Mice receiving these doses of FK-506 or MMF showed 0% mortality and had cleared infection at day 9 post infection (p.i.). But if mice were treated with a regime containing hydrocortisone and FK-506 or MMF, additionally FK-506 treated animals showed increased mortality in comparison to hydrocortisone only treated animals. Histological analysis showed an increase in tracheobronchitis and persistent inflammation in FK-506 treated animals whereas there was no clear difference in fungal burden. To establish infection in FK-506 or MMF only treated animals, the fungal inoculation dose had to be increased.

Conclusions:
These studies indicate that the solid organ immunosuppressive drug tacrolimus causes incremental susceptibility to death from invasive aspergillosis in the steroid-based model of invasive aspergillosis. Death is associated with tracheobronchitis and persistent inflammation. Since mice used in this study are immunological naïve and death is acute, the observed effects of tacrolimus are due to defects in innate immunity, most likely neutrophils.
90 COMPARATIVE VIRULENCE AND SUSCEPTIBILITY OF ASPERGILLUS TERREUS AND ASPERGILLUS ALABAMENSIS

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Purpose:
A. terreus (AT), an Aspergillus species relatively amphotericin (AB)-resistant, is in the taxonomic section Terrei. A second species, A. alabamensis (AA), described by molecular methods as closely related to AT, is also in this section. Both species are clinically relevant. However, the comparative virulence and susceptibility of the species has not been reported previously. The aim of our studies was to determine the comparative virulence and susceptibility of these closely related species.

Methods:
A collection of 139 AT and 11 AA from Europe and N. America was tested by broth dilution against AB. A murine model of systemic disease using 5-wk-old female CD-1 mice was studied. A preliminary study determined that an inoculum of 2 x 10⁷ conidia of AT given i.v. resulted in lethal infection within 14 days; lower inocula were less lethal. Three separate in vivo studies were performed that included 10 isolates of AT and 10 isolates of AA. Mice (n = 10 per group) were infected i.v. and mortality followed for 12 days. CFU in the kidneys of surviving mice was determined by quantitative plating of homogenates.

Results:
Susceptibility testing revealed a geometric mean, median, MIC50, MIC90 for AT of 1.2, 1, 1, 2 μg/mL respectively (range 0.06-16), and for AA of 1.1, 1, 1, 1 (0.25-2). Eight isolates of AT and 4 of AA were tested in the initial in vivo study. Comparative survival showed that 2 strains of AT were significantly (P < 0.03-0.0001) more virulent than any of the other isolates tested. Virulence spanned a range from high to low. Three of the 4 AA tested had the lowest virulence and no isolate of AA was significantly more virulent than an isolate of AT; one AA showed an intermediate level of virulence. None of the AA isolates were in the top third of virulence. The recovery of CFU from the kidneys gave similar results. In the second study 4 isolates of AT and 7 isolates of AA were tested including replicates from the first study spanning high and low virulence. All 4 isolates of AT proved the most virulent (≤30% survival) vs. the 7 isolates of AA (≥80% survival)(P <0.004-0.0001)(any AT vs. any AA, P < 0.02- <0.0001), CFU gave similar results (P <0.04- <0.0001). A third study also replicated some previously high and low virulent strains, and included some new ones, and was confirming.

Conclusions:
Each species had a range of virulence, but in general, isolates of AT were more virulent than isolates of AA. However, some isolates of AT were equivalent in virulence with AA isolates. Furthermore, no association with geographic locale or genetic clade were noted for the AT isolates. The species do not overall differ in susceptibility to AB. Additional studies are warranted to examine the potential difference in virulence in other models of infection and in response to therapy.
VIRULENCE OF TOXIGENIC AND ATOXIGENIC STRAINS OF \textit{ASPERGILLUS FLAVUS}

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Purpose:
Aflatoxin produced by \textit{Aspergillus flavus} is one of the most potent toxins and carcinogens known to man. Aflatoxin contamination of foods represents a significant risk for those ingesting the contaminated foodstuffs. Atoxigenic strains of \textit{A. flavus} are released into the agricultural environment as bioremediators by spraying crops (e.g., corn) with the goal of replacing the toxigenic natural strains with the atoxigenic strain and thus, reducing aflatoxin contamination. However, little about the contribution of aflatoxin to the virulence of \textit{A. flavus} for mammals is known, or about the virulence of atoxigenic strains in murine models. Our aim was to compare the virulence of toxigenic and atoxigenic strains of \textit{A. flavus} in murine systemic infection.

Methods:
Groups of 10, 5-wk-old female, CD-1 mice were infected intravenously with conidia in 3 replicate experiments, at 2-3 different inoculum sizes/experiment, spanning 100-fold range in experiments. A total of 10 strains were studied; 5 toxigenic and 4 atoxigenic (1 atoxigenic from 2 different sources), including 2 atoxigenic strains widely introduced onto crops. Survival was followed through 10 days and CFU in the brain and kidneys of surviving mice determined by plating of organ homogenates. Some mice were preassigned for the collection of blood and tissue samples after 3 days of infection. Samples were extracted and assayed for aflatoxin by thin layer chromatography.

Results:
\textit{A. flavus} strains appeared to be more virulent than \textit{A. fumigatus} strains previously extensively studied in this model. Lethality and infectious burden was proportional to inoculum size, and there was no consistent difference in these virulence attributes between toxigenic and atoxigenic strains, between high toxin producers in vitro and lower producers, or the same atoxigenic strain obtained from two different sources. A toxin producer with reduced virulence to plants (lacking pectinase virulence factor) was similarly virulent in vivo to the other strains. Assessment of tissue samples and blood samples from mice infected with toxigenic strains showed that no aflatoxin could be detected in any sample tested, in contrast to isolated case reports of \textit{A. flavus} human infections.

Conclusions:
Overall our results indicate that there is no significant difference in virulence among the toxigenic and atoxigenic strains of \textit{A. flavus} in our model of systemic infection. Furthermore, our results indicate the capacity to produce aflatoxin is not important to virulence in experimental systemic murine infection. This suggests isolates being released into the environment are not more or less virulent for mammals than wild-type isolates. These results warrant additional studies to determine whether equal virulence would be observed in a pulmonary model of infection.
MOLECULAR VARIABILITY IN AFLATOXIN PRODUCING STRAINS OF RICE ASPERGILLI

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Purpose:
The rice crop exposed to frequent and heavy rainfall, and flood is subjected to contamination by various Aspergillus spp. Current methods for identification of these species still depend primarily on cultural and morphological characteristics. It is often difficult to differentiate these species because the phenotypic differences are not distinct and are confused by the high degree of intra- and interspecies variation. Because of the economic value of members of the Aspergillus section Flavi and importance of differentiating them, studies were undertaken to distinguish these strains and find their genetic variability by RAPD-PCR.

Methods:
The rice samples collected were either from areas exposed to different weather conditions or stored at various storage conditions. Using agar plate method, the Aspergilli were isolated from the seed samples. The cultures were grown for genomic DNA isolation, and DNA was extracted by following the standard methods. To assess the genetic variability in Aspergillus flavius, the isolates were analyzed by using random amplified polymorphic DNA (RAPD) markers. Four primers AP12 h (5´-CGG CCC CTG T-3´), R108 (5´-GTA TTG CCC T-3´), R151 (5´-GCT GTA GTG T-3´) and A1 (5´-GAT AGA TAG ATA GAT A-3´) obtained from Imperial Biomedic were used for RAPD analysis, viz., PCR based amplification of the template DNA of the isolates. To generate a dendrogram of the strains with UPGMA algorithm using NTSYS-PC version 2.0, the marker alleles were converted to binary scores. A pair-wise similarity index (SI) was calculated and the dendrogram obtained is depicted.

Results:
Twenty-two aflatoxin B$_1$ producing A. flavius strains were isolated from 1,200 discolored rice grain samples. Further these isolates were characterized through RAPD. Of the four primers tried, one primer (AP12 h) failed to effect any amplification in the isolates tested, while two other primers (R151 and A1) showed amplification. Primer R108 showed the highest amplification in all the isolates compared to others. A total of six alleles were produced with R108 primer. All the strains produced 1 to 12 bands. The genotypes were grouped into two major clusters, i.e. Cluster I and Cluster II. The Jaccard’s similarity coefficient based on RAPD markers loci ranged from 0.44 to 1.00. In pair-wise comparison, the maximum similarity was obtained between MTCC2799 (standard strain) and DRAf 001, 002, 004 and 011 with a SI of 0.800 (80%), whereas DRAf 006 showed least similarity with a SI of 0.167 (17%). The isolates (DRAf 002, 004 and 011) showed 100% similarity between the isolates from Tamil Nadu State.

Conclusions:
This is the first comprehensive study on the collection of large number of rice grain samples from across the country, isolation of different strains of A. flavius, and studies on genetic variability of A. flavius isolates by RAPD. In this study, RAPD helped to confirm the identity of isolates as A. flavius. The isolates showed 17–80% similarity with standard culture of A. flavius (MTCC 2799). The study in general, revealed the genetic variability of the A. flavius strains by RAPD-PCR. It is concluded that the method is valuable for identification and fast differentiation of strains.
A COMPARATIVE ANALYSIS OF POLYKETIDE SYNTHASES IN *ASPERGILLUS FLAVUS*

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**Purpose:**
Medical, agricultural and biological importance of *Aspergillus* species is due to their potential to synthesize, multifunctional proteins, enzymes, allergens, antigens and secondary metabolites. Recent advances in genomic and proteomic sciences has facilitated identification of novel allergens, antigens, virulent factors and polyketides relevant for various applications. These compounds facilitated development of novel diagnostic and therapeutic molecules for *Aspergillus* induced diseases. Further molecular mechanisms involved in host-pathogen interactions and the important biochemical pathways are better understood today for the *Aspergillus* species. The need and pharmaceutical importance of polyketides of *Aspergillus* species necessitates an insight into the polyketide biosynthetic pathway. The key enzyme of the pathway namely Polyketide synthase has posed important challenges with respect to structure and the function.

**Methods:**
Current work is focused on the gene sequences of polyketide synthase (PKS) of Aspergilli such as *A. fumigatus, A. flavus, A. parasiticus, A. niger, A. terreus, A. oryzae and A. clavatus* with a view to understand the common and variable features and the diversity of the polyketides they synthesize.

**Results:**
Phylogenetic analysis based on domain sequences of PKS enzymes of important *Aspergillus* species resulted in the classification of the PKS proteins into highly reducing, partially reducing and non reducing type of enzymes. This has facilitated to evaluate the potential of the *Aspergillus* species for possible secretion of polyketides of human value.

**Conclusions:**
*A. flavus* is an important human pathogen and is also known to cause economic losses to several crops by contaminating with aflatoxins. Hepatotoxicity, neurotoxicity and nephrotoxicity of the carcinogenic Aflatoxins and the aflatoxicosis in humans are well established. Analysis of polyketide synthase domain sequences of aflatoxigenic and atoxigenic *A. flavus* isolates of Indian origin revealed novel nucleotide changes and a variation in aminoacid sequence of ketosynthase domain in atoxigenic *A. flavus*. The atoxigenic *A. flavus* isolates exhibiting the variations in their gene sequence are being explored for applications. These results are useful in exploration for biocontrol potential and for differential detection of *A. flavus*. 
COMPARATIVE ANALYSIS OF ALLERGEN PRODUCED BY ASPERGILLUS AND PENICILLIUM SPECIES ISOLATED FROM KATHMANDU, NEPAL

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Purpose:
Fungal spores are universal atmospheric components of indoors and outdoors and are now generally recognized as important causes of respiratory allergies. Fungal spores and spore extracts can cause immediate bronchoconstriction in sensitive subjects. More than 80 genera of fungi have been associated with symptoms of respiratory tract allergy. Comparative analysis of allergens was carried out from Aspergillus and Penicillium species which were prevalent fungi in Kathmandu.

Methods:
Fungi were isolated from the atmosphere of Kathmandu valley from the year 2010 to 2011 by plate exposure method. Colonies were identified on the basis of colony morphology and staining techniques. Allergens was extracted from 3 days old cultures of Aspergillus and Penicillium on malt extract agar which was suspended in the 0.01% Tween 80 and was diluted to obtain spore concentration 10^6 spores/ml. One ml of standard inoculum was inoculated in 100 ml of medium (pH 7.0) in a 500 ml Erlenmeyer flask and incubated at 37 °C for 21 days. The allergens were extracted by chemical method (as described by Sambrook J, Fritsch E F and Maniatis T, (1989) Molecular cloning with minor modification). The molecular weight of allergens were determined by running 8% Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE) as described in ( Sambrook J, Fritsch E F and Maniatis T, 1989) using SDS7 (supplied from SIGMA-ALDRICH MW:14,000-66,000), Bovine Serum Albumin, Actin, Carbonic anhydrate and viral protein as standards for protein determination.

Results:
Aspergillus species (7.4%) and Penicillium species (19.7%) were the most predominant fungi. Allergenic bands ranging from 30 to 120 kDa were observed in Aspergillus species and allergenic bands of 20 to 90 kDa were observed in Penicillium species. The fractions of 50-67 kDa and 20-35 kDa were common in both of the most of the species but the most prominent were seen in A. fumigatus.

Conclusions:
Aspergillus and Penicillium species were the predominant fungi isolated from the Kathmandu valley especially from the core residential area. In comparison of allergens produced by Aspergillus and Penicillium species, A. fumigatus was the most allergenic among the species of Aspergillus and also than Penicillium as detected on SDS-PAGE.
THE UGM1 MUTANT OF ASPERGILLUS FUMIGATUS ACTIVATES HUMAN ENDOTHELIAL CELLS UPON HYPHAE-CELL CONTACT

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Purpose:
Besides the emergency of more active and less toxic antifungal agents and the conventional use of antifungal prophylaxis, invasive mold infections have still high mortality rates, especially, invasive aspergillosis (IA). This life-threatening disease is a predominant fungal opportunistic infection for patients with long-term neutropenia, mostly HSCT recipients. Aspergillus fumigatus is the most important species causing IA and is already known as an angioinvasive fungal pathogen. Upon filamentation this fungus can damage and activate human vein endothelial cells (HUVEC) which in turn switch to a pro-thrombotic phenotype. HUVEC activation is known to be mediated by TNF-α once cell-cell contact occurs. To investigate the possible pathogen molecules involved in this interaction, conidia and germlings of several mutants of A. fumigatus were screened by their capacity to adhere to and be internalized by HUVECs in confluent monolayers.

Methods:
Briefly, HUVECs were infected with germ tubes and conidia of A. fumigatus strains and a differential quantitative fluorescence assay was achieved to determine adhesion and internalization rates. The ugm1 mutant, which lacks the AFUGM1 gene encoding UDP-galactopyranose mutase, an enzyme responsible for the conversion of UDP-galactopyranose in UDP-galactofuranose, showed a significant increased in adhesion to HUVECs in comparison to two reference wild type (WT) strains. Galactofuranose is an uncommon 5-membered ring form of galactose and a very important component of glycostructures of the A. fumigatus cell wall. Thus, we further investigated if this increment in the adhesion capacity of ugm1 strain had any correlation with endothelial cell activation. To ascertain this goal a kinetic study of secreted pro-inflammatory cytokines and chemokines in HUVEC conditioned medium was performed by a multiplex assay. The cytokine production was assayed at three time points (4, 8 and 16 hours) using either live or dead germlings for an E:T ratio of 2:1.

Results:
A significant increase in IL-6, IL-8 and TNF-alfa secretion by HUVECs upon interaction with the ugm1 mutant was observed in relation to the AF293 strain which was already known to activate endothelial cells. In contrast, several mutants which showed a decrease in their capacity to adhere to HUVECs did not induce cytokine secretion and the results were similar to non-activated HUVECs, as a negative control. These data are being confirmed by RT-PCR including other intracellular markers.

Conclusions:
Our results indicate that the interaction between HUVECs and the ugm1 mutant strain stimulated at least a 10-fold and 2.5-fold increased of TNF-alfa and IL-6 secretion respectively by this host cell, as compared to the activation profile observed for the WT strains.

Additionally, it’s possible to infer that this increased in cytokine secretion is dependent on cell-cell adhesion and independent on fungal cell viability. Finally, to further investigate HUVEC molecular routes activated in this process a differential proteomics analysis is being accomplished. Financial support: CNPq, Capes, Ministério da Saúde and Faperj.
EXPOSURE OF *ASPERGILLUS FUMIGATUS* TO CASPOFUNGIN LEADS TO LEAKAGE AND *DE NOVO* SYMTHESIS OF GLIOTOXIN

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**Purpose:**
The filamentous fungus *Aspergillus fumigatus* is capable of causing a range of diseases in immunocompromised patients and is responsible for up to 3% of all hospital-based deaths in the EU. Caspofungin is a recently introduced antifungal compound that has good efficacy against this fungus with minimal side effects. The aim of the work presented here was to characterise the response of *A. fumigatus* to caspofungin.

**Results:**
The results obtained demonstrated that caspofungin has potent anti-fungal activity and retards fungal growth. Exposure of *A. fumigatus* hyphae to this drug leads to the release of amino acids and gliotoxin, there is also an increase in internal level of this toxin following exposure to the drug. Prolonged exposure to caspofungin lead to reduced growth but greatly elevated levels of secreted and internal gliotoxin.

**Conclusions:**
The results presented here demonstrate that caspofungin is an effective antifungal agent but that sub-lethal levels lead to the elevated release and synthesis of gliotoxin. Since this toxin is highly immunosuppressive it is possible that *in vivo* elevated levels of this toxin may exacerbate the deterioration in the patient’s condition following caspofungin therapy.
SPECIFIC INDUCTION OF CD203C EXPRESSION IN BLOOD BASOPHILS DISCRIMINATES BETWEEN CF PATIENTS WITH ASPERGILLUS COLONIZATION AND THOSE WITH CF-ABPA

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Purpose:
The opportunistic fungus A. fumigatus (Af) colonizes the airways of 25-50% of cystic fibrosis (CF) patients, with some further progressing towards allergic bronchopulmonary aspergillosis (ABPA). ABPA significantly impacts short- and long-term prognoses in CF, yet may be avoided by early diagnosis and treatment. However, the diagnosis of CF-ABPA is clinically challenging, due in large part to the absence of an objective biological test. Since blood basophils play a major role in allergic responses, we hypothesized that discrete changes on their surface activation pattern could discriminate CF patients with ABPA from those without.

Method:
We used a direct flow cytometry assay to measure surface CD203c and CD63 levels on blood basophils, at baseline and upon rapid in vitro activation with Af allergen, in 5 groups of subjects: (A) CF patients with Af colonization but without ABPA (N=11); (B) CF-ABPA patients (N=8); (C) CF-ABPA patients whose treatment includes anti-immunoglobulin E (omalizumab) (N=2); (D) CF patients without Af colonization and without ABPA (N=5) and (E) healthy controls (N=11). The quantity of histamine in the plasma was measured by mass spectrometry.

Results:
In the CF-ABPA group, basophil CD203c levels and histamine levels increased significantly upon Af allergen stimulation (CD203c levels: P<10^-3, compared to the three other groups of patients). This increase was Af allergen-specific, since stimulation with a non-offending allergen did not increase surface CD203c or plasma histamine from subjects in any of the groups (CD203c levels: P<10^-2). Basophil CD203c levels upon Af allergen stimulation provided very good predictive value for discriminating CF-ABPA from CF patients with Af colonization but without ABPA (area under the receiver operating characteristics curve = 0.92, P<10^-3). In our preliminary results, basophil CD203c levels increased also upon recombinant A. fumigatus allergen 1 (Asp f 1) stimulation.

Conclusions:
These results support the notion that basophils play a role in the pathogenesis of CF-ABPA and can be used to diagnose and monitor this condition clinically with a simple and minimally invasive blood assay.
MORPHOLOGICAL AND GENOTYPIC IDENTIFICATION OF ENVIRONMENTAL ISOLATES OF ASPERGILLUS SPECIES BASED ON SEQUENCING OF B-TUBULIN GENE

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Purpose:
The genus Aspergillus are mycelia fungi consisting various groups and species. Species identification of Aspergillus spp is important in pathogenicity, toxigenicity and industrial aspects. Conventional laboratory methods for species identification of Aspergillus spp are time consuming and need expert technicians. The aim of the present study was sequence analysis of beta tubulin gene for precise identification of various environmental isolates of Aspergillus

Methods:
Totally about 200 Aspergillus strains were isolated from soils and air samples taken from hospital and public and natural places. The isolates were subcultured on sabouraud dextrose agar and Zchapex dox agar and preliminary identified based on slide microculture. Genomic DNA was isolated and purified from all strains by a conical grinder and beta tubulin gene was amplified by polymerase chain reaction (PCR) from each sample. Nucleotide sequence of 21 representative isolates were determined and analysed and compared with sequences existed in GenBank database

Results:
Among 21 Aspergillus isolates studied by sequence analysis, seven isolates were identified as Aspergillus flvus, 3 as Aspergillus fumigatus, 3 as Aspergillus chevalieri, 2 as Aspergillus tubingensis, 2 as Aspergillus niveoglacus, 2 as Aspergillus rubber, one as Aspergillus niger and one as Aspergillus versicolor. The morphological methods failed to identify 9 out of 21 isolates

Conclusions:
Finding of this study showed that PCR sequencing of beta tubulin gene is a valuable tool for identification of environmental isolates of Aspergillus in the species level, more reliable than morphological methods. We recommend the use of this molecular target for more studies on detection or identification of Aspergillus spp.
THE ASPERGILLUS GENOME DATABASE: RECENT DEVELOPMENTS IN COMPREHENSIVE MULTISPECIES CURATION, COMPARATIVE GENOMICS AND COMMUNITY RESOURCES

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Purpose:
The Aspergillus Genome Database (AspGD; http://www.aspgd.org) is a freely available, web-based resource for researchers studying the biology of fungi in the genus Aspergillus, which includes organisms of clinical, agricultural and industrial importance.

Results:
AspGD curators have now completed curation of the entire published literature for both A. nidulans and A. fumigatus. The web site and database have been upgraded to provide streamlined, ortholog-based navigation of the annotation for multiple species concurrently. To facilitate Aspergillus comparative genomics, AspGD provides a full-featured genomics viewer as well as matched, standardized sets of genomic information for the sequenced Aspergilli.

Conclusions:
AspGD is funded by grant R01 AI077599 from the National Institutes of Allergy and Infectious Diseases at the US National Institutes of Health. We welcome and encourage you questions or suggestions, and can be reached at Aspergillus-curator@lists.stanford.edu.
Purpose:
Heterothallism in fungi facilitates genetic studies by allowing recombinational analysis of genes and it has been exploited as a genetic tool in model fungi such as *Saccharomyces cerevisiae*, *Neurospora crassa*, *Cryptococcus neoformans* and *Schizophyllum commune*. In *Aspergillus fumigatus*, the heterothallic sexual cycle when first reported seemed less than ideal as a routine genetic tool due to six-months incubation required for the formation of meiotic products. At the 4th Advances Against Aspergillosis, we reported the identification of a ‘supermater’ pair that successfully completed the sexual cycle within a significantly shorter time. Among 50 strains of *A.fumigatus* screened, a cross between a clinical strain AFB62 (MAT-1) isolated in the US, and AFIR928 (MAT-2), an environmental isolate from Ireland, successfully produced viable ascospores in four wks. We now aimed to determine whether this supermater pair is suitable for genetic studies by investigating the extent of their genome similarity and frequency of recombination between these two strains.

Methods:
Comparative Genomic Hybridization (CGH) based on the AF293 microarray was used to compare the two supermaters genomes. Recombination frequency was analyzed using a cross between AFIR928*alb1Δ*, which produces white conidia and AFB62*abr2Δ*, which produces brown conidia. The mutants *alb1* and *abr2* were generated from deletion of genes AFUA_2G17600 and AFUA_2G17530, both functioning in the DHN-melanin pathway of conidial color and approximately 8 kb apart on chromosome 2.

Results:
Although the CGH approach does not reveal potential divergence between the two supermaters due to the genes which may not be present in the AF293 array, the analysis showed 99% similarity with only 86 genes highly diverged or absent between the two genomes: 46 genes were present in AFB62 but absent or highly diverged in AFIR928, and 40 showed the opposite distribution. The divergent genes were distributed among all chromosomes, with 50% of the genes concentrated in genomic loci known to be highly variable among *A.fumigatus* and other aspergilli. Of the 86 genes, 34 had an unknown function while the function of the remaining 52 genes varied greatly and did not appear to be interdependent.

Progeny from a cross between *alb1Δ* and *abr2Δ* yielded 3 conidial color phenotypes: white, brown and green. The green progeny, which resulted from crossing over between the *alb1* and *abr2* loci during meiosis, comprised 5-7% of the F1 population harvested at 8 wks. The *MAT-1:*MAT-2 ratio among the progeny from the same cross was nearly 1:1, confirming independent assortment of chromosome2 and the mating-type alleles on chromosome3. Interestingly, a lower than expected proportion of white progeny was observed in 4 wk-old crosses, suggesting that *alb1* may be involved in spore maturation and viability.

Conclusions:
Our findings regarding recombination frequency are the first to establish a relationship between the genetic map and physical distance in *A.fumigatus*. The genome similarity and high recombination frequency confirm the suitability of these supermater strains as genetic tools for recombinational analysis in *A.fumigatus*.

**NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION**
IDENTIFICATION OF ASPERGILLUS NIGER AND ASPERGILLUS TUBINGENSIS BY PCR-RFLP METHOD TARGETING THE BETA-TUBULIN (B-TUB) GENE

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Purpose:
Black aspergilli (Aspergillus section Nigri) are an important group of fungi in medicine, biotechnology and food industries. Regarding to the importance of black aspergilli the exact identification of these species is necessary. Morphology identification of these fungi are difficult. Recently phylogenetic analyses indicated that there are several species in section Nigri. The aim of this study was identification of 50 environmental isolates of black Aspergillus by molecular methods.

Methods:
A total of 200 aspergillus strains isolated from environmental samples of soil, air and other habitats in some parts of Iran and Japan. Among these, 50 isolates were identified as black aspergilli according to the culture characteristics on Sabouraud and Czapek Agar medium together with micro-morphology on slide-culture. The isolates and a standard strain of A. japonicus as a black aspergillus species subjected to β-TUB gene amplification using fungal Bt2a/Bt2b universal primers. Eleven strains as the phenotypically representative samples were subjected to sequencing. After sequencing all 50 black isolates and standard strain of A. japonicus were subjected to RFLP analysis by the restriction enzyme. The species, then delineated according to the specific pattern.

Results:
In the PCR a single sharp fragment of 410 bp for A. japonicus and 560 bp for other isolates was amplified. Sequence analysis revealed that TasI is the applicable enzyme for differentiation of the 3 species. In PCR-RFLP by TasI there was no cutting site for A. japonicus standard strain but other isolates produced two different RFLP profiles. 24 isolates with a profile of 336, 141, 78 bp, specific for A. niger and 26 with a pattern of 337, 219 bp characteristic for and A. tubingensis.

Conclusions:
In this study it was found that our isolates belonged to the A. niger and A. tubingensis species and the applied PCR-RFLP system targeting the β-TUB gene is a reliable method to differentiate common member of black aspergilli.
NCE102 HOMOLOGUE IN \textit{ASPERGILLUS FUMIGATUS} IS REQUIRED FOR NORMAL SPORULATION, NOT HYPHAL GROWTH OR PATHOGENESIS

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\textbf{Purpose:}
Here we describe the characterization of Nce102 homologue in the human pathogen, \textit{Aspergillus fumigatus}

\textbf{Methods:}
The Saccharomyces cerevisiae Nce102 sequence (GeneID:856272) was used to identify homologues in the \textit{A. fumigatus} genome using BlastP. A deletion cassette containing 1.8 kb 5' and 3' flanking region of nce102 surrounding the pyrG marker was prepared and used for transformation of \textit{A. fumigatus} AF293 pyrG- strain. A deletion mutant was isolated and characterized. To investigate the intracellular localization of AfuNce102, a C-terminal GFP fusion construct, driven by the glaA inducible promoter, was prepared and transformed into the \textit{A. fumigatus} AF293 parent strain. A murine model for systemic aspergillosis was used to test the virulence of the mutant.

\textbf{Results:}
Nce102 deletion mutant showed a cotton-like colony appearance and a clear delay in conidiation at 37°C. The mutant was also not able to produce any conidia at room temperature in minimal medium. In young mycelia, Nce102 tagged with EGFP was primarily detected in ER with a tip-high gradient. The fluorescence was also detectable at the septum. In old hyphae, the ER localization of EGFP-tagged protein was more clear and the EGFP fluorescence was frequently observed in ring-like structures. DAPI staining of mycelia demonstrated that these ring structures are nuclei. During the conidiophore formation, a faint and diffused fluorescence was detected in the vesicle and later a strong signal was observed in phialides and mature conidia. A variable intensity of EGFP fluorescence was observed among phialides. In statistical analysis of survival percentages using Mann-Whitney test, a significant survival difference was observed between the group infected with wild type spores and the control group which only received cyclophosphamide (P=0.029). The difference of survival between the group infected by AfuNce102 deletant spores and the control group was also significant (P=0.04). However, the difference of survival between two infected groups was not statistically significant (P=0.34). These comparisons support the conclusion that the virulence of fungus has not been affected by AfuNce102 gene deletion.

\textbf{Conclusions:}
we have shown that AfuNce102 is involved in sporulation process in \textit{A. fumigatus}. Although the localization data presented in this study were derived from the expression of AfuNce102-GFP under the control of a strong and non physiological promoter, the targeting of GFP fusion protein to the conidiophores and mature conidia along with an abnormal sporulation in deletion mutant may be relevant to the potential role in sporulation.
FUNCTIONAL ANALYSIS OF HIGH OSMOLARITY GLYCEROL (HOG) PATHWAY IN \textit{ASPERGILLUS FUMIGATUS}: A POTENTIAL TARGET FOR ANTIFUNGAL DRUG

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**Purpose:**
Most fungi have High Osmolarity Glycerol (HOG) pathway that plays a pivotal role for adaptation to environmental osmotic condition. \textit{Aspergillus fumigatus} HOG pathway is composed of His-Asp phosphorelay system and SakA MAPK cascade. Since His-Asp phosphorelay system is not found in mammal cells, it would be a good target for antifungal drug. Our final goal is to develop new antifungals targeting this signal transduction system. To evaluate its possibility and to understand the molecular mechanism of this system, functional analysis of HOG pathway was undertaken in \textit{A. fumigatus}.

**Methods:**
To characterize NikA, a sensor of His-Asp phosphorelay system, and SakA MAP kinase, 1) the gene deletion mutants were constructed. 2) Sensitivity to osmotic and oxidative stresses, fungicides, and cell wall stress reagents were investigate in the mutants. 3) Expression profiles of HOG pathway dependent genes were investigated in the mutants.

**Results:**
1) Deletion mutants of \textit{nikA} and \textit{sakA} were obtained by replacing these ORF with hygromycin resistance marker (HygBr). \textit{nikA} mutant showed growth retardation on GMM (glucose minimal medium) and YGMM (GMM containing 0.1 % Yeast Extract) plates but not on PDA (potato dextrose agar). 2) When grown on YGMM plates containing high osmolarity, radial growth of \textit{nikA} mutant was highly inhibited, whereas \textit{sakA} mutant showed a slight retardation. Among other fungi, HOG pathway is shown to be a target for fludioxonil and iprodione, which are fungicides used for protecting crops from plant pathogenic fungi. Hence, sensitivity to these fungicides was investigated in the mutants. \textit{sakA} mutant was comparable in sensitivity to fludioxonil and iprodione with the wild-type strain. In contrast, \textit{nikA} mutant showed remarkable resistance to both fungicides. When grown on YGMM plates containing cell wall stress reagents, Calcofluor White, Congo Red, and Micafungin, \textit{nikA} mutant showed a significant resistance to those reagents, whereas the sensitivity of \textit{sakA} mutant was comparable to that of wild-type strain. 3) When wild-type strains were challenged with high osmolarity or fludioxonil, expressions of \textit{catA}, \textit{dprA}, \textit{dprB} genes were induced. These inductions were not observed in \textit{sakA} mutant. In \textit{nikA} mutant, these expressions were partly induced in response to osmotic shock and fludioxonil treatment.

**Conclusions:**
In line with the study in \textit{Aspergillus nidulans}, NikA and SakA MAP kinase were required for responses to osmotic stress and certain fungicides in \textit{A. fumigatus}. Particularly, NikA but not SakA seems to play an essential role for osmotic adaptation and growth inhibition effects of fludioxonil and iprodione. In addition, tolerance to the cell wall stress reagents found in \textit{nikA} mutant pointed to a possibility that cell wall composition and (or) structure were differently arranged in the \textit{nikA} mutant. Further study will be focused on NikA function in cell wall synthesis or modification and its contribution to virulence.
104 \textit{ASPERGILLUS FUMIGATUS} CONTAMINATION MONITORING IN A FRENCH TURKEY HATCHERY USING MULTIPLE LOCUS VNTR ANALYSIS (MLVA)

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\textbf{Purpose:}
Avian aspergillosis is a severe respiratory disease which can cause significant economic losses in poultry industry especially in turkey facilities. In hatcheries, strict sanitation programmes are required to avoid brooder pneumonia hazard responsible for high mortality \textit{in ovo} or of few-days-old chicks. Effective prevention of hatchers and incubators contamination by airborne spores involves identification of putative \textit{Aspergillus} sources and circulation circuits.

\textbf{Methods:}
In this perspective, we tested a highly discriminant molecular method, Multiple Locus VNTR (Variable Number Tandem Repeat) Analysis (MLVA) combining 10 different markers in a turkey hatchery located in France.

\textbf{Results:}
A total of 118 \textit{Aspergillus fumigatus} isolates were collected in 4 different hatchery rooms during a 6-months period and subsequently genotyped. Minimum spanning tree analysis of 67 distinct genotypes demonstrated that decontamination protocols appear to be effective but also that multiple contamination of hatchery rooms could occur from a unique origin due to air circuits in the building

\textbf{Conclusions:}
This molecular tool should provide a better understanding of \textit{A. fumigatus} circulation both inside and between poultry farms and contribute to secure commercial transactions.
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Purpose:
Aspergillus fumigatus-related infections, allergic and/or invasive, are increasing. One of the most affected groups is immune-compromised patients, reaching mortality rates of 50-90% in the case of invasive aspergillosis. Published studies show that during A. fumigatus infection, the fungus is able to survive stress conditions inside the host. It is also known that fungal secondary metabolites, such as gliotoxin, play a role in A. fumigatus pathogenicity.

Methods:
Our lab has previously demonstrated a broad role of the veA gene in morphogenesis and secondary metabolism in other Aspergilli, such as A. nidulans, A. flavus, A. parasiticus and also in Fusarium verticillioides. Furthermore, we have shown that veA is important for infection in plant pathogens. Interestingly, veA is conserved in A. fumigatus. Our recent findings indicate a strong correlation between veA and secondary metabolite production in A. fumigatus.

Results:
Gene expression analysis indicates statistically significant differences in the expression of gliP and ftmA genes in ΔveA strains compared to the control strain. Currently, production of gliotoxin, fumitremorgen and other secondary metabolites by this organism is being analyzed. Production of secondary metabolism has been linked to oxidative stress in other fungi. Surprisingly, our current findings indicate that ΔveA strains are less sensitive to oxidative stress induced by menadione supplementation. In addition, we found veA to also control hydrolytic activity in vitro.

Conclusions:
This study contributes to set the basis for the design of strategies for the diagnosis and treatment of this opportunistic pathogen.
NAIL INFECTION BY *ASPERGILLUS CANDIDUS*; THE FIRST REPORT FROM IRAN

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**Introduction and Objectives:**
Onychomycosis, fungal infection of the nail plate, occurs worldwide and accounts for up to 50% of all nail infections. Onychomycosis can be caused by three groups of fungi: dermatophytes, yeasts, and non-dermatophytes filamentous fungi. From the literature review, *Aspergillus*, *Fusarium*, *Acremonium*, *Penicillium* and *Scopulariopsis* species are the most common saprophytic fungi isolated from onychomycosis. Hereby we document a case of nail infection with *Aspergillus candidus* as a causative agent from Iran.

**Materials and Methods:**
A 60-years-old housewife woman with chronic deep brown discoloration of toe nail (suspected to onychomycosis) referred to medical mycology department at Tehran University of Medical Sciences. Discoloration of nail and subungual hyperkeratosis was the common presentation in this case. Sampling from the affected area was done and the obtained samples were subjected to the direct examination with 15% KOH, culture on Sabouraud’s dextrose agar and Czapek agar media together with slide culture. A molecular detection (PCR) of 28S rDNA unit from nail sample and subsequent sequencing was performed to confirm the phenotypical findings.

**Results:**
In the microscopic examination rather broad septate hyphae was seen, which did not correspond to the morphology of dermatophytes. Culture of the samples yielded several white to cream colonies after 6 days. A presumptive identification as *A. candidus* was done based on colony morphology in Czapek and Sabouraud’s media and microscopy of slide culture. As a result in the molecular detection by PCR a single fragment of approximately 630 bp was amplified and sequencing confirmed the morphological finding as *A. candidus*.

**Conclusions:**
Onychomycosis caused by *Aspergilli* species is the most common nail infection in the world. In our knowledge this is the first case of nail infection by *A. candidus* from Iran that confirmed by sequencing.
ONYCHOMYCOSIS DUE TO \textit{ASPERGILII} SPECIES IN 170 PATIENTS IN TEHRAN, IRAN

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Introduction:
Onychomycosis is the fungal infection of the nail, which is caused by various species of dermatophytes, yeasts and moulds. Non-dermatophyte filamentous fungi are the most heterogeneous groups of organisms which may cause onychomycosis. Epidemiological studies have shown that \textit{Aspergillus} \textit{spp.} is emerging fungal agents of toenail onychomycosis. Several \textit{Aspergillus} \textit{species} have been isolated from nails, including \textit{A. fumigatus}, \textit{A. flavus}, \textit{A. terreus}, \textit{A. sclerotiorum}, and \textit{A. nidulans}. Only 2% of onychomycosis cases are caused by \textit{Aspergillus} \textit{species}. This prospective study was undertaken in order to determine the prevalence of aspergilii species as causative agents in onychomycosis.

Material and Methods:
Over a period of 1 year (2009-2010), samples were obtained from 170 patients with clinically suspected fungal nail infections, who were referred to medical mycology department at Tehran University of Medical Sciences. The samples were examined by direct microscopy for fungal elements in 15% KOH. For cultures, nail scrapping were inoculated in Sabouraud-Dextrose-Agar-chloramphenicol medium (SC), with and without cycloheximide (SCC), and incubated at 27°C for 3 weeks. Ten inoculation sites were performed in each plate. Species identification was done by colony characters, pigment production, slide culture and microscopic examination with lactophenol- cotton blue preparation. The number of samples was detected only as mycelium by direct examination and their culture were negative. In order to detect these samples we extracted DNA from nail specimens and amplification of part of the 28S rDNA by PCR was performed with universal primers and the fungal species were identified by sequencing.

Result:
Out of the 170 patients with nail dystrophy, 74 individuals were confirmed with onychomycoses (43.5%), 21 of which were due to non-dermatophytic fungi (28.3%). In 14 cases (8 females, 6 males, aged 20–73 years); the non-dermatophytic onychomycosis was caused by \textit{Aspergillus} \textit{spp.} (18.9% of all cases of onychomycosis). Among these 14 positive cases, 1 (7.1%) was with fingernail and 13 (92.8%) were with toenail onychomycosis. The big toenails were more affected. Age average was 52.4 years that 8 patients in 51-60 years age group (57.1%) were at high risk. The prevalent clinical features were distal form with 10 (71.4%) and distal-lateral form with 4 (28.5%). None of the patients had tinea pedis. Direct and culture positive findings were observed in 11 (78.5%) and 3 samples (21.4%) with positive direct examination and negative cultures were detected by molecular testing. \textit{Aspergillus} \textit{species} isolated from the nails were: \textit{A. flavus}, 7 (50%); \textit{A. oryzae}, 3 (21.4%); \textit{A. niger}, 3 (21.4%); and \textit{A. candidus}, 1 (7.1%).

Conclusions:
In most of the latter, the main non-dermatophyte mould involved in onychomycosis as primary pathogen appears to be Scopulariopsis (1.4–6% of all nail mycotic infections), whereas cases of onychomycosis caused by \textit{Aspergillus} \textit{spp.} are reported with varying frequency depending on geographic area and the criteria used by mycology laboratories. Considering our results, this revealed high frequency of onychomycosis in elders and women. \textit{Aspergillus} \textit{species} could be considered as emergent pathogens causing toenail onychomycosis. Our results showed that the polymerase chain reaction was more sensitive method for detection and identification of onychomycosis than conventional methods.
108 CLASSIFICATION AND RAPID IDENTIFICATION OF ASPERGILLUS FUMIGATUS AND RELATIVE SPECIES

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Purpose:
Aspergillosis is a clinically important mycosis that comprises a wide variety of bronchopulmonary infections. The causative agents of aspergillosis are *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. nidulans*, *A. terreus* and other species in this genus. The most significant causative agent is *A. fumigatus*. Recently some species related *A. fumigatus* have been reported as new species mostly based on phylogeny. It is important to discriminate *A. fumigatus* and relatives in a medical field, because of the difference on in vitro susceptibilities to multiple antifungal drugs. Therefore we tried to discriminate *A. fumigatus* and related species rapidly by using PCR and RAPD analyses.

Methods:
We examined the MICs against antifungal drugs on total about 170 strains of *A. fumigatus* and relative species, *A. lentulus*, *A. udagawae*, and *A. viridinutans*, preserved at the MMRC, Chiba Univ. The species of *Aspergillus* section *Fumigati* were genotyped based on sequence analysis on b-tubulin and other genes and the relationship among phylogeny, morphology and physiological characteristics were examined. Using these sequence data, we designed primer sets to apply specific regions of *A. fumigatus* and related species, respectively by PCR and LAMP methods.

Results:
Among the relative species, *A. viridinutans* specially showed high resistance against voriconazole.

The species of section *Fumigati* were divided into 5 clades. Typical strains of *A. fumigatus* were placed in clade I, with globose and echinulate conidia, and growing at more than 50°C. *A. lentulus* and *A. fimisynnematus* were placed in Clade II, with subglobose and almost smooth conidia, and growing up to 45°C. *A. fimigatiaffinis* and *A. novofumigatus* were placed in Clade III, with ellipsoidal and almost smooth conidia, and growing up to 42°C. *A. viridinutans* and *A. udagawae* were placed in Clade IV, with globose and almost smooth conidia, and growing up to 42°C. The other species were placed in Clade V, separated from typical *A. fumigatus*, so they were considered the out group.

We try to design primer sets to apply specific regions of *A. fumigatus*, *A. lentulus*, *A. udagawae* and *A. fimigatiaffinis*, respectively. The primer pair amplified only *A. fumigatus*, and there were no amplifications in other pathogenic *Aspergillus* species and the other fungi that were distributed in the environment widely. Similarly, the other primer pairs amplified only *A. lentulus*, *A. udagawae* and *A. fimigatiaffinis*, respectively. So we could estimate to identify those species quickly.

We also designed LAMP primer set. Comparing with PCR method, the advantages of LAMP method are short reaction time, isothermal reaction and high sensitivity.

Conclusions:
Among the relative species, *A. viridinutans* specially showed high resistance against voriconazole. The species of the section *Fumigati* were divided into 5 clades. There were correlations regarding phylogeny, morphology and physiological characteristics. We were able to discriminate *A. fumigatus* and related species rapidly by using PCR and RAPD analyses.
COMPARISON OF CULTURE AND NESTED-PCR IN DIAGNOSIS OF AVIAN ASPERGILLOSIS

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Purpose:
Standard culture based methods in diagnosis of aspergillosis has limited sensitivity and specificity and is time consuming. The PCR assay provides a high sensitivity and specificity for detection of fungal DNA and rapidly identifies most of clinically relevant Aspergillus species. Avian aspergillosis is a widespread, common fungal disease. The infection is reported in several avian species. The aim of this study was to compare culture and nested PCR for detection of avian aspergillosis in specimens obtained from infected poultry flocks.

Methods:
During a 12 month period, from July 2009 to July 2010, of 400 suspected cases, Two hundred and fifty three (253) infected poultry which approved by veterinarian were collected from different farms in Mazandaran, Hamedan and Tehran provinces of Iran. The fungal balls isolated from lung lesions, all were going under culture and PCR procedure. Aspergillus isolates were identified in the level of genus on Sabouraud glucose agar. To improve the sensitivity and specificity of routine culture approach for identification of Aspergillus in the level of species, we used four differential media including, czapek dox agar. Fungal DNA isolation methods were adopted. All PCR were the same for each template and set of primers used. PCR products were analyzed by electrophoresis on 2% (wt/vol) agarose gels stained with 0.5 μg of ethidium bromide per ml. Targets for PCR of fungi in the 18S or ITS2 subunits of the ribosomal RNA genes facilitated the design of Aspergillus species probes.

Results:
In cultured method 198 case (78.2%) identified as Aspergillus fumigates, 17 case (6.7%) as A. flavus, 10 (3.9%) as A. niger and 6 (2.3%) as other species. In Nested PCR method, by using specific primers 231 cases (91.3%) identified as Aspergillus fumigates, 10 (3.9%) as A. flavus, 7 (2.7%) as A. niger and 5 (1.9%) as other species. However, PCR are less sensitive than nested PCR assays, and the majority detects only the most common pathogenic species, Aspergillus fumigatus. In this study, we developed a nested Aspergillus-specific PCR. We compared the performance of the nested PCR assay and culture method in infected poultries. The results shows that there are significant differences between culture results and the nPCR result in identifying of Aspergillus spp.

Conclusions:
In this investigation we found that using Developed nested PCR method would be more trustworthy in comparison conventional methods in identifying avian aspergillosis.
MPS1 KINASE IS ESSENTIAL FOR THE GROWTH IN *ASPERGILLUS FUMIGATUS*

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**Purpose:**
Protein kinase is a key enzyme that functionally changes the target protein by phosphorylation. It involves in regulation of the majority of cellular pathways such as signal transduction. The monopolar spindle 1 (MPS1) is a conserved dual specificity protein kinase with various cell cycle functions, which has been well studied in budding yeast or higher eukaryotes. Despite of its importance, it has been still unknown whether MPS1 homolog in filamentous fungi plays similar roles. Here we investigate functions of *Aspergillus fumigatus* Mps1 kinase by chemical and/or genetic inactivation.

**Methods:**
A conditional mutant of *MPS1* was generated by replacing its promoter with hygromycin resistance marker and *NiiA* promoter, which can be repressed in the presence of ammonium ion as a nitrogen source. LY83583, *Candida albicans*-specific Mps1 inhibitor (Tsuda et al, 2011), was used to inhibit kinase activity of *A. fumigatus* Mps1. An analog sensitive MPS1-as1 mutant strain with an engineered ATP-binding pocket to render the kinase uniquely sensitive to an orthogonal inhibitor 1NM-PP1 was generated by replacing wild-type *MPS1* locus with hygromycin resistance marker and *MPS1* gene possessing an analog-sensitive mutation.

**Results:**
Mps1 is considered essential for the growth of *A. fumigatus*, as well as yeast or higher eukaryotes. The essentiality of *MPS1* gene was initially examined using the conditional expression driven by the *NiiA* promoter. Transcriptional downregulation of *MPS1* resulted in a strong growth inhibition associated with abnormal nuclear segregation in the presence of ammonium ion. To investigate whether Mps1 kinase activity is essential for the growth, we used a chemical compound LY83583, which specifically inhibits the kinase activity of *C. albicans* Mps1. Chemical inactivation of Mps1 in *A. fumigatus* showed inhibition of germination. Since LY83583 might have a potential inhibitory effect on kinases other than Mps1, we used chemical genetics strategy. An inhibitor 1NM-PP1 suppressed the growth of MPS1-AS1 analog-sensitive mutant, whereas the compound had no effects on the growth of wild type.

**Conclusions:**
Mps1 kinase may play a crucial role for the growth of *A. fumigatus* by controlling nuclear segregation. Hence, Mps1 kinase could be a potential target of novel antifungal agent against aspergillosis.
PEPTIDE APTAMERS AS NOVEL DIAGNOSTICS, AND FOR BLOCKING PALH-MEDIATED ENVIRONMENTAL SENSING IN A. FUMIGATUS

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Purpose:
Peptide aptamers (PA) are amino acid sequences of variable length and composition, often randomly generated, and conformationally constrained at both ends within a surface-presenting region of an inert scaffold. Aptamer libraries can provide a plethora of unique conformations able to recognise individual combinations of charge, shape and hydrophobicity on target protein or protein complex surfaces. Coupled with a selection strategy addressing the desired effect of a binding PA upon a target system it is possible to select aptamers which moderate biological functions. In order to develop a PA selection tool, which can be used to identify PAs selectively able to moderate the functionality of A. fumigatus signalling cascades, we are exploiting an established split-ubiquitin protein interaction assay, the utility of which has already been demonstrated for analyses of A. fumigatus pH-sensing membrane protein interactions. In Aspergillus species seven gene products, encoded by the palA, B, C, F, H and I genes, are required for growth at alkaline pH.

Method:
In order to assess the signalling requirements for A. fumigatus virulence, we constructed a gene replacement mutant lacking the seven transmembrane (7-TM) domain pH-sensor, PalH. The mutant was assessed, relative to a wild type progenitor for virulence in neutropenic mice. In order to identify interactions between A. fumigatus pH-signalling membrane proteins, we used a split-ubiquitin protein fragment complementation assay, conducted in S. cerevisiae, to assess the interaction between integral membrane proteins and their protein partners in living fungal cells. To address the physiological relevance of these protein-protein interactions in Aspergillus species, we constructed a diploid A. nidulans strain expressing both GFP-tagged and myc-tagged PalH proteins, we then used co-immunoprecipitation to detect interacting protein complexes.

Results:
Here we show that, relative to a wild type progenitor isolate, an A. fumigatus gene replacement mutant lacking the seven transmembrane (7-TM) domain pH-sensor PalH, is intolerant of alkaline pH in vitro, and attenuated for virulence in a murine model of pulmonary aspergillosis. We performed multiple pair-wise split-ubiquitin protein interaction assays, addressing every combination of PalF, H and I bait and prey fusion proteins. We were able to capture a previously described interaction of PalH with its cognate arrestin PalF, and additionally we detected dimerisation of the 7-TM PalH protein suggesting a mechanistic basis for dimerisation of this protein during A. fumigatus pH signalling. We then performed co-immunoprecipitation to assess the presence of the PalH dimer under acidic and alkaline pH. PalH dimerisation was detectable at both acidic and alkaline pH.

Conclusions:
From these analyses we can conclude that PalH is a pH-sensor required for alkaline adaptation and murine virulence. Moreover we have established a model for PalH interaction with its cognate arrestin, and palH dimerisation, which is amenable to inhibitor screening in S. cerevisiae.

Currently we are adapting the split-ubiquitin screen to accommodate a null reporter of protein interactions. We will use the modified screen to identify PA inhibitors of PalH-PalF interactions and of PalH dimerisation. Extending the relevance of such screens to diagnostic applications, we are also establishing a screening platform to select for PAs which selectively bind A. fumigatus biomarkers.
SIGNALLING REQUIREMENTS FOR, AND CELL WALL DEPENDENCY UPON, PACC: A MASTER REGULATOR OF A. FUMIGATUS VIRULENCE FACTORS

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Purpose:
The ability to adapt to external pH is a fundamental requisite for the success of Aspergillus species in multiple niches being relevant to growth in the environment, and also in the infected host. An efficient response of Aspergillus species to extracellular pH flux requires the integrity of seven genes, including the DNA binding transcription factor PacC. Besides its role in adaptation to alkaline conditions, we found that A. fumigatus PacC is required for virulence in neutropenic mice. We therefore sought to identify gene products under PacC regulatory control during early infection, and novel regulators of PacC.

Methods:
To identify gene functions under PacC regulatory control during murine infection we performed transcriptional profiling upon an A. fumigatus ATCC46645 clinical isolate and an isogenic ΔpacC mutant using germlings extracted from bronchoalveolar lavage (BAL), and assessing transcript abundance, on a global scale, at 4, 8 and 16 hours post-infection. To identify novel and crucial Rim101p/PacC regulators, a global phenotypic screen of the Euroscarf S. cerevisiae ORF deletion library was performed to identify mutants having identical cation hypersensitive phenotypes to Δrim101, the S. cerevisiae homologue of PacC. An epistacy screen to address the impact of activated Rim101p upon cation sensitivity was performed, along with a screen for mutants aberrantly regulating a synthetic Rim101p-regulatable promoter.

Results:
Relative to infections with a wild type isolate, microarray analyses revealed aberrant transcription of multiple transporter and secondary metabolism functions during infection with a PacC null mutant. A significant impact upon cell wall biosynthesis was also evident from this analysis, and accordingly, pH-non-sensing A fumigatus mutants were found to be highly susceptible to cell wall-active antifungal drugs. Concordant with these findings, among 10 mutants suffering aberrant Rim101p processing and/or transcriptional activity, we identified mutants deficient in the cell wall integrity regulators Bck1p and Slt2p/Mpk1p. Phenotypic analysis of A. fumigatus null mutants of Bck1 and Mpk1 revealed pH-sensitive phenotypes for these mutants in vitro as well as sensitivity to cell wall damaging agents which was potentiated at alkaline pH.

Conclusions:
In A. fumigatus the pH-responsive transcription factor PacC is required for co-ordinated expression of genes encoding secreted proteins, transporters, gliotoxin and cell wall biosynthetic enzymes. As such it represents a major regulatory hub for expression of virulence determining functions. We hypothesise that PacC acts downstream of the cell wall integrity pathway to direct the regulation of cell wall biosynthesis in accordance with environmental pH. Given that the A. fumigatus cell wall is essential for viability, agents which selectively inhibit the pH-dependent activation of PacC signalling might provide useful adjuncts to existing antifungal therapies.
EXPLORING THE ADDITIVE EFFECTS OF MULTIPLE SECONDARY METABOLITES DURING ASPERGILLUS FUMIGATUS VIRULENCE

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Purpose:
In the two murine models most commonly used to study pulmonary aspergillosis, the Aspergillus fumigatus secondary metabolite gliotoxin has been found to differentially impact virulence in a host-dependent manner, whereby the immune status of the infected host governs outcome of infection. Sequenced A. fumigatus genomes are thought to encode a minimum of 22 secondary metabolite loci, the products of which remain largely unknown, as do their impacts upon the mammalian host.

Methods:
To determine the repertoire of expressed secondary metabolite gene clusters during murine infection we performed a comparative microarray analysis of A. fumigatus gene expression at 4, 8 and 16 hours post-infection in cyclophosphamide and hydrocortisone-treated mice. Among the predicted 22 secondary metabolite loci, five were expressed solely in the corticosteroid model, and expression of eight gene clusters was common to both.

Results:
Relative to laboratory culture, expression of genes located in a putative secondary metabolism supercluster (SC), in the subtelomeric region of chromosome VIII, were upregulated in both murine models of infection. The SC, which is poorly conserved among closely related but differing virulent Aspergillus species, includes genes encoding a putative NRPS (AFUA_8g00170) previously named as ftmA, and the hybrid NRPS/PKS (AFUA_8g00540) previously named psoA. Other studies have shown that FtmA, is required for brevianamide F synthesis, a precursor of tremorgenic mycotoxins like fumitremorgins and PsoA is essential for the biosynthesis of pseurotin A, a competitive inhibitor of chitin synthase, and suppressor of immunoglobulin E production.

In order to determine the role of psoA and ftmA in murine virulence, mutants were infected into cyclophosphamide and corticosteroid treated mice, and fungal burden was quantified. Relative to the Af293 progenitor strain, a ΔpsoA mutant was found to be less abundant in both models of infection (p ≤ 0.02) at 4 and 5 days post-infection respectively, while proliferation of a ΔftmA mutant was less abundant in the neutropenic host (p < 0.002).

To establish the role of this SC in virulence we have generated a series of recombinant A. fumigatus bacterial artificial chromosomes to construct mutants lacking the entire pseurotin A gene cluster, and mutants lacking putative methyltransferase or transport functionalities. Mycelial extracts from these mutants were analysed for pseurotin A levels relative to the wild type, identifying a requirement for the entire gene cluster, and methyltransferase for pseurotin A production. In a Galleria mellonella model of aspergillosis, analysis of deletion mutants for each of the PsoA cluster, transporter, and methyltransferase indicated that neither the methyltransferase nor the transporter were required for virulence. These results suggest that the PsoA cluster is required for virulence but that the bioactive product might be a precursor of pseurotin A.

Conclusions:
We can therefore conclude that a pseurotin A precursor, and a product of the ftmA gene cluster, influence A. fumigatus in the mammalian host. A double mutant lacking the ftmA gene and the PsoA cluster has been constructed and the results of virulence assays will be presented, thereby revealing the role of coordinated secondary metabolite biosynthesis during initiation of murine virulence.
GENETIC DIVERSITY OF *ASPERGILLUS FUMIGATUS* ISOLATED FROM AIR AT MANCHESTER

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**Purpose:**

*Aspergillus fumigatus* is considered by many to be the world’s most harmful mould. It is an opportunistic fungal pathogen which causes Invasive Aspergillosis (IA) in immunosuppressed patients, the air consider the main source for the transporting the conidia and involving in pulmonary lung infection.

**Methods:**

Air sampling airborne fungi from air, carried out once a month over two years (2009 and 2010) close to Manchester city centre, to assess the concentration level of viable *A. fumigatus* conidia and total air fungi (TAF). Temperature, humidity and wind measurements were obtained to correlate the metrological parameters to the concentration level in air. *A. fumigatus* isolates were tested for genetic relatedness using Random amplified polymorphic DNA (RAPD) analysis.

**Results:**

220 isolates of *A. fumigatus* was isolated from the air over the study period at a mean of (>10-20 CFU m-3), and TAF (>10-1333 CFU m-3). The total % of *A. fumigatus* in relation TAF was ~3% and persistent throughout the whole year with a negative correlation found between *A. fumigatus* and metrological factors. Statistically there were no significant differences in either the *A. fumigatus* count or TAF between the two years. The level of *A. fumigatus* varied seasonally (14/17) spring, (11/14) summer, (8/12) autumn & (11/13) winter among two years. However, for TAF the highest count was detected in autumn and summer while the lowest count occurred in the spring and winter. Temperature correlated significantly with the TAF (p=0.536). *A. fumigatus* was found to be stable throughout the whole year with a low count of ca.10 CFU m$^{-3}$. RAPD demonstrated genetic diversity among the environmental isolates.

**Conclusions:**

While some of the isolates from the same month clustered into discrete groups, the majority showed genetic diversity indicating the airborne spores arose from a genetically diverse population rather than from a single discrete source. Currently, the virulence of these strains is being assessed in an insect model and compared to clinical strains isolated from patients.
Purpose:
Because knowledge about the molecular mechanisms governing successful invasive infections by *Aspergillus fumigatus* in a mammalian host is limited, forward genetic analysis is a valuable tool to identify and potentially quantify the roles played by the fungal genes involved in this process. Insertional mutagenesis via AMT is a robust and a powerful tool to implement forward genetics because it allows generation of a large number of mutants and for rapid identification of the mutated gene that yields a particular phenotype. We have developed an improved AMT transformation protocol that allows for the easy construction of mutant libraries containing on the order of 100,000 clones. Our modified AMT protocol has high transformation efficiencies and simplified clone isolation procedures. We have used *A. fumigatus* chromosomal sequences within the Ti plasmid transposon to achieve homologous chromosomal transposon insertions.

Methods:
In performing AMT of *A. fumigatus* we employed two *Aspergillus* recipient strains, CEA10 and CEA10ΔKU80, and two transposon donor strains, *Agrobacterium tumefaciens* EHA105/pDHt/hph and EHA105/pDHt/alb1::hph. The first *Agrobacterium* strain contains the Ti plasmid pDHt2/hph, which confers resistance in *A. fumigatus* to the antibiotic hygromycin. The second construct, pDHt/alb1::hph, contains a Ti plasmid with the hygromycin resistance gene flanked by the *A. fumigatus* alb1 flanking sequences. The latter construct was used to delete the alb1 gene from the *A. fumigatus* genome via homologous recombination, which will result in white colonies bearing white non-pigmented conidia that indicate homologous integration events.

Results:
We optimized the transformation protocol to efficiently obtain a large number of mutant colonies. A key to this protocol was the use of *A. fumigatus* germlings for the transformation in place of dormant spores. We also observed that the pDHt/hph transposon was essentially incapable of transforming the CEA10ΔKU80 *A. fumigatus* strain, while transformation of the CEA10ΔKU80 strain by pDHt/hph/alb1 resulted in essentially 100% of the transformed colonies being white. These observations support the notion that a functional KU80 protein is required for AMT T-DNA integration via the Ti border repeats.

Conclusions:
Our findings demonstrate that AMT modification of the *A. fumigatus* genome provides a powerful methodology for generating random loss-of-function insertions or for targeted deletions or insertions into *A. fumigatus*. 
ROLE OF NOVEL ASPERGILLUS FUMIGATUS GENE CLUSTERS IN HOST ADAPTATION AND VIRULENCE

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Purpose:
Previous microarray studies have implicated a requirement for the co-ordinate expression of multiple clusters of *A. fumigatus* genes during murine infections. Among these, two novel gene clusters designated as 13 and 24 were analysed for their possible role in host adaptation and virulence during infection. Cluster 13 consists of 8 genes (Afu1g17130 - Afu1g17200) localized at the subtelomeric region of chromosome I and cluster 24 consists of 9 genes (Afu2g05230 - Afu2g05310) on group linkage II.

Methods:
To determine the role of these clusters in *A. fumigatus* virulence we generated gene cluster deletion strains using a recombineering-based approach which allows construction of replacement cassettes from bacterial artificial chromosome clones (BACs). The respective gene clusters were replaced with a biselectable resistance marker (zeocin and pyrithiamine) by λ-bacteriophage–mediated recombination in *E.coli*. The replacement of gene clusters was subsequently confirmed by PCR amplification of the pyrithiamine (PtrA) gene. Recombineered BACs were linearised using restriction enzymes and were then used for transformation of the *akuB<sup>KL200</sup>* strain of *A. fumigatus*. DNA extracted from the spores of these transformants was used for diagnostic PCR to confirm the presence of the PtRA gene.

Results:
Two independent deletion mutants for each gene cluster were characterised by phenotypic analyses in *vitro*. Radial growth, and susceptibility to oxidative stress, as measured by serial dilution and spot assays, did not reveal any divergences from the behavior of the progenitor isolate.

The mutants were assessed for virulence in corticosteroid and neutropenic murine models of pulmonary aspergillosis, revealing no differences in mortality among mice infected with the wild type or mutant isolates.

Conclusions:
In order to ascertain the existence of more subtle effects upon virulence fungal burden of the mutants is being tested at four days post-infection. From the available data, however, we can draw the preliminary conclusion that neither gene cluster is required for virulence of *A. fumigatus.*
SURVEY THE EFFECT OF LICORICE EXTRACT ON *ASPERGILLUS PARASITICUS* GROWTH AND AFLATOXIN PRODUCTION BY MIC AND HPLC TECHNIQUE

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Introduction:
Mycotoxins are toxic secondary metabolites produced by molds, an important example of mycotoxins are the aflatoxins. Aflatoxins are potent carcinogenic and mutagenic metabolites mainly produced by the fungal species *Aspergillus flavus* and *A. parasiticus*. These species can contaminate several food commodities including cereals, peanuts and crops. In recent years researches on extracts and medicinal herb such as licorice extract lead to reduce the microbial growth and also have a particular effect on production of aflatoxins as carcinogenic compounds. A positive correlation between aflatoxin contamination of agricultural commodities and primary human hepatocellular carcinoma has been documented. For this reason, efforts to reduce and eventually eliminate aflatoxin contamination from food and feed have been undertaken by scientists worldwide. The aim of this study is to explore the antifungal and cytotoxic activity of Glycyrrhiza glabra extract by using MIC and HPLC technique.

Methods:
In this study after culture fungi (standard strain of A. Parasiticus ATCC 15517) in SDA and SDB media, serial dilutions of extracts were produced for fulfilling the MIC technique. In the other hand for determining the rate of aflatoxin produces by the fungi alone and in combination with different dilutions of extract (0.5ml- 2.5ml- 5ml- 10ml) was done by HPLC technique.

Results:
Results demonstrated that licorice extract can inhibited the mentioned fungus growth at 0.5gr/ml. However, HPLC results showed that the mentioned fungi produced the amount of 28960.00 ppb without licorice extract. In addition, the amount of produced toxin was about 14.60 ppb with 10ml of licorice extract. Regards to this concentration, 5ml of extract shows less inhibitory efficiency than 10ml of it but more efficiency than 2.5 ml. However, the antifungal activity of licorice extract was increased with 10ml of the extract.

Discussion:
Since licorice Glycyrrhiza glabra extract has an antifungal activity and also it can inhibit the *Aspergillus* growth, therefore, it may be mentioned as an antibiotic staff. knowledge about activity and determining the effect of licorice extract can help to produce antifungal and anti-aflatoxin drugs.
MUTATION AND EXPRESSION PROFILING OF SEQUENTIAL PATIENT ISOLATES THAT DEVELOPED AZOLE RESISTANCE

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Purpose:
Azole drugs are the primary and most effective therapy against invasive aspergillosis, although resistance is emerging in its main causative agent, Aspergillus fumigatus. Early treatment significantly improves patients’ outcomes, providing there is no resistance to the specific azole being used. Therefore, prompt resistance detection and categorization is important in guiding a clinician’s choice for the primary therapy. Molecular diagnostic tests hold promise to detect resistant infections by identifying specific mutations in A. fumigatus such as single nucleotide polymorphisms in the CYP51A gene, which encodes the azole target. However, 10% to 50% of resistant isolates carry non-CYP51A mutations, which remain unidentified. This fact represents the biggest obstacle to developing comprehensive azole susceptibility tests for invasive aspergillosis.

Methods:
To identify candidate genes that play a role in acquired drug resistance, we have performed genome sequencing of eight sequential clinical isolates of A. fumigatus that developed resistance in vivo as a result of azole therapy. The strains were obtained from the Mycology Reference Centre Manchester culture collection in Manchester, UK, and tested for susceptibility to azoles. The isolates had the minimum inhibitory concentrations of 8 mg/L and higher for voriconazole, 4-8 mg/L for itraconazole, and 1-8 mg/L for posaconazole. Subsequently, genomic DNA extracts from these isolates were sequenced using the Illumina HiSeq instrument. To identify mutations associated with resistance, resistant strains were compared to the susceptible strains by aligning sequence reads against genome assemblies using CLC Genomic Workbench (CLC Bio). If a mutation was located within 200 nt of a differentially expressed gene, it was annotated as gene-associated and classified as a promoter, exonic, or intronic mutation based on its position. Candidate mutations associated with azole resistance in A. fumigatus were further characterized by RNA-Seq to measure gene expression.

Results:
Preliminary comparative analysis has identified multiple genetic variations among the clonal strains isolated from the same patient over the span of two years. Single nucleotide polymorphisms were detected in dozens of genes including bZIP transcription factor HacA (AFUA_3G04070), beta-ketoacyl synthase (AFUA_2G05760) and phosphoglycerate kinase PgkA (AFUA_1G10350). HacA, which is major regulator of the unfolded protein response, has been previously shown to function in virulence and antifungal susceptibility in A. fumigatus. AFUA_2G05760 is homologous to mitochondrial beta-keto-acyl synthase CEM1, which is essential for fatty acid biosynthesis in Saccharomyces cerevisiae. Comparison of the mutational and expression profiles of A. fumigatus genes in resistant and susceptible isolates allowed us to link genes mutations and differentially expressed genes including AFUA_2G05760. It was up-expressed in strains resistant to posaconazole in comparison to sensitive ones.

Conclusions:
The results show that the fungus continues to evolve inside the human host. It can accumulate mutations that may allow it to better adjust to selective pressures including exposure to azole drugs. Constitutive expression is likely to plays a key role in the initial stages of resistance development in A. fumigatus.
SIMPLE AND HIGHLY DISCRIMINATORY VNTR-BASED MULTIPLEX PCR FOR THE TYPING OF ASPERGILLUS FLAVUS ISOLATES FROM DIFFERENT GEOGRAPHIC ORIGINS AND DIFFERENT HOSTS

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Purpose:
In countries with arid dry conditions, including most of the Middle East, Africa and Southeast Asia, Aspergillus flavus is the predominant Aspergillus pathogen accounting for most of the cases of sinusitis, keratitis and cutaneous infections in humans. In a recent epidemiological survey in avian farms in the South of China (Guangxi Province), we demonstrated that A. flavus represented the major fungal contaminant. The genetic relationship between clinical and environmental isolates of A. flavus can be studied using genetic fingerprinting with DNA-based methods. The objective of the present study was to develop a new typing method based on the detection of VNTR (Variable number tandem repeat) markers in A. flavus.

Methods:
The availability of sequences of A. flavus (strain NRRL3357) allowed us to search for tandem-repeat sequences using the Tandem Repeat Finder on-line software (http://tandem.bu.edu/trf/trf.html). Loci with tandem repeats consisting of more than 20 nucleotides and more than 3 repeats were selected. In order to evaluate the polymorphism of selected tandem repeats, primers were chosen on both sides of the repeats and a first group of 30 unrelated isolates was analyzed. Primer sets were further associated by pairs to develop a multiplex technique, which was used to type 90 isolates from avian farms in China or human cases of aspergillosis in Tunisia.

Results:
We finally selected 8 VNTR markers located on 6 different chromosomes (1, 2, 3, 5, 7 and 8) of A. flavus. The Simpson index for individual markers ranged from 0.380 to 0.807. A combined loci index calculated with all the markers yielded an index of 0.995. The MLVA technique was proved to be specific and reproducible. Only one major cluster of isolates could be defined by using the graphing algorithm termed Minimum Spanning Tree. This cluster comprised most of the avian isolates collected in a poultry farm in China.

Conclusions:
Despite the growing challenge due to A. flavus, the molecular epidemiology of this fungus has not been well studied. Recently, two techniques based on Microsatellite Length Polymorphism have been described. MLVA is a more simple method, which does not require the use of capillary electrophoresis. MLVA displayed an excellent discriminatory power and could be used to track the sources of contamination in different epidemiological situations.
IDENTIFICATION OF LEVELS OF *ASPERGILLUS* SPP CONIDIA IN DIFFERENT SEASONS AND MOLECULAR PATTERN OF TWO SPECIES *ASPERGILLUS* (FUMIGATUS AND FLAVUS) IN THE HOSPITAL

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Purpose:
*Aspergillus* spp can cause various diseases. invasive aspergillosis (IA), aspergilloma and allergic aspergillosis being the most frequent forms. IA is the most life-threatening one which recently poses a threat not only to patients suffering from hematological malignancies. it also occurs in a much broader patient population in the classical immunocompromised hosts patients and patients receiving corticosteroids for treatment of chronic lung diseases. Most IA caused by *Aspergillus fumigates*. *Aspergillus flavus* being often reported as the second most frequent species. Nosocomial infections occur worldwide. They are important contributors to morbidity and mortality and *Aspergillus fumigatus* play an important role in these infections. Clinical microbiology laboratories in tertiary-care hospitals should be able to perform epidemiological analysis and surveillance of aspergilla in a hospital environment on routine basis.

Methods:
We collected samples of air from outdoor and indoor environment in the hospital in summer, autumn, winter and spring. Czapeck agar plates were used as culture medium. Among the methods suitable for identification RAPD offers the best potential in terms of obtaining discriminatory data in a rapid simple and cost-efficient way.

Results:
The autumn collection yielded more isolates than that of the other seasons. Several RAPD primers and protocols have been published for using in different species of the genus *Aspergillus*. Among the primers tested R108 show an unequivocal differentiation of *Aspergillus fumigatus* and *Aspergillus flavus*.

Conclusions:
The higher mortality which is seen in the infections with *A. fumigatus* appears to be due to weakened immune response, to the virulence capacity of the microorganism itself and also, probably to the delay in establishing a diagnosis, which can prevent the success of the treatment. So, A reliable simple and rapid molecular method with high discriminatory power is necessary for identifying the molecular pattern of each species.
COMPARATIVE ANALYSIS OF METABOLIC PATHWAYS TO IDENTIFY AND CHARACTERIZE THE PUTATIVE DRUG TARGETS FOR ASPERGILLUS

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Purpose:
The genus *Aspergillus* consorting with a prevalent air born filamentous fungal pathogen causing morbidity and mortality in immune compromised patients. To identify and prioritize antifungal drug targets, against these pathogens is required to develop new pharmaceuticals. In present study we have attempted to find the putative target and their characterization and virtual screening.

Methods:
We have analyzed various enzymes from different biochemical pathways of pathogen using KEGG database server and compared with human protein. The structure of candidate enzyme KARI was modeled based on predicted structural homology to characterized KARI. The generated model had been validated by PROCHECK and WHAT IF programs. The Zinc library was generated within the limitation if Lipinski rule of five, for docking study. Based on the dock-score six molecules have been studied for ADME/TOX analysis and subjected for pharmacophore model generation. This systematic evaluation of metabolic enzymes of pathogens reliable than conventional bioinformatics methods and can be extended to other pathogens of clinical interest.

Results:
We have identified eight enzymes as the potential target for drug design and are Anthranilate phosphoribosyl transferase /2.4.2.18, Anthranilate synthase/4.1.3.27, Urate oxidase /1.7.3.3, Keto-acid-reductoisomerase /1.1.1.86, Chitin synthase /2.4.1.16, 3,4-dihydroxy-2-butanone 4-phosphate synthase /4.1.99.12, alpha-alpha-trehalose-phosphate synthase / 2.4.1.15 and Dihydroxy-acid dehydratase/ 4.2.1.9 respectively. The enzyme KARI was found to be unique by comparing to host proteome through BLASTp analysis. The Zinc ID of those seems like inhibitors ZINC00720614, ZINC01068126, ZINC09291743, ZINC02090678, ZINC00663057 and ZINC02284065 and found to be pharmacologically active agonist and antagonist of KARI.

Conclusions:
This systematic evaluation of metabolic enzymes of pathogens reliable and conventional bioinformatics methods and can be extended to other pathogens of clinical interest. The target and their inhibitor which is reported in this paper could be the better alternative for synthesis of the new kind of drug against Aspergilli.
FETUIN A, A SERUM GLYCOPROTEIN, PROMOTES THE GROWTH AND THE BIOFILM FORMATION OF ASPERGILLUS FUMIGATUS

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Purpose:
When \textit{Aspergillus fumigatus} invades human organs in aspergillosis, it has direct contact with blood and its components such as serum proteins, which might affect the growth of \textit{A. fumigatus}. At the 4th AAA conference, we showed that WGA-trapped glycoproteins worked as an accelerator of the growth of \textit{A. fumigatus} and, at least in part, participated in the biofilm formation. In the present study, we identified a serum glycoprotein, fetuin A, as a promoter of the fungal growth and the biofilm formation of \textit{A. fumigatus}.

Methods:
To identify the serum glycoprotein in the fraction trapped by WGA lectin, the fraction was separated by SDS-PAGE and one of the proteins was identified as fetuin A by mass spectrometry. To confirm the effect of fetuin A, conidia of \textit{A. fumigatus} were cultured with 2mg/ml fetuin A at 37 °C for 30 hours in the presence of 5 % CO2 and then were examined the morphology and the weight of fungal community. Specimens were also observed with fluorescent microscope after staining with FungiFlora Y. Additionally, to analyze the growth rates of hyphae, hyphal tips were traced and recorded by the Bio-Cell Tracer system.

Results:
Results of SDS-PAGE and mass spectrometry suggested fetuin A as a candidate to promote the growth and the biofilm formation of \textit{A. fumigatus}. When 2 mg/ml of fetuin A was added to the culture medium, \textit{A. fumigatus} formed well-networked thick fungal community. The Bio-Cell Tracer analysis showed that fetuin A significantly accelerated the growth of \textit{A. fumigatus} as seen when cultured with BSA. Then, we examined the biomass of the fungal community cultured in DME with BSA or fetuin A. The biomass of \textit{A. fumigatus} cultured with fetuin A was larger than that formed in DME only or DME with BSA. The observation by fluorescent microscopy disclosed the abundant branching of hyphae when cultured with fetuin A.

Conclusions:
Fetuin A in the serum works as an accelerator of the growth of \textit{A. fumigatus} and an inducer of hyphal branching as well, forming the well-networked thick fungal community. These results suggest that fetuin A is deeply involved in the formation of biofilm by \textit{A. fumigatus} in vivo and the pathogenesis of the fungus.
INTERACTION STUDIES OF ASPERGILLUS AND HUMAN AIRWAY CELLS

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Purpose:
Aspergillosis is the name used for a series of infections caused by *Aspergillus* species. Among pathogenic strains of *Aspergillus*, *A. fumigatus* is the major cause of aspergillosis; especially invasive aspergillosis all over the world.

Throughout microbial infections microorganisms and their hosts are known to communicate with each other via a range of signals. The cell-cell communication in microorganisms is called Quorum Sensing (QS). Recent studies show that QS is also implicated in communication between microorganisms and their host. This field of research has evolved from the initial interspecies findings about QS between microorganisms with each other.

This research project aim to investigate the interactions of *Aspergillus fumigates* (pathogen) and human airways (host) via inter-kingdom signalling by focussing on the potential molecules that are likely to be effective in the host pathogen interactions during aspergillosis. Amongst different molecules secreted by pathogenic strains of *Aspergillus*, gliotoxin has been the subject of many investigations for its role as a putative virulence factor. In addition, corticosteroid therapy in immunodeficient patients is associated with an increase in invasive aspergillosis.

Methods:
In this research gliotoxin (as a pathogen signal) and hydrocortisone (as the host signal) were chosen for investigation. The effect of gliotoxin and hydrocortisone on human lung carcinoma epithelia and their response and sensitivity to gliotoxin and hydrocortisone was evaluated through quantification of changes in relative metabolic activity and changes in morphology.

Results:
As a result, the cells show a dose dependent response to gliotoxin and this mycotoxin caused cell death and change in morphology of the cells, hydrocortisone on the other hand did not cause any cell death as expected nor did it cause any significant changes in the morphology of the lung epithelial cells. Furthermore, gliotoxin was extracted and detected from the clinical isolate of *A. fumigatus* by both TLC and HPLC techniques and the effect of hydrocortisone at 10-5 and 10-6 M concentrations on the level of gliotoxin production was examined. However this study did not show a significant change in the level of gliotoxin production as a consequence of hydrocortisone addition.

Conclusions:
The work carried out so far has led to the conclusion to further direct the research towards proteomics. At present the effect of hydrocortisone on the growth of *Aspergillus fumigatus* is under investigation via proteomic studies.
MICROEVOLUTION OF ASPERGILLUS FUMIGATUS IN ASPERGILLOMAS

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Purpose:
Aspergillomas (fungal balls) form over weeks or months when spores germinate on the bronchial or cavity wall, where mycelia and debris attach to form an amorphous mass. Aspergillomas are most commonly seen in chronic pulmonary aspergillosis (CPA). Most patients with simple pulmonary aspergillomas are asymptomatic initially, although symptoms are often severe when aspergillomas are associated with multi-cavity CPA. A characteristic feature is haemoptysis, which varies from trivial to fatal in severity. Furthermore aspergillomas are a risk factor associated with the development of azole resistance.

Methods:
Aspergillomas were removed from 3 patients; Patients 1 and 2 during surgery, and Patient 3 at autopsy. Overall 92 Aspergillus fumigatus were isolated from throughout the dissected fungal balls, with individual colony picking. Microsatellite typing was conducted to determine the genetic type, and a phylogenetic tree was constructed using this data. Minimum inhibitory concentrations (MICs) were performed by modified European Committee for Antibiotic Susceptibility Testing (mEUCAST) method, against itraconazole, voriconazole and posaconazole. The entire coding region of the cyp51A gene was amplified in 22 isolates.

Results:
Isolates from Patient 1 revealed azole susceptible and resistant A. fumigatus, although cyp51A sequences were all wild-type, so the mechanism of resistance remains ill-defined. In these isolates significant genetic differences were observed in 5 of 6 microsatellite loci, splitting the isolates into at least 2 distinct clades. In Patient 2, isolates were less variable; all were azole susceptible, and microvariation was only seen in 2 microsatellite loci. Finally only azole resistant strains were isolated from Patient 3, although the pattern of cross-resistance was notably different for posaconazole, ranging from 0.125->8mg/L. Interestingly two different amino acid alterations were found in this aspergilloma, both at codon 220 (M220K and M220T). The phylogenetic tree, constructed using microsatellite data, revealed isolates from different aspergilloma patients did not cluster.

Conclusions:
Diverse microevolutionary alterations were observed in this set of aspergilloma isolates; revealing differences in azole susceptibility, mechanisms of resistance and genetic type (sometimes even within the same aspergilloma).
GENETIC DIVERSITY OF *ASPERGILLUS FUMIGATUS* AND *ASPERGILLUS FLAVUS* FROM POULTRY FARMS IN SOUTHERN CHINA, GUANGXI PROVINCE

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**Purpose:**
Aspergillosis may create severe economic losses in the poultry industry worldwide. *Aspergillus* are fast-growing moulds that find optimal conditions for growth in avian farms, especially in tropical regions. The presence of *Aspergillus* moulds can also be detected in eggs and birds. The purpose of this study was to isolate and genetically characterize *A. fumigatus* and *A. flavus* from avian farms in Guangxi, a province of southern China.

**Methods:**
Epidemiological investigations were conducted in 6 avian farms in 2009 and 2010. The farms represented different avian production types: outdoor or indoor breedings with ducks, broiler chickens or hens. The farms were successively examined during a 2 month-study period and contamination by thermophilic fungal species was assessed by culture. All the samples were inoculated on malt medium and incubated at 37°C for 7 days to detect the growth of *Aspergillus*. To analyze the genetic polymorphism of *A. fumigatus* and *A. flavus*, a total number of 102 and 55 isolates were genotyped using 10 and 8 specific VNTR (variable-number tandem repeat) markers, respectively.

**Results:**
*Aspergillus* moulds were frequently detected in avian farms. However, the level of contamination seems to vary according to the period of sampling and according to the production type. The species *A. fumigatus* was more frequently isolated than *A. flavus* in farms where hens were bred in cages.

The analysis of 102 *A. fumigatus* isolates using 10 specific VNTR markers led to the resolution of 85 distinct genotypes. Clusters of isolates could be defined by using the graphing algorithm termed minimum spanning tree (MST). The genetic structuration of *A. fumigatus* isolates clearly depended on the type of poultry production. Clusters were defined for isolates collected in avian farms where chickens were kept indoor. On the contrary, isolates collected from open breeding systems (for ducks or broiler chickens) were not associated in the minimum spanning tree. The analysis of 55 *A. flavus* isolates using 8 specific VNTR markers led to the resolution of 45 distinct genotypes. The genetic structuration of *A. flavus* isolates was similar to that of *A. fumigatus* isolates.

**Conclusions:**
Contamination by *Aspergillus* moulds is common in poultry farms from southern China. For the analysis of the genetic diversity of the more prevalent species *A. fumigatus* and *A. flavus*, MLVA was confirmed to be a very useful method and the graphing algorithm MST revealed an interesting clustering with a clear separation between isolates according to the level of confinement of the breeding birds.
Purpose:
Human platelets are essential components in haemostasis, yet they also play a role in defence against microorganisms. We found that germination, hyphal elongation and galactomannan release of *Aspergillus* spp. were significantly affected when exposed to platelets. However, the exact mode of action is still unknown. Of interest, several publications show that reactive oxygen species (ROS) represent a new modulator of platelet activity, as they themselves can generate phagocyte-dependent “burst-like” production of high quantities of ROS. *Aspergillus fumigatus* demonstrates the presence of an alternative oxidase (AOX) to prevent mitochondrial damage due to ROS. This alternative cyanide-insensitive metabolic pathway behaves as a short-circuit in the main respiratory chain. AOX contributes to both reduction of the ROS and may play a role in the antioxidant defense mechanism in *A. fumigatus*.

Methods:
Therefore, we investigated fungal intracellular ROS production and gene expression of AOX under platelet treatment to detect the formation of intracellular ROS. Furthermore, hyphal damage to *A. fumigatus* and fungal gene expression level of fungal mitochondrial coded cytochrome B (cytB) were investigated under platelet treatment.

Therefore, human platelets were co-incubated with *A. fumigatus* for 30 and 90 minutes prior to immunofluorescence detection of intracellular ROS by use of H$_2$DCFDA (Sigma) at 10µM. Gene expression levels of fungal AOX and cytB were performed with qRT-PCR. Hyphal damage was performed by measuring the fungal mitochondrial dehydrogenase activity with XTT (Sigma).

Results:
We found increased intracellular ROS production and an up-regulation of fungal AOX gene expression after 30 min and a down-regulation after 90 min of platelet incubation. We also found a time independent damage to hyphae, and a down-regulation of fungal cytB gene expression.

Conclusions:
Roilides et al. showed that stimulation of neutrophils with either conidia or hyphae of *A. fumigatus* resulted in increased ROS, leading to inhibition of germination. As human platelets are activated by *Aspergillus*, it seems possible that direct or indirect alteration of fungal mitochondria may result from platelets’ ROS production. In conclusion, the present in vitro data provide deeper insights into the biological antifungal characteristics of human platelets against *A. fumigatus*. The identification of this pathway could give new insights into and deepen the role of human platelets in innate immunity.
127 SECONDARY SOLUBILE METABOLITES OF ASPERGILLUS FUMIGATUS AND IT’S INFLUENCE ON INHIBITION OF HUMAN WISH, CACO AND DU-145 CANCER CELL PROLIFERATION

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Purpose:
Culture filtrate (CF) of Aspergillus fumigatus obtained under aerobic conditions contains numerous mycotoxines with a variety of effects on mammalian cells and a potent cytotoxic effect on different cell lines. Gliotoxin has been well studied as a candidate for that activity and it was shown that production of gliotoxin depends on well aerated and oxygenated conditions. In contrast, influence of A. fumigatus CF obtained under anaerobic conditions on mammalian cells and human cancer cells lines are not described despite of the fact that inside of aspergilloma, fungus cells grow in the non-aerated conditions. The aim of the study was to determine the influence of A. fumigatus CF obtained under anaerobic conditions on different human cancer cell lines.

Methods:
A. fumigatus environmental isolate was grown for 5 days on Sabouraud dextrose agar at 30 °C and the conidia were collected and resuspended in MAM culture medium at concentration of 1 x 10⁶ /ml. A. fumigatus CF were prepared under oxygen and non-oxygen conditions at 37 °C and supernatants were collected after 24 h, 3 and 6 days and tested for prostaglandin E2 concentration by ELISA. WISH, CaCo and DU-145 cells lines were treated with different CF and colorimetric and cologenic assays was done in order to detect inhibition and stimulation of cells lines proliferation.

Results:
Data demonstrated inhibitions of WISH cells line and inhibition of proliferation of human cancer cell lines CaCo and DU-145 (100%) when treated with CF obtained in aerobic conditions. In contrast, A. fumigatus CF obtained under anaerobic conditions stimulated human cancer cell lines proliferation (50%), without any effect on WISH cell line.

Conclusions:
This is the first study that demonstrates that A. fumigatus obtained under anaerobic conditions contains secondary metabolites which stimulate proliferation of human cancer cell lines, so the nature of A. fumigatus product obtained under anaerobic conditions needs to be determined.
GENETICS OF HETEROKARYON INCOMPATIBILITY IN *ASPERGILLUS FUMIGATUS*

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Purpose:
Filamentous fungi have developed genetic self/non-self recognition systems to prevent hyphal fusion between unrelated individuals. Heterokaryon incompatibility (HI) is dependent on matching het alleles for formation of a heterokaryon. Incompatibility results in localised compartmentalisation and rapid programmed cell death (PCD) at the site of hyphal fusion. The HET domain proteins involved in the HI system in *Aspergillus fumigatus*, the leading causative agent of aspergillosis, differ from those studied in the characterised models of *Neurospora crassa* and *Podospora anserina*. An understanding of which genes play important HI roles in *A. fumigatus* would provide useful insight into apoptotic triggers, the evolutionary background of HI and expand the scope for potential drug targets.

Methods:
Compatibility grouping between various environmental and clinical *A. fumigatus* isolates has been defined through pair-wise crosses as a base for further genetic research. An ongoing impala-based transposon mutagenesis method is in use to randomly interrupt the *A. fumigatus* genome and characterise any individual spores from the recovered mutant library exhibiting altered HI characteristics. Use of fusion-PCR genetic manipulation techniques to knockout HI genes has been performed, and a similar technique has been employed for promoter replacement and GFP-tagging of HI genes.

Results:
The HI genes were identified via CADRE-genomics and AspGD databases, and through homology to genes involved in HI in *N. crassa* and *P. anserina*.

Conclusions:
Further work will include RNA sequencing of *A. fumigatus* mycelia undergoing PCR from early stationary phase cultures to identify important gene expression changes.

This work is funded by AlerGenetica SL and BBSRC.
CHARACTERIZATION OF A SIALIDASE (KDNASE) FROM THE OPPORTUNISTIC FUNGAL PATHOGEN, \textit{ASPERGILLUS FUMIGATUS}

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\textbf{Purpose:}
\textit{Aspergillus fumigatus} is the most prevalent airborne fungal pathogen in developed countries and it is the main cause of invasive pulmonary aspergillosis in immunocompromised patients. Previous research in our laboratory identified \(\alpha2,6\)-linked N-acetylneuraminic acid (Neu5Ac) on \(A. fumigatus\). Sialic acids are charged monosaccharides known to play important roles in microbial pathogenesis and in host immune systems. We have shown that \(A. fumigatus\) sialic acids mediate binding of conidia to fibronectin, laminin and other proteins in the lung cell basal lamina. In addition, the removal of sialic acids significantly decreased conidial uptake by cultured murine macrophages and type 2 pneumocytes compared to sham controls. This indicates that the sialylated molecules on the conidia surface act as ligands for both professional and non-professional phagocytes. Although sialic acids are present on the fungal surface, the mechanism of their biosynthesis/acquisition is unknown and examination of the \(A. fumigatus\) genome shows no homologues to known sialic acid biosynthetic machinery; therefore, the overall aim of our research is to determine the mechanism of sialic acid display on conidia of \(A. fumigatus\).

\textbf{Methods:}
Recently, we identified a gene in \(A. fumigatus\) encoding a sialidase, an enzyme that cleaves terminal sialic acids from glycoconjugates.

\textbf{Results:}
The \(A. fumigatus\) sialidase was cloned and characterized, showing that it can cleave the synthetic sialic acid substrate, 4-methylubelliferyl \(\alpha\)-D-N-acetylneuraminic acid (Mu-nana) and has a preference for \(\alpha2,3\)-linked sialic acid. qPCR studies showed that exposure to human serum induced sialidase expression. A crystal structure of the enzyme was determined to a resolution of 1.84 Å using SAD phasing on a selenomethionine-derivatized form of the recombinant enzyme. This is the first fungal sialidase to have its structure determined. \(A. fumigatus\) sialidase shares the typical sialidase canonical 6-bladed \(\beta\)-sheet propeller topology but with an extended “cap” domain above the active site. The closest structural homolog to \(A. fumigatus\) sialidase is the sialidase from \textit{Micromonospora viridifaciens}, a soil-borne bacterium.

\textbf{Conclusions:}
An examination of the active site of \(A. fumigatus\) sialidase suggested that it can better accommodate a sialic acid with a smaller substitution at the fifth carbon. We have shown by kinetics that another naturally occurring sialic acid, 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN), is the preferred substrate and we have determined the structure of \(A. fumigatus\) sialidase in complex with KDN. This sugar is found in almost all types of glycoconjugates where KDN residues are often in place of Neu5Ac. Interestingly, we have determined that KDN can be used as a sole carbon source for \(A. fumigatus\), while Neu5Ac is not. A KDNase knockout \(A. fumigatus\) strain has been successfully created in our laboratory for further phenotype analysis in comparison with wild-type \(A. fumigatus\). Our data suggests that the \(A. fumigatus\) sialidase (KDNase) could play an important role in sialic acid metabolism in \(A. fumigatus\).
THE IMPORTANCE OF SECRETED SIDEROPHORES IN THE DIAGNOSIS OF INVASIVE ASPERGILLOSIDIS

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Purpose:
Iron is essential for growth, and in low iron environments, such as in serum, *Aspergillus fumigatus* secretes iron-chelating molecules called siderophores. In *A. fumigatus* the major secreted siderophore is the hydroxamate, $N,N',N''$-triacetylfusarinine C (TAFC). Siderophore biosynthesis is required for virulence of *A. fumigatus*. The aim of our research is to investigate whether TAFC can be detected in serum, urine or bronchoalveolar lavage fluids from patients with invasive aspergillosis (IA). This could represent a novel means to diagnose invasive aspergillosis.

Methods:
An LC-MS/MS detection method was developed for detecting TAFC in serum and bronchoalveolar lavage fluid. Solvent precipitation was used to remove proteins from the samples followed by LC-MS/MS analysis. A standard curve, constructed by spiking known amounts of TAFC into human serum, was used to quantify the amount of TAFC in patient samples. An enzyme-linked immunosorbent assay (ELISA) was investigated as an alternative detection method for TAFC. Coprogen, a hydroxamate siderophore, was base hydrolyzed yielding dimerum acid and N-acetyl fusarinine. Fragments were conjugated to bovine serum albumin and used as antigens for monoclonal antibody production. Briefly, BALB/c mice were immunized with the protein conjugates and boosted intravenously at three, six and nine weeks. Test bleeds were taken and tested for TAFC cross-reactivity before proceeding with fusion. Hybridomas were plated on semi-solid HAT selective media and clones were screened for cross reactivity to TAFC using an indirect ELISA. Positive clones were maintained in culture for two weeks to ensure stability and were then stored in liquid nitrogen.

Results:
TAFC was extracted from human serum samples with a percent recovery ranging from 117-120%. LC-MS/MS analysis of TAFC showed upper and lower limits of quantitation to be 750 ng/ml and 5 ng/ml, respectively. The detection limit for the method was less than 1 ng/ml of TAFC. Out of nine galactomannan-positive human serum samples, LC-MS/MS of TAFC confirmed six positive cases (66.7%). Bronchoalveolar lavage samples from six patients who had had at least one positive *A. fumigatus* culture were analyzed by LC-MS/MS and two had detectable TAFC levels.

Development of an immune response directed against TAFC in BALB/c mice was monitored via indirect ELISAs. Mouse test bleeds, taken after the second and third boosts, were tested for activity against hydrolyzed coprogen, with and without a carrier protein, and TAFC without a carrier protein. Results indicate that pre-fusion bleeds were reactive towards both hydrolyzed coprogen and TAFC but not reactive towards siderophores that did not possess the N-acetyl fusarinine moiety.

Conclusions:
Current diagnostic tests for IA suffer from significant interference which reduces their sensitivity. Preliminary data indicates that the fungal siderophore, TAFC is detectable in bronchoalveolar lavage and serum samples from patients with suspected IA; however, a prospective study of patients is required to determine whether this method provides improved detection of IA, particularly during the early stages of infection.
CONTRIBUTION OF PROTEIN KINASE C (PKAC) AND ALB1 GENES INVOLVED IN CONIDIATION/PIGMENTATION PATHWAY IN WILD TYPE (WT) AND PIGMENTLESS, NON-CONIDIATING VORICONAZOLE-RESISTANT (VCZ-R) ASPERGILLUS FLAVUS

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Background:
Mechanisms unrelated to cyp51 mutations have been demonstrated to be responsible for triazole resistance in clinical isolates of A. fumigatus. Studies on signaling pathways and their role if any in triazole resistance in Aspergillus species is limited. Protein kinase C is a central component of the cyclic AMP signaling cascade in the human opportunistic mold A. flavus that regulates cellular processes by phosphorylation of specific proteins. It has been shown to be involved in both conidiation/pigmentation pathways (via alb1 gene) in fungi. Recent studies have also evaluated the role of these pathways in virulence in A. fumigatus. However the role of pkaC and alb1 gene mutations in triazole resistance in A. flavus is unknown.

Aim:
To characterize and study the expression of pkaC and alb1 genes in WT and pigmentless, non-conidiating VCZ-R (MIC 4 mcg/ml) clinical isolate of A. flavus isolate that lacks cyp51A mutation.

Methods:
Fresh cultures of WT and VCZ-R A. flavus were used for DNA and RNA isolation (Qiagen kit). Gene specific primers were designed and used to amplify the complete transcriptional unit of pkaC and alb1 genes using standard PCR and qRT-PCR (Taqman probes) conditions as appropriate. The amino acid sequences of pkaC and alb1 genes in WT and VCZ-R A. flavus were deduced from the nucleotide sequence and compared. Any change in expression of pkaC was tested using qRT-PCR using 18sRNA as the housekeeping gene.

Results:
We have characterized the pkaC gene of A. flavus for the first time in an albino clinical isolate of A. flavus that is VCZ-R (MIC 4 mcg/ml). The pkaC gene in WT A. flavus demonstrated 99% homology (3 exons, 3 introns, 1158bp, 385aa) with the published NRRL3357 pkaC gene sequence. No mutations in pkaC were demonstrated in VCZ-R albino A. flavus although there were amino acid mutations involving the alb1 or the pigmentation gene. Differential expression of pkaC was observed in various VCZ-R isolates of A. flavus.

Conclusion:
Lack of conidiation and pigmentation were associated with alb1 but not pkaC mutations in VCZ-R albino A. flavus. However differential expression of pkaC was demonstrated in pigmentless, non-conidiating VCZ-R A. flavus. Both pkaC and alb1 genes are involved in the conidiation and pigmentation pathways respectively and may contribute to triazole resistance in VCZ-R A. flavus. The contribution of the protein kinase pathway to conidiation/pigmentation and triazole resistance in A. flavus needs to be further explored. The virulence potential of these albino mutants of A. flavus needs to be investigated using an in vivo model.
EFFECT OF PHARMACOLOGICAL AND GENETIC INHIBITION OF THE HEAT-SHOCK PROTEIN 90 (HSP90) IN ASPERGILLUS FUMIGATUS

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Purpose:
Hsp90 is a key molecular chaperone present in large amount in the cytosol of most living species, including fungi. We investigated the effect of the pharmacological and genetic inhibition of Hsp90 in Aspergillus fumigatus (Af).

Methods:
Effect of Hsp90 inhibitors (geldanamycin, 17-AAG, 17-DMAG) alone or in combination with other antifungal drugs (caspofungin, micafungin, anidulafungin, voriconazole, amphotericin B, nikkomycin Z, FK506) was assessed against Af by in vitro susceptibility testing according to CLSI standards. To control the expression of Hsp90, the endogenous promoter was substituted by an inducible nitrogen-regulatable promoter (pniia) to generate the pniia-Hsp90 strain. An Af strain expressing a C-terminal EGFP-labelled Hsp90 at its native locus (Hsp90-EGFP) was also generated to localize Hsp90 under normal growth and stress conditions.

Results:
Hsp90 inhibitors alone were poorly effective against Af. Their combination with echinocandins (fungistatic against Af) resulted in a fungicidal effect at high doses (4 µg/ml geldanamycin, 32 µg/ml 17-AAG/17-DMAG). Combination with FK506 also showed increased efficacy at high doses. No additive effect was observed in combination with other antifungal drugs. Under genetic repression conditions, the pniia-Hsp90 strain exhibited reduced hyphal growth and a lack of conidiation. These effects were much more pronounced in the presence of caspofungin. While under normal growth conditions the localization of Hsp90 in the Hsp90-EGFP strain was mainly cytosolic, accumulation near the cell wall and at the sites of septa formation was observed in the presence of caspofungin.

Conclusions:
Genetic repression of Hsp90 was achieved for the first time in Af and resulted in severe morphological alterations and an increased susceptibility to echinocandins. Further characterization of the pniia-Hsp90 and Hsp90-EGFP strains will help better define the role of Hsp90 in Af pathogenesis.
PHENOTYPIC ANALYSIS OF *ASPERGILLUS FUMIGATUS* STRAIN LACKING THE SODIUM ATPASE ENCODING GENE ENA**A**

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**Purpose:**
To examine the role of the gene enaA in pH sensing and ion homeostasis in the opportunistic pathogen *Aspergillus fumigatus*.

**Methods:**
*Aspergillus fumigatus* gene enaA was replaced with the hygromycin resistance gene hph by homologous recombination. The replacement construct was transformed to the *A. fumigatus* wild-type strain AF293 and the transformants were selected for growth in the presence of hygromycin B. Deletion was confirmed by realtime PCR and Southern blot. Phenotypic analysis was performed by measuring colony diameters after exposure to different stresses.

**Results:**
The ΔenaA and the wild-type strains showed comparable hyphal growth when exposed to sorbitol as osmotic stress, as well as to high level of Na+, K+ and Ca2+. Similar results were observed with acidic and alkaline pH. However, the ΔenaA strain appeared to be more susceptible to manganese stress, since the hyphal growth of the mutant strain reached about 50% of those of the wild-type strain.

**Conclusions:**
Deletion of the gene enaA in *A. fumigatus* moderately increased susceptibility to Mn2+, suggesting a possible role in manganese homeostasis. Further investigations in term of gene responses under high Mn2+ conditions are necessary to clarify the role of enaA.
DIVERSITY AND COMPARISON OF SHORT TANDEM REPEATS WITHIN ASPERGILLUS SECTION FUMIGATI

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Purpose:
Microsatellites (or short tandem repeats) are frequently employed for molecular epidemiology of Aspergillus fumigatus due to its reproducibility and high discrimination power. Nevertheless, the specificity of A. fumigatus microsatellite markers have never been tested in a group of closely related isolates. The aim of this study was to test a previously described and validated panel of microsatellite markers in a set of species belonging to section Fumigati, namely Aspergillus fumigatus affinis, Aspergillus lentulus, Aspergillus novofumigatus, Aspergillus unilateralis, Neosartorya hiratsukae, Neosartorya pseudofischeri and Neosartorya udagawae.

Methods:
The reference A. fumigatus ATCC 46645 was genotyped under the standard conditions.

Results:
An electrophoretic profile with 8 expected peaks was observed. Inversely, no peaks were observed for the other species belonging to section Fumigati; a single exception was the marker MC6b in A. unilateralis. By screening the complete genome sequence of Neosartorya fischeri NRRL 181, markers MC3, MC6a and MC7 were found with more than 3 repeats of each motif. The accumulation of insertions and deletions was frequently observed in the genomic region surrounding the studied microsatellites what may explain the difficulties to amplify microsatellite markers in taxonomical species close to A. fumigatus. When the microsatellite multiplex was tested in less stringency PCR conditions, few markers were amplified and a distinct electrophoretic profile was observed for species within section Fumigati.

Conclusions:
The microsatellite-based PCR multiplex was confirmed as a highly specific panel for A. fumigatus under the recommended conditions and might allow the identification of this species. With a slight modification of temperature conditions, the microsatellite markers allowed the discrimination of other pathogenic species within section Fumigati, particularly A. lentulus and N. udagawae.
A RAPID APPROACH TOWARDS SCREENING OF ASPERGILLUS FUMIGATUS POPULATION STRUCTURE USING A SINGLE NUCLEOTIDE POLYMORPHISM BASED METHOD

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**Purpose:**
*Aspergillus fumigatus* is a ubiquitous fungus responsible for several human diseases. Invasive aspergillosis is considered the most threatening *Aspergillus*-related disease due to high mortality rates in severely immunocompromised patients. Several methods have been developed over the last few years to evaluate genetic diversity, to recognize the population structure and to understand the epidemiology of *A. fumigatus*. Multilocus Sequence Typing (MLST) is a very reliable method, portable and robust, able to provide a unified characterization approach that generates data for evolutionary and population studies. By screening a set of seven housekeeping genes, Bain et al. (2007) genotyped 100 clinical and environmental *A. fumigatus* isolates and 30 sequence types were clearly distinguished. Moreover, it was determined a discriminatory power of 0.93 for this technique. However, MLST remains very expensive and unpractical for routine examination of *A. fumigatus* genotype. Thus, the main goal of this study was to develop a new Single Nucleotide Polymorphism (SNP) based method with a set of 21 polymorphic sites selected from MLST scheme.

**Methods:**
The 21 markers were successfully grouped into a single reaction and tested in a population sample of 45 isolates of *A. fumigatus*. The diversity index for each one of the SNPs was calculated, ranging from 0.03 to 0.51.

**Results:**
The discrimination power of the methodology was 0.995 in comparison to MLST. The 45 *A. fumigatus* isolates were compared with a group of 26 strains of *A. fumigatus* from United Kingdom from the MLST database (http://www.mlst.net). A set of 7 polymorphisms were exclusively found in the Portuguese collection (markers A9, C123, C185, C540, L48, L340 and S329) and 2 other polymorphisms were specific to the UK population (B129 and C403).

**Conclusions:**
This new SNP based method was engaged in a much shorter period of time than MLST and the final cost was much lower. Therefore, it represents an alternative to the MLST scheme in order to assess the population structure and genotypic profile for *A. fumigatus* isolates.

**References:**
GENETIC ASSOCIATIONS WITH PLASMINOGEN AND RELATED GENES WITH CHRONIC CAVITARY (CCPA) AND ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA) COMPARED WITH HEALTHY AND ASTHMATIC CONTROLS

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Purpose:
Various genes and SNPs have been identified as associated with invasive aspergillosis (IA), but much less is known about the genetic factors influencing susceptibility to CCPA, ABPA and severe asthma and fungal sensitisation (SAFS). Plasminogen (PLG) binds to *A. fumigatus*, and can be converted to plasmin while bound. Expression of genes encoding proteins that are involved in this conversion (PLAU and PLAUR) is upregulated in monocytes exposed to *A. fumigatus*. Plasmin can act as a chemoattractant for monocytes and induces expression of inflammatory cytokines and chemokines by these cells. Plasminogen activator tissue (PLAT) also acts to promote the conversion of plasminogen to plasmin.

Method:
The Sequenom® MassArray® iPLEX™ Gold system was used to genotype HapMap tagging SNPs in the PLG and PLAT genes. 23 SNPs in PLG and 11 SNPs in PLAT were analysed in 95 ABPA patients, 47 SAFS patients and 112 CCPA patients (±aspergilloma), and in 152 atopic asthmatics and 279 healthy controls. Genotype and allele frequencies were compared using χ² and Fisher’s exact tests. Frequencies in the CCPA group was compared to the healthy group, and frequencies in the ABPA and SAFS groups were compared to both the healthy group and the atopic asthmatic group.

Results:
Four SNPs in PLAT were significantly associated with CCPA compared to the healthy group. The GG genotype of rs879293 (G/A) was significantly less common in the CCPA subjects (OR 1.65). The AA and GG genotypes of rs8178880 (A/G; OR 3.26) and rs8178890 (G/A; OR 2.66) respectively, were more common in the CCPA subjects. The G allele of rs2070712 (A/G; OR 1.52) was more common in CCPA. It is interesting that some of these associations are with the common allele. The AA genotype of rs8178880 (A/G; OR 2.73-3.82) was also significantly associated with ABPA, and the G allele of rs2070712 (A/G) showed a trend towards association with ABPA, but did not reach significance. All of these are novel associations not been described previously. The SNPs are intronic and their function is currently unknown. One SNP in PLG was associated with SAFS; the AA genotype of rs4252200 (A/G; OR 6.20-6.23) is significantly more common in the SAFS group compared to the healthy and atopic asthma groups. This SNP in 3’ of PLG and this is again a novel association. No significant association was found with any of the diseases tested and rs4252125, which has previously been associated with IA.

Conclusions:
The SNPs identified in PLAT and PLG may influence susceptibility to aspergillosis by affecting the conversion of plasminogen to plasmin in response to *A. fumigatus*, or may affect the binding of plasminogen to this fungus. Remarkably SNPs in PLG and PLAT have been found associated with each of the disease phenotypes tested raising fundamental questions of pathogenesis and susceptibility to non-invasive forms of aspergillosis.
IMPAIRED SWITCHING OF THE NORMAL IL1 RESPONSE TO A. FUMIGATUS IN CHRONIC CAVITARY PULMONARY ASPERGILLOSIS (CCPA)

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Purpose:
Aspergillus fumigatus causes invasive aspergillosis (IA) in immunocompromised subjects, but can also cause CCPA in immunocompetent subjects. Little is known about the genetic factors influencing susceptibility to CCPA. Studies have demonstrated that the IL1 pathway is involved in the response to A. fumigatus, with alveolar macrophages co-cultured with this fungus shown to produce the proinflammatory cytokines IL1α, IL1β and IL6, and to induce NFκB activation. IL1RN is a negative regulator of the IL1 pathway.

Method:
For the expression work, monocyte-derived macrophages were generated from blood from 10 healthy volunteers and 10 CCPA patients attending our clinic. These were co-cultured with live A. fumigatus conidia (Af293 strain) and RNA was extracted at various timepoints (0hr-9hr). RNA from the patients and the healthy subjects was pooled. cDNA was generated and RT-PCR was completed on 84 test genes using the Human Innate and Adaptive Immune Responses RT² Profiler PCR Array (SABiosciences). Expression was normalised to HPRT1, RPL13A and GAPDH and fold changes were generated compared to the Healthy 0hr (baseline) expression level. For the genotyping work, the Sequenom® MassArray® iPLEX™ Gold system was used to genotype HapMap tagging SNPs in the IL1B and the IL1RN genes. Six SNPs in IL1B and 10 SNPs in IL1RN were analysed, in 112 CCPA (+aspergilloma) patients and 279 healthy controls. Genotype and allele frequencies were compared using χ² and Fisher’s exact tests.

Results:
In the CCPA group, expression of IL1A and IL1B increased after exposure to A. fumigatus until 9hr, while in the healthy group expression initially increased until 3hr and then decreased at 6hr and 9hr. Expression of IL1F8 did not alter much in either group until 9hr, when it increased dramatically in the healthy group but remained low in the CCPA group. Expression of IL1RN increased in both groups after exposure to A. fumigatus. SNPs in IL1B (rs3136558, A/G, intronic) and IL1RN (rs4252041, C/T, 3’ UTR) were significantly associated with CCPA. The AA genotype of rs3136558 (OR 1.75, 95% CI 1.08, 2.84) and CC genotype of rs4252041 (OR 4.38, 95% CI 1.31, 14.65) were more common in the CCPA group compared to the healthy group.

Conclusions:
The expression profiles observed in the macrophages from healthy subjects for IL1A and IL1B suggest an initial pro-inflammatory response to the presence of conidia, and then a reducing inflammatory response at 6hr-9hr as germtubes and hyphae become the predominant fungal morphology. As expression did not reduce in the CCPA cells, it may be that these cells do not respond to the change in fungal morphology, or, alternatively, show a delayed response. As IL1F8 expression increased in the healthy group as IL1A and IL1B expression decreased, it appears that in the healthy macrophages a switching of the proinflammatory response from an IL1A and IL1B driven response to an IL1F8 driven response occurs. These differential gene expression profiles, and the SNPs identified support a role for the IL1 pathway in the response to A. fumigatus and in susceptibility to aspergillosis.
NEW DIAGNOSTIC TARGETS FOR *ASPERGILLUS FUMIGATUS* INFECTIONS

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**Purpose:**
*Aspergillus fumigatus* is commonly isolated from respiratory samples from patients with cystic fibrosis with reported incidences varying from 7-45% depending upon the culture methods employed and the age of the population studied. The common clinical problem however is evaluating the significance of these isolates in patients without a clinical exacerbation of their disease and differentiating contamination organisms from those that are clinically relevant. We propose that in patients who are chronically colonised with *aspergillus*, the *aspergillus* is present as a biofilm form and as such a different pattern of gene expression should be detectable compared with those who are transiently infected.

**Methods:**
Using a validated in vitro analysis method of distinctive biofilm development morphologies samples were analysed by microarray and confirmed by real-time reverse transcriptase PCR (qRT-PCR). Several targets were identified which vary in a phase dependant manner and could potentially provide useful diagnostic targets.

**Results:**
The validation of expression patterns was confirmed in an animal model where respiratory samples were analysed using qRT-PCR including genes that encoded, cell wall structure, immunological responses and virulence. A subset of the identified genes were found in detectable levels of clinical respiratory samples from cystic fibrosis patients who provided *Aspergillus fumigatus* culture positive specimens at routine clinic visits.

We have identified and confirmed the presence and phase dependence of these a number of potential targets in a rat model, and have also been able to demonstrate the expression of two selected genes gliT and Cat2 in the RNA from sputum samples from patients with cystic fibrosis.

**Conclusions:**
We propose that by examining these markers in parallel it may be possible to distinguish between transient infection with *Aspergillus fumigatus* and patients who have developed a stable biofilm with this organism. This would provide the clinician with clinically pertinent information and allow them to tailor treatment strategies accordingly.
ASPERGILLUS PROFICIENCY TESTING - EXTERNAL QUALITY ASSURANCE (EQA) FOR OPTIMISATION OF DIAGNOSTIC MOLECULAR TESTING

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**Purpose:**
The use of molecular testing for *aspergillus* sp. in high risk patients with potential invasive fungal disease has been used for many years, however, this testing has mainly been developed ‘in-house’ and this lack of standardisation has resulted in this form of testing not being recognised by bodies such as the European Organisation for Research and Treatment (EORTC). The use of a recognised EQA scheme will hopefully allow proficiency of individual assays to be evaluated and standards maintained that give reassurance to clinically reported results.

**Methods:**
A panel of samples were produced in collaboration between the European Aspergillus PCR Initiative (EAPCRI) and Quality Control for Molecular Diagnostics (QCMD). The panel consisted of *Aspergillus fumigatus* DNA or conidia spiked into two matrixes, TE buffer (analytical) and plasma (clinical), to test laboratory proficiencies in both analysis and extraction protocols. Five core samples were selected as required to be achieved to determine a lab was proficient at testing. Samples were blinded by QCMD and distributed on dry ice to participating laboratories for processing.

**Results:**
The performance of participants who returned all five core samples correctly was 48%. Performance of analysis DNA samples in clinical or analytical matrixes showed little difference. 19 datasets recorded a positive result for the 3,000 G.eq/ml sample in plasma compared to 13 for the 3,000 G.eq/ml sample in TE buffer [P=0.097]. Additionally, when samples containing *A. fumigatus* DNA and comparable concentrations of conidia in plasma were compared no statistical difference in the number of positive results was observed 16 datasets recorded a positive result for the sample containing 100 Con/ml compared to 19 datasets for the sample containing 3,000 G.eq/ml [P=0.393]. It was however shown that only real-time PCR assays were capable of detecting the lowest concentration samples. The number of false positives however was a cause for concern with between 15% and 22% false positivity rates. These results were regardless of the samples being a clinical or analytical negative.

**Conclusions:**
In conclusion there is a range of proficiency levels in testing laboratories real-time PCR assays performed better than conventional PCR. The major issue of concern was the false positivity rates and this would have to be improved to allow confidence in this methodology to be achieved.
MODELING OF ASPERGILLUS FUMIGATUS VIRULENCE: OPPORTUNITIES FOR NOVEL THERAPEUTIC TARGET DISCOVERY

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Purpose:
For several years we have used genome-wide transcriptomic and comparative genomic studies to understand the genetic mechanism underlying A. fumigatus virulence. The experiments have yielded a wealth of data that by necessity has been reduced to small sets of genes associated with virulence, while ignoring whole-cell physiological changes that might also be essential for virulence and fitness in a host.

Methods:
Here, we have used an integrative, systems biology approach to combine data from gene expression experiments from mouse infections and neutrophils from normal and CGD patients to get a cell-wide picture of metabolic fluctuations in A. fumigatus that occur during infection. We used the data to model the interactions between metabolic networks in A. fumigatus using the concept of Biological Process Linkage Networks (BPLN).

Results:
We found that transport of carbohydrates and cations (including Zn2+ and Fe3+), and catabolism of amino acid and lipids were up-regulated in the fungi during growth in whole animals. In contrast, ribosomal function, ergosterol biosynthesis, and aerobic respiration were down-regulated in the host. A detailed metabolic analysis shows down-regulation of steroid biosynthesis, the TCA cycle, and synthesis of N-glycans, consistent with a reduced cell proliferation program due to propionate accumulation. The degradation of propionate in A. fumigatus has been shown to be necessary for full virulence in macrophages and establishment of disease in mice. The metabolic processes were highly interconnected in the inferred network suggests the metabolic program of A. fumigatus in the host fits a “small-world” network. The network correctly predicted high connectivity between fatty acid metabolism, amino acid catabolism, and peroxisomal function. Interestingly, the network predicted a high correlation between fatty acid metabolism and penicillin and cephalosporin biosynthesis, and other secondary metabolites that have been implicated in virulence. We also used our gene expression profiling data from A. fumigatus infection in mouse lungs to model candidate regulatory networks beside known metabolic pathways, with the hypothesis that there exists an ‘Aspergillus fumigatus virulence pathway’. In addition, we predicted that we could then identify potential regulators that could be responsible for such regulation. The data for over 1100 differentially regulated genes was used to infer four distinct correlation networks containing 748, 253, 89, and 12 genes respectively. Eight different transcriptional regulators were identified in the two intermediate-size networks (5 and 3, respectively). Interestingly, all these regulators belong to the zinc finger C2H2 or C6 class of transcriptional regulators.

Conclusions:
Thus, network modeling allows the prediction of novel metabolic interconnections and identification of regulatory nodes implicated in virulence that cannot be identified in conventional data analysis. In addition, clarification of network ‘hubs’ and transcriptional regulators associated with such networks expands our repertoire of potential drug targets because of their significant impact on the pathogen’s physiology.
CHEMICAL GENETIC PROFILING AND DRUG MECHANISM OF ACTION STUDIES IN *ASPERGILLUS FUMIGATUS*

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**Purpose:**
Treatment options for *Aspergillus* infections are limited and those drugs currently in use suffer from a variety of shortcomings including low efficacy, toxicity and increasing resistance. There is therefore a pressing need to identify novel classes of antifungal agents for the treatment of *Aspergillus* infections. Despite the discovery of numerous promising drug targets, very few lead compounds have been discovered by target based approaches. This can be partly attributed to the attrition rate of compounds that show promising activity against an enzyme but are either not active against the whole cell or are toxic and consequently most of the antimicrobials presently on the market were originally discovered by random screening of compounds against whole cell screens. A solution to this problem is to employ technologies that allow the identification of gene targets from compounds that already show antifungal activity and have clean toxicity profiles.

**Methods:**
Haploinsufficiency is observed when an abnormal phenotype results from the loss of a single gene copy in a diploid organism. This has been utilised as the basis of chemical genetic profiling techniques in drug mechanism of action studies as strains that lack a single copy of a drug’s target are hypersensitive to that drug. *S. cerevisiae* and knock out libraries have been used to successfully identify the mechanism of action of several promising compounds, however, haploinsufficiency screens and chemical genetic profiling in *A. fumigatus* has been hindered by the difficulty in generating an adequate set of heterozygous *A. fumigatus* strains. This difficulty is a result of lengthy, low-throughput transformation procedures, unreliable gene replacement techniques and the requirement for a suitable diploid *A. fumigatus* strain.

**Results:**
By optimizing the common transformation protocol for *A. fumigatus* high-throughput gene disruption, employing fusion-PCR to generate targeted gene disruption cassettes and utilising a diploid *Ku80-/*Ku80- mutant to facilitate more reliable homologous recombination, a high-throughput targeted gene knock out method for *A. fumigatus* has been established. Consequently, we have been able to demonstrate that chemical genetic haploinsufficiency studies are possible in filamentous fungi.

**Conclusions:**
This offers new possibilities for antifungal research, enables high-throughput methods for surveying the genome of *A. fumigatus* for new drug targets and supports unveiling the mechanisms of action of antifungal drugs.
ISOLATION AND IDENTIFICATION OF \textit{ASPERGILLUS} SPP FROM AIRBOURNE OF SULAIMANI CITY

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\textbf{Methods:} 
Airborne fungi were isolated by settle plate method (gravitation method) in different areas of Sulaimani city during two seasons; Autumn October 2008 and Spring April 2009, in which plate with Sabouraud dextrose agar, containing chloramphenicole were exposed to air for 1 hour.

\textbf{Results:} 
A total of 2409 colonies isolated, belonging to twenty two genera and twenty four species. The most predominant fungi isolated in the environment of different locations of Sulaimani city were \textit{Penicillium} spp. 28.1\%, \textit{Aspergillus} spp. 20.25\%, \textit{Yeast} 13.33\%, \textit{Cladosporium} spp. 12.1\%, and \textit{Alternaria} spp. 6.72\%.

\textit{Aspergillus niger} and \textit{Aspergillus flavus} are the predominant species of \textit{Aspergillus} isolated, while the most common. The highest number of fungi were isolated during spring 1492 colony forming units compare to Autumn were 917 CFU isolated.

\textbf{Conclusions:} 
Depending on sampling locations, the highest number of fungi were isolated in dietary factories 637 CFU followed by houses 471 CFU, factories 421 CFU, dormitories 396 CFU, schools 332 CFU, and hospitals 152 CFU.
COMPARISON OF ANTIMICROBIAL POTENTIAL OF SOME *ASPERGILLUS FUMIGATUS* STRAINS

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**Purpose:**
During the present investigation seven *Aspergillus fumigatus* strains, isolated from different sources were screened for antimicrobial activity against some bacterial (*Escherichia coli, Baccillus fortis, B. faragiris, Pseudomonas flourescence* and *P. malophilia*) and pathogenic fungal species (*Alternaria alternata, Derslera biseptata, Curvularia lunata Fusarium oxysporium, Fusarium solani, and Cladosporium cladosporides*).

**Results:**
*Aspergillus fumigatus* strains showed promising antagonistic features. All test bacterial isolates were inhibited by *A. fumigatus*. Culture filtrates of *A. fumigatus* strains were tested for their antifungal potential by well diffusion assays. Cultural filtrate of *A. fumigatus* 893 showed high antifungal effects on mycelial growth of *F. oxysporum, F. solani*, and *C. cladosporioides*, inhibited the fungal growth from 45 to 68%. However in case of *Derslera biseptata*, all *A. fumigatus* strains were equally effective and reduce the colony growth up to 68%. On the other hand filtrate of all strains of *A. fumigatus* were least effective against *E. coli*.

**Conclusions:**
*A. fumigatus* 867 and 985 strains showed promising results against *P. flourescence*. 
Case Report:
We report a case of primary subcutaneous aspergillosis in a 75-years-old otherwise healthy woman, a tea plantation worker in Rize, in Black Sea Region of Turkey. She had the first lesion four years ago as a small erythematous papule in the right hand middle finger, then it drained, however subsequently new papules occured. She had visiting different hospitals for management and received unsuccessful courses of antibacterial therapy. She was admitted to Cerrahpasa Medical Faculty Dermatology Department at 06 December 2010 with gradually extending series of squamous itchy nodules erupted on her hand and arm. The Routine clinical laboratory data was unremarkable. There was no history of steroid use. An excisional biopsy was performed on one of the lesions for pathological and microbiological examinations. Histological findings were consistent primarily with deep mycoses and secondarily with atypical mycobacterial infection. Treatment with Doxycycline (200 mg, 2x1) and itraconazole (200 mg, 2x1) was started. Gram, Ehrlich- Ziehl-Neelsen and Periodic acid-Schiff stained imprint preparations revealed no bacteria. No bacterial organisms were cultured from the specimen. Mycobacterial cultures were also negative. Giemsa stained tissue was negative for Leishmania amastigotes. Mycological examination of Giemsa stained imprint preparations revealed septated hyphae. Aseptically divided pieces of tissue specimen were inoculated into Sabouraud dextrose agar, Brain heart infusion agar, chocolate agar plates and incubated at 30 and 35oC. All cultures yielded pure growth of *Aspergillus flavus*. On follow up at 24 December 2010 the patient showed some regression of enduration and puritis. Treatment was continued 3 months. Then, the patient completely responded to therapy and no recurrence occured within 10 months.

Conclusions:
Primary subcutaneous aspergillosis is a rare entity and usually occures secondary to systemic or disseminated aspergillosis. Primary subcutaneous *aspergillus* infections most commonly related to and affects the exposed areas of intravenous cannulation, venipunctur or surgical or burn wounds. Although subcutaneous infections with a sporotrichoid pattern in an otherwise healthy patient could be due to relatively limited aetiologies including *Sporothrix schenckii*, *Nocardia brasiliensis*, *Mycobacterium marinum*, *Aspergillus* spp. can also be responsible for primary subcutaneous lesions mimicking sporothricosis as was in this case.
# AUTHOR INDEX

<table>
<thead>
<tr>
<th>Author</th>
<th>Poster No.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abastabar, M</td>
<td>98, 101</td>
<td>169, 172</td>
</tr>
<tr>
<td>Abbasi, F</td>
<td>23</td>
<td>87</td>
</tr>
<tr>
<td>Abdalla, W</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>Abdellah, F</td>
<td>3</td>
<td>67</td>
</tr>
<tr>
<td>Abdi, R</td>
<td>35</td>
<td>101</td>
</tr>
<tr>
<td>Agbetile, J</td>
<td>58</td>
<td>126</td>
</tr>
<tr>
<td>Aggad, H</td>
<td>4</td>
<td>68</td>
</tr>
<tr>
<td>Ahmadi, B</td>
<td>7, 98, 106, 107</td>
<td>71, 169, 177, 178</td>
</tr>
<tr>
<td>Aimanianda, V</td>
<td>83</td>
<td>154</td>
</tr>
<tr>
<td>Akay, MO</td>
<td>37</td>
<td>103</td>
</tr>
<tr>
<td>Akoumianaki, T</td>
<td>80</td>
<td>151</td>
</tr>
<tr>
<td>Akova, M</td>
<td>26 Jan, 13.45-14.45</td>
<td>36</td>
</tr>
<tr>
<td>Aksit, F</td>
<td>37</td>
<td>103</td>
</tr>
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<td>Alachkar, H</td>
<td>81</td>
<td>152</td>
</tr>
<tr>
<td>Al-Barrag, A</td>
<td>124</td>
<td>195</td>
</tr>
<tr>
<td>Alcazar-Fuoli, L</td>
<td>113, 116</td>
<td>184, 187</td>
</tr>
<tr>
<td>Alcorn, J</td>
<td>72</td>
<td>143</td>
</tr>
<tr>
<td>Al-Hatmi, A</td>
<td>60</td>
<td>128</td>
</tr>
<tr>
<td>Alialy, M</td>
<td>26, 68</td>
<td>90, 139</td>
</tr>
<tr>
<td>Al-shair, K</td>
<td>59</td>
<td>127</td>
</tr>
<tr>
<td>Alshareef, FO</td>
<td>114</td>
<td>185</td>
</tr>
<tr>
<td>Alvarado, D</td>
<td>90</td>
<td>161</td>
</tr>
<tr>
<td>Alzahrany, HA</td>
<td>3</td>
<td>67</td>
</tr>
<tr>
<td>Amorim, A</td>
<td>134, 135</td>
<td>205, 206</td>
</tr>
<tr>
<td>Amvam Zollo, PH</td>
<td>2</td>
<td>66</td>
</tr>
<tr>
<td>Anand, R</td>
<td>70</td>
<td>141</td>
</tr>
<tr>
<td>Anders, M</td>
<td>124</td>
<td>195</td>
</tr>
<tr>
<td>Ansari, S</td>
<td>106</td>
<td>177</td>
</tr>
<tr>
<td>Araujo, R</td>
<td>134, 135</td>
<td>205, 206</td>
</tr>
<tr>
<td>Araviysky, RA</td>
<td>62</td>
<td>132</td>
</tr>
<tr>
<td>Arikans-Akdagli, S</td>
<td>28 Jan, 14.10-14.35</td>
<td>59</td>
</tr>
<tr>
<td>Armstrong-James, D</td>
<td>89</td>
<td>160</td>
</tr>
<tr>
<td>Arnaud, MB</td>
<td>99</td>
<td>170</td>
</tr>
<tr>
<td>Arnc, P</td>
<td>8, 104, 119, 125</td>
<td>72, 175, 190, 196</td>
</tr>
<tr>
<td>Arroyo, J</td>
<td>74</td>
<td>145</td>
</tr>
<tr>
<td>Arsic Arsenijevic, V</td>
<td>73, 127</td>
<td>144, 198</td>
</tr>
<tr>
<td>Aryal, M</td>
<td>64, 94</td>
<td>135, 165</td>
</tr>
<tr>
<td>Aslan, M</td>
<td>37</td>
<td>103</td>
</tr>
<tr>
<td>Atherton, GT</td>
<td>54, 59, 119, 125</td>
<td>122, 127, 158</td>
</tr>
<tr>
<td>Atia Mhfuod</td>
<td>87</td>
<td>158</td>
</tr>
<tr>
<td>El-Muzyghi, A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aversa, F</td>
<td>40, 42, 85, 86</td>
<td>106, 110, 156, 157</td>
</tr>
<tr>
<td>Ayadi, A</td>
<td>119</td>
<td>190</td>
</tr>
<tr>
<td>Azizi, M</td>
<td>102</td>
<td>173</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td></td>
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</tr>
<tr>
<td>Badali, H</td>
<td>7</td>
<td>71</td>
</tr>
<tr>
<td>Bafadhel, M</td>
<td>58</td>
<td>126</td>
</tr>
<tr>
<td>Bai, H</td>
<td>61</td>
<td>129</td>
</tr>
<tr>
<td>Bakhotmah, BA</td>
<td>3</td>
<td>67</td>
</tr>
<tr>
<td>Balajee, SA</td>
<td>90</td>
<td>161</td>
</tr>
<tr>
<td>Barac, A</td>
<td>73, 127</td>
<td>144, 198</td>
</tr>
<tr>
<td>Barber, PV</td>
<td>55</td>
<td>123</td>
</tr>
<tr>
<td>Barozzi, P</td>
<td>74</td>
<td>146</td>
</tr>
<tr>
<td>Barthakur, B</td>
<td>30</td>
<td>96</td>
</tr>
<tr>
<td>Bassiri, A</td>
<td>35</td>
<td>101</td>
</tr>
<tr>
<td>Batista, MV</td>
<td>52</td>
<td>120</td>
</tr>
<tr>
<td>Baxter, C</td>
<td>26 Jan, 12.00-12.25, 33</td>
<td>36, 99, 102</td>
</tr>
<tr>
<td>Bayat, M</td>
<td>120</td>
<td>191</td>
</tr>
<tr>
<td>Bebb, R</td>
<td>74</td>
<td>145</td>
</tr>
<tr>
<td>Beauvrais, A</td>
<td>6, 74</td>
<td>70, 145</td>
</tr>
<tr>
<td>Beebe, DJ</td>
<td>79</td>
<td>150</td>
</tr>
<tr>
<td>Beli, S</td>
<td>87</td>
<td>158</td>
</tr>
<tr>
<td>Ben Chaaban, T</td>
<td>53</td>
<td>121</td>
</tr>
<tr>
<td>Ben-Ami, R</td>
<td>26 Jan, 09.15-09.40</td>
<td>28</td>
</tr>
<tr>
<td>Bennet, A</td>
<td>129</td>
<td>200</td>
</tr>
<tr>
<td>Berthier, E</td>
<td>79</td>
<td>150</td>
</tr>
<tr>
<td>Bertruzzi, M</td>
<td>111, 112</td>
<td>182, 183</td>
</tr>
<tr>
<td>Besbes, G</td>
<td>53</td>
<td>121</td>
</tr>
<tr>
<td>Bhaduri, A</td>
<td>93</td>
<td>164</td>
</tr>
<tr>
<td>Bhetariya, P</td>
<td>93</td>
<td>164</td>
</tr>
<tr>
<td>Bignell, EM</td>
<td>26 Jan, 09.40-10.05</td>
<td>29</td>
</tr>
<tr>
<td>Binder, U</td>
<td>13, 126</td>
<td>77, 197</td>
</tr>
<tr>
<td>Binkley, G</td>
<td>99</td>
<td>170</td>
</tr>
<tr>
<td>Binkley, J</td>
<td>99</td>
<td>170</td>
</tr>
<tr>
<td>Binnie, M</td>
<td>78</td>
<td>149</td>
</tr>
<tr>
<td>Blum, G</td>
<td>15</td>
<td>79</td>
</tr>
<tr>
<td>Bocanegra, R</td>
<td>12</td>
<td>76</td>
</tr>
<tr>
<td>Bogomolova, TS</td>
<td>62</td>
<td>132</td>
</tr>
<tr>
<td>Borow, R</td>
<td>56</td>
<td>124</td>
</tr>
<tr>
<td>Borzova, YV</td>
<td>62</td>
<td>132</td>
</tr>
<tr>
<td>Botterel, F</td>
<td>8, 104, 119</td>
<td>72, 175, 190</td>
</tr>
<tr>
<td>Boukraa, L</td>
<td>3</td>
<td>67</td>
</tr>
<tr>
<td>Bouzani, M</td>
<td>83</td>
<td>154</td>
</tr>
<tr>
<td>Bowyer, P</td>
<td>20, 118, 124, 136, 137</td>
<td>84, 189, 195, 207, 208</td>
</tr>
<tr>
<td>Boychenko, EG</td>
<td>62</td>
<td>132</td>
</tr>
<tr>
<td>Boyle, K</td>
<td>26 Jan, 15.35-15.45</td>
<td>39</td>
</tr>
<tr>
<td>Boyom, FF</td>
<td>1</td>
<td>65</td>
</tr>
<tr>
<td>Boyton, R</td>
<td>26 Jan, 12.50-13.15</td>
<td>35</td>
</tr>
<tr>
<td>Brakhage, A</td>
<td>88</td>
<td>159</td>
</tr>
<tr>
<td>Brightling, CE</td>
<td>58</td>
<td>126</td>
</tr>
<tr>
<td>Bright-Thomas, R</td>
<td>33</td>
<td>99</td>
</tr>
<tr>
<td>Brock, M</td>
<td>69</td>
<td>140</td>
</tr>
<tr>
<td>Bromley, M</td>
<td>27 Jan, 10.30-10.55, 20</td>
<td>141, 212</td>
</tr>
</tbody>
</table>
## AUTHOR INDEX

<table>
<thead>
<tr>
<th>Author</th>
<th>Poster No.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buchheidt, D</td>
<td>45</td>
<td>113</td>
</tr>
<tr>
<td>Busca, A</td>
<td>40, 42, 43</td>
<td>106, 110, 111</td>
</tr>
<tr>
<td>Caira, M</td>
<td>40, 41, 42, 43</td>
<td>106, 108, 110, 111</td>
</tr>
<tr>
<td>Cairns, T</td>
<td>112, 113</td>
<td>183, 184</td>
</tr>
<tr>
<td>Calvo, AM</td>
<td>105</td>
<td>176</td>
</tr>
<tr>
<td>Campbell, B</td>
<td>91</td>
<td>162</td>
</tr>
<tr>
<td>Campos, SV</td>
<td>52</td>
<td>120</td>
</tr>
<tr>
<td>Candoni, A</td>
<td>40, 42, 43, 74</td>
<td>106, 110, 111, 1145</td>
</tr>
<tr>
<td>Cao, L</td>
<td>61</td>
<td>129</td>
</tr>
<tr>
<td>Caramalho, R</td>
<td>135</td>
<td>206</td>
</tr>
<tr>
<td>Caramatti, C</td>
<td>40, 42, 43</td>
<td>106, 110, 111</td>
</tr>
<tr>
<td>Carroll, CS</td>
<td>130</td>
<td>201</td>
</tr>
<tr>
<td>Carvalho, A</td>
<td>77, 85, 86</td>
<td>148, 156, 157</td>
</tr>
<tr>
<td>Casagrande, A</td>
<td>77</td>
<td>148</td>
</tr>
<tr>
<td>Castagnola, C</td>
<td>43</td>
<td>111</td>
</tr>
<tr>
<td>Cattaneo, C</td>
<td>40, 42</td>
<td>106, 110</td>
</tr>
<tr>
<td>Cavaillon, JM</td>
<td>69</td>
<td>140</td>
</tr>
<tr>
<td>Cerqueira, GC</td>
<td>99</td>
<td>170</td>
</tr>
<tr>
<td>Chaiprasert, A</td>
<td>133</td>
<td>204</td>
</tr>
<tr>
<td>Chaker, E</td>
<td>53</td>
<td>121</td>
</tr>
<tr>
<td>Chamilos, G</td>
<td>80</td>
<td>151</td>
</tr>
<tr>
<td>Chandrasekar, PH</td>
<td>131</td>
<td>202</td>
</tr>
<tr>
<td>Chang, J</td>
<td>129</td>
<td>200</td>
</tr>
<tr>
<td>Chang, YC</td>
<td>100</td>
<td>171</td>
</tr>
<tr>
<td>Chen, V</td>
<td>90, 91</td>
<td>161, 162</td>
</tr>
<tr>
<td>Chermette, R</td>
<td>8, 104, 119, 125</td>
<td>72, 175, 190, 196</td>
</tr>
<tr>
<td>Chernoppyatova, RM</td>
<td>62</td>
<td>132</td>
</tr>
<tr>
<td>Chibucos, MC</td>
<td>99</td>
<td>170</td>
</tr>
<tr>
<td>Chierichini, A</td>
<td>40</td>
<td>106</td>
</tr>
<tr>
<td>Chishimba, L</td>
<td>50</td>
<td>118</td>
</tr>
<tr>
<td>Chiusolo, P</td>
<td>51</td>
<td>108</td>
</tr>
<tr>
<td>Chowdhary, A</td>
<td>11</td>
<td>75</td>
</tr>
<tr>
<td>Chrdle, A</td>
<td>33</td>
<td>99</td>
</tr>
<tr>
<td>Clark, LP</td>
<td>44</td>
<td>112</td>
</tr>
<tr>
<td>Clements, M</td>
<td>123</td>
<td>194</td>
</tr>
<tr>
<td>Clemens, KV</td>
<td>90, 91</td>
<td>161, 162</td>
</tr>
<tr>
<td>Cleverley, JO</td>
<td>46</td>
<td>114</td>
</tr>
<tr>
<td>Comoli, P</td>
<td>74</td>
<td>145</td>
</tr>
<tr>
<td>Constância, C</td>
<td>52</td>
<td>120</td>
</tr>
<tr>
<td>Cooley, J</td>
<td>50</td>
<td>118</td>
</tr>
<tr>
<td>Costa, SF</td>
<td>52</td>
<td>120</td>
</tr>
<tr>
<td>Cotty, PJ</td>
<td>26 Jan, 17.15-17.40, 42, 91</td>
<td>162</td>
</tr>
<tr>
<td>Crabtree, J</td>
<td>99</td>
<td>170</td>
</tr>
<tr>
<td>Cuenca-Estrella, M</td>
<td>28 Jan, 15.10-15.35</td>
<td>61</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Author</th>
<th>Poster No.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cunha, C</td>
<td>77, 85, 86</td>
<td>148, 156, 157</td>
</tr>
<tr>
<td>D</td>
<td></td>
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<tr>
<td>da Mata, FMR</td>
<td>17</td>
<td>81</td>
</tr>
<tr>
<td>da Silva, DL</td>
<td>17</td>
<td>81</td>
</tr>
<tr>
<td>Dabas, Y</td>
<td>32</td>
<td>98</td>
</tr>
<tr>
<td>Daci, R</td>
<td>106, 107</td>
<td>177, 178</td>
</tr>
<tr>
<td>Dai, Y</td>
<td>61</td>
<td>129</td>
</tr>
<tr>
<td>Dam, S</td>
<td>6</td>
<td>70</td>
</tr>
<tr>
<td>Davari, B</td>
<td>5</td>
<td>69</td>
</tr>
<tr>
<td>Davis, Z</td>
<td>97</td>
<td>168</td>
</tr>
<tr>
<td>De Paolis, MR</td>
<td>43</td>
<td>111</td>
</tr>
<tr>
<td>DeBenedictis, FM</td>
<td>77</td>
<td>148</td>
</tr>
<tr>
<td>Debnath, S</td>
<td>30</td>
<td>96</td>
</tr>
<tr>
<td>Delia, M</td>
<td>40, 42</td>
<td>106, 110</td>
</tr>
<tr>
<td>DeLuca, A</td>
<td>77</td>
<td>148</td>
</tr>
<tr>
<td>Denning, DW</td>
<td>20, 33, 36, 50, 51, 84, 99</td>
<td>118, 124, 136, 137, 119, 123, 124, 125, 127, 152, 189, 195, 207, 208</td>
</tr>
<tr>
<td>Desai, D</td>
<td>58</td>
<td>126</td>
</tr>
<tr>
<td>Dhingra, S</td>
<td>105</td>
<td>176</td>
</tr>
<tr>
<td>Diaz-Torres, MR</td>
<td>128</td>
<td>199</td>
</tr>
<tr>
<td>Dimopoulos, G</td>
<td>26 Jan, 12.25-12.50</td>
<td>34</td>
</tr>
<tr>
<td>Djebli, N</td>
<td>4</td>
<td>68</td>
</tr>
<tr>
<td>Djokic, J</td>
<td>87</td>
<td>158</td>
</tr>
<tr>
<td>Doffinger, R</td>
<td>81</td>
<td>152</td>
</tr>
<tr>
<td>Donato, R</td>
<td>85</td>
<td>156</td>
</tr>
<tr>
<td>Donnelly, JP</td>
<td>28 Jan, 09.40-10.05</td>
<td>53-54</td>
</tr>
<tr>
<td>Doser, K</td>
<td>71</td>
<td>142</td>
</tr>
<tr>
<td>Dubey, LK</td>
<td>84</td>
<td>155</td>
</tr>
<tr>
<td>Duddy, N</td>
<td>57</td>
<td>125</td>
</tr>
<tr>
<td>Dulley, FL</td>
<td>52</td>
<td>120</td>
</tr>
<tr>
<td>Dunn, C</td>
<td>97</td>
<td>168</td>
</tr>
<tr>
<td>Dunn-Coleman, N</td>
<td>128</td>
<td>199</td>
</tr>
<tr>
<td>Durmaz, G</td>
<td>37</td>
<td>103</td>
</tr>
<tr>
<td>Dusane, DH</td>
<td>6</td>
<td>70</td>
</tr>
<tr>
<td>Dyer, PS</td>
<td>100</td>
<td>171</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Author</th>
<th>Poster No.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echtenacher, B</td>
<td>71</td>
<td>142</td>
</tr>
<tr>
<td>Edinger, M</td>
<td>71</td>
<td>142</td>
</tr>
<tr>
<td>Einsele, H</td>
<td>83, 88</td>
<td>154, 159</td>
</tr>
<tr>
<td>Ekberov, E</td>
<td>49</td>
<td>117</td>
</tr>
<tr>
<td>Enayati, S</td>
<td>102</td>
<td>173</td>
</tr>
<tr>
<td>Erbeznik, T</td>
<td>15</td>
<td>79</td>
</tr>
<tr>
<td>Eshwika, A</td>
<td>96</td>
<td>167</td>
</tr>
<tr>
<td>Everson, C</td>
<td>97</td>
<td>168</td>
</tr>
</tbody>
</table>
## AUTHOR INDEX

<table>
<thead>
<tr>
<th>Author</th>
<th>Poster No.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fairs, A</td>
<td>58</td>
<td>126</td>
</tr>
<tr>
<td>Fanci, R</td>
<td>40, 43</td>
<td>106, 111</td>
</tr>
<tr>
<td>Federova, N</td>
<td>20, 112, 113, 118</td>
<td>84, 183, 184, 189</td>
</tr>
<tr>
<td>Fekam Boyom, F</td>
<td>2</td>
<td>66</td>
</tr>
<tr>
<td>Felton, T</td>
<td>27 Jan, 13.05-13.15, 33</td>
<td>99</td>
</tr>
<tr>
<td>Ferjaoui, M</td>
<td>53</td>
<td>121</td>
</tr>
<tr>
<td>Fidan, I</td>
<td>75</td>
<td>146</td>
</tr>
<tr>
<td>Flechsig, C</td>
<td>83</td>
<td>154</td>
</tr>
<tr>
<td>Fonseca, CA</td>
<td>52</td>
<td>120</td>
</tr>
<tr>
<td>Fonseca, GH</td>
<td>52</td>
<td>120</td>
</tr>
<tr>
<td>Foongladda, S</td>
<td>133</td>
<td>204</td>
</tr>
<tr>
<td>Forghieri, F</td>
<td>74</td>
<td>145</td>
</tr>
<tr>
<td>Fortwendel, JR</td>
<td>132</td>
<td>203</td>
</tr>
<tr>
<td>Fouad, A</td>
<td>75</td>
<td>146</td>
</tr>
<tr>
<td>Fraczek, M</td>
<td>20, 55</td>
<td>84, 123</td>
</tr>
<tr>
<td>França, IL</td>
<td>52</td>
<td>120</td>
</tr>
<tr>
<td><strong>G</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamba, CM</td>
<td>52</td>
<td>120</td>
</tr>
<tr>
<td>Garzia, MG</td>
<td>40</td>
<td>106</td>
</tr>
<tr>
<td>Gasbarrino, C</td>
<td>40</td>
<td>106</td>
</tr>
<tr>
<td>Gaur, SN</td>
<td>11</td>
<td>75</td>
</tr>
<tr>
<td>Geoffrey, RD</td>
<td>114</td>
<td>185</td>
</tr>
<tr>
<td>Gerami-Shoar, M</td>
<td>106, 107</td>
<td>177, 178</td>
</tr>
<tr>
<td>Gernez, Y</td>
<td>97</td>
<td>168</td>
</tr>
<tr>
<td>Gholami, S</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>Ghosh, A</td>
<td>116</td>
<td>187</td>
</tr>
<tr>
<td>Ghoubontini, A</td>
<td>53</td>
<td>121</td>
</tr>
<tr>
<td>Giannatou, EL</td>
<td>46</td>
<td>114</td>
</tr>
<tr>
<td>Giovannini, G</td>
<td>77, 85</td>
<td>148, 156</td>
</tr>
<tr>
<td>Glamoclija, J</td>
<td>87</td>
<td>158</td>
</tr>
<tr>
<td>Gono, T</td>
<td>103</td>
<td>174</td>
</tr>
<tr>
<td>Gregson, L</td>
<td>124</td>
<td>195</td>
</tr>
<tr>
<td>Gresnigt, MS</td>
<td>80</td>
<td>151</td>
</tr>
<tr>
<td>Grice, C</td>
<td>111</td>
<td>182</td>
</tr>
<tr>
<td>Gricourt, M</td>
<td>8</td>
<td>72</td>
</tr>
<tr>
<td>Grigoleit, GU</td>
<td>83</td>
<td>154</td>
</tr>
<tr>
<td>Groll, A</td>
<td>27 Jan, 12.40-13.05</td>
<td>51</td>
</tr>
<tr>
<td>Guillot, J</td>
<td>8, 104, 119, 125</td>
<td>72, 175, 190, 196</td>
</tr>
<tr>
<td>Guinea Ortega, JV</td>
<td>26 Jan, 11.35-12.00</td>
<td>32</td>
</tr>
<tr>
<td>Gumral, G</td>
<td>49</td>
<td>117</td>
</tr>
<tr>
<td>Gunzer, M</td>
<td>26 Jan, 15.10-15.35</td>
<td>38</td>
</tr>
<tr>
<td>Gupta, A</td>
<td>32</td>
<td>98</td>
</tr>
<tr>
<td>Gusmão, L</td>
<td>134, 135</td>
<td>205, 206</td>
</tr>
<tr>
<td>Gut, I</td>
<td>20</td>
<td>84</td>
</tr>
<tr>
<td>Gyotoku, H</td>
<td>38</td>
<td>104</td>
</tr>
<tr>
<td><strong>H</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haas, H</td>
<td>112</td>
<td>183</td>
</tr>
<tr>
<td>Habibi, MR</td>
<td>26, 68</td>
<td>90, 139</td>
</tr>
<tr>
<td>Hada, MS</td>
<td>64, 94</td>
<td>135, 165</td>
</tr>
<tr>
<td>Hadrich, I</td>
<td>119</td>
<td>190</td>
</tr>
<tr>
<td>Haesebrouck, F</td>
<td>31</td>
<td>97</td>
</tr>
<tr>
<td>Haghibi, M</td>
<td>23</td>
<td>87</td>
</tr>
<tr>
<td>Hagiwara, D</td>
<td>103</td>
<td>174</td>
</tr>
<tr>
<td>Hagleitner, M</td>
<td>66, 67</td>
<td>137, 138</td>
</tr>
<tr>
<td>Hajikhodadad, S</td>
<td>109</td>
<td>180</td>
</tr>
<tr>
<td>Haleem Khan, AA</td>
<td>48</td>
<td>116</td>
</tr>
<tr>
<td>Hammmond, MA</td>
<td>84</td>
<td>155</td>
</tr>
<tr>
<td>Hammoudi, SM</td>
<td>3</td>
<td>67</td>
</tr>
<tr>
<td>Harris, C</td>
<td>51, 54, 81</td>
<td>119, 122, 152</td>
</tr>
<tr>
<td>Harris, SD</td>
<td>26 Jan, 16.25-16.50</td>
<td>40</td>
</tr>
<tr>
<td>Harrison, E</td>
<td>124</td>
<td>195</td>
</tr>
<tr>
<td>Hartigan, EG</td>
<td>72</td>
<td>143</td>
</tr>
<tr>
<td>Hashemi, J</td>
<td>109</td>
<td>180</td>
</tr>
<tr>
<td>Hashemi, SJ</td>
<td>106, 107</td>
<td>177, 178</td>
</tr>
<tr>
<td>Hedayati, MT</td>
<td>26, 35, 68</td>
<td>90, 101, 139</td>
</tr>
<tr>
<td>Henriet, S</td>
<td>26 Jan, 17.40-17.50, 76</td>
<td>147</td>
</tr>
<tr>
<td>Herbrecht, R</td>
<td>28 Jan, 11.15-11.30</td>
<td>56</td>
</tr>
<tr>
<td>Herbst, SH</td>
<td>89</td>
<td>160</td>
</tr>
<tr>
<td>Hermans, PWM</td>
<td>76</td>
<td>147</td>
</tr>
<tr>
<td>Herzzenberg, LA</td>
<td>97</td>
<td>168</td>
</tr>
<tr>
<td>Hidir, Y</td>
<td>49</td>
<td>117</td>
</tr>
<tr>
<td>Hirano, K</td>
<td>10, 38, 39</td>
<td>74, 104, 105</td>
</tr>
<tr>
<td>Hoenigl, M</td>
<td>27, 28, 29</td>
<td>91, 93, 94</td>
</tr>
<tr>
<td>Hoffmann, P</td>
<td>71</td>
<td>142</td>
</tr>
<tr>
<td>Hoffmann, WK</td>
<td>45</td>
<td>113</td>
</tr>
<tr>
<td>Hohaus, S</td>
<td>41</td>
<td>108</td>
</tr>
<tr>
<td>Holland, SM</td>
<td>44</td>
<td>112</td>
</tr>
<tr>
<td>Holmskov, U</td>
<td>84</td>
<td>155</td>
</tr>
<tr>
<td>Hooper, LV</td>
<td>78</td>
<td>149</td>
</tr>
<tr>
<td>Hörtmagl, C</td>
<td>15</td>
<td>79</td>
</tr>
<tr>
<td>Hosogaya, N</td>
<td>38</td>
<td>104</td>
</tr>
<tr>
<td>Hosseinl, N</td>
<td>120</td>
<td>191</td>
</tr>
<tr>
<td>Hosseini, SV</td>
<td>5</td>
<td>69</td>
</tr>
<tr>
<td>Hosseinpour, L</td>
<td>106, 107</td>
<td>177, 178</td>
</tr>
<tr>
<td>Howard, SJ</td>
<td>124</td>
<td>195</td>
</tr>
<tr>
<td>Howarth, C</td>
<td>99</td>
<td>170</td>
</tr>
<tr>
<td>Huang, W</td>
<td>8, 119, 125</td>
<td>72, 190, 196</td>
</tr>
<tr>
<td>Hummel, M</td>
<td>45</td>
<td>113</td>
</tr>
<tr>
<td>Hzounda Fokou, JB</td>
<td>2</td>
<td>66</td>
</tr>
</tbody>
</table>
## AUTHOR INDEX

<table>
<thead>
<tr>
<th>Author</th>
<th>Poster No.</th>
<th>Page</th>
<th>Author</th>
<th>Poster No.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iannitti, RG</td>
<td>77</td>
<td>148</td>
<td>Khostelidi, SN</td>
<td>62</td>
<td>132</td>
</tr>
<tr>
<td>Ibrahim-Granet, O</td>
<td>69</td>
<td>140</td>
<td>Khwakhali, US</td>
<td>64, 94</td>
<td>135, 165</td>
</tr>
<tr>
<td>Ide, S</td>
<td>10, 38, 39</td>
<td>74, 104, 105</td>
<td>Kibbler, CC</td>
<td>46</td>
<td>114</td>
</tr>
<tr>
<td>Ignatyeva, SM</td>
<td>62</td>
<td>132</td>
<td>Kim, EK</td>
<td>121</td>
<td>192</td>
</tr>
<tr>
<td>Ikeda, H</td>
<td>38</td>
<td>104</td>
<td>Kiraz, N</td>
<td>37</td>
<td>103</td>
</tr>
<tr>
<td>Imamura, Y</td>
<td>10, 38, 39</td>
<td>74, 104, 105</td>
<td>Kirkpatrick, WR</td>
<td>12</td>
<td>76</td>
</tr>
<tr>
<td>Imanishi, Y</td>
<td>108</td>
<td>179</td>
<td>Kirwan, MB</td>
<td>54, 55</td>
<td>122, 123</td>
</tr>
<tr>
<td>Inglis, DO</td>
<td>99</td>
<td>170</td>
<td>Klaassen, CH</td>
<td>11</td>
<td>75</td>
</tr>
<tr>
<td>Invernizzi, R</td>
<td>40, 43</td>
<td>106, 111</td>
<td>Klimko, NN</td>
<td>62</td>
<td>132</td>
</tr>
<tr>
<td>Ivanovic, D</td>
<td>127</td>
<td>198</td>
<td>Kniemeyer, O</td>
<td>88</td>
<td>159</td>
</tr>
<tr>
<td>Iwanaga, N</td>
<td>39</td>
<td>105</td>
<td>Koester-Eiserfunke, N</td>
<td>88</td>
<td>159</td>
</tr>
<tr>
<td>Izumikawa, K</td>
<td>27 Jan, 11.50-12.15</td>
<td>49, 74</td>
<td>Kohno, S</td>
<td>10, 38, 39</td>
<td>74, 104, 105</td>
</tr>
<tr>
<td>Jafarpour, M</td>
<td>109</td>
<td>180</td>
<td>Kolbin, AS</td>
<td>62</td>
<td>132</td>
</tr>
<tr>
<td>Jalali-Zand, N</td>
<td>98, 101</td>
<td>169, 172</td>
<td>Kolls, JK</td>
<td>26 Jan, 10.30-10.55, 31, 143, 72, 78</td>
<td>149</td>
</tr>
<tr>
<td>Jazet Dongmo, PM</td>
<td>2</td>
<td>66</td>
<td>Kontoyiannis, DP</td>
<td>28 Jan, 15.35-16.00, 62, 151</td>
<td>80</td>
</tr>
<tr>
<td>Jensen, JB</td>
<td>84</td>
<td>155</td>
<td>Kordbach, P</td>
<td>98, 101</td>
<td>169, 172</td>
</tr>
<tr>
<td>Jones, AM</td>
<td>36</td>
<td>102</td>
<td>Kovalevskaya, E</td>
<td>45</td>
<td>133</td>
</tr>
<tr>
<td>Jones, B</td>
<td>14, 138, 139</td>
<td>78, 209, 210</td>
<td>Krause, R</td>
<td>27, 28, 29</td>
<td>91, 93, 94</td>
</tr>
<tr>
<td>Jukic, E</td>
<td>15</td>
<td>79</td>
<td>Kreindler, J</td>
<td>72</td>
<td>143</td>
</tr>
<tr>
<td>Julvadi, PR</td>
<td>132</td>
<td>203</td>
<td>Krishnan Natesan, S</td>
<td>131</td>
<td>202</td>
</tr>
<tr>
<td>Kabsay, MR</td>
<td>9</td>
<td>73</td>
<td>Kumararatne, DS</td>
<td>81</td>
<td>152</td>
</tr>
<tr>
<td>Kakeya, H</td>
<td>10, 38, 39</td>
<td>74, 104, 105</td>
<td>Kurihara, S</td>
<td>10, 39</td>
<td>74, 105</td>
</tr>
<tr>
<td>Kalkanci, A</td>
<td>75</td>
<td>146</td>
<td>Kurs-Lasky, M</td>
<td>72</td>
<td>143</td>
</tr>
<tr>
<td>Kalkum, M</td>
<td>27 Jan, 10.55-11.20</td>
<td>48</td>
<td>Kwon-Chung, KJ</td>
<td>44, 100, 115</td>
<td>112, 171, 186</td>
</tr>
<tr>
<td>Kamei, K</td>
<td>103, 122</td>
<td>174, 193</td>
<td>Kymrizi, E</td>
<td>80</td>
<td>151</td>
</tr>
<tr>
<td>Kanda, T</td>
<td>38</td>
<td>104</td>
<td>Khabnadideh, S</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>Kantarcioğlu, AS</td>
<td>144</td>
<td>215</td>
<td>Kawamoto, S</td>
<td>103</td>
<td>174</td>
</tr>
<tr>
<td>Kapushesky, M</td>
<td>20</td>
<td>84</td>
<td>Keller, NP</td>
<td>79</td>
<td>150</td>
</tr>
<tr>
<td>Kataranovski, D</td>
<td>87</td>
<td>158</td>
<td>Kenfack Tsague, IF</td>
<td>2</td>
<td>66</td>
</tr>
<tr>
<td>Kataranovski, M</td>
<td>87</td>
<td>158</td>
<td>Kennedy, DL</td>
<td>54</td>
<td>122</td>
</tr>
<tr>
<td>Kathuria, S</td>
<td>11</td>
<td>75</td>
<td>Kent, P</td>
<td>57</td>
<td>125</td>
</tr>
<tr>
<td>Kavanagh, K</td>
<td>96</td>
<td>167</td>
<td>Keshavarz, T</td>
<td>123</td>
<td>194</td>
</tr>
<tr>
<td>Kawamoto, S</td>
<td>103</td>
<td>174</td>
<td>Khabnadideh, S</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>Keller, NP</td>
<td>79</td>
<td>150</td>
<td>Khalaj, V</td>
<td>102</td>
<td>173</td>
</tr>
<tr>
<td>Kenfack Tsague, IF</td>
<td>2</td>
<td>66</td>
<td>Khodavaisy, S</td>
<td>26, 68</td>
<td>90, 139</td>
</tr>
<tr>
<td>Kennedy, DL</td>
<td>54</td>
<td>122</td>
<td>Khorasanizadeh, D</td>
<td>102</td>
<td>173</td>
</tr>
<tr>
<td>Kent, P</td>
<td>57</td>
<td>125</td>
<td>Khoosravi, AR</td>
<td>18, 19</td>
<td>82, 83</td>
</tr>
</tbody>
</table>
# AUTHOR INDEX

<table>
<thead>
<tr>
<th>Author</th>
<th>Poster No.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macdonald, DA</td>
<td>141</td>
<td>212</td>
</tr>
<tr>
<td>Machado, KN</td>
<td>17</td>
<td>81</td>
</tr>
<tr>
<td>MacKay, WG</td>
<td>139</td>
<td>210</td>
</tr>
<tr>
<td>Madan, T</td>
<td>93</td>
<td>164</td>
</tr>
<tr>
<td>Maertens, J</td>
<td>74</td>
<td>145</td>
</tr>
<tr>
<td>Magalhães, TFF</td>
<td>17</td>
<td>81</td>
</tr>
<tr>
<td>Mahdavi Omran, S</td>
<td>26</td>
<td>90</td>
</tr>
<tr>
<td>Majumder, T</td>
<td>90</td>
<td>161</td>
</tr>
<tr>
<td>Makimura, K</td>
<td>98</td>
<td>169</td>
</tr>
<tr>
<td>Mancinelli, M</td>
<td>41, 42</td>
<td>108, 110</td>
</tr>
<tr>
<td>Manna, A</td>
<td>43</td>
<td>111</td>
</tr>
<tr>
<td>Marasca, R</td>
<td>74</td>
<td>145</td>
</tr>
<tr>
<td>Maresca, M</td>
<td>41</td>
<td>108</td>
</tr>
<tr>
<td>Marr, K</td>
<td>28 Jan, 11.45-12.00</td>
<td>58</td>
</tr>
<tr>
<td>Martel, A</td>
<td>31</td>
<td>97</td>
</tr>
<tr>
<td>Martin, J</td>
<td>55</td>
<td>123</td>
</tr>
<tr>
<td>Martinez, M</td>
<td>90, 91</td>
<td>161, 162</td>
</tr>
<tr>
<td>Martino, B</td>
<td>40, 43</td>
<td>106, 111</td>
</tr>
<tr>
<td>Martins, CVB</td>
<td>17</td>
<td>81</td>
</tr>
<tr>
<td>Mateus, V</td>
<td>24</td>
<td>88</td>
</tr>
<tr>
<td>Matsuzawa, T</td>
<td>108</td>
<td>179</td>
</tr>
<tr>
<td>Maurer, E</td>
<td>13</td>
<td>77</td>
</tr>
<tr>
<td>McAleer, JP</td>
<td>78</td>
<td>149</td>
</tr>
<tr>
<td>McCulloch, E</td>
<td>128, 139</td>
<td>209, 210</td>
</tr>
<tr>
<td>Mckeena, S</td>
<td>58</td>
<td>126</td>
</tr>
<tr>
<td>Meis, JF</td>
<td>11</td>
<td>75</td>
</tr>
<tr>
<td>Melillo, L</td>
<td>40, 42</td>
<td>106, 110</td>
</tr>
<tr>
<td>Menkem, EZ</td>
<td>1</td>
<td>65</td>
</tr>
<tr>
<td>Menut, C</td>
<td>2</td>
<td>66</td>
</tr>
<tr>
<td>Mihara, T</td>
<td>38</td>
<td>104</td>
</tr>
<tr>
<td>Mikacil, A</td>
<td>47</td>
<td>115</td>
</tr>
<tr>
<td>Mikimura, K</td>
<td>101</td>
<td>172</td>
</tr>
<tr>
<td>Miljkovic, D</td>
<td>87</td>
<td>158</td>
</tr>
<tr>
<td>Minematsu, T</td>
<td>38, 39</td>
<td>104, 105</td>
</tr>
<tr>
<td>Minooeianbaghighi, M</td>
<td>18, 19</td>
<td>82, 83</td>
</tr>
<tr>
<td>Mirahzadeh, E</td>
<td>102</td>
<td>173</td>
</tr>
<tr>
<td>Mirhendi, H</td>
<td>98, 101</td>
<td>169, 172</td>
</tr>
<tr>
<td>Mirkov, I</td>
<td>87</td>
<td>158</td>
</tr>
<tr>
<td>Mistry, V</td>
<td>58</td>
<td>126</td>
</tr>
<tr>
<td>Mitra, ME</td>
<td>40, 42, 43</td>
<td>106, 110</td>
</tr>
<tr>
<td>Mitsunaga, E</td>
<td>97</td>
<td>168</td>
</tr>
<tr>
<td>Miyasato, SR</td>
<td>99</td>
<td>170</td>
</tr>
<tr>
<td>Miyazaki, T</td>
<td>10, 38, 39, 110</td>
<td>74, 104, 105, 181</td>
</tr>
<tr>
<td>Mol, JC</td>
<td>84</td>
<td>155</td>
</tr>
<tr>
<td>Mofo, CT</td>
<td>1</td>
<td>65</td>
</tr>
<tr>
<td>Mohseni, R</td>
<td>117</td>
<td>188</td>
</tr>
<tr>
<td>Moman, M</td>
<td>27 Jan, 09.40-10.05, 45, 76</td>
<td>147</td>
</tr>
<tr>
<td>Monod, M</td>
<td>74</td>
<td>145</td>
</tr>
<tr>
<td>Moore, CB</td>
<td>55, 57</td>
<td>123, 125</td>
</tr>
<tr>
<td>Moore, MM</td>
<td>129, 130</td>
<td>200, 201</td>
</tr>
<tr>
<td>Morinaga, Y</td>
<td>10, 38, 39</td>
<td>74, 104, 105</td>
</tr>
<tr>
<td>Morley, J</td>
<td>58</td>
<td>126</td>
</tr>
<tr>
<td>Morris, J</td>
<td>56</td>
<td>124</td>
</tr>
<tr>
<td>Morselli, M</td>
<td>74</td>
<td>145</td>
</tr>
<tr>
<td>Morten, C</td>
<td>88</td>
<td>159</td>
</tr>
<tr>
<td>Morya, VK</td>
<td>121</td>
<td>192</td>
</tr>
<tr>
<td>Moss, RB</td>
<td>27 Jan, 12.15-12.40, 97</td>
<td>50, 168</td>
</tr>
<tr>
<td>Motaharinia, Y</td>
<td>5</td>
<td>69</td>
</tr>
<tr>
<td>Mounaud, S</td>
<td>118</td>
<td>189</td>
</tr>
<tr>
<td>Mousavi, B</td>
<td>5, 7, 35</td>
<td>69, 71, 101</td>
</tr>
<tr>
<td>Moussa, A</td>
<td>4</td>
<td>68</td>
</tr>
<tr>
<td>Mridha, A</td>
<td>65</td>
<td>136</td>
</tr>
<tr>
<td>Muhktar, I</td>
<td>143</td>
<td>214</td>
</tr>
<tr>
<td>Mustakim, S</td>
<td>33</td>
<td>99</td>
</tr>
<tr>
<td>Nadali, G</td>
<td>40</td>
<td>106</td>
</tr>
<tr>
<td>Nagayoshi, Y</td>
<td>38</td>
<td>104</td>
</tr>
<tr>
<td>Nagl, M</td>
<td>15</td>
<td>79</td>
</tr>
<tr>
<td>Naik, R</td>
<td>6</td>
<td>70</td>
</tr>
<tr>
<td>Najafi, N</td>
<td>35</td>
<td>101</td>
</tr>
<tr>
<td>Najvar, LK</td>
<td>12</td>
<td>76</td>
</tr>
<tr>
<td>Nakamura, S</td>
<td>10, 38, 39</td>
<td>74, 104, 105</td>
</tr>
<tr>
<td>Nakibi, F</td>
<td>74</td>
<td>145</td>
</tr>
<tr>
<td>Nascimento, AM</td>
<td>17</td>
<td>81</td>
</tr>
<tr>
<td>Nasrollahi, A</td>
<td>109, 117</td>
<td>180, 188</td>
</tr>
<tr>
<td>Nazari, S</td>
<td>47</td>
<td>115</td>
</tr>
<tr>
<td>Nesbitt, J</td>
<td>129</td>
<td>200</td>
</tr>
<tr>
<td>Netea, MG</td>
<td>26 Jan, 14.45-15.10, 80</td>
<td>37, 151</td>
</tr>
<tr>
<td>Neves, GWP</td>
<td>95</td>
<td>166</td>
</tr>
<tr>
<td>Newton, P</td>
<td>51, 81</td>
<td>119, 152</td>
</tr>
<tr>
<td>Nguyen, N</td>
<td>72, 78</td>
<td>143, 149</td>
</tr>
<tr>
<td>Nierman, W</td>
<td>20, 100, 112, 113, 115, 118, 140</td>
<td>84, 171, 183, 184, 186, 189, 211</td>
</tr>
<tr>
<td>Nikaeen, M</td>
<td>98, 101</td>
<td>169, 172</td>
</tr>
<tr>
<td>Niven, R</td>
<td>50</td>
<td>118</td>
</tr>
<tr>
<td>Nogueira, LJ</td>
<td>17</td>
<td>81</td>
</tr>
<tr>
<td>Noorbakhsh, F</td>
<td>107, 117</td>
<td>178, 188</td>
</tr>
<tr>
<td>Nosari, A</td>
<td>40, 42</td>
<td>106, 110</td>
</tr>
<tr>
<td>Nuri, N</td>
<td>144</td>
<td>215</td>
</tr>
<tr>
<td>Nussbaumer, W</td>
<td>126</td>
<td>197</td>
</tr>
</tbody>
</table>
## AUTHOR INDEX

<table>
<thead>
<tr>
<th>Author</th>
<th>Poster No.</th>
<th>Page</th>
<th>Author</th>
<th>Poster No.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>O</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Offidani, M</td>
<td>43</td>
<td>111</td>
<td>Rajendran, R</td>
<td>28 Jan, 15.00-15.10, 78, 209</td>
<td></td>
</tr>
<tr>
<td>O’Gorman, CM</td>
<td>100</td>
<td>171</td>
<td>Ramadan, N</td>
<td>142</td>
<td>213</td>
</tr>
<tr>
<td>Olno, H</td>
<td>110</td>
<td>181</td>
<td>Ramage, G</td>
<td>14, 138, 139</td>
<td>78, 209, 210</td>
</tr>
<tr>
<td>Orvis, J</td>
<td>99</td>
<td>170</td>
<td>Rambach, G</td>
<td>66, 67</td>
<td>137, 138</td>
</tr>
<tr>
<td>Osherov, N</td>
<td>26 Jan, 13.45-14.45</td>
<td>36</td>
<td>Ran, X</td>
<td>61</td>
<td>129</td>
</tr>
<tr>
<td>Ott, H</td>
<td>66, 82</td>
<td>137, 153</td>
<td>Ran, Y</td>
<td>61</td>
<td>129</td>
</tr>
<tr>
<td>Oz, Y</td>
<td>37</td>
<td>103</td>
<td>Randhawa, HS</td>
<td>11</td>
<td>75</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td></td>
<td></td>
<td>Ranque, S</td>
<td>119</td>
<td>190</td>
</tr>
<tr>
<td>Pakala, S</td>
<td>118</td>
<td>189</td>
<td>Rashidi, A</td>
<td>5</td>
<td>69</td>
</tr>
<tr>
<td>Pakshir, K</td>
<td>16</td>
<td>80</td>
<td>Rauteamaa-Richardson, R</td>
<td>57</td>
<td>125</td>
</tr>
<tr>
<td>Pancholi, M</td>
<td>58</td>
<td>126</td>
<td>Ray, A</td>
<td>72</td>
<td>143</td>
</tr>
<tr>
<td>Paolini, A</td>
<td>74</td>
<td>145</td>
<td>Razaei, MA</td>
<td>5</td>
<td>69</td>
</tr>
<tr>
<td>Paris, L</td>
<td>42</td>
<td>110</td>
<td>Reddy, CS</td>
<td>21, 22, 92</td>
<td>85, 86, 163</td>
</tr>
<tr>
<td>Parlato, M</td>
<td>69</td>
<td>140</td>
<td>Reinwald, M</td>
<td>28 Jan, 10.30-10.40, 113, 45</td>
<td></td>
</tr>
<tr>
<td>Parvin, M</td>
<td>35</td>
<td>101</td>
<td>Resende-Stoianoff, MA</td>
<td>17</td>
<td>81</td>
</tr>
<tr>
<td>Paschley, CH</td>
<td>58</td>
<td>126</td>
<td>Rezaei, S</td>
<td>98, 101, 106, 107, 169, 172, 177, 178</td>
<td></td>
</tr>
<tr>
<td>Pasmans, F</td>
<td>31</td>
<td>97</td>
<td>Rezaei, Z</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>Pasqualeotto, A</td>
<td>124</td>
<td>195</td>
<td>Rezaeimatekola, A</td>
<td>98, 101</td>
<td>169, 172</td>
</tr>
<tr>
<td>Patterson, TF</td>
<td>12</td>
<td>76</td>
<td>Rezaie, S</td>
<td>117</td>
<td>118</td>
</tr>
<tr>
<td>Pavor, ID</td>
<td>58</td>
<td>126</td>
<td>Richardson, MD</td>
<td>55, 57</td>
<td>123, 125</td>
</tr>
<tr>
<td>Pekmezovic, M</td>
<td>127</td>
<td>198</td>
<td>Ricks, DM</td>
<td>78</td>
<td>149</td>
</tr>
<tr>
<td>Perkhofer, S</td>
<td>82, 126</td>
<td>153, 197</td>
<td>Rijjs, AJMM</td>
<td>76</td>
<td>147</td>
</tr>
<tr>
<td>Perlin, DS</td>
<td>28 Jan, 14.35-15.00</td>
<td>60</td>
<td>Riva, G</td>
<td>74</td>
<td>145</td>
</tr>
<tr>
<td>Peterson, SW</td>
<td>44</td>
<td>112</td>
<td>Robson, GD</td>
<td>128</td>
<td>199</td>
</tr>
<tr>
<td>Pfaller, K</td>
<td>66</td>
<td>137</td>
<td>Rockette, H</td>
<td>72</td>
<td>143</td>
</tr>
<tr>
<td>Phukian, I</td>
<td>30</td>
<td>96</td>
<td>Rogers, T</td>
<td>88</td>
<td>159</td>
</tr>
<tr>
<td>Picardi, M</td>
<td>40, 43</td>
<td>106, 111</td>
<td>Rolides, E</td>
<td>28 Jan, 09.15-09.40, 52</td>
<td></td>
</tr>
<tr>
<td>Piccirillo, N</td>
<td>41</td>
<td>108</td>
<td>Rokas, A</td>
<td>27 Jan, 10.05-10.30, 46</td>
<td></td>
</tr>
<tr>
<td>Pilewski, J</td>
<td>72</td>
<td>143</td>
<td>Romani, L</td>
<td>26 Jan, 08.00-08.45, 27, 145, 74, 77, 85, 86, 148,156, 157</td>
<td></td>
</tr>
<tr>
<td>Pinchail, N</td>
<td>133</td>
<td>204</td>
<td>Rossi, G</td>
<td>40, 43</td>
<td>106, 111</td>
</tr>
<tr>
<td>Pinto, LJS</td>
<td>130</td>
<td>201</td>
<td>Roudharmohammadi, SH</td>
<td>120</td>
<td>191</td>
</tr>
<tr>
<td>Ponpinit, T</td>
<td>133</td>
<td>204</td>
<td><strong>R</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Popov, A</td>
<td>87</td>
<td>158</td>
<td>Saad, A</td>
<td>4</td>
<td>68</td>
</tr>
<tr>
<td>Popova, MO</td>
<td>62</td>
<td>132</td>
<td>Sabino, R</td>
<td>24, 25</td>
<td>88, 89</td>
</tr>
<tr>
<td>Potenza, L</td>
<td>26 Jan, 15.45-15.55, 110, 145</td>
<td>42, 74</td>
<td>Safari, M</td>
<td>123</td>
<td>194</td>
</tr>
<tr>
<td>Pourcel, C</td>
<td>104, 119</td>
<td>175, 190</td>
<td>Sahili, N</td>
<td>142</td>
<td>213</td>
</tr>
<tr>
<td>Powell, GL</td>
<td>54, 56</td>
<td>122, 124</td>
<td>Salutari, P</td>
<td>40, 43</td>
<td>106, 111</td>
</tr>
<tr>
<td>Prentice, AR</td>
<td>46</td>
<td>114</td>
<td>Salzer, H</td>
<td>27, 28, 29</td>
<td>91, 93, 94</td>
</tr>
<tr>
<td>Punt, PJ</td>
<td>26 Jan, 16.50-17.15</td>
<td>41</td>
<td>Samonis, G</td>
<td>80</td>
<td>151</td>
</tr>
<tr>
<td>Quintavalle, C</td>
<td>40</td>
<td>106</td>
<td>Saracli, MA</td>
<td>49</td>
<td>117</td>
</tr>
<tr>
<td>Rahmani, MR</td>
<td>5</td>
<td>69</td>
<td>Sarma, PU</td>
<td>93</td>
<td>164</td>
</tr>
<tr>
<td>Raggam, R</td>
<td>27, 28, 29</td>
<td>91, 93, 94</td>
<td>Savers, A</td>
<td>69</td>
<td>140</td>
</tr>
<tr>
<td>Rahmani, MR</td>
<td>5</td>
<td>69</td>
<td>Schlosser, A</td>
<td>84</td>
<td>155</td>
</tr>
<tr>
<td>Schmich, AL</td>
<td>83</td>
<td>154</td>
<td>Schrettl, M</td>
<td>112</td>
<td>183</td>
</tr>
</tbody>
</table>
# AUTHOR INDEX

<table>
<thead>
<tr>
<th>Author</th>
<th>Poster No.</th>
<th>Page</th>
<th>Author</th>
<th>Poster No.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeber, K</td>
<td>27, 29</td>
<td>91, 93, 94</td>
<td>Teutschbein, J</td>
<td>88</td>
<td>159</td>
</tr>
<tr>
<td>Séguin, D</td>
<td>8</td>
<td>72</td>
<td>Thierry, S</td>
<td>104, 119</td>
<td>125, 175, 190</td>
</tr>
<tr>
<td>Shah, P</td>
<td>99</td>
<td>170</td>
<td>Thornton, CR</td>
<td>12</td>
<td>76</td>
</tr>
<tr>
<td>Shankar, J</td>
<td>90</td>
<td>141</td>
<td>Tirouvanziam, R</td>
<td>97</td>
<td>168</td>
</tr>
<tr>
<td>Shaunak, S</td>
<td>89</td>
<td>160</td>
<td>Tiwary, BN</td>
<td>70</td>
<td>141</td>
</tr>
<tr>
<td>Sheppard, D</td>
<td>27 Jan, 09.15-09.40, 44, 149</td>
<td>78</td>
<td>Topp, MS</td>
<td>28 Jan, 10.05-10.30</td>
<td>55</td>
</tr>
<tr>
<td>Sherlock, G</td>
<td>99</td>
<td>170</td>
<td>Tortorano, AM</td>
<td>90</td>
<td>161</td>
</tr>
<tr>
<td>Shidfar, MR</td>
<td>106, 107</td>
<td>177, 178</td>
<td>Toto, A</td>
<td>67</td>
<td>138</td>
</tr>
<tr>
<td>Shikanai-Yasuda, MA</td>
<td>52</td>
<td>120</td>
<td>Toyotome, T</td>
<td>103, 122</td>
<td>174, 193</td>
</tr>
<tr>
<td>Shankar, J</td>
<td>99</td>
<td>170</td>
<td>Tsukamoto, M</td>
<td>10, 39</td>
<td>74, 105</td>
</tr>
<tr>
<td>Séguin, D</td>
<td>8</td>
<td>72</td>
<td>Topp, MS</td>
<td>28 Jan, 10.05-10.30</td>
<td>55</td>
</tr>
<tr>
<td>Shah, P</td>
<td>99</td>
<td>170</td>
<td>Tortorano, AM</td>
<td>90</td>
<td>161</td>
</tr>
<tr>
<td>Shankar, J</td>
<td>90</td>
<td>141</td>
<td>Toto, A</td>
<td>67</td>
<td>138</td>
</tr>
<tr>
<td>Shaunak, S</td>
<td>89</td>
<td>160</td>
<td>Toyotome, T</td>
<td>103, 122</td>
<td>174, 193</td>
</tr>
<tr>
<td>Sheppard, D</td>
<td>27 Jan, 09.15-09.40, 44, 149</td>
<td>78</td>
<td>Topp, MS</td>
<td>28 Jan, 10.05-10.30</td>
<td>55</td>
</tr>
<tr>
<td>Sherlock, G</td>
<td>99</td>
<td>170</td>
<td>Tortorano, AM</td>
<td>90</td>
<td>161</td>
</tr>
<tr>
<td>Shidfar, MR</td>
<td>106, 107</td>
<td>177, 178</td>
<td>Toto, A</td>
<td>67</td>
<td>138</td>
</tr>
<tr>
<td>Shikanai-Yasuda, MA</td>
<td>52</td>
<td>120</td>
<td>Toyotome, T</td>
<td>103, 122</td>
<td>174, 193</td>
</tr>
<tr>
<td>Shankar, J</td>
<td>99</td>
<td>170</td>
<td>Tsukamoto, M</td>
<td>10, 39</td>
<td>74, 105</td>
</tr>
<tr>
<td>Séguin, D</td>
<td>8</td>
<td>72</td>
<td>Topp, MS</td>
<td>28 Jan, 10.05-10.30</td>
<td>55</td>
</tr>
<tr>
<td>Shah, P</td>
<td>99</td>
<td>170</td>
<td>Tortorano, AM</td>
<td>90</td>
<td>161</td>
</tr>
<tr>
<td>Shankar, J</td>
<td>90</td>
<td>141</td>
<td>Toto, A</td>
<td>67</td>
<td>138</td>
</tr>
<tr>
<td>Shaunak, S</td>
<td>89</td>
<td>160</td>
<td>Toyotome, T</td>
<td>103, 122</td>
<td>174, 193</td>
</tr>
<tr>
<td>Sheppard, D</td>
<td>27 Jan, 09.15-09.40, 44, 149</td>
<td>78</td>
<td>Topp, MS</td>
<td>28 Jan, 10.05-10.30</td>
<td>55</td>
</tr>
<tr>
<td>Sherlock, G</td>
<td>99</td>
<td>170</td>
<td>Tortorano, AM</td>
<td>90</td>
<td>161</td>
</tr>
<tr>
<td>Shidfar, MR</td>
<td>106, 107</td>
<td>177, 178</td>
<td>Toto, A</td>
<td>67</td>
<td>138</td>
</tr>
<tr>
<td>Shikanai-Yasuda, MA</td>
<td>52</td>
<td>120</td>
<td>Toyotome, T</td>
<td>103, 122</td>
<td>174, 193</td>
</tr>
<tr>
<td>Shankar, J</td>
<td>99</td>
<td>170</td>
<td>Tsukamoto, M</td>
<td>10, 39</td>
<td>74, 105</td>
</tr>
<tr>
<td>Séguin, D</td>
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<td>72</td>
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**T**

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<th>Author</th>
<th>Poster No.</th>
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<tbody>
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<td>154</td>
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<td>179</td>
<td>Warris, A</td>
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<td>193</td>
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### AUTHOR INDEX

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<th>Author</th>
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<th>Page</th>
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<tbody>
<tr>
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<td>Williams, C</td>
<td>14, 138, 139</td>
<td>78, 209, 210</td>
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<td>126</td>
<td>197</td>
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<td>27, 28, 29</td>
<td>91, 93, 94</td>
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<td>215</td>
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<td>70</td>
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<td>62</td>
<td>132</td>
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<td>27, 28, 29</td>
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<td>62</td>
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