7th ADVANCES AGAINST ASPERGILLOSIS
Manchester, United Kingdom
3 - 5 March 2016
Manchester Central Convention Complex
www.AAA2016.org
Dear Advances Against Aspergillosis Colleague,

This is now the 7th Advances Against Aspergillosis conference and the meeting continues to grow and change with the field. The previous six meetings were overwhelmingly successful, including the first meeting in 2004 (San Francisco) where we had 364 attendees from 28 countries, the second meeting in 2006 (Athens) with 464 attendees from 44 countries, the third meeting in 2008 (Miami) with 351 attendees from 48 countries, the fourth meeting in 2010 (Rome) with 533 attendees from 41 countries, the fifth meeting in 2012 (Istanbul) with 375 attendees from 39 countries, and the sixth meeting in 2014 (Madrid) with 342 attendees from 33 countries. Because of all of you, this conference has now established itself as the premier forum for discussion of all aspects of Aspergillus infection and research.

The Aspergillus field continues in a state of rapid advancement, including the publication of numerous post-genomic papers and substantial advances in translational, immunologic, and diagnostic research. We have seen the launch of another effective antifungal for invasive aspergillosis (Isvavuconazole) and anticipated clinical trials of newer compounds is an exciting time for mycology. Itraconazole, pan-azole, and echinocandin resistance has emerged, and combination therapy following the large trial remains an important area of interest. Greatly increased awareness of allergic aspergillosis has opened new market opportunities for both antifungal agents and immunotherapies. There is a continuing high death toll from invasive aspergillosis, particularly among patient groups not usually associated with this opportunistic infection. This meeting is another chance to gather the world’s aspergillosis experts in one venue. A fundamental tenet of this 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 top centers in the world, with a focus on contemporary topics. 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 again increased the number of oral presentations from submitted abstracts to represent the wider community.

We also thank all the speakers and poster presenters for contributing to the success of this effort. Please also join us at the welcome reception, the Basilea symposia, 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 7th meeting will once again be published in Medical Mycology, creating what we hope will be highlights of the newer insights from the many disciplines that encompass Aspergillus research and care. As Advances Against Aspergillosis has become the leading global meeting for basic and clinical science regarding Aspergillus, its efforts form one of the foundations of the repository of knowledge about this pathogen; 229 papers have been published in 7 Supplements, comprising 1,739 pages of full papers, as well as 1,061 abstracts from the meetings (not including this meeting). 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 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
We would like to offer very special thanks to the following organizations for their generous educational grants. Their financial support makes this conference possible.

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SCHOLARSHIP AWARDS

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Narjes Alfuraiji, Iraq
Rajesh Anand, India
Graham Atherton, UK
Jennifer Bartholomew, UK
Maria Christina R. Batac, Philippines
Raj Kumar Bhatta, Nepal
Felix Bongomin, Uganda
Anuradha Chowdhary, India
Abigail Cowley, UK
Yubhisha Dabas, India
José Rodolfo Fretes Rivas, Paraguay
Sara Gago, Spain
Rocio Garcia-Rubio, Spain
John Guto, Kenya
Gina Hong, USA
Joe L. Hsu, USA
Bhavna T. Jishnu, India
Natarajaswamy Kalleda, India
Aiah Khateh, Saudi Arabia
Sadegh Khodavaisy, Iran
Ushana Shrestha Khwakhali, Nepal
Georgia Koltsida, Greece
Helen Le Sueur, UK
Sophie Loeffert, France
Hasan Nazik, Turkey
Maria Noni, Greece
Vasileios Oikonomou, Greece
Ehimwenma Okungbowa, Nigeria
Rita Oladele, Nigeria
Iain Page, UK
Giuseppe Paolicelli, Italy
Irum Perveen, Pakistan
Vijay Raghavan Pooja, India
Nadeem Ramadan, Iraq
Ramya Ramamurthy, India
Hilary Renshaw, USA
Raquel Sabino, Portugal
Gabriele Sass, Germany
Findra Setianingrum, Indonesia
Jata Shankar, India
Elliot Shwab, USA
Nicola Smith, UK
Maiko Umemura, Japan
Jose Vargas-Muniz, Puerto Rico
Sergio Velasquez, Mexico
Can Zhao, China
SCHOLARSHIP AWARDS

Advances Against Aspergillosis gratefully thanks the donors who made Scholarships possible:

International Society for Human and Animal Mycology

Gilead Sciences

Cystic Fibrosis Foundation

Journal of Fungi

Fungal Infection Trust

Dean Winslow, MD

Northwest Lung Centre Charity

and the Co-Organizers, Scientific Committee, and invited speakers, who all forego honoraria so as to make funding for scholarships available.
## PROGRAMME

### THURSDAY 3 MARCH

*All sessions will take place in Exchange Auditorium unless otherwise stated*

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**Special Morning Session: Meet the Professor**

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<td>08.00 - 08.50</td>
<td><strong>30 years of battling the cell wall</strong></td>
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<td>Jean-Paul Latgé, PhD</td>
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<td>08.00 - 08.50</td>
<td><strong>Difficult cases of allergic and chronic pulmonary aspergillosis</strong></td>
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<td>Eavan Muldoon, MBCh MD MPD</td>
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<td>Chris Kosmidis, MD PhD</td>
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<td>09.00 - 09.10</td>
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<td>David W. Denning, FMedSc</td>
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**Session 1: How *Aspergillus* Turns from Trivial Colonizer into a Pathogen**

*Moderators: Jean-Paul Latgé, PhD & Dimitrios P. Kontoyiannis, MD ScD FACP FIDSA*

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<td><strong>How <em>Aspergillus</em> invades the respiratory epithelium</strong></td>
<td>Elaine Bignell, PhD</td>
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<td>09.35 - 10.00</td>
<td><strong>Lung mycobiota and aspergillosis</strong></td>
<td>Laurence Delhaes, MD PhD</td>
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<td><strong>Selected Abstract:</strong> Regulation of in vivo fitness and virulence through the <em>Aspergillus fumigatus</em> transcription factor CreA</td>
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<td><strong>Selected Abstract:</strong> A pH-responsive molecular switch required for <em>Aspergillus fumigatus</em> pathogenicity</td>
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<td>Richard B. Moss, MD</td>
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<td>Are allergic fungal rhinosinusitis and ABPA lifelong conditions?</td>
<td>Ritesh Agarwal, MD DM</td>
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<td>12.45 - 13.00</td>
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<td>Anthony de Soyza, PhD MBChB</td>
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<td>Andrew J. Ullmann, MD</td>
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16.45 - 17.00  Selected Abstract:
Combination therapy with isavuconazole and micafungin for treatment
of experimental invasive pulmonary aspergillosis: preliminary report
Vidmantas Petraitis, MD

17.00 - 17.25  Salvage strategies for IA - how to develop meaningful evidence
Jörg Janne Vehreschild, MD

17.25 - 18.15  Welcome Reception

Session 4: Basilea Satellite Symposium
Moderator: David W. Denning, FMedSci

Challenges and recent developments in the management of invasive
aspergillosis

18.15 - 18.55  Invasive aspergillosis - are we making progress in reducing mortality?
Livio Pagano, MD

18.55 - 19.35  Pharmacological aspects of the management of invasive aspergillosis
William Hope, FRACP PhD

19.35 - 20.15  Recent developments in the management of invasive aspergillosis and
mucormycosis
Johan Maertens, MD PhD

20.15  Supper
FRIDAY 4 MARCH

All sessions will take place in Exchange Auditorium unless otherwise stated

Special Morning Session: Meet the Professor

08.00 - 08.50 Six papers of great interest in 2015-2016
William J. Steinbach, MD
Sean Doyle, PhD

08.50 - 09.00 Resources for researchers
Rory A. Duncan, MS

Session 5: Host Response
Moderators: Robert A. Cramer, PhD & Stuart M. Levitz, MD

09.00 - 09.25 Calcineurin orchestrates macrophage cell death during fungal germination
Darius Armstrong-James, FRCP MSc PhD DipMedMycol

09.25 - 09.50 Inborn errors of antifungal immunity
Michail S. Lionakis, MD ScD

09.50 - 10.05 Selected Abstract: Innate antifungal effector mechanisms of cystic fibrosis phagocytes
Shan Brunel

10.05 - 10.20 Selected Abstract: Compartment-specific activation of the inflammasome by Aspergillus fumigatus
Joshua Obar, PhD

10.20 - 10.50 Coffee Break

Exchange Hall
Session 6: Diagnostics

Moderators: David S. Perlin, PhD & Johan Maertens, MD PhD

10.50 - 11.15  Barcoding, phylogeny, taxonomy and novel methods in biodiversity
Sybren de Hoog, PhD

11.15 - 11.40  Aspergillus antibody testing - have we arrived?
Malcolm Richardson, PhD

11.40 - 11.55  Selected Abstract:
Clinically significant cryptic Aspergillus species in a referral chest hospital Delhi, India: MALDI-TOF identification, sequencing and antifungal susceptibility profiling
Anuradha Chowdhary, MD PhD

11.55 - 12.10  Selected Abstract:
Dectin-2 is a receptor for galactomannan
Jatin M. Vyas, MD

12.10 - 12.25  Selected Abstract:
Selective-fungal culture media associated with high prevalence of Aspergillus in cystic fibrosis
Gina Hong, MD

12.25 - 12.50  Performance evaluation of multiplex PCR including Aspergillus - not so simple!
Stéphane Bretagne, MD PhD

12.50 - 14.45  Lunch and Poster Session 2

CONFERENCE SOCIAL EVENT:
Quarry Bank Mill Tour and Concorde Dinner

14.45 - 15.00  Coaches depart Manchester Central Convention Complex for those attending the Quarry Bank Mill Tour AND Concorde Dinner

16.45 - 17.00  Coaches depart Manchester Central Convention Complex for anyone attending the Concorde Dinner ONLY
SPECIAL MORNINGS SESSION: MEET THE PROFESSOR

08.00 - 08.50  Difficult cases of invasive mycoses
Thomas J. Walsh, MD
Frederic Lamoth, MD

Moderator: David A. Stevens, MD

08.00 - 08.50  Lipid rafts and epigenetics in fungal infection biology
Axel A. Brakhage, PhD

Session 7: Resistance

Moderators: Donald Sheppard, MD & Thomas J. Walsh, MD

09.00 - 09.25  Global azole resistant Aspergillus fumigatus
Jacques F. Meis, MD PhD

09.25 - 09.40  Selected Abstract:
CYP51-independent isavuconazole-induced resistance in Aspergillus fumigatus
Cristina Jiménez-Ortigosa, PhD

09.40 - 09.55  Selected Abstract:
Autophagy in Aspergillus fumigatus infection and immunity
Vasilis Oikonomou, PhD

09.55 - 10.20  Echinocandin resistance in Aspergillus - does it exist?
David S. Perlin, PhD

10.20 - 10.45  Resistant species
Ana Alastruey-Izquierdo, PhD

10.45 - 11.15  Coffee Break

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Session 8: Aspergillus and Disease Establishment
Moderators: Thomas F. Patterson, MD & Karl V. Clemons, PhD

11.15 - 11.40  Global impact of non-human vertebrate cases of aspergillosis
Seyedmojtaba Seyedmousavi, DVM PhD

11.40 - 11.55  Selected Abstract:
Impact of echinocandin exposure in the interactome of Aspergillus fumigatus protein kinase A
Keats Shwab, PhD

11.55 - 12.10  Selected Abstract:
Corticosteroids impair neutrophils but not other CD11b+ myeloid cells to control pulmonary Aspergillus fumigatus infection
Natarajaswamy Kalleda

12.10 - 12.35  Lung transplantation
Mark R. Nicolls, MD

12.35 - 13.00  Orchestrating gene switches during infection by Aspergillus fumigatus
Robert A. Cramer, PhD

13.00 - 14.30  Lunch

Session 9: Genetics
Moderators: Sean Doyle, PhD & Stéphane Bretagne, MD PhD

14.30 - 14.55  CRISPR in Aspergillus fumigatus
Sven Krappmann, PhD

14.55 - 15.10  Selected Abstract:
Poly-ICLC confers protection against invasive aspergillosis caused by Aspergillus fumigatus and Aspergillus tanneri
Seyedmojtaba Seyedmousavi, DVM PhD

15.10 - 15.25  Selected Abstract:
The immunoregulatory role of microbiota tryptophan metabolites in fungal allergy
Giuseppe Paolicelli

15.25 - 15.40  Selected Abstract:
Phenotypic maturation of dendritic cells is impaired by the calcineurin inhibitor FK506 and an in vitro model of invasive aspergillosis in lung transplant recipients
Amid Adlakha
Transcriptional regulation and drug resistance in *Aspergillus fumigatus*
Michael Bromley, PhD

Coffee Break

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**Session 10: Looking into the Future**
Moderators: Malcolm Richardson, PhD & Axel A. Brakhage, PhD

Anti-*Aspergillus* antifungal pipeline
Dimitrios P. Kontoyiannis, MD ScD FACP FIDSA

*Aspergillus* vaccines: hardly worth studying or worthy of hard study?
Stuart M. Levitz, MD

Fungal diagnostics - an essential component of the AMR attack plan
David W. Denning, FMedSci

Closing remarks
David A. Stevens, MD

Farewell Drinks, Snacks and Discussion about AAA 2018
ABSTRACTS
OF
INVITED
FACULTY
30 YEARS OF BATTLING THE CELL WALL

Jean-Paul Latgé, PhD

Institut Pasteur - France

THURSDAY 3 MARCH 2016 (08.00 - 08.50)

A major characteristic of the fungal cell is to be surrounded by a cell wall. In \textit{Aspergillus fumigatus}, like in other pathogenic fungi, the cell is essential for fungal growth as well as for resisting environmental stresses such as phagocytic killing.

Most of the chemical analysis undertaken on the cell wall of \textit{A. fumigatus} are focused on the mycelial cell wall because it is the vegetative stage of the fungus. However, the cell walls of the mycelium and conidium (which is the infective propagule) are different especially at the level of the surface layer which plays a significant role in the interaction between \textit{A. fumigatus} conidia and phagocytic cells of the immune system. The cell wall of the conidia is covered by an outer layer of rodlets and melanin which imparts immunological inertness and confers hydrophobic properties. As soon as the conidia germinate (\textit{in vivo as in vitro}), melanin and rodlets disappeared, exposing polysaccharides which play an essential role during infection and can either promote or inhibit protective immunity.

In spite of the essential function of the cell wall in fungal life and in spite of recent progresses in the area, we are at the beginning of our understanding of the three dimensional organization of the cell wall. A major difficulty is the fact that the composition and structural organisation of the cell wall is not immutably set and is constantly reshuffled depending on the environmental conditions as well as depending on the morphotype. The data presented will show that that in contrast to an old postulate, the cell wall is not an inert skeleton but a live organelle that responds to modification of the environment in the most appropriate way to protect the fungal cell in vivo and in vitro.
DIFFICULT CASES OF ALLERGIC AND CHRONIC PULMONARY ASPERGILLOSIS

Eavan Muldoon, MBBCh MD MPD
University Hospital of South Manchester - UK

Chris Kosmidis, MD PhD
University Hospital of South Manchester - UK

THURSDAY 3 MARCH 2016 (08.00 - 08.50)

Chronic pulmonary aspergillosis (CPA) is a progressive, debilitating infection associated with substantial morbidity and mortality. Diagnosis may be missed because of the non-specific symptoms and poor sensitivity of culture. Oral antifungals offer an improvement in quality-of-life parameters, but prolonged treatment is often required as recurrences are common. Prolonged antifungal treatment is associated with high cost and an extensive side effect profile frequently leading to discontinuation. In addition, resistance developing during treatment is a common problem that limits our therapeutic options. Challenging CPA cases will be presented, and the step-by-step management will be discussed, focusing on the difficulties in recognition, diagnosis and treatment.
HOW ASPERGILLUS INVADES THE RESPIRATORY EPITHELIUM

Elaine Bignell, PhD

University of Manchester - UK

THURSDAY 3 MARCH 2016 (09.10 - 09.35)

Destruction of the pulmonary epithelium is a major feature of lung diseases caused by the mould pathogen Aspergillus fumigatus. Although it is widely postulated that tissue invasion is governed by fungal proteases, A. fumigatus mutants lacking individual or multiple enzymes remain fully invasive, suggesting a concomitant requirement for other pathogenic activities during host invasion. In favour of the view that tissue invasion is a genetically regulated trait, we recently described a tissue non-invasive A. fumigatus phenotype in a mutant lacking the pH-responsive transcription factor PacC. PacC null mutants are defective in both contact-mediated epithelial entry and protease and gliotoxin expression, and significantly attenuated for pathogenicity in leukopenic mice. We exploited this phenotype as a tool to dissect the mechanistic basis of invasive growth. Our study reveals a combinatorial mode of tissue entry dependent upon sequential, and mechanistically distinct, perturbations of the pulmonary epithelium and demonstrates, for the first time a protective role for Dectin-1 blockade in epithelial defences.
LUNG MYCOBIOTA AND ASPERGILLOSIS
Laurence Delhaes, MD PhD
University of Bordeaux - France

THURSDAY 3 MARCH 2016 (09.35 - 10.00)

The lung microbiome, which is believed to be stable or at least transient in healthy people, is now considered as a poly-microorganism component (composed of bacteria, archaea, viruses, and fungi) contributing to disease pathogenesis. Most research studies on the respiratory microbiome have focused on bacteria and their impact on lung health, but there is evidence that other nonbacterial organisms, namely composing the mycobiota, are also likely to play an important role in healthy people as well as in patients with chronic pulmonary diseases such as COPD, asthma and cystic fibrosis (CF).

In the last few years, the respiratory mycobiota has drawn closer attention. Thanks to advances in culture independent methods, especially high-through put sequencing, a number of fungi not detected by culture methods have been molecularly identified in human lungs. Whereas there is growing evidences that lung mycobiota has a significant impact on clinical outcome of chronic pulmonary diseases, the recent data on the human respiratory mycobiota, its diversity, richness, and variation among different populations (healthy and different diseased individuals) are still limited. By interacting with the bacteriome and/or virome, the respiratory mycobiota appears to be a cofactor in inflammation as well as in the host immune response, and therefore may contribute to the decline of the lung function and the pulmonary disease progression. Given the prevalence of Aspergillus diseases in chronic pulmonary diseases, we will discuss the potential respiratory microbiota’s connections and interactions with Aspergillus.

The current studies suggest several outlooks for this emerging research field, which will certainly call for a renewal of our understanding of chronic pulmonary diseases in one hand, and for a better knowledge of Aspergillus disease from lung colonization to aspergillosis on the other hand.
MODELLING WHAT GOES ON IN THE AIRWAYS

Donald Sheppard, MD

McGill University - Canada

THURSDAY 3 MARCH 2016 (10.45 - 11.10)

*Aspergillus fumigatus* frequently colonizes the airways of patients with chronic lung diseases such as cystic fibrosis or bronchiectasis. The natural history and outcome of airway infection is variable, and includes asymptomatic colonization, colonization with worsening of airway inflammation, allergic bronchopulmonary aspergillosis and progression to acute or chronic invasive disease. Although *A. fumigatus* infections in patients with chronic lung disease are far more common than invasive aspergillosis in the immunocompromised host, the fungal and host factors that contribute to the diversity of disease following airway infection remain largely unknown.

To begin to probe the molecular mechanisms underlying the pathogenesis of these conditions we have developed a mouse model of chronic airway infection in order to study the interactions of live hyphae with the host within the airways of immunocompetent mice. Intratracheal infection of healthy mice with agar beads containing *A. fumigatus* led to the colonization of the airways with hyphae for up to 28 days. Infection was associated with the early induction of pro-inflammatory cytokines including IL-1α and IL-1β, and the recruitment of neutrophils to the site of infection. IL-1 receptor signaling was found to play a critical role in restricting hyphal growth to within beads and the airways. This anti-fungal effect was mediated by IL-1α, and IL-1β and the caspase 1/11 inflammasome were dispensable for control of fungal growth within the airways. IL-1α was not required for leukocyte recruitment to the site of infection, suggesting that this cytokine mediates protection through enhancing the antifungal activity of host cells. An evolution of the adaptive immune response to airway colonization was observed over time. Evidence for an early, transient Th2 response was observed with the production of IgE, IL-4 producing T cells, and increased pulmonary IL-4. This response waned over time and was replaced by an increase in Th17 cells and pulmonary IL-17 levels as well as a sustained increase in pulmonary Foxp3+ T regulatory cells.

Our understanding the molecular pathogenesis of *A. fumigatus* chronic pulmonary infections has lagged behind that of invasive aspergillosis. The availability of this and other novel models of infection in immunocompetent mice may permit the dissection of the host and fungal factors underlying the pathogenesis of these conditions.
Fungal allergy is common in atopic individuals and has been increasingly linked to more severe phenotypes of asthma, recently differentiated under the classification of severe asthma with fungal sensitization (SAFS) and culminating in its most extreme clinical manifestation as allergic bronchopulmonary mycosis (ABPM). While *Alternaria* and *Cladosporium* spp are fungal genera most commonly associated with simple fungal allergy in general, *Aspergillus fumigatus* (*Af*) is the dominant pathogen in SAFS (>80% of cases) and ABPM (>90% of cases, i.e., ABPA). The ontogeny of SAFS and ABPA has, however, received relatively little attention. Although ABPA generally is diagnosed in the teen or early adult years, reports of recognition in people with asthma or cystic fibrosis (CF) as early as infancy have appeared for decades; and while SAFS was initially described in adults, recent studies have revealed a surprisingly common prevalence in children (40-50% of children with severe asthma). Onset of fungal sensitization and associated respiratory disease has been much better delineated in people with cystic fibrosis than in the general population (where fungal sensitization rates are 6-10%) or in asthma (where *Af* sensitization may occur in up to 30%). In CF, atopic responses to *Af* have been associated with accelerated disease progression and are found in 35-70% of patients; ABPA is diagnosed in 10%; and elevated IgG antibody responses to *Af* are seen in the majority of children by age 5 years, indicating early respiratory exposure to this ubiquitous fungus in people with CF that causes immune and potentially allergic responses early in life. Hence, annual serologic screening for ABPA in CF has been recommended and employed in US CF Centers beginning at school age. Similar studies in children with asthma could clarify whether screening might lead to earlier, broader and more effective therapeutic interventions for SAFS and ABPA in people with asthma.
ARE ALLERGIC FUNGAL RHINOSINUSITIS AND ABPA LIFELONG CONDITIONS?

Ritesh Agarwal, MD DM

Postgraduate Institute of Medical Education and Research - India

THURSDAY 3 MARCH 2016 (12.05 - 12.30)

Allergic bronchopulmonary aspergillosis (ABPA) is a complex pulmonary disorder caused by immunological reactions mounted against antigens of *Aspergillus fumigatus*, a ubiquitous fungi colonizing the tracheobronchial tree of patients with asthma and cystic fibrosis. On the other hand, allergic fungal rhinosinusitis is a related disorder representing an allergic hypersensitivity response to the presence of extramucosal fungi within the sinus cavity, akin to ABPA. The natural history of ABPA and AFRS remains unclear.

Both ABPA and AFRS are chronic disorders with an unpredictable course characterized by recurrent episodes of remission and exacerbation. Although long-term remissions are known in both the disorders, patients can experience exacerbations of their disorder, years after apparent clinical stability. Thus, a close followup is warranted to detect exacerbations of these disorders as early as possible. In ABPA, patients may also develop asymptomatic exacerbations characterized by rise in total IgE and worsening of radiological opacities. About 15-25% of patients with ABPA have recurrent exacerbations and treatment-dependent ABPA, and these are seen in patients with extensive bronchiectasis, presence of high-attenuation mucus on high-resolution computed tomography of the chest and existence of aspergilloma.

In AFRS, the principles of treatment include reduction of the antigenic load and inflammatory response primarily with surgical debridement of fungal mucin and polyps, as well as the use of saline irrigations and systemic and topical glucocorticoids. Systemic antifungal therapy is thought to have little role in the treatment of AFRS. On the other hand, in ABPA, the initial treatment of choice is oral glucocorticoids. Patients who experience exacerbations are treated with a combination of oral glucocorticoids and itraconazole. In contrast to AFRS, the preferred long-term treatment approach in ABPA is the use of oral azoles to clear the fungi from the airways.

As both the entities are lifelong conditions, the aim is to maintain the patient with best possible physical, social and occupational functioning with a therapy that is both cost-effective and devoid of major side-effects.
ASPERGILLUS AND BRONCHIECTASIS - ARE THEY DIRECTLY LINKED?

Anthony de Soyza, PhD MBChB

Newcastle University - UK

THURSDAY 3 MARCH 2016 (13.00 - 13.25)

This talk will highlight the complexities of Aspergillus related diseases in bronchiectasis. A mixture of case presentations and literature review will demonstrate the complexities of managing patients with bronchiectasis and pose key questions on the challenges on diagnostics, therapeutics and monitoring.
CHALLENGES OF THE GRADE CLASSIFICATION IN DEVELOPING THE IDSA ASPERGILLUS GUIDELINES

Thomas F. Patterson, MD

University of Texas Health Science Center at San Antonio - USA

THURSDAY 3 MARCH 2016 (15.15 - 15.40)

Guidelines for the treatment of invasive aspergillosis have become important tools for practitioners managing patients with these infections. Grading of Recommendations, Assessment, Development and Evaluation (GRADE) is a systematic approach to guideline development that has also been used in meta-analyses and reviews to provide a transparent and consistent framework to develop clinical guidelines so that recommendations can be compared and analyzed. The IDSA/HIVMA adopted GRADE in 2008. In the GRADE system, the guideline panel assigns each recommendation with separate ratings for the underlying quality of evidence supporting the recommendation and for the strength with which the recommendation is made. Data from randomized controlled trials begin as “high” quality, and data from observational studies begin as “low” quality. However, the panel may judge that specific features of the data warrant decreasing or increasing the quality of evidence rating, and GRADE provides guidance on how such factors should be weighed. The strength assigned to a recommendation reflects the panel’s confidence that the benefits of following the recommendation are likely to outweigh potential harms and are categorized as “weak” or “strong”. While the quality of evidence is an important factor in choosing recommendation strength, it is not prescriptive. Challenges for these criteria for guideline development in invasive aspergillosis are several, including the lack of extensive randomized clinical trial data, few comparative data for diagnostic strategies, antifungal drugs and treatment strategies and many areas in which expert opinion forms the basis of clinical practice decisions. These challenges are compounded by a heterogeneous group of clinical conditions ranging from rapidly lethal disease in highly immunocompromised patients to saprophytic colonization or allergic manifestations of disease. These difficulties are further complicated by diverse clinical populations at risk for these infections. Key areas in which limited data exist include management strategies, including combination therapies as well as specific approaches in infections that have not been extensively studied, among others. Despite these limitations, guidelines can be developed using GRADE which are beneficial to patient management.
WHERE EXPERT OPINION IS UNSUPPORTED BY THE EVIDENCE - OUR BIGGEST EVIDENCE GAPS IN EUROPEAN GUIDELINES

Andrew J. Ullmann, MD

Julius Maximilians University - Germany

THURSDAY 3 MARCH 2016 (15.40 - 16.05)
AZOLE CONCENTRATION AND TOXICITY - IS THERE A RELATIONSHIP?

Li-Ping Zhu, PhD

Fudan University - China

THURSDAY 3 MARCH 2016 (16.05 - 16.30)

Invasive aspergillosis is still a great challenge due to its high morbidity and mortality. Azoles, especially voriconazole are recommended as a first-line antifungal drug for the treatment of invasive aspergillosis. However, a number of studies indicated the influence of voriconazole’s wide inter-patient variability of trough concentration on efficacy and adverse events. Also, there is a significant relationship between CYP2C19 genetic polymorphisms and voriconazole concentrations. Therefore, the use of therapeutic drug monitoring (TDM) is recommended and the proposal of TDM-guided and CYP2C19 genotype-guided dosing of voriconazole is supported to increase the probability of achieving efficacy while avoiding toxicity. But, several other clinical investigators failed to demonstrate an association between voriconazole concentration and toxicity/efficacy as well as CYP2C19 genotype. This raises the question of the utility of routine clinical monitoring of voriconazole concentration. Here, we review the contribution of drug concentration and CYP2C19 genotype in relation to the toxicity and efficacy.
SALVAGE STRATEGIES FOR IA - HOW TO DEVELOP MEANINGFUL EVIDENCE

Jörg Janne Vehreschild, MD

University Hospital of Cologne - Germany

THURSDAY 3 MARCH 2016 (17.00 - 17.25)

Even with next-generation antifungal drugs, failure rates in patients with invasive aspergillosis as high as 60% have been reported. While numerous trials have been performed to determine efficacy of new drugs as salvage treatment for patients with failing or intolerable first-line treatment, there are no randomized clinical trials to guide our choices. This talk will explore strategies to overcome current shortcomings for a better understanding of salvage strategies for IA.
Invasive aspergillosis (IA) represents one of the major cause of morbidity and mortality for infectious complications among immunocompromised patients (i.e. hematological malignancies, hemopoietic stem cell recipients (HSCTs), solid organ transplants, autoimmune diseases, and more).

Particularly, among hematological malignancies patients, acute myeloid leukemia patients (AMLs) and in HSCTs were in the past those with a dramatically high mortality rate. In fact, in the years between 1980-2000, it has been reported to be about 60% and 90% respectively in AML/lymphoma and in HSCTs (1).

However in the recent years the availability of more effective antifungal drugs (lipid compounds of amphotericin B, azoles of new generation, echinocandins) favored an improve of the outcome of these patients reducing the attributable mortality.

Comparing the attributable mortality (AM) rate among patients with AML in Italian multicentric studies in different period 1988-1997, 1999-2003, 2004-2007 the attributable mortality rate is progressively fell from 48% to 38% and to 27% respectively (2-4). These data demonstrated that there is a marked reduction of mortality for aspergillosis probably due not only to antifungal drugs but also to the improvement of diagnostic tools that allow us to better and timely treatments.

In a recent analysis of data from SEIFEM 2010 study, among a population of 879 newly diagnosed AMLs, collected between 2010 and 2012, the incidence of AM due to invasive aspergillosis was 9% (17/191 patients). These good data reflect the efficacy of posaconazole, added as golden standard in the last years for antifungal prophylaxis in AMLs. In this kind of patients it seems able not only to reduce the incidence of IA cases but also to have an impact on the following breakthrough IA. On the other hand, even if the AM in the posaconazole arm is lower than that observed in AMLs treated with other prophylaxis (2/510 cases vs. 15/369 cases), in any case in both groups the AM rate was marked lower respect the past (data in progress).

We must take in account that in the majority of these cases, AMLs were at first diagnosis of the underlying malignancy, but when diagnosis of IA was made in advanced stage of malignancy (relapsed/refractory disease) the outcome is not so good.

In fact the incidence of mortality in the study performed between 1988 and 1987 was 57% in AMLs that developed IA in induction and only 44% in relapsed/refractory AMLs (69/133 Vs. 24/54)(2). In the following studies the mortality in induction drop down to 30% and 19% in induction (32/107 and 16/85 respectively) while it was dramatically high in relapsed/refractory AMLs 54% and 43% (67/123 and 22/41 respectively) (3,4).

These data show that during the years there is an improvement of IA in those patients where there is the possibility of a remission of malignancy with an high efficacy of the antifungal treatments. On the contrary the higher mortality in unresponsive patients to chemotherapy reflects the compromising of immune status of AMLs in these stages and, in spite of effective antifungal drugs, the outcome is poor.
The next step will be not to further reduce the mortality in induction but to reduce the impact of an invasive aspergillosis on the treatment schedule of underlying malignancy.

References
PHARMACOLOGICAL ASPECTS OF THE MANAGEMENT OF INVASIVE ASPERGILLOSIS

William Hope, FRACP PhD

University of Liverpool - UK

THURSDAY 3 MARCH 2016 (18.55 - 19.35)
RECENT DEVELOPMENTS IN THE MANAGEMENT OF INVASIVE ASPERGILLOSIS AND MUCORMYCOSIS

Johan Maertens, MD PhD

University Hospitals Leuven - Belgium

THURSDAY 3 MARCH 2016 (19.35 - 20.15)
SIX PAPERS OF GREAT INTEREST IN 2015-2016

William J. Steinbach, MD
*Duke University* - *USA*

Sean Doyle, PhD
*National University of Ireland* - *Maynooth*

**FRIDAY 4 MARCH 2016 (08.00 - 08.50)**
RESOURCES FOR RESEARCHERS

Rory A. Duncan, MS

National Institutes of Health (NIH) - USA

FRIDAY 4 MARCH 2016 (08.50 - 09.00)

The USA’s National Institutes of Health/National Institute of Allergy and Infectious Diseases/Division of Microbiology and Infectious Diseases offers a wide range of preclinical and clinical gap-filling resources to reduce risk in the translation of potential commercial products - vaccines, therapeutics and diagnostics - into licensed products. These services include testing against multi-drug resistant fungi and bacteria. This presentation will provide a high level overview of the resources with a more detailed look at those for fungal diseases. A summary of fungal diseases-support we have provided to date will be included.
CALCINEURIN ORCHESTRATES MACROPHAGE CELL DEATH DURING FUNGAL GERMINATION

Darius Armstrong-James, FRCP MSc PhD DipMedMycol

Imperial College London - UK

FRIDAY 4 MARCH 2016 (09.00 - 09.25)

Aspergillus fumigatus (Af) is a lethal fungal pathogen in transplant recipients. We report a major role for the calcineurin pathway, which is the primary target of transplant immunosuppression, in initial fungal control of Af in human macrophages. Calcineurin was required for phagocytosis, reactive oxygen species production, and killing of Af. Ultimately, progressive fungal germination within macrophages led to calcineurin-dependent necroptotic cell death, and was coupled to lateral transfer of Af to recipient macrophages which enabled fungal control. Cell-cell transfer occurred through a vasodilator-stimulated phosphoprotein (VASP)-actin encapsulated late endosomal compartment. These observations with a model mould pathogen identify calcineurin-dependent programmed cell death as a primary innate response to fungi in the lung, which facilitates cooperative control of infection by macrophages through cell-cell transfer. To our knowledge this is the first description of a host-mediated pathogen cell-cell transfer mechanism.
INBORN ERRORS OF ANTIFUNGAL IMMUNITY

Michail S. Lionakis, MD ScD

National Institute of Allergy and Infectious Diseases - USA

FRIDAY 4 MARCH 2016 (09.25 - 09.50)

Chronic granulomatous disease (CGD), caused by mutations in any of the 5 subunits of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and complete myeloperoxidase (MPO) deficiency, both disorders of the phagocyte oxidative machinery, have been long-known to lead to inherited susceptibility to invasive fungal infections (IFIs), predominantly caused by molds and Candida, respectively. A recent surge in newly described mutations in CARD9, STAT1, STAT3, GATA2, NEMO, IL-12R and IFNgR has significantly expanded the spectrum of inherited susceptibility to IFIs and has greatly enhanced our understanding of the cellular and molecular basis of antifungal immunity. Characterization of single-gene defects that predispose to various combinations of infections caused by Aspergillus and other molds, Candida, Cryptococcus, and dimorphic fungi has unmasked the critical role of novel molecules and signaling pathways in systemic antifungal host defense promoted by monocytes, macrophages, neutrophils and lymphocytes. Some of these defects can be late in onset and limited in scope suggesting that such defects should be sought in patients who develop IFIs without iatrogenic predisposing risk factors. Collectively, these experiments of nature offer a unique opportunity for developing new knowledge in immunological research and form the foundation for devising immune-based therapeutic approaches for patients suffering from IFIs.
BARCODING, PHYLOGENY, TAXONOMY AND NOVEL METHODS IN BIODIVERSITY

Sybren de Hoog, PhD

Institute of the Royal Netherlands Academy of Arts and Sciences (KNAW) - The Netherlands

FRIDAY 4 MARCH 2016 (10.50 - 11.15)

The leading principle of modern fungal taxonomy is molecular phylogeny. The main reason is that fungi that take close positions in phylogenetic trees are expected to bear ecological and medical similarities. This aspect has stimulated a discussion on an eventual subdivision of Aspergillus in smaller entities, which should be more homogeneous than the large umbrella-type of classification as used today. Examples will be provided showing that ecological consistency cannot be reached by splitting up the genus. Extensive chemotaxonomic analyses have demonstrated that many species share similar and analogous secondary metabolites across the genus Aspergillus, indicating that a broad generic concept is better. The molecular revolution has generated three types of fundamental changes in taxonomy: (1) recognition of novel molecular siblings due to the fact molecular methods provide more detailed insight than morphology; (2) generic rearrangements because phenotypically similar species appeared to be phylogenetically remote; (3) abandoning pleomorphism with dual naming required reconsidering of choice of generic names, which were based either on anamorphs or on telemorphs. Obviously the renewed methodology continues to lead to numerous taxonomic and nomenclatural changes. It should be realized, however, that these are not excessive, as in other areas of medical mycology historic changes on the basis of phenotypic characters have been much more prolific. Taxonomy on the basis of phylogeny alone has a number of severe drawbacks, and therefore a polyphasic approach is maintained in Aspergillus, combining e.g. multilocus sequencing and the use of extrolites. For routine fungal diagnostics the use of a single gene (rDNA ITS) has been recommended as barcode, but in many groups, among which is Aspergillus, this gene is insufficiently variable and secondary barcodes are applied. Species delimitation and the potential use of novel approaches will be discussed.
ASPERGILLUS ANTIBODY TESTING - HAVE WE ARRIVED?

Malcolm Richardson, PhD

University of Manchester - UK

FRIDAY 4 MARCH 2016 (11.15 - 11.40)

Chronic and allergic forms of pulmonary aspergillosis are estimated to affect over three million people worldwide. Anti-Aspergillus antibody detection (serology) has been performed for over 50 years for the diagnosis of different chronic Aspergillus infections, starting with aspergilloma and later with chronic necrotizing pulmonary aspergillosis. A wide variety of techniques have been used. Serology is also a cornerstone for defining allergic broncho-pulmonary aspergillosis (ABPA) and contributes to the initial diagnosis of Aspergillus-sensitized asthma patients without ABPA, to the follow-up of treatment or to the detection of exacerbations. Raised Aspergillus-specific IgG in chronic pulmonary aspergillosis and raised Aspergillus-specific IgE in allergic aspergillosis are characteristic of these diseases. For acute invasive aspergillosis, antibody detection has low utility compared to galactomannan antigen detection. Serology results have to be interpreted together with other clinical, radiological and biological, mycological criteria.

The diagnostic criteria of ABPA and chronic pulmonary aspergillosis are based on the patient’s immunological reactivity to crude extracts of Aspergillus fumigatus. However, because of cross-reactivity between crude allergen extracts from different fungi, apparent sensitization to crude A. fumigatus extracts does not always indicate genuine A. fumigatus sensitization. Recently, allergen components purified from the various morphological forms of fungi or produced as recombinant proteins have been introduced into a battery of new tests available for the diagnosis of allergic diseases, generally known as component-resolved diagnostics. More recently, molecular-based allergy diagnostics have been applied to fungal disease diagnostics.

Many methods exist to detect Aspergillus-specific antibodies, but there are limited published data regarding comparative efficacy and reproducibility. Recent studies have shown that the levels of IgE to Aspergillus allergens (Asp f 1 and/or Asp f 2) can effectively differentiate ABPA from A. fumigatus-sensitized asthma. Using appropriate panels of Aspergillus allergens will also lead to an understanding of genuine versus cross-reactive sensitization in A. fumigatus-sensitized patients. It is anticipated that great strides will be made in fine-tuning antibody-based diagnostics for allergic and chronic aspergillosis. Serology is ‘on approach’ with an expected time of arrival in the not too distant future.

Suggested reading:


PERFORMANCE EVALUATION OF MULTIPLEX PCR INCLUDING \textit{ASPERGILLUS} - NOT SO SIMPLE!

Stéphane Bretagne, MD PhD

\textit{Institut Pasteur - France}

FRIDAY 4 MARCH 2016 (12.25 - 12.50)

Direct examination and culture are often reported as insufficient for the diagnosis of invasive aspergillosis. That is why numerous PCR assays have been developed, mainly to improve sensitivity, knowing that detection of molds in respiratory specimens is always debatable between true infection, colonization or bystander recovery. The generalization of the real-time quantitative PCR format (qPCR) allows some consensus recommendations for the assay itself. Issues remain for the extraction step from respiratory specimens and should be kept in mind when comparing PCR assays. In respiratory specimens, the wall of fungal elements (hyphae, spores) must be broken to release their DNA content. There is currently no satisfactory control for this step for all the molds, in contrast to the amplification itself, which should include an internal control.

Along with the improvement of the PCR assays, it is logical to address not only the detection of the fungus but also the detection of resistance. Azole resistance is becoming a clinical issue in some countries, which could challenge the therapeutic strategies. This resistance is mainly caused by mutations in the CYP51A gene of \textit{Aspergillus fumigatus}. Since the CYP51A gene is single copy and \textit{A. fumigatus} is haploid, several options are available for the detection of the known mutations, such as PCR-sequencing, multiplex assay, or punctual mutation detection.

The first question is the sensitivity of such tests knowing that detection of \textit{A. fumigatus} usually targets multicopy genes (rDNA genes) to improve sensitivity. Therefore, if the fungal load is low, the CYP51A gene amplification fails and no possible answer on the presence or the absence of azole resistance mutation can be provided. This is particularly true when switching to serum samples, where the fungal load is usually very low, which limits the interest of using a single copy gene. On the other hand, when the fungal load is high, mutations can be detected. However, the assay should allow determination of the resistant/wild copy number ratio. Indeed, since several \textit{A. fumigatus} isolates can be involved in pulmonary IA, it is still not clear whether the disease is due to the susceptible isolates or the resistant ones. This issue can be secondary for therapeutic decision, but of importance for the physiopathology of the diseases since mutants can be different in virulence from the wild types.

Therefore, the issues of quantification are becoming pivotal, not only for the quantification of \textit{A. fumigatus} in respiratory specimens to define a positivity threshold as requested by clinicians, but also for the relative quantification between mutated and non-mutated CYP51A. Moreover, variability does not come only from PCR assays, but also from the heterogeneity of the respiratory specimens between studies and between patients, which is impossible to control but should be integrated as a limit of the assays.

The present developments of qPCR-based assays, alone or multiplexed, are a first advancement in the more complete microbiological diagnosis of aspergillosis. These first assays are stimulating to develop tests taking into account all the limits of diagnostic PCR for the specific question of \textit{A. fumigatus} DNA and azole-resistance detection.
DIFFICULT CASES OF INVASIVE MYCOSES

Moderator: David A. Stevens, MD
Stanford University - USA

Thomas J. Walsh, MD
Weill Cornell Medical Center of Cornell University - USA

Frederic Lamoth, MD
Lausanne University Hospital - Switzerland

SATURDAY 5 MARCH 2016 (08.00 - 08.50)

See pages 67 - 72.
LIPID RAFTS AND EPIGENETICS IN FUNGAL INFECTION BIOLOGY

Axel A. Brakhage, PhD

Leibniz Institute for Natural Product Research - Germany

SATURDAY 5 MARCH 2016 (08.00 - 08.50)

The filamentous fungus *Aspergillus fumigatus* is the most important air-borne fungal pathogen causing 90% of all systemic *Aspergillus* infections in humans. The most severe disease caused by *A. fumigatus* is invasive aspergillosis which almost exclusively occurs in immunocompromised patients. A lack of reliable diagnostic tools and effective treatment options results in high mortality rates despite therapy. In my laboratory, we aim at identifying pathogenicity determinants and mechanisms how *A. fumigatus* can overcome the response of immune effector cells. For addressing these questions we apply transcriptome and (immune)proteome analyses including systems biological analyses with subsequent generation of deletion mutants and their analysis in processing by the immune system like neutrophilic granulocytes, alveolar macrophages and complement. Furthermore, we test mutants in a mouse infection model.

*A. fumigatus* has developed immune evasion mechanisms which interfere at the different levels of the infection process with the response of the human host. These include recognition of conidia, modulation of phagocytosis, intracellular processing, neutrophil extracellular trap formation, and complement activation. We have identified several molecules including surface components such as proteins and dihydroxynaphthalene melanin as well as secondary metabolites, which manipulate the immune response. Furthermore, fungus-specific T cells were identified which allow novel approaches for diagnosis and therapy. A recent interesting finding is that lipid rafts appear to be important for the interaction of *A. fumigatus* with immune effector cells. Also, in a study with *A. nidulans* and *A. fumigatus* we found the concept that bacteria are able to reprogram the fungal metabolism by targeting the chromatin modification system.
GLOBAL AZOLE RESISTANT *ASPERGILLUS FUMIGATUS*

Jacques F. Meis, MD PhD

Canisius Wilhelmina Hospital - The Netherlands

SATURDAY 5 MARCH 2016 (09.00 - 09.25)

*Aspergillus fumigatus*, a ubiquitously distributed opportunistic pathogen, is the global leading cause of aspergillosis. Azole antifungals play an important role in the management of aspergillosis. However in the last decade azole resistance in *A. fumigatus* isolates has been increasingly reported, especially in Europe, and this is potentially complicating the effective management of aspergillosis. The higher mortality rates observed in patients with invasive aspergillosis caused by azole resistant *A. fumigatus* isolates pose serious challenges to the mycologist for timely identification of resistance and appropriate therapeutic interventions. The ‘TR$_{34}$/L98H’ mutation in the cyp51A gene of *A. fumigatus* is responsible for most multi-azole resistance seen in many European countries, the Middle East, China, Australia and India. Azole-resistant isolates carrying this mutation have been reported from both patients and the environment. In addition, a newly emerging resistance mechanism, TR$_{46}$/Y121F/T289A, conferring high voriconazole and variable itraconazole MICs was lately described in several European countries, Asia and the American continent. Environmental screening and routine antifungal susceptibility testing of clinically significant isolates should be considered in order to develop guidelines for local and national purposes. Considering that azole antifungal drugs are the mainstay of (oral) therapy, especially for chronic invasive and allergic aspergillosis, emergence of resistance will have profound impact on healthcare. This presentation highlights the global development of azole resistance in *A. fumigatus* and the possible relation with environmental fungicide use.
ECHINOCANDIN RESISTANCE IN ASPERGILLUS - DOES IT EXIST?

David S. Perlin, PhD

UMDNJ – New Jersey Medical School - USA

SATURDAY 5 MARCH 2016 (09.55 - 10.20)

The echinocandin drugs are increasingly being used for prophylaxis with patients at high risk for development of invasive fungal disease, as well as for therapy with patients with proven or probable invasive aspergillosis who fail conventional therapy. Unlike Candida species like C. glabrata, which show resistance levels between 3-12%, clinical breakthrough of Aspergillus species following echinocandin exposure is extremely rare. Surveillance studies show prominent susceptibilities of Aspergillus species to all three echinocandin drugs. However, as echinocandin usage continues to broaden, there is likelihood that Aspergillus species may acquire resistance in some clinical settings. But what types of mechanisms are expected? Target site modification involving hot spot regions of Fks subunits is responsible for clinical resistance in Candida species. Although this mechanism has not yet been observed in clinical isolates of A. fumigatus, a highly resistant strain can be engineered by introducing characteristics FKS mutations into homologous sequences of FKS1 in A. fumigatus. Target site upregulation may also play a role in reduced susceptibility, as well as other non-FKS mechanisms involved in maintaining cell wall integrity. Mono-resistance has been demonstrated following selection with caspofungin, which also appears to be independent of the classical FKS mechanism. Drug-induced stress responses in Aspergillus appear to play an important role in promoting drug adaptation with a potential for breakthrough.

Literature Cited


RESISTANT SPECIES

Ana Alastruey-Izquierdo, PhD

Instituto de Salud Carlos III - Spain

SATURDAY 5 MARCH 2016 (10.20 - 10.45)

Secondary resistance to azoles in *Aspergillus fumigatus* has emerged in the last years as a global health problem. However resistance in *Aspergillus* is not only limited to this species. The decreased susceptibility to amphotericin B of *A. terreus* it is also well known and strains of *A. niger* with high itraconazole MICs (Minimal Inhibitory Concentrations) have also been reported. In addition, polyphasic taxonomical studies published in the last decade, have revealed the presence of “cryptic” species of *Aspergillus* that are almost indistinguishable by each other by classical identification methods. Case reports and epidemiological studies have demonstrated the presence of these cryptic species in human clinical samples. Although there are no many studies dealing with the susceptibility profile of these species, the scarce data available indicate that some of these species are resistant. Thus, *A. lentulus* and *A. fumigatiainfis* (cryptic species of *A. fumigatus*) show high MICs to azoles and amphotericin B and have been related with poorer outcomes. *Aspergillus alliaceus* shows elevated MICs to amphotericin B and echinocandins. Species of *A. ustus* complex are resistant to most antifungals and species of *A. niger* complex show a strain-dependent susceptibility profile, with some strains showing elevated MICs to itraconazole. Hence antifungal resistance in *Aspergillus* is not only limited to *A. fumigatus*. Moreover, some epidemiological studies have shown that resistance in *Aspergillus*, is more frequently due to intrinsically resistant non-*fumigatus* Aspergillus species than to secondary resistance in *A. fumigatus* in some countries. Therefore, clinicians should be aware of the presence of other species of *Aspergillus* with different susceptibility profiles in clinical samples and antifungal susceptibility testing is still an essential tool to identify the optimal antifungal agent to treat aspergillosis.
GLOBAL IMPACT OF NON-HUMAN VERTEBRATE CASES OF ASPERGILLOSIS

Seyedmojtaba Seyedmousavi, DVM PhD

National Institutes of Health (NIH) - USA

SATURDAY 5 MARCH 2016 (11.15 - 11.40)

The importance of aspergillosis in humans and various animal species has increased over the last decades. *Aspergillus* species are found worldwide in humans and in almost all domestic animals and birds as well as in many wild species, causing a wide range of diseases from localized infections to fatal disseminated diseases, as well as allergic responses to inhaled conidia.

In humans, *Aspergillus fumigatus* is the most common and life-threatening airborne opportunistic fungal pathogen, especially significant among immunocompromised hosts.

In animals, aspergillosis is primarily a respiratory infection that may become generalized; however, tissue predilection is highly variable among species. Some prevalent forms of animal aspergillosis are pulmonary and air sac infections in poultry and other birds, mycotic abortion and mammary gland infections in cattle, guttural pouch (auditory tube diverticulum) mycosis in horses, sinonasal infections in dogs and cats, invasive pulmonary and cerebral infections in marine mammals and non-human primates.

The goal of this lecture is to provide an overview of the most common infections reported by *Aspergillus* species and the corresponding diseases in various types of animals.
LUNG TRANSLANTATION

Mark R. Nicolls, MD

Stanford University - USA

SATURDAY 5 MARCH 2016 (12.10 - 12.35)

*Aspergillus*-related pulmonary diseases plague lung transplant recipients. This talk will discuss research performed by Dr. Joe Hsu in our laboratory who has discovered an important risk factor for *Aspergillus* invasiveness in transplant tissue that is intimately connected with the viability of airway microvessels. In recent years, our group has focused on the state of airway microvasculature as an important determinant of transplant health. Loss of blood flow during acute rejection episodes sets up a series of events that not only favors pathogen invasion but also predisposes transplant recipients to chronic rejection. This talk will conclude with a brief discussion about how these ideas can influence future research and therapeutics to limit infection and promote patient survival.
ORCHESTRATING GENE SWITCHES DURING INFECTION BY *ASPERGILLUS FUMIGATUS*

Robert A. Cramer, PhD

*Geisel School of Medicine at Dartmouth - USA*

**SATURDAY 5 MARCH 2016 (12.35 - 13.00)**

Despite the increasing significance of invasive fungal infections, our understanding of fungal pathogenesis mechanisms remains ill defined. Simply put, the *in vivo* microenvironment remains a black box waiting to be fully defined to better understand what mechanisms specific fungi require for *in vivo* proliferation and tissue damage. Therefore, one under studied area of fungal-host interactions is the impact of *in vivo* fungal and host bioenergetics on fungal virulence and disease outcome. Our laboratory is interested in the effects of *in vivo* microenvironments on the ability of human fungal pathogens to cause disease. From the fungal perspective, we are characterizing the role of metabolic pathways and their transcriptional regulators in *in vivo* acclimation, growth, and virulence. We have identified important transcription factors and metabolic pathways that mediate *A. fumigatus* adaptation to the *in vivo* microenvironment and are required for fungal virulence. In addition, we have observed that changes in fungal metabolism *in vivo* alter the expression and exposure of key fungal pathogen associated molecular patterns, particularly those in the fungal cell wall, that alter interactions with host immune effector cells. Thus, a natural translational research question that arises from our data is how can we manipulate the *in vivo* microenvironment to thwart pathogen growth and improve treatment outcomes from these too often lethal infections. In this presentation, we will provide an overview of research from our laboratory that supports a key role for oxygen in determining the outcome of *Aspergillus*-host interactions.
CRISPR IN *ASPERGILLUS FUMIGATUS*

Sven Krappmann, PhD

*University Hospital Erlangen - Germany*

SATURDAY 5 MARCH 2016 (14.30 - 14.55)
TRANSCRIPTIONAL REGULATION AND DRUG RESISTANCE IN _ASPERGILLUS FUMIGATUS_

Michael Bromley, PhD

_University of Manchester - UK_

**SATURDAY 5 MARCH 2016 (15.40 - 16.05)**

The azoles have provided the cornerstone of systemic antifungal therapy for the last 30 years. However, resistance to these compounds, particularly in the major human mould pathogen _Aspergillus fumigatus_, is emerging and reaching levels that have prompted some centres to move away from azoles as a sole first line therapeutic. The primary mechanism of resistance is modification of the azole drug target cyp51A, a gene required for the biosynthesis of the cell membrane component ergosterol. In some centres however around 50% of resistant isolates are as the result of other factors. It is critical that we understand how resistance develops so that we can develop better strategies to improve therapeutic outcomes.

The regulatory mechanisms governing resistance to the azoles in filamentous fungi are poorly understood. Two transcription factors have been associated with modified azole tolerance in _A. fumigatus_. The best studied of these is the sterol regulatory element SrbA which has been shown to act as a positive regulator of ergosterol biosynthesis and specifically controls the expression of cyp51A. Loss of SrbA function leads to azole sensitivity. A recent report has also implicated the CCAAT binding complex (CBC), as modification of one of its subunits, HapE (to HapE<sup>P88L</sup>) has been identified as the cause of resistance in a clinical isolate.

Here I reveal our recent work to expand our understanding of the transcriptional mechanisms that contribute to azole resistance and explore how and why SrbA and the CBC contribute to clinical azole resistance.
ANTI-ASPERGILLUS ANTIFUNGAL PIPELINE

Dimitrios P. Kontoyiannis, MD ScD FACP FIDSA

University of Texas MD Anderson Cancer Center - USA

SATURDAY 5 MARCH 2016 (16.35 - 17.00)

Acute, subacute and chronic aspergillosis remain major threats for human health, especially in immunosuppressed hosts and patients with underlying structural lung disease. Despite significant strives, only three classes of molecules are currently used in clinical practice. The emerging azole resistance threats to devitalize the activity of the most commonly used drugs for that disease, the triazoles. In my lecture, I will summarize the unmet clinical needs of current antifungal therapy for aspergillosis, discuss the emerging pipeline and agents belonging to new molecular classes, the challenges inherent to antifungal drug discovery and development, and offer some perspectives for the future.
ASPERGILLUS VACCINES: HARDLY WORTH STUDYING OR WORTHY OF HARD STUDY?

Stuart M. Levitz, MD

*University of Massachusetts Medical School - USA*

**SATURDAY 5 MARCH 2016 (17.00 - 17.25)**

Arguably, vaccines have been the greatest advance in the history of public health. Yet, despite the great need, there are no licensed vaccines to protect humans against fungal diseases, including aspergillosis. The major scientific and logistical challenges to developing *Aspergillus* vaccines will be discussed. Promising approaches will be reviewed, ranging from pan-fungal vaccines to patient-specific therapeutic vaccines. To keep the audience entertained, a few jokes will be admixed with the talk, all politically correct so as not to offend anyone. Please laugh even if you don’t find the jokes funny as my ego is very fragile and feeds on titters running through the audience when I speak. My crystal ball will be wiped free of dust and greasy fingerprints and predictions about the future will be rendered to those brave enough to listen.
Fungal diagnostics - an essential component of the AMR attack plan

David W. Denning, FMedSci

University of Manchester - UK

Saturday 5 March 2016 (17.25 - 17.50)

The global burden of serious fungal diseases is thought to exceed 15 million cases annually with over 1.6 million deaths. Deaths from fungal infection in AIDS is estimated to exceed 700,000, nearly 50% of the total AIDS deaths. All those patients that present for care will be administered antibiotics, most without any effect. Antifungal therapy may also be given, but without a specific fungal diagnosis may be incorrect and will often be administered too late. Antimicrobial resistance (AMR) is a major public health concern developing largely from the excess use of antibacterial and antifungal drugs. The lack of fungal disease diagnostic tests exacerbates the problem of antimicrobial empiricism, both antibiotic and antifungal. For example, the failure to diagnose chronic pulmonary aspergillosis in those with smear-negative TB by not testing for Aspergillus antibody, typically means unnecessary and ineffective first or second line antituberculous therapy is given. Another example is mis-diagnosing ‘fungal asthma’ and hence omitting a trial of oral antifungal therapy avoiding repetitive courses of antibiotics. Not diagnosing Aspergillus bronchitis will usually mean repetitive oral courses of antibiotics and corticosteroids. All communities should have access to fungal diagnostics which will have a substantial benefit for antimicrobial stewardship and AMR control.
DIFFICULT CASES OF INVASIVE MYCOSES
FATAL PNEUMONIA IN A PAEDIATRIC HSCT RECIPIENT

J Ratner*, R Barton
The Leeds Mycology Reference Centre, Leeds General Infirmary, Leeds, United Kingdom

An eight year old female with a history of β-thalassemia was admitted to Leeds General Infirmary to undergo a sibling donor HSCT. She began spiking fevers with rigours 2 days following transplantation and treated empirically with IV piperacillin/tazobactam (TZP). She was switched to meropenem after an extended spectrum beta-lactamase producing *E.coli* was isolated from peripheral blood cultures, which was TZP resistant. The source of the *E. coli* was thought to be from the gut, as she had abdominal pain and loose stools, however stool samples were negative for common enteric pathogens, including *C. difficile*. Five days after transplant, she developed pneumonia with a small pleural effusion which was too small to drain. Clarithromycin was added to cover pneumonia and Ambisome with stat Amikacin if required. Samples were sent for *Mycoplasma, Legionella* and a viral panel which were all negative and clarithromycin was stopped on day 7.

The patient continued to spike fevers and on day 11, a CT chest showed right upper-lobe consolidation and pulmonary thrombus with ground glass consolidation, and anticoagulants were started. Weekly *Aspergillus* antigen (galactomannan) screens were all negative. A bronchoscopy was performed and a soft grey mass of tissue was seen occluding the upper right bronchus. A bronchoalveolar lavage (BAL) was sent for bacterial and fungal culture, which grew coagulase-negative *Staphylococci* but no fungi.

Day 21, the neutrophil count began to return, however the patient was still spiking fevers and had increasing respiratory distress. CT and chest X-ray show a convex shape into a fissure affecting the whole right upper lobe, which was thought to be fungal and caspofungin was added in for 7 days. A sample was tested for β-D glucan, and was negative.

Day 29, the patient underwent a right upper lung resection, which was a difficult procedure with friable necrotic lung tissue showing mycelia on microscopy with calcaflour. *Aspergillus* antigen (serum) remained negative and samples from the lung biopsy were sent for culture, which was negative apart from a coagulase-negative *Staphylococcus*. She continued to have increasing oxygen requirements and worsening of chest radiograph, which now showed left sided lower lung consolidation with a small pleural effusion and patchy perihilar opacity of the right apex (which was removed). Voriconazole was added.

Day 31, CRP continued to increase and the patient was believed to have severe pulmonary aspergillosis and ambisome was stopped, whilst voriconazole was continued with caspofungin added in. Day 33, she had ongoing pulmonary haemorrhage and a serum sample was sent to Bristol for B-D glucan, which was positive. On day 35, the patient passed away from a sudden hypotensive and hypoxive episode.
CUTANEOUS DISEASE IN HIV INFECTED PATIENT DURING ANTIRETROVIRAL THERAPY

R Wahyuningsih1,3*, I Irfani2, W Kurniawan2, M Yulianti2, E Yunihastuti2, A Rozaliyani1, S Djauzi2
1Parasitology, Universitas Indonesia, Jakarta, Indonesia
2Internal Medicine, Universitas Indonesia/ Cipto Mangunkusumo Hospital, Jakarta, Indonesia
3Parasitology, Indonesian Christian University, Jakarta, Indonesia

Introduction:
HIV infected patients are susceptible to opportunistic infections, caused by various organisms such as fungi, bacteria, protozoa and virus. Without proper diagnosis and treatment the mortality is quite high. Unfortunately opportunistic infections in the HIV population may be caused by more than one organism, which causes difficulties in establishing diagnosis, and ultimately inappropriate treatment. Here we describe a fatal case of an HIV infected patient undergoing antiretroviral treatment with unexplained skin disease.

Case report:
A 37 year old HIV infected male presented with fever, cough and acute diarrhea with nausea and vomitus. The patient had been treated for pulmonary tuberculosis for 9 months, and was diagnosed with CMV retinitis. Combination antiretroviral (ARV) therapy, zidovudine (AZT), lamivudine (3TC), and nevirapine (NVP) was started a month before he was hospitalized, when his CD4+ count was 4 cells/µl. He had discreet multiple hyperpigmented lenticular papules on both arms and legs that was labeled a pruritic papular eruption. Pancytopenia developed (hemoglobin/hematocrit 9.4, 24.6; leucocyte count 1980; and thrombocyte count 86 000) and did not resolve, even after changing AZT to stavudine (d4T) drug. Chest X-ray showed bilateral infiltrates, and the sputum culture revealed Klebsiella pneumonia, a sputum smear revealed neither fungi nor Pneumocytis jiroveci. There was significant improvement after 2-weeks course of antibiotics as gauged by the sputum culture result. Bone marrow puncture was performed due to unimproved pancytopenia and it demonstrated decreased cellularity, suspected caused by infection, and the smear revealed microbes most suggestive of Pneumocytis jiroveci. High dose cotrimoxazole was then started. A touch skin smear was taken from a leg skin lesion, stains were performed, and cultures made.
EUMYCETOMA; CHALLENGES IN DIAGNOSIS AND TREATMENT

S Ahmed, M Abbas, G Jouvion, A Al-Hatmi*, G de Hoog, A Kolecka, E Mahgoub

1Medical Microbiology, UofK/CBS, Khartoum, Sudan
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3Human Histopathology and Animal Models Unit, Institute Pasteur, Paris, France
4Directorate General of Health Services, Ibri Hospital, Muscat, Oman
5Medical Fungi, CBS, Utrecht, Netherlands
6Yeast, CBS, Utrecht, Netherlands
7Pathology, UofK, Khartoum, Sudan

Clinical Case Text:
We report a case of eumycetoma in a 55-year-old male from Darfur State, Sudan. An eumycetoma on his left foot had been noted for a period of 17 years. He developed swelling, sinuses and white grain discharge. He was diagnosed initially on the basis of histological and radiological examination as having white grain mycetoma caused by Scedosporium boydii. The patient underwent surgical operations nine times. In addition, he was treated with several courses of ketoconazole and itraconazole but without improvement. He had type 2 diabetes mellitus diagnosed 1 year after swelling of the foot began. A biopsy was taken from the infected foot in July 2013 and cultured.
BARKING UP THE WRONG TREE!

S Sibartie*, E Muldoon, D Denning

*University Hospital of South Manchester, UK

A 63 year old school caretaker was admitted to Stepping Hill Hospital, referred by his general practitioner (GP), with fever, shortness of breath, dry cough, right sided chest pains and a painful and pruritic maculo-papular rash on his lower back and upper torso. These symptoms persisted despite a week of antibiotics and antihistamines prescribed by his GP. He felt quite unwell and lost weight due to poor appetite. He attributed his illness as a progression of ‘flu-like’ symptoms that occurred 24 hours after carrying out extensive manual labour over a cold and wet weekend.

He had previously been fit and well with no significant illnesses and was on no medications. He had no allergies and had not suffered from chest infections despite a significant smoking history. He previously worked as a machine engineer in the oil industry. He lives with his wife and only drinks alcohol occasionally. His dog is his only pet. He is a ‘lawn bowling’ enthusiast.

On admission his heart rate was 106, temperature 37.6°C, blood pressure 118/80 mmHg, respiratory rate 16 and oxygen saturation 95%. His CXR revealed a right apical shadow. He had a leucocytosis and an elevated C-reactive protein (CRP).

He was treated with piperacillin/tazobactam and azithromycin. He also received chlorpheniramine with faster resolution of the rash on his torso than his lower back. His chest symptoms failed to improve. On day 10, CT showed a right apical consolidation with cavitation. Bronchoalveolar lavage (BAL) samples grew yeasts, mycobacterial microscopy and culture were negative, galactomannan was positive and Aspergillus PCR was negative. Blood cultures, serum galactomannan and bacterial urinary antigens were negative. Aspergillus IgG (AF IgG) was elevated at 276 mg/L (normal, <40mg/L), Aspergillus fumigatus IgE was 0.8 kAU/L (normal, <0.4 kAU/L) and total IgE was raised at 1300 kU/L (normal, <113 kU/L).

Day 14, he was discharged on voriconazole with significant improvement of his symptoms. On day 24, he was seen at the National Aspergillosis Centre. He still had a bothersome dry cough and a repeat chest radiograph showed persistent right apical shadowing. His white cell count and CRP had normalised, AF IgG was 300 mg/L, Aspergillus fumigatus precipitins were positive and total IgE was 450 kU/L with unremarkable Aspergillus IgE. At 3 months, his cough had resolved. After 4 months, his AF IgG was 50 mg/L and total IgE 180 kU/L. Repeat CT showed significant reduction in the volume of the right apical consolidation and cavitation.

At 6 months, he was asymptomatic and his AF IgG had normalised and voriconazole was stopped. At 1 year, chest radiograph showed no residual active lung lesions. He remains well after 3 years of follow-up.
POSTER ABSTRACTS
PROSPECTIVE EVALUATION OF AZOLE RESISTANCE IN ASPERGILLUS FUMIGATUS CLINICAL ISOLATES IN PAKISTAN

I Perveen*, S Sehar, I Naz, S Ahmed

Microbiology, Quaid-i-Azam University, Islamabad, Pakistan

**Purpose**

*Aspergillus fumigatus*, a ubiquitously distributed opportunistic pathogen, is the global leading cause of aspergillosis and causes one of the highest numbers of deaths among patients with fungal infections. The triazole antifungals are recommended first-line drugs in the treatment and prophylaxis of aspergillosis, however azole resistance in *A. fumigatus* isolates is increasingly reported with variable prevalence in Europe, the United States, South America, China, Japan, Iran, and India, but very few data are available in Pakistan.

Our study aimed to determine the resistance prevalence in *A. fumigatus* isolates recovered from clinical samples over a period of two years in two university hospital centers.

**Methods:**

All *A. fumigatus* isolates were screened for azole resistance using RPMI agar plates supplemented with itraconazole and voriconazole. Resistance was then confirmed by the EUCAST method. A part of the beta-tubulin gene was amplified for resistant isolates to confirm the *A. fumigatus* species, and the Cyp51A gene and its promoter were afterward sequenced to detect mutations potentially responsible for this resistance.

**Results:**

Two hundred and ten *A. fumigatus* isolates were recovered from patients. Fourteen isolates were found resistant with MICs of >9 mg/l, 5 mg/l, and 1 mg/l for itraconazole, voriconazole, and posaconazole, respectively. The TR34/L98H mutation, was detected in the three isolates.

**Conclusion:**

Our study demonstrated the occurrence of azole resistance among clinical isolates of *A. fumigatus*, with an overall prevalence of 6.6%.
IN VITRO ACTIVITY EXTRACTS OF SYZYGIUM AROMATICUM AGAINST ASPERGILLUS SPECIES

I Zurak*

Microbiology, University Hospital Zagreb, Croatia

Purpose:
Treatment for fungal disease is limited to a small number of antifungal drugs. Increase resistance of the *Aspergillus* species towards antifungal compounds; claim the search of new therapeutic alternative. Among medical herb species to have that which is show antifungal activity drugs? The objective of this study was to examine in the vitro susceptibility and antifungal activity of *Aspergillums* species to *Syzygium aromaticum*, in 60% ethyl alcohol extracts. Develop of new medical herbs agents against *Aspergillums* species.

Methods:
*Aspergillums* species were isolated from different hospitals patient samples. In this, experimental works use this *Aspergillums* species: *A. niger; A. fumigates; A. flavus; A. terreus; A. clavatus; A. glaucus and A. nidulans*. *Syzygium aromaticum* extracted by 60% ethyl alcohol in concentrations of 5g/50 ml (50mg/ml.). On the Mueller Hinton agar inoculated with *Aspergillus* species and after inoculation made in the agar 10mm in diameter wells. Extract of 50mg/ml *Syzygium aromaticum* used directly and into each well-added (50 to 100 µl). In one, well add 60% ethyl alcohol without *Syzygium aromaticum* served as control. Plates incubated between 18/36 hour at 24°C.

Results:
Zone of inhibition made in the mm. The highest of the alcohol extracts dilution, which incorporated into well on agar medium, and showing no visible growth *Aspergillums* species after incubate, regarded and referred to as the minimum inhibitory concentration or MIC. The zone of inhibition test shows varies among different *Aspergillums* species. All *Aspergillums* species that where isolated clinically from patients is 100% sensitivity towards tested of *Syzygium aromaticum.*

Conclusion:
The findings of this study showed that *Syzygium aromaticum* have anti-fungal valid alternative function against *Aspergillums* species. Therefore, they can be use in pharmacology. The in vitro sensitive of various *Aspergillums* species suggest the possibility of making pharmaceuticals preparations, medical products to suppress infection, priority of the skin (soap, creams, curative water, oil preservative) and mucosal surface (have tea, dentifrice and cavity washings) caused by *Aspergillums* species.
CLINICALLY SIGNIFICANT CRYPTIC ASPERGILLUS SPECIES IN A REFERRAL CHEST HOSPITAL DELHI, INDIA: MALDI-TOF IDENTIFICATION, SEQUENCING AND ANTIFUNGAL SUSCEPTIBILITY PROFILING

A Chowdhary1*, C Sharma1, A Masih1, PK Singh1, S Kathuria1, JF Meis2,3

1Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, India
2Medical Microbiology & Infectious Diseases, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands
3Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands

Purpose:
Aspergillus is a ubiquitous fungus causing a wide spectrum of infections and most threatening clinical manifestation being invasive aspergillosis is associated with high morbidity and mortality. Aspergillus species are easily misdiagnosed based on morphology making it difficult to ascertain the clinical and epidemiological peculiarities of the infections they cause. Also, these species may exhibit variable in vitro susceptibility to antifungal drugs; therefore, accurate identification of species is critical. The aim of the present study was to molecularly identify and to evaluate the MALDI-TOF spectral profile of cryptic Aspergillus species obtained from patients in a referral chest hospital, Delhi, India during 2011-2015.

Methods:
The clinical specimens included bronchial aspirates, bronchoalveolar lavage, nasal washings, nasal polyps and cerebrospinal fluid from 5 hospitals in Delhi, India. Aspergillus species were preliminarily identified based on macro- and micro morphological characteristics on Czapek dox agar plates incubated at 28°C. Their identification were confirmed by sequencing β-tubulin and calmodulin genes and spectral profiles determined by MALDI-TOF MS. Antifungal susceptibility testing was performed using broth microdilution method (CLSI M38-A2).

Results:
Of 11,242 samples processed, 25.7% (n=2421) were positive for Aspergillus species, which included 46% (n=1113) A. flavus, 32.5% (n=786) A. fumigatus and 6.4% (n=155) A. terreus. The remaining 15% (n=367) were Aspergillus species. A selection of 79 Aspergillus spp. isolates were studied with molecular characterization, which included species, found to be atypical in terms of growth and sporulation. They were identified belonging to 24 species by β-tubulin and calmodulin sequencing. The species distribution included three isolates each of A. niveus, A. melleus, Eurotium amstelodami, A. sydowii followed by two each of A. fijiensis, A. tamarii, A. ochraceus, A. hortai, A. tritici, Neosartorya fischeri and A. aculeatus. Other species included A. chevalieri, A. clavatus, A. egyptiacus, A. unguis, A. wentii, Emericella corrugata, E. nidulans and its variety, E. dentata. Among the azoles, posaconazole (GM MIC, 0.06 µg/ml) showed excellent antifungal activity followed by itraconazole (GM MIC, 0.17 µg/ml), isavuconazole (GM MIC, 0.34 µg/ml) and voriconazole (GM MIC, 0.68 µg/ml). Among echinocandins micafungin and anidulafungin exhibited lower MICs than caspofungin against all species. Twelve (15%) isolates excluding A. terreus exhibited high MICs of amphotericin B (MIC ≥2µg/ml). Further 9 isolates (11%) had voriconazole MICs ≥2µg/ml. Also 4 and 3 isolates demonstrated isavuconazole and posaconazole MICs in the range of 4->8µg/ml, respectively. Of the 79 isolates, 62 were identified up to species level by MALDI with a score value of >2. The clinical profile of the majority of patients ranged from allergic, chronic and invasive aspergillosis including a solitary case of brain abscess. However, in 16% of cases Aspergillus species were isolated from respiratory specimens of pulmonary tuberculosis patients with damaged lungs.

Conclusion:
The present report extends the spectrum of rare Aspergillus species involved in aspergillosis. Accurate molecular identification is taxonomically and clinically relevant as many of these species exhibit resistance to amphotericin B and voriconazole. Although Bruker MALDI-TOF database is limited to only 19 Aspergillus species, it correctly identified all the species present in the database.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
AZOLE RESISTANCE IN CLINICAL ASPERGILLUS FLAVUS ISOLATES WITH NOVEL S196F, A324P, N423D AND V465M SUBSTITUTIONS IN CYP51C GENE

C Sharma, S Kathuria, A Chowdhary*

Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, India

Purpose:

Aspergillus flavus is the second leading cause of invasive aspergillosis in immunocompromised patients, and is also an important causative agent of fungal rhinosinusitis and fungal keratitis. Voriconazole (VRC) is being used as primary and empiric therapy for the treatment of invasive aspergillosis. As in A. fumigatus prior azole exposure causes the strain to acquire resistance, similarly, long term exposure may predispose A. flavus to acquire azole resistance. Although, the mechanism of azole resistance in A. fumigatus is well studied and is mainly attributed to hotspot mutation in Cyp51A gene but in A. flavus the resistant mechanism is not well understood and so far only two reports of azole resistance in A. flavus suggesting Cyp51C to be the target gene for mutations are on record. In this study we analyzed the mechanism of azole resistance in A. flavus isolates originating from clinical specimens of patients in a tertiary Chest referral hospital, Delhi, India.

Methods:

A total of 120 A. flavus isolates originating from patients of suspected bronchopulmonary aspergillosis and rhinosinusitis were screened for antifungal susceptibility testing against azoles using CLSI broth microdilution method (CLSI M38-A2). The isolates with VRC MIC values higher than epidemiological cut off values (ECVs) along with wild type isolates were subjected to molecular identification by amplification and sequencing of β-tubulin and calmodulin genes. The Cyp51C gene of the VRC non-wild type isolates was amplified and sequenced for mutation analysis and compared with the reference A. flavus strain (NRRL3357). The homology modeling and molecular dynamic simulations were performed to assess the role of the substitutions.

Results:

Of the 120 A. flavus isolates, 3 (2.5%) had VRC MIC above ECV (> 1 µg/ml). Of the three isolates, two had MICs 2 µg/ml of VRC but low MICs for itraconazole and posaconazole. However, a solitary isolate exhibited cross-resistance to itraconazole (>16 µg/ml), VRC (>16 µg/ml) and posaconazole (>8 µg/ml). A comparison of the nucleotide and amino acid sequences of cyp51C homologs of non-WT (n=3) and WT (n=2) with the reference strain (NRRL3357) showed the presence of three missense mutations, namely, M54T, S240A and D254N. In a solitary non-WT isolate, in addition to the above mentioned mutations, four novel substitutions, namely, S196F, A324P, N423D and V465M were observed. The three isolates originated from individual patients of COPD, and nasal polyposis. Of the three patients, two were on VRC therapy for 2 weeks and no information on antifungal therapy was available for a solitary patient.

Conclusion:

This study identified the presence of four novel substitutions in voriconazole resistant A. flavus isolate. The azole resistance in A. flavus remains unexplored and many other mechanisms may have been responsible for the elevated MICs in the other two non-WT isolates observed in this study. Future studies are warranted to assess the impact of these novel substitutions on the general structure of A. flavus Cyp51C.
NOVEL MECHANISMS OF AZOLE RESISTANCE IN ASPERGILLUS FUMIGATUS INVOLVING TRANSFER-RNA

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Purpose:
Fungal diseases cause approximately 2 million deaths each year. There are few effective antifungal agents, and their widespread use has led to the development of drug resistance increasing the associated mortality and healthcare costs. Azoles are the ‘gold standard’ for treatment of the most common airborne fungal pathogen Aspergillus fumigatus, however azole resistance rates of up to 20% have been reported. Most resistant isolates in the UK carry unknown resistance mechanisms. Moreover, mortality exceeds 85% in individuals who are infected with resistant isolates. This study aims to understand a new mechanism of antifungal drug resistance based on tRNA and tRNA modification enzymes as translation regulatory elements in A. fumigatus. We previously identified 8 tRNA genes and several tRNA modification enzymes including General Control of amino-acid synthesis 2 (GCN2) and Eukaryotic Initiation Factor 2 (eIF2) in a screen for azole resistance in A. fumigatus.

Methods:
An A. fumigatus strain with a tRNA modification enzyme (tRNA-dihydrouridine synthase) deletion was generated using A1160 (ΔKu80 pyrG+) as parental strain. Growth rate on solid media containing inhibitory levels of itraconazole (ITZ) and MICs in liquid media (EUCAST protocol) were tested.

Results:
The strain had a radial growth rate 30% higher than the wild type on 1mg/L ITZ. In addition, the knockout strain demonstrated an MICITZ 4 fold higher than the parental strain.

Conclusion:
We hypothesise that resistance is linked to an altered tRNA regulation in stress response in the A. fumigatus mutant. This regulation may influence the translation process in two ways. The first involves alteration of tRNA and tRNA modification enzymes creating the wobble phenomenon, which may generate additional mis-regulation of genes involved in azole resistance. Alternatively, the tRNA mediates the initiation of the translational process via GCN2 and eIF2. Thus, modification of tRNA-dihydrouridine synthase may be involved in antifungal resistance mechanism and may provide a new therapeutic or diagnostic target.
CLINICOMYCOLOGICAL CORRELATION OF INFECTIONS CAUSED BY ASPERGILLUS SPECIES – A RETROSPECTIVE AND PROSPECTIVE STUDY IN A TERTIARY CARE CENTRE IN SOUTH INDIA

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Purpose:
• To isolate the Aspergillus from various clinical samples
• To speciate the rare Aspergilli using gene sequencing
• To find the prevalence of infection caused by Aspergillus in a tertiary care center
• To determine the antifungal susceptibility patterns of the identified Aspergillus species
• To analyse demography and clinical outcome of the patients

Methods:
For the retrospective study the lab records has been referred to find out the isolates of Aspergillus. Hospital numbers were noted for the year 2012-2014. The profoma was filled from the Medical Records Department. For the prospective study samples which grew Aspergillus were taken up for the study. Histopathological samples which were positive for Aspergillus were also taken up for the study. The clinical isolates were subcultured and speciation confirmed after which antifungal susceptibility test has been put as per the CLSI –M 38 A2 guidelines.

The drugs like voriconazole, itraconazole, posaconazole, amphotericin B and caspofungin have been used. The demographic details and the treatment modalities has been noted from the patients case sheet.

Results:
Samples commonly received were ear swab from CSOM patients (10), nasal swab (6), sputum (2), pus from wound (2), tissue from nose (1). Most commonly identified species were Aspergillus flavus (10 were from ear swab) followed by Aspergillus niger (7), Aspergillus fumigatus (3) and Aspergillus terreus (1). Demographic analysis of the patients showed that only one patient was between the age group 10-20, 10 patients in the age group 20-30, 3 patients in the age group 30 to 40, 4 patients in the age group 40 to 50 and 3 patients in the age group 50 to 60. Among the 21 patients 15 were females and 6 were males. Five patients were identified to have co–morbid conditions like diabetes mellitus and hypertension, two patients expired due to sepsis.

Conclusion:
Antifungal susceptibility testing was done for 2 isolates one of which was bronchial wash was identified as Aspergillus flavus. The minimum inhibitory concentration values were 1 µg/ml for posaconazole, 2 µg/ml for itraconazole, 2 µg/ml for voriconazole, 2µg/ml for amphotericin-B and 0.25 µg/ml for caspofungin.

Second isolate which was from nose was identified as Aspergillus fumigatus. Antifungal susceptibility testing showed minimum inhibitory concentration values of 0.25 µg/ml for posaconazole, 0.25 µg/ml for itraconazole, 0.25 µg/ml for voriconazole, 0.25 µg/ml for amphotericin-B and 0.25 µg/ml for caspofungin.

Antifungal susceptibility testing of the rest of the isolates is going to be performed and the results will be ready by February 2016.
**IN VITRO Antifungal Susceptibility Profiles of Clinical and Environmental Species of Aspergillus Section Flavi Isolated from Iran During 2008 to 2014**

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**Purpose:**

Aspergillus species are the most common and life threatening fungal pathogens among both immunocompetent and immunocompromised patients, causing a wide range of infections depending on the immune status of the host. Notably, *A. flavus* is the leading cause of invasive aspergillosis in tropical and sub-tropical countries like Iran. We therefore aimed to evaluate *in vitro* antifungal susceptibility (AFST) of nine antifungal against clinical and environmental *Aspergillus* section *Flavi* isolates collected from Iran during 2008 to 2014.

**Methods:**

Two hundred strains belonging to *Aspergillus* section *Flavi* were identified down to species level by using PCR-sequencing of β-tubuline rDNA gene. *In-vitro* AFST was performed against nine antifungal using CLSI-M38-A2 protocol for filamentous fungi.

**Results:**

Sequencing analysis were resulted in total, 118/200 (59%) *A. flavus*, 69/200 (34.5%) *A. oryzae*, 10 (5%) *A. nomius* and 3 (1.5%) *A. tamarii*. Isavuconazole was the most effective antifungal tested followed by posaconazole, itraconazole and voriconazole. All isolates showed susceptible profile to echinocandins: caspofungin (MEC50/90 / GMMEC: 0.032, 0.125 / 0.047), anidulafungin (MEC50/90 / GMMEC: 0.016, 0.032 / 0.023) and imidazole group including luliconazole (MEC50/90 / GMMEC: 0.00013, 0.00013 / 0.00012) and lanoconazole (MEC50/90 / GMMEC: 0.016, 0.032 / 0.013). Amphotericin B was not effective against any of the tested isolates. In addition, there were not significant differences between the AFST of environmental versus various clinical isolates.

**Conclusion:**

*A. flavus* was the most prevalent species in *Aspergillus* section *Flavi*, and there was not significant differences in AFST between identified species. The new azole, isavuconazole can be recommended as the most effective antifungal against systemic infections caused by these fungi. Luliconazole and lanoconazole showed a high efficacy against *Aspergillus* section *Flavi*, which make them promising antifungal agents against topical infections such as non-dermatophytes onychomycosis.
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Purpose:
CD101, a novel echinocandin with long-acting pharmacokinetics and chemical stability, is being developed as an IV, once-weekly administered antifungal for serious fungal infections. CD101 IV is currently in clinical development for the treatment of candidemia. Given the potent in vitro activity of CD101 against A. fumigatus, this study was conducted to evaluate the in vivo efficacy of CD101 IV for treatment of aspergillosis using a disseminated infection model in neutropenic mice.

Methods:
The susceptibility of the A. fumigatus test strain ATCC 13073 was evaluated by measuring the minimal effective concentration (MEC) for changes in the hyphal morphology (CLSI protocol M38-A2). The in vivo efficacy was assessed using a mouse model of disseminated aspergillosis in which neutropenic animals were infected by injecting a suspension of A. fumigatus strain ATCC 13073 into the tail vein with an inoculum size of 10⁴ CFU/mouse. Test article and vehicle were administered to groups of 10 mice twice daily by IV injection starting 2 h after infection for five days (BID×5). Survival was monitored for 10 days after infection. The Fisher’s exact test was performed to assess the significance of the differences between the test article and vehicle treatment groups.

Results:
CD101 demonstrated potent in vitro activity against A. fumigatus strain ATCC 13073 with an MEC value of 0.0078 µg/ml. CD101 administered IV to infected neutropenic mice at 0.2, 1 and 5 mg/kg BID for 5 days was associated with a significant increase in 10-day survival compared to vehicle group (p < 0.05; Figure). Amphotericin B was used as the positive control treatment at 0.3 mg/kg BID for 5 days. Animal survival rate of CD101 at the lowest dose tested, 0.2 mg/kg, was comparable to amphotericin B at 0.3 mg/kg.

Conclusion:
CD101 IV was shown to be effective when administered by the IV route, using a mouse model of disseminated A. fumigatus infection. The efficacy supports the utility of CD101 IV for the treatment of aspergillosis.
CYP51-INDEPENDENT ISAVUCONAZOLE-INDUCED RESISTANCE IN ASPERGILLUS FUMIGATUS

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Purpose:
Drug resistance to triazole drugs in A. fumigatus is largely due to a limited spectrum of mutations in CYP51A, which encodes the lanosterol 14 α-demethylase in the ergosterol biosynthetic pathway. However, CYP51A-independent resistance is increasingly being reported. Isavuconazole (ISA) is a next generation highly active azole agent with potent activity on the mold A. fumigatus. Known mutations that confer resistance to itraconazole (ITR), voriconazole (VRC) and posaconazole show some cross-resistance with ISA. However, it is not known to what extent ISA induces azole resistance in CYP51A and what spectrum of mutations is formed. In a previous study in our lab, sixteen stable ISA resistant (IR) mutants with MICs ≥ 4-16 µg/ml were obtained after exposure to increasing concentrations of ISA. No characteristic CYP51A or CYP51B mutations were identified nor were prominent drug efflux transporters overexpressed. All IR isolates showed cross-resistance with ITR and VRC but retained sensitivity to amphotericin B and the three echinocandin drugs. In order to further investigate what is the molecular basis of resistance to ISA, whole-genome sequencing (WGS) was performed for high-resolution single-nucleotide polymorphism (SNP) analysis in two A. fumigatus isolates, one IR mutant and the parental susceptible strain.

Methods:
Conidia from A. fumigatus parental strain ATCC13073 and the IR mutant isolate A1 were collected from PDA plates after 5 days of growth. Antifungal susceptibility testing was performed following the guidelines described in the CLSI document M38-A2. Genomic DNA was extracted following the protocol published by Calera et al., 1997. The isolated DNA was sequenced by next generation sequencing technology (Illumina MiSeq). For each isolate, two technical replicates were analyzed. The reads obtained from sequencing were mapped on the reference genome of A. fumigatus Af293.

Results and Conclusions:
WGS analysis confirmed the absence of mutations in both CYP51A and CYP51B genes in the IR mutant analyzed. However, some SNPs were found in other genes involved in the biosynthesis of the ergosterol (ERG27, ERG4, ERG6, ERG25B, ERG3C). Among them, the most dramatic change was found in ERG3C one of the three C-5 sterol desaturases that exists in A. fumigatus. We found an insertion of 7 nucleotides (nt642) that lead to a frame shift ending up in a truncated protein of 204 amino acids instead of 320. We sequenced the ERG3C gene in the other IR mutants and only the ones with MIC ≥ 4 mg/L showed the insertion. Currently, we are analyzing the viability of the truncated protein and checking the content in ergosterol in these IR mutants.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
SUSCEPTIBILITY TO AZOLES IN CLINICAL ISOLATES OF *ASPERGILLUS FUMIGATUS* AND *A. TUBINGENESIS* FROM OBIHIRO, JAPAN

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**Purpose:**
Azole antifungals are used not only clinically for fungal infections but also as agricultural fungicides. Recently, azole-resistant *Aspergillus fumigatus* containing a tandem repeat (TR) in the promoter region of *cyp51A* combined with amino acid substitution(s) appeared in the environment in Eurasia. Although azole fungicides have been used for more than 10 years in Japan, particularly in Hokkaido, the surveillance and characterization of *A. fumigatus* in Hokkaido has not been reported. We collected soil samples from farms that used an azole fungicide in the Tokachi area of Eastern Hokkaido, but did not detect TR-containing resistant *A. fumigatus*. In this study, we collected clinical isolates of both *A. fumigatus* and *A. tubingensis* and determined the minimal inhibitory concentration (MIC) of medical azoles.

**Methods:**
*A. fumigatus* and *A. tubingensis* were isolated from clinical specimens from a hospital in Obihiro, Japan. To determine MICs of medical azoles, the broth microdilution method based on CLSI M38-A2 was used with a slight modification.

**Results:**
Almost all *A. fumigatus* and *A. tubingensis* isolates retained susceptibility to medical azoles. MICs of *A. fumigatus* strains OKH34 and OKH6 for voriconazole were 8 and 2 µg/mL, respectively. The MIC of voriconazole against *A. tubingensis* OKH5 strain was 4 µg/mL. OKH34 and OKH6 strains did not contain the TR in the *cyp51A* promoter region. The OKH34 strain contained the G448S mutation in *cyp51A*, conferring voriconazole resistance, which is the first report from Japan.

**Conclusion:**
TR-containing resistant strains were not identified in our clinical isolates. Our data suggest that in this area, this type of resistance has not been introduced.
COMBINATORIAL EFFECTS OF AZOLES AND HYPOXIA ON STEROL BIOSYNTHESIS AND STEROL COMPOSITION OF *ASPERGILLUS* SPP.

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**Purpose:**
Invasive aspergillosis represents a serious life threatening disease in patient cohorts with impaired immune response. During infection, fungal pathogens must adapt to microenvironmental stresses, and such conditions are usually not taken into account in the assessment of antifungal sensitivities. Hypoxia is one stress which occurs during fungal infection *in vivo*. Ergosterol, the most important fungal cell membrane component, or its biosynthesis are the main targets of azoles, and additionally, the ergosterol pathway is dependent on oxygen availability.

We therefore investigated the influence of azole treatment together with hypoxia on (1) the effectivity of azoles, (2) transcriptional changes of ergosterol biosynthetic genes (qPCR), (3) total ergosterol amount and (4) sterol accumulation in clinically relevant Aspergilli.

**Methods:**
Antifungal susceptibility was evaluated under variable oxygen conditions by EUCAST format and MFCs were determined under both oxygen conditions. Total ergosterol amount and sterol pattern of treated mycelia was determined by HPLC and GC-MS analysis, and qPCR was used to check for transcriptional changes in ergosterol biosynthetic genes.

**Results:**
MICs of voriconazole and posaconazole did not change due to oxygen availability. A significant decrease of total ergosterol was observed during azole treatment in normoxia, while no changes were visible under hypoxic conditions. Additionally, azole treatment led to changes in the relative amount of sterols at both oxygen concentrations compared to untreated controls. Interestingly, with lower abundance in hypoxia, indicating lower efficacy of azoles under low oxygen conditions. Azole exposure elicited increased levels of *erg11A, erg11B, erg6* and *erg7C* mRNA abundance in normoxia, while transcript levels in hypoxia were not altered in comparison to untreated controls. These observations are well correlating with results from GC-MS and HPLC.

**Conclusion:**
This study emphasizes the combinatorial effect of hypoxia and azole treatment on antifungal activity and highlights the important link between azole interactions and ergosterol biosynthesis.
REVIEW OF *ASPERGILLUS* SPP. COLLECTED FROM BRAIN, CEREBRAL SPINAL FLUID, VITREOUS FLUID AND CORNEAS IN THE UNITED STATES

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Purpose:
Although pulmonary disease is the main manifestation of invasive aspergillosis, dissemination to the central nervous system can occur, and this is associated with significant morbidity and mortality. *Aspergillus* spp. can also cause infections of the eye. Rarely, endophthalmitis following dissemination may also occur. More commonly, *Aspergillus* isolates can infect the cornea through external inoculation resulting in keratitis. Our objective was to review the species distribution and antifungal susceptibility profiles against *Aspergillus* isolates collected from the brain, cerebral spinal fluid, and vitreous fluid, and corneas of humans and animals in the US.

Methods:
The isolate and antifungal susceptibility database at the University of Texas Health Science Center at San Antonio Fungus Testing Laboratory was queried for *Aspergillus* spp. isolates collected from eyes (cornea and vitreous fluid), cerebral spinal fluid (CSF), and brain tissue that had been sent to our reference mycology laboratory from institutions across the U.S. between 2001 and 2015. In addition, antifungal minimum inhibitory concentration (MIC) results were also reviewed. Susceptibility testing was performed according to CLSI M38-A2 reference methods. The MIC50, MIC90, and geometric mean (GM) MIC values were determined.

Results:
230 *Aspergillus* isolates that were collected from eyes (cornea and vitreous fluid), cerebral spinal fluid (CSF), and brain tissue were sent to our laboratory between 2001 and 2015. The majority of the isolates came from corneas of humans and animals (148; 64.3%), 60 (26.1%) were cultured from brain tissue, 18 (7.8%) from vitreous fluid, and 4 from CSF (1.8%). *A. fumigatus* was the most prevalent species (93 isolates, 40.4%), followed by *A. flavus* (40 isolates, 17.4%), which was primarily isolated from corneas. Other species included *A. niger* (4), *A. sydowii* (3), *A. terreus* (3), *A. versicolor* (5), and one each of *A. granulosus*, *A. penicillioides*, and *A. tamarii*. Six *Aspergillus* Section Usti isolates were also collected, including 5 *A. calidoustus* isolates from brain tissue. Identification to only the genus level was provided for 70 isolates (30.4%). The species distribution per specimen type is shown in the Table. Of the antifungals tested, posaconazole was the most potent agent (GM MIC 0.3289 mg/L, MIC50 and MIC90 values 0.25 and 1 mg/L, respectively), followed by itraconazole (0.4811 mg/L, 0.5 and 1 mg/L), voriconazole (0.5724 mg/L, 0.5 and 1 mg/L), and amphotericin B (0.9538 mg/L, 1 and 2 mg/L). Miconazole and natamycin were also tested against isolates from the cornea and demonstrated higher MIC values (GM MICs 1.554 mg/L and 11.88 mg/L, respectively).

Conclusion:
Similar to what is observed in invasive pulmonary aspergillosis, *A. fumigatus* was the most predominant species in isolates collected from the central nervous system and eyes of patients and animals in our reference collection. However, other species, including more rare causes of disease and those with reduced antifungal susceptibility, were also found. Identification to the species level and susceptibility testing may be warranted in *Aspergillus* infections in these tissues when feasible.
<table>
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<th>Species</th>
<th>Brain Tissue – all from humans (n = 60)</th>
<th>CSF – all from humans (n = 4)</th>
<th>Cornea (Human; n = 98)</th>
<th>Cornea (Animal; n = 50)</th>
<th>Vitreous Fluid – all from humans (n = 18)</th>
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IDENTIFICATION AND SUSCEPTIBILITY STUDY OF ASPERGILLUS SPP. ISOLATED FROM INTENSIVE CARE UNIT PATIENTS IN SIX HOSPITAL AND ENVIRONMENTAL SAMPLES FROM JAKARTA, INDONESIA


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7Pulmonology, Soelianti Saroso Hospital, Jakarta, Indonesia
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9Community Medicine, Universitas Indonesia, Jakarta, Indonesia
10Microbiology, Canisius Wilhelmina Hospita, Nijmegen, The Netherlands
11Parasitology, Indonesian Christian University, Jakarta, Indonesia

Purpose:
To determine the etiology of invasive pulmonary aspergillosis (IPA) and identify environmental isolates collected from several part of Jakarta and hospitals where the patients admitted. A susceptibility study also conducted for all isolates.

Methods:
This study is part of a multicenter cohort study to determine IPA incidence in ICU admitted patients at six hospitals in Jakarta. Collection of clinical and environmental samples was done from October 2012 – February 2014. Isolation of the fungus and phenotypic screening was done in Mycology Laboratory, Department of Parasitology, University of Indonesia, Faculty of Medicine. Microsatellite typing and susceptibility study were conducted in Microbiology Laboratory Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands. The strains were tested for amphotericine-B (AMB), fluconazole (FLC), itraconazole (ITC), voriconazole (VRC), posaconazole (PSC), isavuconazole (ISA), and micafungin (MICA) using CLSI method.

Results:
Out of 405 patients investigated 31 (7.7%) was diagnoses as probable IPA and seven (1.7%) with Aspergillus airways colonization but not accompanied by symptoms of pneumonia. There were 45 Aspergillus isolates derived from those patients. The classical identification method showed 42 out of 45 isolates of Aspergillus fumigatus and Aspergillus flavus, and three isolates of Aspergillus niger and Aspergillus nidulans. Molecular identification with microsatellite typing/short tandem repeat method showed 27 isolates of A. flavus (60.0%) and 8 isolates of A. fumigatus (17.8%), and seven other species of Aspergillus isolates (15.6%) were suspected of cryptic species or mixed isolates. Further investigation is needed to confirm the cryptic species. The molecular profiles of A. flavus and A. fumigatus showed that Indonesian isolates had different genotypes compared to other countries, and there was genotypic diversity among isolates. There were some clinical isolates that showed genotypic similarity to environmental isolates that might reflect the possibility of nosocomial infection, but further study is needed to prove it. All strains tested for their susceptibility are susceptible for all antifungal tested. Only one isolate of Aspergillus section Fumigati is resistant against itraconazole, voriconazole, and fluconazole. Further investigation of the resistant strain showed that the isolate consist of two different species, Penicillium citrinum and A. tamarii.

Conclusion:
The main cause of invasive aspergillosis in Jakarta is Aspergillus flavus, followed by Aspergillus fumigatus. Indonesian isolates showed diverse clonality and the result also showed a possibility of environment as the source of infection. Susceptibility study showed that almost all isolates in the sensitive range, except one strain of A. fumigatus is resistant, but further identification showed that the isolate is mixed of two different species and we could not determined its pattern of susceptibility yet.
IMPACT OF THE NOVEL OROTOMIDE ANTIFUNGAL F901318 ON VIABILITY OF ASPERGILLUS FUMIGATUS

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Purpose:
Worldwide approximately 1.7 billion people suffer from fungal diseases. Per year around 1.5 million of these infections result in death of the patient. The emerging resistance of fungal pathogens to the available drugs poses an increasing threat to global health. Hence, there is an urgent need for the development of new kinds of antifungal therapeutics with a novel mode of action.

F901318 is the lead candidate of the novel class of orotomide antifungals with excellent \textit{in vitro} activity against all the clinically significant \textit{Aspergillus} spp. F901318 also shows excellent \textit{in vivo} efficacy in murine models of invasive aspergillosis together with good pharmacokinetics, tissue distribution and oral bioavailability.

In this study we investigated the impact of F901318 treatment on the viability of \textit{A. fumigatus}.

Methods:
Susceptibility of \textit{A. fumigatus} to F901318 was determined using CLSI methodology. Live-cell imaging was used to observe the effect of F901318 on the elongation rate of \textit{A. fumigatus} germinated spores and hyphae. Confocal microscopy was employed to determine viability of \textit{A. fumigatus} after exposure to F901318, using fluorescent viability dyes propidium iodide, DiBac and FUN-1. The impact of the drug on \textit{A. fumigatus} was also determined by quantitation of the intracellular ATP concentration. Furthermore, biomass assays were established to evaluate the ability of \textit{A. fumigatus} hyphae to recover from treatment with F901318.

Results:
\textit{A. fumigatus} is highly susceptible to F901318 with a minimal inhibitory concentration (MIC) of <0.03 mg/L. In addition, F901318 displays a rapid antifungal effect by inhibiting growing germlings after approx. 30 min. exposure and growing hyphae after approx. 60 min exposure to concentrations just above the MIC. Staining F901318 treated hyphae with the viability dyes showed that F901318 had a detrimental effect on \textit{A. fumigatus} hyphae which appeared to be irreversible. This was confirmed by growth assays that showed that removal of the compound and supply of fresh medium only allows minimal to no regrowth depending on the concentration of F901318.

Conclusion:
Although it is difficult, particularly for filamentous fungi, to definitively conclude that an agent is fungicidal, a combination of approaches indicate that F901318 causes damage to \textit{Aspergillus fumigatus} hyphae that leads to cell death.
ANALYSIS OF UPTAKE AND INTRACELLULAR DISTRIBUTION OF THE NOVEL OROTOMIDE ANTIFUNGAL AGENT F901318 USING FLUORESCENT ANALOGUES

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Purpose:
Invasive fungal infections are responsible for an estimated 1.5 million deaths per year. At the moment, azole antifungal therapy is the first line treatment option, but issues such as drug-drug interactions, toxicity, variable PK and emerging resistance make the discovery of new antifungal agents acting via new mechanisms an important task. F901318 is the lead candidate of the novel class of orotomide antifungals and acts via inhibition of DHODH, a key enzyme in pyrimidine biosynthesis, a mechanism not shared with current antifungals. F901318 has a mould-only spectrum and has excellent in vitro activity against all the clinically significant Aspergillus spp. with MICs below 0.03 µg/ml.

In this study we synthesised several fluorescent analogues of F901318 and investigated their antifungal activity, uptake and cellular localisation and distribution.

Methods:
A total of five fluorescent variants of F901318 have been synthesised by conjugating an F901318 analogue to a fluorophore. These analogues have been tested for spectrum and antifungal activity using fluorescence measurements, MIC tests and DHODH inhibition assays. Uptake, localisation and distribution of the most active compound, F901848, by Aspergilli was further analysed using confocal fluorescence microscopy.

Results:
Although displaying a good fluorescent signal, most analogues lacked potent antifungal activity compared to F901318. F901848 showed the best activity with MICs of 0.8 µg/ml against A. niger and A. flavus and inhibited A. niger DHODH with an IC50 of 37 nM. This compound is conjugated to fluorophore 4-chloro-7-nitrobenzofurazan (NBD-Cl) and fluorescence was observed at an excitation/emission of 490/550 nm. Confocal microscopy with A. flavus showed that F901848 was rapidly taken up by A. flavus within minutes and was spread throughout the hyphae.

Conclusion:
Together our results show that fluorescent compound analogues can be used to study uptake, localisation and distribution of compounds by Aspergillus spp. The successful design of a fluorescent version of F901318 that retains some DHODH inhibition and antifungal activity has provided a useful tool for further investigation of orotomide action.
COMPARATIVE GENOMICS AND GENETIC INSPECTION IN ASPERGILLUS FUMIGATUS TOWARD IDENTIFICATION OF MULTIAZOLE RESISTANCE MECHANISMS EMERGED DURING AZOLE TREATMENT

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Purpose:
We serially isolated several multiazole resistant Aspergillus fumigatus strains from a patient during voriconazole treatment (>3 years) for chronic pulmonary aspergillosis. The aim of this study is to identify the responsible azole resistance mechanisms in the strains.

Methods:
To determine if the strains have identical genetic lineage, microsatellite analysis was undertaken. Drug susceptibility was investigated and the Cyp51A gene sequence was determined in the strains. To identify further genetic mutations, genome of each strain was sequenced by Illumine NGS technology and compared each other.

Results:
According to microsatellite analysis, the strains were derived from a clonal strain. Whereas the strain initially isolated (designated 1st strain) was susceptible to itraconazole, voriconazole, and posaconazole, the strains isolated 3 years later (as 2nd strain) and another 2 months later (as 3rd strain) showed elevated MICs for all the azoles. The 2nd strain had G448S mutation in Cyp51A, however no mutations were found in Cyp51A of the 3rd strain. From a genome-wide comparison, we found 9 mutations specific to the 2nd strain and 7 mutations to the 3rd strain. Notably, 2 of these mutations were commonly found in both 2nd and 3rd strains. One of the common mutations was a SNP in hmg1 encoding hydroxymethylglutaryl-CoA (HMG-CoA) reductase.

Conclusion:
Among the azole-susceptible and multiazole resistant strains, which were likely derived from a clonal strain and diverged during infection, we found several mutations which may be potentially responsible for the azole resistance mechanism. Genetic transformation tests for clarifying the role of SNP in hmg1 are underway.
ACTIVITY OF THE NOVEL OROTOMIDE ANTIFUNGAL F901318 AGAINST AZOLE RESISTANT ASPERGILLUS SPP.

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Purpose:
Invasive aspergillosis represents a serious life-threatening disease especially for immuno-compromised patients and triazole therapy is frequently the first line treatment option. The emerging resistance to triazoles in the fungal pathogens causing Aspergillosis poses a growing threat to global health. Since 2001, no new classes of antifungal agents have been developed.

F901318 represents the first member of the novel orotomide antifungal class and is currently in clinical development for the treatment of serious systemic fungal infections in particular invasive aspergillosis. F901318 acts via a novel mechanism of action that is different to that of the members of theazole class which all act through inhibition of the sterol biosynthetic enzyme Cyp51. Multiple cyp51A mutations conferring azole resistance have been identified, with the most common being TR34/L98H found in Aspergillus fumigatus. The TR34/L98H mutation is associated with significantly reduced itraconazole, voriconazole, and posaconazole susceptibility.

In this study a panel of Aspergillus spp. strains, isolated in the North West of England and Tyrol, Austria, that showed resistance or elevated MICs to single or multiple azoles was tested for susceptibility to F901318. The study also included several isolates with defined cyp51A mutations.

Methods:
A total of 40 Aspergillus isolates were tested for susceptibility to F901318. MICs were determined by CLSI methodology outlined in document M38-A2. All organisms were tested in microdilution format. Voriconazole, posaconazole, itraconazole and amphotericin B were tested as comparators.

Results:
All tested Aspergillus species (A. fumigatus n= 19, Aspergillus terreus n = 17 and Aspergillus flavus n=4) were highly susceptible to F901318 with all MICs < 0.03 mg/L. The F901318 MICs for these isolates were identical to those for azole susceptible ones indicating a lack of cross resistance withazole drugs.

Conclusion:
The obtained data underline the high in vitro effectiveness of F901318 against both azole susceptible and azole resistant Aspergillus spp. The great potential of F901318 as an antifungal agent is further supported by the fact that it shows significant activity against both A. terreus and A. flavus, which are frequently found resistant to amphotericin B.
ASPERGILLUS SPECIES GROUNDNUT SEED INVASION AS INFLUENCED BY
SOIL SOLARIZATION AND TIME OF PLANTING

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Purpose:
Aflatoxin contamination of groundnut (Arachis hypogaea L.) is a serious problem in most groundnut-producing countries where frequent end season drought (soil water stress) and/or the crop is grown under rain-fed conditions. Laboratory and field experiments were conducted to determine the effect of soil solarization on Aspergillus species inocculum in the soil and to evaluate the effect of soil solarization and time of planting on Aspergillus species seed invasion and yield of groundnut varieties.

Methods:
Groundnut seed samples and soil samples were taken in three rounds and analyzed for aflatoxin producing Aspergillus population. Soil solarization reduced fungal inoculum and increased groundnut yields. Individual and total (colony forming unit) cfu g⁻¹ of soil was determined before, after solarization and at harvest.

Results:
Four Aspergillus species namely, A. flavus, A. parasiticus, A. niger and A. terreus were identified and their densities were significantly (P<0.05) reduced at 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. terreus were detected. Three Aspergillus species namely, A. flavus, A. niger and A. parasiticus were isolated from seed samples plated on Czapek-Dox Agar medium.

Conclusion:
Early planting of the varieties showed the lowest level of seed infection by A. flavus (22.8%). Whereas from delayed planted verities showed higher in infection of A. flavus (65.7%). Generally, aflatoxin levels of 5 to 300 μg/kg in groundnut seeds were analyzed.
AZOLE SUSCEPTIBILITY OF A CLINICAL COLLECTION OF 206 ISOLATES OF *ASPERGILLUS FUMIGATUS* ISOLATED ACROSS 6 CENTRES IN INDIA

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**Purpose:**

We determined the susceptibility of 206 isolates of *Aspergillus fumigatus* isolated from patients across six centres in India.

**Methods:**

The susceptibility was checked for commonly employed triazoles including itraconazole (ITR), voriconazole (VOR) and posaconazole(POS). The susceptibility was determined by broth microdilution method as per the CLSI M38-A2 guidelines. Briefly, the cultures were grown on Potato dextrose agar with chloramphenicol (50mg/L) for 3-5 days at 37°C or until there was good amount of sporulation. The inoculum was harvested in sterile normal saline with 0.01% tween-20 to make homogenous suspension. The inoculum was set between 0.09-0.13 absorbance units at 530 nm. The plates were incubated at 35°C for 48 hrs. Genomic DNA was extracted by phenol chloroform method and PCR amplification of the upstream promoter region was done using oligo sequences available from the literature.

**Results:**

All the triazoles exhibited excellent in vitro activity against *Aspergillus fumigatus* with itraconazole (MIC50:0.03mg/L, MIC90: 0.12 mg/L and GM: 0.04 mg/L) and posaconazole (MIC50:0.03mg/L, MIC90: 0.12 mg/L and GM: 0.04 mg/L) showing most potent activity. The MIC of Voriconazole (MIC50:0.25mg/L, MIC90: 0.5 mg/L and GM: 0.04 mg/L) were slightly more as compared to itraconazole and posaconazole. Only one isolate which was isolated from the southern part of India was pan-azole resistant (ITR :16mg/L, VOR: 4 mg/L and POS: 1 mg/L). This strain was isolated from the bronchial wash of a patient presenting with fever and respiratory distress. PCR amplification of the upstream promoter region of cyp51A of this isolate showed presence of TR34 mutation.

**Conclusions:**

These findings are part of an ongoing study to determine the prevalence and mechanism of azole resistance in *Aspergillus fumigatus*. This study indicates that the emergence of azole resistance is not so frequent in *Aspergillus fumigatus*. Only a single isolate showed resistance to azoles and the route of resistance development (TR34/L98H) in this isolate was environmentally driven.
LIVE-CELL IMAGING OF THE DYNAMICS OF CELL WALL COMPOSITION AND β-1,3-GLUCAN SYNTHASE ASSOCIATED WITH THE PARADOXICAL GROWTH EFFECT IN ASPERGILLUS FUMIGATUS

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Purpose:
The importance of invasive fungal infections in humans has been grossly underestimated. Indeed, every year > 1.5 million people die of fungal infections. Unfortunately, few antifungal drugs are available and resistance against these drugs is increasing. The most important fungal lung disease is caused by *Aspergillus fumigatus* and the mortality rates from aspergillosis in immunocompromised patients are typically > 50%. Caspofungin, which targets the cell wall synthesising enzyme β-1,3-glucan synthase, is one of the main antifungal drugs used to treat aspergillosis. However, at high concentrations, caspofungin shows reduced activity against *A. fumigatus*, a phenomenon called paradoxical growth effect. Here, we describe the dynamic changes in cell wall composition and localization of β-1,3-glucan synthase complex associated with the paradoxical growth effect in *A. fumigatus*.

Methods:
Advanced live-cell imaging methodologies were used in combination with different fluorescent cell wall stains and a reporter strain in which the β-1,3-glucan synthase catalytic subunit had been labelled with GFP.

Results:
Initially, cells treated with either 0.5 or 4 µg/ml of caspofungin showed similar abnormalities, such as swollen cells, highly branched hyphae, and abnormal septum distribution. Furthermore, caspofungin induced changes in the distribution and content of the main cell-wall components, such as chitin, galactomannans, α-glucans and β-1,3-glucans. However, after 48-h of continuous exposure to a high concentration of caspofungin (4 µg/ml), the leading hyphae of *A. fumigatus* recovered their normal morphology (paradoxical growth), as well as their normal content and distribution of galactomannans, α-glucans and β-1,3-glucans, but not chitin, which remained continuously high. Live-cell imaging of the β-1,3-glucan synthase FKSA labelled with GFP showed exclusive localisation in the growing tips of untreated hyphae. However, treatment with either 0.5 or 4 µg/ml caspofungin resulted in GFP-FKSA being transported from the tips to the vacuoles and this was associated with growth inhibition. Later, when hyphae treated with 4 µg/ml caspofungin resumed their paradoxical growth, GFP-FKSA was restored to the hyphal tips.

Conclusion:
Our findings highlight the multiple changes in cell-wall composition and the dynamics of β-1,3-glucan synthase resulting in the paradoxical growth effect in *A. fumigatus* exposed to caspofungin.
IN VITRO ACTIVITIES OF ANTIFUNGAL DRUGS AGAINST 199 CLINICAL AND ENVIRONMENTAL ISOLATES OF ASPERGILLUS FLAVUS, AN OPPORTUNISTIC AGENT

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Purpose:
Aspergillus flavus is the second leading cause of invasive and non-invasive aspergillosis, also most common cause of fungal sinusitis, cutaneous, and endophthalmitis in tropical countries. Since resistance to antifungal drugs has been seen in patients, susceptibility testing can helpful in defining the activity spectrum of an antifungal and determining the appropriate drug for treatment.

Methods:
The antifungal susceptibilities of clinical (n=171) and environmental (n=28) isolates of A. flavus to amphotericin B, itraconazole, voriconazole, posaconazole, and caspofungin were determined in accordance with CLSI document M38-A2.

Results:
In clinical samples, A. flavus (87.5%) was significantly more recovered from sinus and cutaneous specimens. Caspofungin followed by posaconazole showed the lowest MICs. All isolates had caspofungin MEC90 (0.063 µg/ml) lower than epidemiologic cutoff values, and 3.5% of the isolates had amphotericin B MICs higher than epidemiologic cutoff values.

Conclusion:
This study demonstrates that, all of A. flavus strains showed a uniform pattern of low MICs for all antifungal agents. Caspofungin and triazoles had better in vitro activity against the A. flavus strains.
GENOTYPING OF CLINICAL AND ENVIRONMENTAL *ASPERGILLUS FLAVUS* ISOLATES FROM IRAN USING MICROSATellites

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Purpose:

*Aspergillus flavus* has been described as the second most important *Aspergillus* species causing human infections in tropical countries. Despite an increasing number of infections of *A. flavus* in Iran, the molecular epidemiology of clinical and environmental strains has not been well studied.

Methods:

We used a panel of nine microsatellite markers to analyze the genetic relatedness of 143 (119 clinical and 24 environmental) *A. flavus*.

Results:

Microsatellite typing of all isolates demonstrated 118 different genotypes. A possible outbreak at a pulmonary ward was discovered. The discriminatory power for the individual markers ranged from 0.4812 to 0.9457 and the panel of all nine markers combined yielded a diversity index of 0.9948.

Conclusion:

This high resolution typing method yielded a better understanding of the molecular epidemiology of *A. flavus* complex.
A STUDY OF THE NUCLEOTIDE SEQUENCE OF THE PROMOTER AREA OF THE CYP51A GENE AND PHOSPHOLIPASE B1 ENZYME TO SENSIVITY AND RESISTANCE TO ITRACONAZOLE IN ASPERGILLUS FUMIGATUS ISOLATED FROM THE ICU OF HOSPITALS IN NORTHERN IRAN

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Purpose:
Invasive aspergillosis is the most threatening disease affecting the patients suffering from immune system defects which causes iatrogenic fungal diseases and high mortality in them. One of the main factors influencing the pathogenesis of this fungus is its capacity to produce and secrete B group phospholipases which causes tissue damages and destruction of cytoplasmic membranes of invaded cells. A survey of molecules has demonstrated that the main resistance against the azole antifungals in Aspergillus fumigatus is related to substitution of amino acid in Cyp51A gene.

Methods:
Extraction of DNA from Aspergillus fumigatus was performed by use of cTAB method, identification of molecules by use of primers for Cyp51A gene, and the primer of B₁ phospholipase by utilizing the PCR technique. After determination of the sequence, Aspergillus fumigatus was separated and the results were compared with the similar species in the gene bank. Afterwards, the test of drug sensitivity to itraconazole via micro-dilution method and by use of the NLCCCLS guideline was performed and its MIC rate was surveyed after incubation for 72 hours.

Results:
Both Cyp51A and PLb1 gene segments were matched after identifying the sequences; and several mutations were observed in the various nucleotide sequences of their promoter region which demonstrated the sensitivity and resistance to itraconazole.

Conclusion:
The Aspergillus fumigatus species may have several mutations in Cyp51A gene which causes resistance and sensitivity to itraconazole. Aspergillus fumigatus species examined were extracted from the ICU air and the presence of the wild type of Aspergillus fumigatus is likely. The mutations happened in the survey of phospholipase B1 is indicative of high virulence of Iranian Aspergillus fumigatus and its resistance to itraconazole.
**ASPERGILLUS NOSOCOMIAL INFECTIONS - DO CRYPTIC SPECIES FOUND IN HOSPITAL ENVIRONMENT MATTER?**

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**Purpose:**
*Aspergillus* is a major threat causing nosocomial infections in immunocompromised patients, especially those subjected to transplantation. Until recently, species identification relied on morphological features. Advances in molecular methods allowed species identification through sequencing of specific genes, allowing high discrimination amongst isolates, which enables the genetic differentiation to species level of morphologically identical isolates. These are the so-called cryptic or sibling species. Different *Aspergillus* species have different susceptibilities to antifungals and several cryptic species have been described as less susceptible to specific antifungals. Therefore, we addressed the possible influence of hospital environmental isolates in the overall situation of *Aspergillus* antifungal resistance.

**Methods:**
During one year, 101 air and 99 surface samples were collected from Hematology, Oncology and Intensive Care units of the Portuguese Central Hospital of Lisbon. *Aspergillus* isolates were identified morphologically and by molecular methods. Genomic DNA was prepared from each isolate and the sequencing of the Internal Transcribed Spacers (ITS) regions was used to determine the species complex. Sequencing of the β-tubulin and calmodulin genes was done to achieve the correct species identification. Determination of the antifungal susceptibility of selected isolates was performed by microdilution (CLSI M38-A2). The antifungal agents studied were deoxycholate amphotericin B, itraconazole, voriconazole, and posaconazole.

**Results:**
From the 200 samples collected, 75 isolates of *Aspergillus* were isolated and identified to section by ITS sequence; cryptic species were identified by β-tubulin and calmodulin sequencing. Ten different sections within the *Aspergillus* genus were identified: *Versicolores* (N=20), *Nigri* (N=11), *Flavi* (N=10), *Circumdati* (N=10), *Fumigati* (N=8), *Usti* (N=4), *Terrei* (N=4), *Nidulantes* (N=4), *Aspergilli* (N=3) and *Cremei* (N=1). From these, 25 different *Aspergillus* species were identified by β-tubulin and calmodulin sequencing, and a high percentage of cryptic species (not sensu stricto) was found (59%). Sections *Usti*, *Versicolores* and *Circumdati* harbored the highest proportion of cryptic species [100% (4/4), 95% (19/20) and 90% (9/10), respectively]. From the 75 isolates, 22 were tested for their antifungal susceptibility. Of the 8 *Fumigati* isolates, there was 1 cryptic species (*Neosartorya hiratsukae*). The *Circumdati*, *Versicolores* and *Nigri* complexes contained isolates of cryptic species with reduced susceptibility to some of the antifungals used in clinical therapeutics. In the *Circumdati* complex, 3/8 isolates had MIC to amphotericin B >8µg/ml (*A. westerdjikae*) and 1/8 MIC >8µg/ml to itraconazole (*A. sclerotium*); 1/5 isolates from *Versicolores* complex had MIC to itraconazole >8 µg/ml (*A. sidowii*), all 4 isolates from *Nigri* (*A. tubigensis, phoenicis and niger sensu stricto*) complex had MIC to itraconazole= 4 µg/ml.

**Conclusion:**
Although aspergillosis caused by cryptic species remain less frequent than infections caused by “sensu stricto” isolates, in a recent study of *Aspergillus* clinical isolates collected in different Portuguese health institutions, we found that 19% of those were cryptic species. Since *Aspergillus* infections are mainly nosocomial, the knowledge of the molecular epidemiology and determination of the susceptibility profile of environmental isolates, which have a much higher frequency of cryptic isolates, may allow preventive or corrective measures to be taken. As a consequence, a decreased exposure to those organisms and a better prognosis is expected.
IDENTIFICATION AND FUNCTIONAL CATEGORIZATION OF PROTEINS INVOLVED IN GERMINATION OF CONIDIA OF *ASPERGILLUS FLAVUS*

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**Purpose:**
The ability of the *Aspergillus flavus* to adapt within host environment is crucial for its colonization. Other than *Aspergillus fumigatus*, inhalation of *A. flavus* conidias resulted in the second most leading cause of aspergillosis. Onset of germination of conidia is one of the crucial events, thus in order to gain insight of the *A. flavus* adaptation while germinating, intracellular cellular protein profile of *A. flavus* MTCC9367 was obtained.

**Methods:**
About more than 90% of conidia showed germination after 7h of incubation at 30°C in Sabouraud dextrose broth. Thus, samples were collected at this time-point for protein extracted from two biological samples. Protein profiling was performed in triplicate from the protein sample on a nanoflow LC Q-TOF mass spectrometer. The spectral data from MS scan from *A. flavus* MTCC9367 protein sample were search using Protein Lynx Global Server (PLGS 2.2.5) software against the *A. flavus* and *Aspergillus* species of UniProt databases.

**Results:**
A total 412 proteins were identified from different *Aspergillus* species databases, out of which 152 proteins were from *A. flavus*. Orthologs of *A. flavus* proteins were observed in *A. fumigatus*, *A. parasiticus*, *A. niger*, *A. terreus*, *A. clavatus* etc. Number of proteins in our analysis showed match with proteins sequence from other *Aspergillus* species but not to *A. flavus* protein sequence available at Uniprot database. Functional categorization of proteins resulted majorly to cell wall biogenesis/organization, carbohydrate and amino acid metabolism, energy, transcription, protein synthesis and degradation. Proteins/enzymes associated with secondary metabolite production, aflatoxin biosynthesis were also observed. We also observed Dicer-like protein 1 & 2 involved in post-transcriptional regulation. Proteins involved in cell signaling such as serine threonine protein kinases (e.g., nek1, nek2, nek3, ste20, MARK2, atg1, ssn3, cbk1) & serine threonine protein phosphatase were also observed.

**Conclusion:**
Overall the data presents the catalogue of proteins/enzymes, transcriptional regulator or activator involved in germination of *A. flavus* conidia and could be applied to other *Aspergillus* species.
RESPONSE OF THE BRONCHIAL EPITHELIUM TO *ASPERGILLUS FUMIGATUS* - A TRANSCRIPTOME ANALYSIS

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Purpose:
Inhalation of *A. fumigatus* spores represents the primary mode of human infection, disease manifestations being dependent on the underlying state of the host immune system. The bronchial epithelium is increasingly recognised as the first line of defence against inhaled *Aspergillus* however the transcriptional/translational responses of the epithelium are poorly defined. We describe the change in the transcriptional response of the bronchial epithelium to challenge with *A. fumigatus* over 12 hours establishing key biological pathways and processes involved in the early epithelial response to infection.

Methods:
Confluent monolayers of the transformed epithelial cell line 16HBE were challenged with live *A. fumigatus* conidia. RNA was harvested at 0.5 hours, 3 hours, 6 hours, 9 hours and 12 hours and sequenced using the Illumina TruSeq® platform. Data analysis was undertaken using a Tophat2 – DESEQ2 pipeline then by Ingenuity Pathway Analysis using a filter of p<0.05, any log2fold change, and comparison of infected samples to the corresponding uninfected time point control.

Results:
11,022 genes were statistically viable for analysis. At 0.5 hours 23 genes were differentially regulated with fold changes between -1.625 and +1.113. All genes mapped to processes involved in normal cellular function and processing. At three hours the transcriptional response changed little with 24 genes being upregulated, most genes involved in maintenance of the basal cell state.

At 6 hours the transcriptional response changed significantly with 305 genes upregulated and 152 downregulated. Network analysis at this time point demonstrates strong upregulation of pathways involved in organisation of the cytoskeleton (79 genes), organisation of the cytoplasm (84 genes) and endocytosis (22 genes). Further interrogation demonstrates genetic upregulation in microtubule dynamics, the formation of lamellipodia, and the organisation and formation of actin filaments. This strongly suggests changes occur in the cytoskeleton of the bronchial epithelium in response to germinating conidia, perhaps facilitating conidial internalisation. Further canonical pathway analysis demonstrates epithelial adherens junction signalling and epidermal growth factor signalling, known to be involved in MAPK and subsequent JAK-STAT signalling, are enriched in the data set at this time point. No genes were significantly up or down regulated at 9 hours.

At 12 hours 31 genes were upregulated, 62 were down regulated. Genes mapped to pathways involved in cellular apoptosis, recognition of cell damage and reduction in chemotaxis of myeloid and phagocytic cells (activation Z scores of -2.159 and -1.709 respectively).

At no time point was a prominent inflammatory transcriptional response demonstrated. Network analysis of upstream regulators across the time points has demonstrated a prominent role for MAPK driven signalling pathways at 3, 6 and 12 hours.

Conclusion:
The transcriptional response of the bronchial epithelium to challenge with *A. fumigatus* is greatest at 6 hours. Associated changes in the cytoskeleton and the cytoplasm may suggest a phagocytic epithelial response, or represent cellular responses to early damage to the monolayer. The lack of a significant transcriptional inflammatory response at any time point may suggest the bronchial epithelium presents a muted and permissive inflammatory response to early *Aspergillus* infection allowing latent reservoirs of infection to develop.
THE ASPERGILLUS FUMIGATUS SCHASCHE9 KINASE MODULATES SAKA MAP KINASE ACTIVITY, IS IMPORTANT FOR OSMOTIC AND IRON STRESSES, SPHINGOLIPIDS BIOSYNTHESIS AND IT IS INVOLVED IN VIRULENCE

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Purpose:
Calcium signalling is essential for A. fumigatus pathogenicity and is regulated by the CrzA transcription factor. Recently, we used ChIP-seq (Chromatin Immunoprecipitation DNA sequencing) to explore gene targets of the A. fumigatus transcription factor. The PhkB histidine kinase and the SskB MAP kinase kinase kinase of the HOG pathway were regulated by CrzA. Several members of the two-component system (TCS) and the HOG pathway were more sensitive to calcium. CrzA::GFP was translocated to the nucleus upon osmotic stress. We observed that CrzA is important for the phosphorylation of the SakA HOG1 MAPK in response to osmotic shock. These results suggest there is a genetic interaction between the A. fumigatus calcineurin-CrzA and HOG pathway that is essential for full virulence. One of the gene targets identified in this screening was the Sch9 homologue, named SchA.

Methods:
We have used a series of procedures, such as, construction of deletion and GFP mutants, extensive phenotypic assays, immunoblot analysis, label-free quantitative proteomics and lipid analysis by mass spectrometry, Metabolite profiling analysis by gas chromatography, RNA sequencing, virulence studies by using a murine model of pulmonary aspergillosis, lung histopathology and fungal burden, aiming to characterize the biological functions of A. fumigatus SchA.

Results:
Here, we showed that ΔschA mutation is more sensitive to rapamycin, high concentrations of calcium, hyperosmotic stress, and it is involved in iron metabolism. We evaluated through transcriptomic and proteomics possible direct or indirect targets of SchA upon exposure to hyperosmotic stress. The schA null mutant has an increased SakA phosphorylation. SchA::GFP is localized in the mitochondria during non-stressing conditions, and upon osmotic or iron stresses it remains in the mitochondria, but these treatments induce a fission process in the mitochondrial morphology. Finally, ΔschA is avirulent in a low dose murine infection model.

Conclusion:
This study revealed novel functions for A. fumigatus SchA, suggesting its involvement with in several cell functions and virulence. In addition, evidences linking the HOG, Ca²⁺-calcineurin/CrzA, sphingolipids biosynthesis, and iron assimilation pathways were presented. Importantly, we propose that SchA serves as a mediator of the TOR and HOG pathways. Further studies are necessary to fully understand biochemical interaction and how these two pathways cross-talk during a response to different environmental stresses and pathogenicity. In A. fumigatus, both the HOG and calcium-calcineurin/CrzA pathways are important to stress responses and virulence in a mammalian host. Therefore, the identification of the link between these central pathways and TOR will provided new avenues for research into the identification of novel targets for disease intervention.

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MITOGEN ACTIVATED PROTEIN KINASES SAKA\textsuperscript{HOG1} AND MPKC COLLABORATE FOR \textit{ASPERGILLUS FUMIGATUS} VIRULENCE

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Purpose:
In filamentous fungi the conserved MAPK pheromone response, filamentous growth, osmotic stress response and cell wall integrity (CWI) pathways have been shown to influence numerous virulence traits including invasive growth, biofilm formation, mycotoxin production and antifungal tolerance. \textit{A. fumigatus} has four MAPKs: (i) MpkA, the central regulator of CWI pathway also plays a role in oxidative stress, (ii) MpkB is the putative homologue of filamentous growth/pheromone response pathway, still uncharacterized and (iii) MpkC and SakA, homologues of the \textit{Saccharomyces cerevisiae} Hog1, constitute the main regulator of the high osmolarity glycerol response (HOG) pathway. The MpkC protein sequence is very similar to that of SakA. SakA and MpkC have also been shown to play a role in caspofungin adaptation and carbon source utilization, respectively. Our laboratories have been investigating \textit{A. fumigatus} MAPKs and their importance for the establishment of virulence/pathogenicity and mediation of drug resistance.

Methods:
We have used a series of procedures, such as, construction of deletion and GFP mutants, extensive phenotypic assays, immunoblot analysis, and virulence studies by using a murine model of pulmonary aspergillosis, lung histopathology and fungal burden, aiming to characterize the biological functions of \textit{A. fumigatus} SakA and MpkC.

Results:
Here, we investigated which stress responses were influenced by the MpkC and SakA mitogen-activated protein (MAP) kinases of the high-osmolarity glycerol (HOG) pathway in the fungal pathogen \textit{Aspergillus fumigatus}. The \textit{ΔsakA} and the double \textit{ΔmpkC ΔsakA} mutants were more sensitive to osmotic and oxidative stresses, and to cell wall damaging agents. Both MpkC::GFP and SakA::GFP translocated to the nucleus upon osmotic stress and cell wall damage, with SakA::GFP showing a quicker response to both stresses. The phosphorylation state of MpkA was determined post exposure to high concentrations of Congo Red and Sorbitol. In the wild-type strain, MpkA phosphorylation levels progressively increased in both treatments. In contrast, the \textit{ΔsakA} mutant had reduced MpkA phosphorylation, and surprisingly, the double \textit{ΔmpkC ΔsakA} had no detectable MpkA phosphorylation. \textit{A. fumigatus} \textit{ΔsakA} and \textit{ΔmpkC} were virulent in mouse survival experiments, but they had a 40 % reduction in fungal burden. In contrast, the \textit{ΔmpkC ΔsakA} double mutant showed highly attenuated virulence, with approximately 50 % mice surviving and a 75 % reduction in fungal burden. We propose that both CWI and HOG pathways collaborate, and that MpkC could act by modulating SakA activity upon exposure to several types of stress and during cell wall biosynthesis.

Conclusion:
In summary, we have identified an interaction between MpkC and SakA to counteract osmotic, oxidative, high temperature stresses, and also to regulate cell wall biosynthesis. Furthermore, this
interaction is essential for virulence and macrophage recognition. It remains to be investigated how MpkC and SakA are affecting MpkA phosphorylation and the organization of the cell wall. Since most of the phenotypes observed for ΔmpkC were milder than for ΔsakA mutant, we propose that both the CWI and HOG pathways collaborate, and that MpkC could act by modulating SakA activity upon exposure to several different types of stress and during cell wall biosynthesis.

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MODELLING ASPERGILLOSIS SENSITIVITY DETERMINANTS
RECONSTRUCTED IN BRONCHIAL EPITHELIAL CELLS

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Purpose:
Aspergillosis comprises a wide spectrum of fungal diseases with clinical manifestations that range from disseminated, chronic or colonization. One form of clinical disease may evolve into another over time depending on the immune status of the host but it is not known why all patients with comparable immune impairment do not suffer from disease. The aim of this study is to re-create genetic variants associated with susceptibility to allergic bronchopulmonary aspergillosis (ABPA) and chronic pulmonary aspergillosis (CPA) in human bronchial epithelial cells and, to study their importance in susceptibility to Aspergillus exposure.

Methods:
Variants involved in susceptibility to ABPA and CPA were identified in a large exome sequencing study. The four most important genetic variants in three genes involved in epithelium maintenance, were recreated in 16HBE bronchial epithelial cells by using CRISPR-Cas9. To construct CRISPR-Cas9 plasmids targeting human genes, sense and antisense oligonucleotides were designed and cloned into an all-in-one vector containing OFP as a reporter. Forty-eight hours after transfection, cells were analysed and sorted by FACS and cell cultures propagated for 3 weeks. Sequence analysis was performed to confirm gene modification. Changes in the morphology of the epithelial monolayer and in its transmembrane resistance were determined. Moreover, CRISPR cell lines were challenged with Aspergillus fumigatus spores (CEA10) and incubated at 37C for 4h and changes in the epithelial integrity were microscopically observed.

Results:
We have standardized a CRISPR-Cas9 genome-editing system to introduce genetic variants associated with ABPA and CPA in human bronchial epithelial cell lines. The efficiency of the transfection was higher than 70% and the re-created cells could be propagated in culture. Genome editing was confirmed by sequencing a 500bp of the target region. Striking differences in the structure of the epithelial monolayer in CRISPR cell lines were observed when comparing to 16HBE cells suggesting a role for these genes in epithelium maintenance. Herein, the re-created epithelial cells showed a 30-40% reduction in transepithelial resistance compared with non-transfected cells. Moreover, the CRISPR cell lines were more susceptible to Aspergillus exposure and epithelial desquamation 4 hours after infection with multiplicity of infection ranging from 1-0.1.

Conclusion:
Our study reveals novel insight into genetic susceptibility to fungal disease and provides proof-of-concept for the generation on in-vitro models of aspergillosis via CRISPR-Cas9 mediated gene targeting. More studies are needed to functionally confirm the role of these mutations in the different clinical forms of aspergillosis.
THE ASPERGILLUS FUMIGATUS FARNESYLTRANSFERASE β-SUBUNIT, RAM1, REGULATES SPATIOTEMPORAL ACTIVATION OF RASA AND IS REQUIRED FOR VORICONAZOLE SENSITIVITY

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Purpose:
The Ras post-translational modification (PTM) pathway is a highly studied series of protein modifications required for proper spatiotemporal control of Ras network signaling. One important Ras PTM that has been targeted for anti-cancer therapies is the addition of a 15-carbon isoprenoid group (a farnesyl moiety) to the conserved CAAX motif of Ras proteins. Farnesylation of a nascent Ras proteins in the cytosol drives their localization to the endomembranes for further processing. Thus, when farnesylation is defective, Ras proteins are typically mislocalized and Ras-mediated signal transduction is diminished. The farnesyltransferase (FTase) enzyme complex is composed of two subunits: the α-subunit (Ram2), an essential protein shared with the geranylgeranyltransferase complex; and a β-subunit, termed Ram1 in fungal organisms. Previous data from our lab indicate that A. fumigatus RasA localizes primarily to the plasma membrane, where it functions in processes controlling morphogenesis and virulence.

Methods:
To explore the impact of protein farnesylation on Ras-mediated processes in A. fumigatus, we generated a deletion mutant lacking the FTase β-subunit (Δram1). This mutant was analyzed for changes in growth characteristics and ability to properly localize a GFP-tagged RasA fusion protein.

Results:
Conidial germination rate was reduced in the Δram1 mutant, with a concomitant reduction in conidial viability of 45%. Morphogenesis defects were apparent, as the Δram1 mutant displayed reduced radial growth rate, increase in hyphal width, and altered nuclear positioning in growing hyphae versus the wild-type background strain. Furthermore, loss of ram1 resulted in an increased resistance to voriconazole that was independent of triazole target gene upregulation. Despite the observed growth defects, no significant difference in virulence between the wild type and Δram1 was noted in a murine model of invasive aspergillosis. To define the contribution of Ras-mediated signaling to Δram1-associated phenotypes, we generated strains expressing GFP- tagged RasA in the Δram1 mutant. The absence of ram1 resulted in mislocalization of RasA from the plasma membrane. However, mutation of the RasA CAAX motif (RasA^{M213L}) to enhance selectivity for geranylgeranylation as an alternative membrane targeting mechanism, restored RasA plasma membrane localization in the Δram1 background. Interestingly, growth kinetics of the Δram1/ RasA^{M213L} strain were not complemented to wild type levels.

Conclusion:
These findings point to a crucial role for the FTase complex in mediating A. fumigatus growth and suggest that Ras-independent mechanisms may contribute to defective hyphal morphogenesis in the farnesylation-deficient mutant.
IDENTIFICATION OF Δ9-DESATURASE AS THE TARGET OF A NOVEL POTENT ANTIFUNGAL AGENT

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Purpose:
Triazole antifungals represent the gold standard for treatment of infections caused by the fungal pathogen Aspergillus fumigatus. Resistance to this class is growing due to prolonged exposure of patients to these drugs and the widespread use of azoles in agriculture. The mortality rate for individuals who are infected with a resistant isolate exceeds 85%. Novel antifungal drugs are urgently needed. In this study we identify and validate the molecular target of a novel and potent antifungal agent.

Methods:
A variety of genetic, genomic as well as biochemical approaches including chemical genomics, transcriptional profiling and, phenotypic profiling were used to identify delta9-desaturase (sdeA) as the likely drug target of the novel antifungal. To analyse the suitability of delta9-desaturase as potential drug target in A. fumigatus, a tetracycline/doxycycline inducible sdeA strain (sdeA\text{TET}) was created to assess its role in virulence.

Results:
We confirmed the association between the antifungal compound and sdeA with both targeted gene expression analysis and quantification of the substrate:product ratio (stearic acid:oleic acid) of delta9-desaturation in cells exposed to the compound. sdeA\text{TET} was unable to grow in the absence of the inducer doxycycline indicating that sdeA is essential for viability. Supplementation with oleic acid only partially reversed the growth defect of the strain. sdeA\text{TET} was avirulent in both an insect (Galleria melonella) and a murine model of infection. This lack of virulence was reversed by doxycycline treatment.

Conclusion:
Taken together, this study shows that SdeA is the target of the novel antifungal compound and demonstrates the target is critical for virulence, which further validates its suitability as antifungal drug target.
SRBA AND THE CCAAT BINDING COMPLEX – UNVEILING NOVEL TRANSCRIPTIONAL REGULATORY MECHANISMS IN ASPERGILLUS FUMIGATUS AZOLE RESISTANCE

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Purpose:
Triazoles provide the first line therapy for treatment of Aspergillus disease however, resistance to this class is emerging with some centres reporting that up to 20% of infected individuals harbour resistant isolates. The mortality rate for these individuals exceeds 85%. The primary cause of triazole resistance in clinical isolates of A. fumigatus is modification of cyp51A or its promoter. Although several mutations have been shown to promote resistance, isolates that harbor a tandem repeat of a 34 mer within the 5’ non-translated region of cyp51A and an L98H amino substitution (designated TR34/L98H) represent the predominant cause of resistance. However, in some clinical centres the cause of resistance in around 50% of strains is not directly linked to modification of cyp51A. One alternative resistance mechanism has been proposed based on the amino acid substitution P88L within the HapE subunit (HapE_P88L) of the heterotrimeric CCAAT binding complex (CBC).

Methods:
We apply various molecular approaches such as gene deletion, site-directed promoter mutagenesis, protein/DNA interaction analysis to elucidate TR34 and HapE_P88L driven azole resistance.

Results:
We demonstrate that TR34 driven azole resistance is based on increased binding of SrbA, a transcriptional regulator known to activate cyp51A gene expression, to the cyp51A promoter. In addition, we show that CBC mutation caused azole resistance is a result of upregulation of multiple ergosterol biosynthetic genes and increased sterol production.

Conclusion:
Taken together, this study unveils molecular mechanisms by which TR34 and CBC mutation cause increased resistance to clinically administered antifungal azole drugs.
THE PLASMA MEMBRANE H\(^{+}\)-ATPASE PMA-1 IS A KEY TARGET PROTEIN OF THE ANTIFUNGAL PEPTIDE PAF26

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Purpose:
The significance of fungal infections has been grossly underestimated. Only a few drugs are clinically available to treat life-threatening fungal infections, and resistance against these drugs is rising. Natural and synthetic antifungal peptides (AFPs) are being actively explored as novel pharmaceuticals. PAF26 is a de novo-designed hexapeptide possessing high antifungal activity and low cytotoxicity against mammalian cells. Despite its good drug-like properties, the identification of its target protein(s) within the fungal cell has been elusive. The main aim of this study was to identify a key target protein of PAF26 in filamentous fungi and to use this knowledge to design improved AFPs for therapeutic use.

Methods:
The range of proteins from \(N. \ crassa\) that interact either directly or indirectly with PAF26 was first narrowed down using a pull down approach with fluorescently labelled PAF26. A comparative computational analysis of one of these potential target proteins (the well characterised, fungus-specific and essential plasma membrane H\(^{+}\)-ATPase, PMA-1) was performed to identify its hypothetical PAF26 binding site in the fungal model \(Neurospora \ crassa\). Four novel peptides (CMN01-CMN04) derived from PAF26 were computationally designed to increase the binding affinity of PAF26 to PMA-1 in \(N. \ crassa\). These peptides were synthesised and their antifungal activities were tested against \(N. \ crassa\) and fungal pathogens \(Aspergillus \ fumigatus\) and \(Fusarium \ oxysporum\). The influence of PAF26 on cytoplasmic pH dynamics in single living \(N. \ crassa\) hyphae was analysed by using confocal ratio imaging of the pH probe SNARF-1.

Results:
The likely binding site of PAF26 and the conformation and orientation of the PAF26 bound to the PMA-1 protein were demonstrated by the in-silico analysis. The predicted affinities of the four CMN AFPs rationally designed to target \(N. \ crassa\) PMA-1 and its orthologs in the two human pathogens correlated well with their measured IC\(_{50}\) values against each fungus. A cytoplasmic acidification was observed after the addition of PAF26 indicating a likely inhibition of PMA-1. These results provide very strong evidence that PMA-1 is one of the major binding targets of PAF26 in fungal cells.

Conclusion:
Our study has provided novel mechanistic insights into the mode-of-action of PAF26 by discovering that PMA-1 is a key target protein of it. It also demonstrates how structural modification of PAF26 can lead to an improvement in its antifungal activity.
SPECIES IDENTIFICATION AND AZOLE SUSCEPTIBILITY OF *ASPERGILLUS* SECTION *FLAVI* CLINICAL ISOLATES FROM FRANCE

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**Purpose:**
Fungi belonging to *Aspergillus* section *Flavi* are of great economic importance due to their ability to produce carcinogenic mycotoxins. They may also be responsible for invasive aspergillosis in humans and animals. The high number of species with section *Flavi* and their morphological similarity make it difficult to ascertain their clinical and epidemiological particularities. In this study we present molecular characterization and azole susceptibility of clinical isolates belonging to section *Flavi* and collected from patients in two French hospitals.

**Methods:**
Eighty-two isolates were included in the study. These clinical isolates were recovered from different specimens (essentially respiratory specimens) over a 15-year period (2001-2015) and stored frozen. The isolates were initially identified as members of *Aspergillus* section *Flavi* by morphological characteristics. After subculture, each isolate was identified to the species level by sequencing a part of the β-tubulin and calmodulin genes. The isolates were also screened for their susceptibility to itraconazole and voriconazole.

**Results:**
Among the 82 isolates, molecular analysis of the partial β-tubulin and calmodulin sequences showed that 80 isolates were *A. flavus* and 2 isolates were *A. parasiticus* and *A. tamarii*, respectively. These 2 isolates were isolated from sputa and were not resistant to azoles. Among *A. flavus* isolates, a limited polymorphism was observed for the partial β-tubulin gene: for 75 isolates (91%), sequences were identical to the reference sequence (*A. flavus* isolate NRRL 1957 beta-tubulin gene, partial cds, ACCESSION EF661485). For partial calmodulin gene, several polymorphisms were observed with only 14 sequences (17%) identical to the reference sequence (*A. flavus* isolate NRRL 1957 calmodulin gene, partial cds, ACCESSION EF661508). Two *A. flavus* isolates were resistant to voriconazole but not to itraconazole. Analysis of *CYP51A* gene polymorphism for these isolates is in progress.

**Conclusion:**
For the first time in France, we molecularly characterized a large collection of clinical isolates belonging to *Aspergillus* section *Flavi*. Most of the isolates were identified as *A. flavus* and were susceptible to azole antifungal drugs. Nevertheless, the occurrence of a few resistant isolates highlights the importance of antifungal susceptibility testing.
A FATAL INVASIVE PULMONARY ASPERGILLOSIS DUE TO *ASPERGILLUS PSEUDODEFLECTUS* IN A LIVER TRANSPLANT PATIENT: A FIRST CASE REPORT

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Purpose:
Liver transplant patients are a population at risk for invasive aspergillosis. *Aspergillus fumigatus* is the most responsible species. However, other non-*fumigatus* species can be involved with reduced sensitivity to antifungal drugs. Accurate identification, based mainly on molecular biology, is thus essential to adapt the therapy. We report an invasive pulmonary aspergillosis due to *Aspergillus pseudodeflectus* in a liver transplant patient. To our knowledge, this is the first reported case of invasive aspergillosis due to this species.

Clinical case:
In May 2013, a 64 year-old woman was admitted in surgical intensive care for monitoring after a liver transplantation for cirrhosis post-hepatitis C. Six weeks after transplantation, CT scan showed a right pulmonary opacity associated with an increase of galactomannan antigen and β-D-glucan. A BAL was then performed. Direct examination of BAL showed *Aspergillus*-like branching hyphae. Several colonies of a white to brown filamentous fungus with a velvety appearance were obtained in culture on Sabouraud media at 30° and 37°C. Microscopic examination of the colonies showed *Aspergillus* biseriate conidial heads with curved conidiophores. A molecular identification was done, based on partial β-tubulin and calmodulin genes. A BLAST search in GeneBank and MycoBank revealed a sequence identity to 99.78% to the *A. pseudodeflectus* reference sequence CBS 596.65. This species belongs to *Aspergillus* section Usti and is very close to *Aspergillus calidoustus* previously reported in human pathology. The antifungal susceptibility tests revealed low MICs to echinocandines and amphotericin B but high MICs to azoles. After these results, the patient, initially treated with voriconazole was switched for amphotericin B. Unfortunately she died one month after diagnosis.

Conclusion:
We diagnosed an invasive pulmonary aspergillosis in a liver transplant patient due to a new species *A. pseudodeflectus* described in human pathology. This species showed less susceptibility to azoles. This highlights the significance of molecular identification of *Aspergillus* species regarding the reduced susceptibility profile.
VALIDATION OF AN IN VITRO MODEL OF MIXED BIOFILM ASSOCIATING ASPERGILLUS FUMIGATUS AND STENOTROPHOMONAS MALTOPHILIA

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Purpose:
Biofilms are communal structures of microorganisms which have been associated with a variety of persistent infections that may respond poorly to conventional antibacterial or antifungal therapy. The aim of the present study was to develop an in vitro model for a mixed biofilm associating a bacterium, Stenotrophomonas maltophilia (Sm) and a fungus, Aspergillus fumigatus (Af). These microorganisms coexist, especially in a biofilm, in the respiratory tract of patients with cystic fibrosis or immunocompromised patients.

Methods:
We used an Af strain expressing a Green Fluorescent Protein (GFP) (ATCC 13703) and Sm strain ATCC 13637. Fungal and/or bacterial cultures (10⁵ spores/mL and 10⁶ bacteria/mL respectively) (4 days and 1 day, respectively) suspended in RPMI 1640 medium supplemented with 10% SVF were used for the development of in vitro mixed biofilm in 8-well polystyrene chambers Labteck® or in 96-well plates at 37°C. Bacteria were deposited simultaneously with Af on the support. The biofilm formation was monitored microscopically by fluorescent microscopy and by scanning electron microscopy and quantified by qPCR and crystal violet.

Results:
Analytic methods revealed Sm and Af structures typical of biofilm formation with presence of bacteria and fungal hyphae embedded in an extracellular matrix. Quantitative methods showed a significant growth of the biofilm with a slight inhibition of the fungus in the multispecies biofilm.

Conclusion:
For the first time, a mixed biofilm Af - Sm was validated by various analytical and quantitative methods. The development of this mixed biofilm made it possible to evaluate the activity of antimicrobial agents and analyze the interactions between the biofilm and epithelial cells.
ASPERGILLUS FUMIGATUS CYP51A POLYMORPHISMS: SNPS WITH OR WITHOUT SIGNIFICANCE?

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Introduction:
The triazole antifungal compounds are the first treatment of choice for invasive aspergillosis (IA). However, in the last decade azole resistance has increased worryingly. The main resistance mechanisms are well-defined and mostly related to the azole antifungal target 14α-sterol demethylase (Cyp51A). Several points mutations in cyp51A (G54, G138, M220, G448) and/or its promoter (TR34/L98H, TR46/Y121F/T289A, TR53) have been described. In addition, around 10% of Aspergillus fumigatus strains show a combination of eight, missense and silent, Cyp51A mutations (F46Y, G89G, M172V, N248T, D255E, L358L, E427K, and C454C) and have been reported in many European countries, EEUU, Turkey, India, China and Australia. Some authors have pointed out that the distribution of isolates harbouring cyp51A polymorphisms (cyp51A-SNPs) could be influenced by azole pre-exposure. In general, these strains show increased azole MICs, although based on the azole susceptibility breakpoints they would not be considered as resistant.

Purpose:
The aim of this study was to determine the role of these cyp51A polymorphisms in their azole susceptibility profile.

Methods:
Azole susceptibility testing, using E-test and EUCAST broth microdilution method, was performed in a panel of twelve A. fumigatus strains with the described SNPs. One strain was selected for cyp51A gene deletion. In addition, cyp51A gene expression was performed using qRT-PCR. The potential correlation between every SNPs and the azole susceptibility profile was evaluated, modeling both A. fumigatus proteins, WTCyp51A and Cyp51A-SNP, by homology based on the crystal structure of A. fumigatus Cyp51B in complex with voriconazole.

Results:
All strains were slightly less susceptible to azole drugs compared to wild-type (WT) strains without SNPs, reaching voriconazole MICs of 0.5-1. The deleted mutant (deltacyp51A) strain was morphologically indistinguishable from its parental strain, regarding macroscopic and microscopic morphology as well as colony radial growth. The cyp51A deleted mutant showed an azole hypersusceptible phenotype with a decreased in azole MICs of eight fold times. No differences in cyp51A gene expression between WTCyp51A and WTCyp51A-SNP, by homology based on the crystal structure of A. fumigatus Cyp51B in complex with voriconazole.

Conclusion:
A. fumigatus isolates with a combination of missense mutations (F46Y, M172V, N248T, D255E, and E427K) show slightly higher azoles MICs than WT strains. This phenotype might be due to some of these SNPs, being F46Y a good candidate. Molecular dynamics of Cyp51A–azole complexes homology models to assess the implications of these mutations in voriconazole response should be done. Further studies are warranted to better assess the clinical outcome of patients infected with these strains.
MALDI-TOF-MASS-SPECTROMETRY IS A UNIVERSAL INSTRUMENT FOR ROUTINE IDENTIFICATION AND RESEARCH OF PHYLOGENY AND STRAINS VARIABILITY OF ASPERGILLUS SPP.

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Purpose:
The purpose of this study is the development of application of mathematical methods for processing of mass-spectra collected by MALDI-TOF-MS for investigation the similarities and differences between 	extit{Aspergillus} spp. at the levels including whole genus, selected sections and groups of strains.

Methods:
150 clinical isolates of 	extit{Aspergilli} belonging to 20 species from Russian Collection of Pathogenic Fungi (Saint-Petersburg, Russia) were identified by morphology, by MALDI-TOF-MS as described previously (Riabinin I.A. et al., Advances Against Aspergillosis – 2014) and by DNA-sequencing of \(\beta\)-tubuline locus according CLSI MM18 protocol. 384 mass-spectra (MS) of protein extracts were collected from chosen strains by repeated procedures. All amount of MS was investigated by principal component analysis in MALDI Biotyper OC 3.1 (Bruker Daltonics, Germany) with construction of 3D-cluster. For selection of the optimal methods of hierarchical clustering model group of MS was selected with following criteria: for \textit{A. fumigatus}, \textit{A. flavus}, \textit{A. niger} and \textit{A. terreus} – 5 MS with “score value” not less than 2,0; for \textit{A. niger}, \textit{A. awamori} and \textit{A. niger/awamori} (intermediate variants) – 5 MS with the highest level of score value; for other species – all MS with “score value” not less than 1,7. 56 different dendrograms of hierarchical clustering were constructed by combining of 8 methods of distance measurement and 7 linkage algorithms. Created dendrograms were compared with DNA-sequence-based phylogeny of \textit{Aspergillus} spp.

Results:
PCA-3D-cluster shown that MS were grouped in accordance with sections of the genus \textit{Aspergillus} (e.g. MS of \textit{A. flavus}, \textit{A. oryzae} and \textit{A. tamarii} were placed in aggregated area according to their membership in the section \textit{Flavi}). MS of each analyzed sections was distributed along axis, where the lower ends closely converged and areasnear them consisted of MS with predominance of species-specific peaks. In contrast, upper ends diverged and areas near them consisted of MS with predominance of strains-specific peaks. In result of our study the combination of distance measurement by Minkowski and linkage algorithm by Ward was chosen as the best concordant to modern phylogeny of \textit{Aspergillus} spp. Particularly MS of \textit{A. fumigatus}, \textit{A. terreus} and \textit{A. clavatus} were collected to conjoint clade as subgenus \textit{Fumigati}, MS of \textit{A. nidulans}, \textit{A. ustus}, \textit{A. calidoustus}, \textit{A. versicolor} and \textit{A. sydowii} strains were aggregated to conjoint clade as section \textit{Nidulantes sensu lato} (including “\textit{A. nidulans} – group”, sections \textit{Versicolores} and \textit{Usti}). Further features of chosen algorithm of hierarchical clustering are shown on Fig. 1.

Conclusion:
Our research proved that the MALDI-TOF-mass spectrometry and associated mathematical analysis the aggregate of mass-spectra allow to carry out investigations of \textit{Aspergillus} spp. related to the establishment of their phylogenetic relationships. Also succeeded to prove that the mass-spectra of protein extracts, despite of their variability, include stable markers, reflecting belonging of aspergilli to species, sections and subgenera.
Figure 1. Dendrogram obtained by chosen algorithm of hierarchical clustering for selected MS of *Aspergillus* spp.
Purpose:
Pa and Af co-habit airways of immunocompromised hosts. Pa, or its soluble culture filtrate, have been shown to inhibit Af or its biofilm. However, the relative contributions of inhibitory Pa products are unknown. We studied a series of mutants defective in the production of most known virulence factors, bioactive molecules and secondary metabolites previously reported to interact with eukaryotic cells, and compared their inhibitory activities against Af to that of the parent strain, PA14.

Methods:
Live Pa cells or culture filtrates from planktonic Pa growth or growth as Pa biofilm were incubated with Af during Af biofilm formation or on preformed Af biofilm (thus 6 possible conditions compared). A 96-well plate, RPMI1640 medium, and XTT readout of Af inhibition were used as previously described, with 12-28 replicate wells/experimental condition each time, and compared to a concurrent Af control (no Pa exposure). \( P < 0.05 \) was considered significant.

Results:
In 12 experiments, PA14 was shown equivalent to Pa10, a markedly inhibitory cystic fibrosis isolate. Sixteen Pa mutants were studied the 6 ways. Only 1 mutant failed (in 2/6 conditions) to significantly inhibit Af compared to Af control, \( \text{pvdD-/pchE-} \), a pyoverdin-pyochelin double siderophore mutant.

Then in comparison with the parent, \( \text{pvdD-/pchE-} \) mutant was significantly less inhibitory (4/6 conditions), \( \text{mvfR-} \) (underproduction of various quorum sensing-regulated factors related to loss of 4-hydroxy-2-alkyl quinolones (HAQs), and hydrogen cyanide (HCN)) (3/6); and (2/6 each) \( \text{lasR-} \) mutant (lacks several quorum sensing-regulated factors), \( \text{lasR-/rhlR-} \) (double mutant defective for most quorum sensing-regulated metabolites, including phenazines, rhamnolipids, acyl homoserine lactones, HAQs, proteases, HCN, chitinase), \( \text{rsmA-} \) mutant (global post-transcriptional regulator mutant). A few other mutants were significantly inhibitory in only 1/6 conditions, these included \( \text{pvdD-} \) (pyoverdin mutant), \( \text{rhlR-} \), HSI I/II- double mutant (defective in 2 out of 3 of the type VI secretion systems).

Of note, the double phenazine \( \text{phzC1-/phzC2-} \) mutant, and \( \text{pchE-} \) (pyochelin mutant), were not significantly less inhibitory than the parent. The most inhibitory mutants, \( \text{pvdD-/pchE-} \), \( \text{mvfR-} \), \( \text{lasR-} \), \( \text{lasR-/rhlR-} \), \( \text{pvdD-} \), \( \text{pvdC-} \), \( \text{phzC1-/phzC2-} \) all grew as well as the parent in RPMI, indicating slower growth would not explain the less inhibitory mutants.

Conclusion:
Of the putative Af inhibitors, the simultaneous elimination of both siderophores has the most profound and consistent effect. Other mutations contribute less to loss of inhibition. This is concordant with demonstration that Af inhibition by Pa can be reversed with iron, and that denial of iron markedly inhibits Af in this model of Af biofilm.

**ARE CYSTIC FIBROSIS ASPERGILLUS FUMIGATUS ISOLATES DIFFERENT?**

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**Background:**

*Pseudomonas aeruginosa* (Pa) and *Aspergillus fumigatus* (Af) are the leading bacterial and fungal pathogens in cystic fibrosis. We have previously shown that Af biofilm formation or preformed biofilm is susceptible to inhibition by Pa cells, planktonic growth culture filtrate or biofilm growth culture filtrate, with a hierarchy of cystic fibrosis (CF) nonmucoid Pa, CF mucoid Pa, non-CF Pa most inhibitory to least inhibitory. These studies were performed largely with a reference virulent non-CF Af, 10AF, though comparison with a few non-CF Af showed no difference in susceptibility compared to 10AF. Given differences in CF Pa from other Pa, this study addressed whether CF Af, whether as a result of residence in the unusual (consistency, ionic) and inflammatory CF airway milieu, with exposure to other airway microbes, or exposure to therapeutic antimicrobials, might show a different susceptibility to Pa.

**Methods:**

A nonmucoid CF Pa, Pa10, known to inhibit 10AF, was studied. Live Pa cells or culture filtrates from planktonic Pa growth or growth as Pa biofilm were incubated with each of 12 sputum-derived CF Af or 12 non-CF Af, during Af biofilm formation or on preformed Af biofilm (thus 6 possible conditions compared/isolate). 10AF was included in every assay as a positive control. Comparisons were made with each Af in the absence of Pa or Pa product, and expressed as % of this control. A 96-well plate, RPMI1640 medium, and XTT readout of Af inhibition were used as previously described, with 8 replicate wells/experimental condition each time. *P*<0.05 was considered significant.

**Results:**

(Table; mean values of % of control shown). CF and non-CF Af isolates did not differ in any of the 6 sets of studies (*P*≥0.4); laboratory strain 10AF was slightly more inhibited than either wild-type in some assays (*P*>0.05).

**Conclusion:**

CF and non-CF Af isolates form biofilm, and did not differ with respect to intermicrobial inhibition by an inhibitory CF Pa. A caveat is that it is unknown how long an Af isolated from a CF patient’s sputum has been in residence in the airway.


<table>
<thead>
<tr>
<th>Target</th>
<th>Af biofilm formation</th>
<th>Preformed Af biofilm</th>
</tr>
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<tbody>
<tr>
<td><strong>Challenge</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pa planktonicfiltrate</td>
<td>70.7</td>
<td>62.2</td>
</tr>
<tr>
<td>Pa biofilmfiltrate</td>
<td>62.2</td>
<td>6.1</td>
</tr>
<tr>
<td>Live Pa cells</td>
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</tr>
<tr>
<td>Pa planktonicfiltrate</td>
<td>84.7</td>
<td>89.7</td>
</tr>
<tr>
<td>Pa biofilmfiltrate</td>
<td>89.7</td>
<td>65.2</td>
</tr>
<tr>
<td>Live Pa cells</td>
<td>65.2</td>
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</tr>
<tr>
<td>12 CF Af</td>
<td>70.8</td>
<td>61.2</td>
</tr>
<tr>
<td>12 non-CF Af</td>
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</tr>
<tr>
<td>10AF</td>
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<td>82.1</td>
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</table>
FEED PRODUCTION, SWINE AND SLAUGHTERHOUSE: WHERE IS THE HIGHEST OCCUPATIONAL EXPOSURE TO *ASPERGILLUS* SPP. IN THIS PRODUCTION LINE?

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Purpose:
This study intended to characterize fungal contamination in two swine farms, in one feed production unit, and also in one swine slaughterhouse. We aimed to identify where the highest occupational exposure to *Aspergillus* spp. was detected during the production line.

Methods:
Twenty two air and 22 surfaces samples were collected from the four units through impaction and swabbing methods, respectively. After laboratory processing and incubation of the collected samples, quantitative and qualitative results were obtained, with the identification of the isolated fungal species. For molecular analysis, 300L of air were also collected from each same sampling site using the impinger method. Real Time PCR (RT-PCR) was done to perform the molecular detection of the *Aspergillus* sections *Circumdati*, *Fumigati* and *Flavi* (only the toxigenic strains).

Results:
Eleven species of filamentous fungi were identified in air samples from the feed production unit, with a total of 1666 isolates. None *Aspergillus* species was isolated. Seven species of filamentous fungi were found from the slaughterhouse, with a total of 810 isolates and only 10 isolates from the *Aspergillus fumigatus* complex (section *Fumigati*) were isolated. Twelve species were found in the air from one of the analyzed swine farms with a total of 3080 isolates. *Aspergillus* genus showed low prevalence (6.5%), being the sections *Candidi*, *Circumdati* and *Terrei* the ones isolated. Regarding the other assessed swine farm, 15 fungal species were identified, in a total of 5080 isolates. *Aspergillus* genus presented the higher prevalence (15.7%). Among this genus, *Eurotium herbariorum* (section *Restricti*) was the most prevalent (45.0%). *Fumigati* and *Circumdati* sections were also isolated.

Regarding the results obtained from molecular methods, *A. fumigatus* complex was detected in 10 sampling sites where this species-complex was not isolated by conventional methods: 2 in feed production, 4 in slaughterhouse and 2 in each swine farm assessed.

Conclusion:
Cultural methods showed that swine farms were the settings with the highest fungal load, presenting also the highest number of isolates belonging to *Aspergillus* spp., and more diversified number of species-complexes. The applied molecular tools enabled to target selected fungal indicators of higher occupational risk, belonging to the same genus, allowing a more accurate characterization on occupational exposure to *Aspergillus*.

Conventional and molecular methods showed to be complementary methodologies and should always be applied together in occupational environments with high fungal load in order to ensure the best possible characterization of fungal burden in a given sampling source.
UNDERSTANDING THE ROLE OF SEPTINS REGULATORS IN *ASPERGILLUS FUMIGATUS* GROWTH, DEVELOPMENT AND PATHOGENESIS

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2Pediatrics, Duke University Medical Center, Durham, USA
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**Purpose:**

*Aspergillus fumigatus* is the major etiology of invasive aspergillosis, a leading cause of death in immunocompromised patients. Septins are conserved GTPases involved in septation, conidiation, and cell wall organization. The *A. fumigatus* genome contains five genes encoding for septins: AspA, AspB, AspC, AspD, and AspE. While we previously demonstrated that the *A. fumigatus* septin AspB is dispensable for hyphal extension under normal growth conditions, it is required for regular septation, conidiation, and conidial cell wall organization. Strains lacking AspB exhibited hypervirulence in a *Galleria* model of invasive aspergillosis, but not in a murine model. In this study, we explored the phosphorylation status of septin AspB, as well as the role of Gin4, Cla4, and ParA as AspB regulators of *A. fumigatus* growth, development, and pathogenesis.

**Methods:**

In order to understand the mechanism of AspB regulation, we deleted two putative septin kinases (Gin4 and Cla4) and one protein phosphatase 2A subunit (ParA). Radial growth was assessed on solid media daily for five days, conidiation quantified, and inter-septal distances measured by staining with 0.1% aniline blue. To determine if septin regulators contribute to AspB localization, we generated AspB-GFP tagged strains under the native aspB promoter in each of the deletion strains and imaged using fluorescent microscopy. Virulence of each strain was tested using an intranasal murine model of invasive aspergillosis.

**Results:**

This approach revealed that Cla4 and ParA are required for hyphal extension under normal growth conditions (2.9 and 1.5-folds radial growth reduction, respectively [p<0.001]). Deletion of gin4 results in larger inter-septal distances in the apical compartment (p<0.001), while deletion of cla4 and parA resembles the length of the parent strain (p>0.05). Nonetheless, the Δcla4 and the ΔparA exhibit hyperseptation, and the Δgin4 retains the larger interseptal distance in more basal compartments. Gin4, Cla4, and ParA are each required for full conidiation (4.4-, 5.6-, 20.7-folds reduction in conidia production per cm², respectively [p<0.001]). In our murine model of invasive aspergillosis, the Δgin4 strain was hypervirulent (0% survival; day +6; p<0.001), while the Δcla4 and ΔparA strains were not significantly different from the wild-type strain (50-65% survival; day +6, p>0.05). We show that AspB localization is altered in the Δgin4 and ΔparA strain background. Furthermore, LC-MS/MS phosphoproteomics revealed that AspB is phosphorylated in vivo at eight residues, and the phosphorylation profile is altered in each deletion strains.

**Conclusion:**

We demonstrated that Cla4 and ParA are required for basal growth of the fungus. Gin4, Cla4, and ParA are important for inter-septal distances and conidiation, and our Δgin4 strains results in hypervirulence. Deletion of septin regulators results in mislocalization of AspB. AspB is phosphorylated in vivo in multiple sites, and deletion of septin regulators results in a differential phosphorylation profile, reinforcing the notion of their role as septin post-translational regulators. This is the first exploration of septins regulators in a pathogenic filamentous fungus, and understanding this unique aspect of *A. fumigatus* biology will provide critical insight into disease pathogenesis that could lead to identification of novel drugs targets to ultimately improve clinical outcome.
EFFECT OF ANAEROBIASIS OF *PSEUDOMONAS AERUGINOSA* (PA) ON INHIBITION OF *ASPERGILLUS FUMIGATUS* (AF) BIOFILM

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²Infectious Diseases Research Laboratory, California Institute for Medical Research, San Jose, CA, USA

Purpose:
In cystic fibrosis (CF) lungs, Pa and Af are the commonest pathogens found, often concurrently. Pa may be located within very hypoxic mucopurulent masses in airway lumen. In previous studies (PLoS ONE 10(8):e0134692, 2015) we showed particular potency of CF Pa in inhibiting Af biofilm under aerobic conditions. This led to study the inhibitory activity of Pa growing under anaerobic conditions.

Methods:
A clinical CF isolate of Pa, Pa10, and a virulent isolate of Af, 10AF, were used. For anaerobic studies, Trypticase Soy Agar (TSA) plates containing 1% NaNO₃ (under anaerobic conditions, for growth Pa utilizes nitrate as the terminal electron acceptor, in place of oxygen) were used and anaerobic conditions generated using gas pack (GasPak™ EZ Anaerobe Pouch System, BD), resulting in O₂ concentrations of ≤1%. Three types of Pa inocula: (a) anaerobic from nitrate-TSA plates (An), (b) aerobic from TSA plate (AT), (c) aerobic from nitrate plate (ATn; to control for effect of nitrate), were used as live cells (LC) and to produce culture filtrates (0.22 µm). Both planktonic (PCF) and biofilm culture filtrates (BCF) of Pa were prepared as described (PLoS ONE). For studies of formation of Af biofilm (FB), 2X10³ conidia/well was co-cultured with 5X10⁶ viable Pa or filtrates in 96 well tissue culture plates for 16hrs at 37°C, shaking 65-70 RPM in RPMI medium. For study of preformed biofilm (PB), Pa or filtrates were added only after the 16 hrs, then incubated for 24 hrs. After 40 hrs the metabolic activity of 10AF biofilm was measured with XTT assay; OD values from replicate wells compared with respective Af controls using Student’s unpaired t test. In individual experiments, P value ≤0.05 considered significant, and consistently significant differences defined as significant in ≥50% of experiments and marked with “*” below. To study filtrates and compensate for slow An growth, we also compared An vs. AT by diluting AT PCF with fresh media to an absorbance (A: 610nm) of that of the An, before filtering, followed by Af challenge.

Results:
For all 6 combinations studied (LC, PCF, BCF vs. FB or PB), in 2-4 expts./combination, An, AT & ATn significantly inhibited Af compared to respective controls in 42/47 experiments. As table below shows, inhibition of FB and PB by LC was similar, but PCF or BCF of An was markedly less inhibitory than AT or ATn. Adjusting AT PCF by dilution to compensate for reduced An growth, and comparing to An PCF, suggested lessened An growth alone does not account for lower Af inhibition by An filtrates.

Conclusion:
The LC results suggest that when anaerobically grown Pa is shifted to aerobic conditions for the Af challenges, it behaves similar to aerobically grown Pa (whether LC would show differences if the challenge occurred under anaerobic conditions awaits further anaerobic study conditions under which Af can grow sufficiently). However, filtrates of Pa grown anaerobically are deficient in inhibition, and this appears related to underproduction of inhibitors. This suggests that in hypoxic CF plugs, Pa may be less inhibitory to Af.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Anaerobic</th>
<th>AT</th>
<th>ATn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live Pa cells - Af biofilm formation</td>
<td>79</td>
<td>86</td>
<td>75</td>
</tr>
<tr>
<td>Live Pa cells - Preformed Af biofilm</td>
<td>8</td>
<td>25</td>
<td>11</td>
</tr>
<tr>
<td>Planktonic culture filtrate - Af biofilm formation</td>
<td>10*</td>
<td>38</td>
<td>33</td>
</tr>
<tr>
<td>Planktonic culture filtrate - Preformed Af biofilm</td>
<td>9*</td>
<td>18</td>
<td>25</td>
</tr>
<tr>
<td>Biofilm culture filtrate - Af biofilm formation</td>
<td>5*</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Biofilm culture filtrate - Preformed Af biofilm</td>
<td>6*</td>
<td>26</td>
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SMALL COLONY VARIANTS OF *PSEUDOMONAS AERUGINOSA* (PA) DISPLAY HETEROGENEITY IN INHIBITING *ASPERGILLUS FUMIGATUS* (AF) BIOFILM

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**Purpose:**
Pa and Af are the major microbes isolated from cystic fibrosis (CF) patient lungs. In previous studies¹ we reported nonmucoid Pa are more inhibitory to Af than mucoid. A third CF Pa phenotype, small colony variants (SCVs), produce more exotoxins and less siderophores, and are associated with worsening clinical condition. We wished to now compare SCVs in the Af intermicrobial competition.

**Methods:**
Five clinical isolates of Pa SCVs, a reference CF nonmucoid isolate Pa10, and Af strain 10AF were utilized. 4/5 SCVs were derived from culture plates with nonmucoid colonies, #15-71 from a mixture. The live cell (LC) Pa inocula and generation of filtrates, planktonic (PCF) and biofilm culture (BCF), were as described¹. For Af biofilm formation (FB), a conidial suspension of 2x10³ with Pa inoculum of 5x10⁶, or 1:1 PCF or BCF to RPMI1640 medium, was used in 96-well tissue culture plates and incubated 16 hrs at 37°C with shaking at 65-70 RPM. For preformed biofilm study (PB), LC, BCF or PCF were added only after 16 hrs Af culture. Then, in all, after 24 hrs more incubation, XTT measured the metabolic activity of Af biofilm. Differences from controls were assessed by Student’s unpaired t test. A P value of ≤0.05 was considered significant.

To adjust for growth differences, we also studied equalizing the PCF OD of Pa10 or SCVs (depending on which had grown to greater OD) by adding fresh media before filtration.

**Results:**
Pa10 and 15-75 produce green pigment that has been associated with virulence. SCVs 15-75, 15-76 grew better than Pa10 in RPMI1640 medium, the others less well than Pa10. In the 6 types of studies (LC, BCF or PCF vs. FB or PB), the 6 Pa’s each inhibited Af in 154/161 assays, the few exceptions being some 15-71 and 15-74 assays. 15-71, 15-73, 15-74 were less inhibitory than Pa10 in most assays, 15-75 and 15-76 equal to Pa10 or more inhibitory. Adjusting PCFs for growth differences suggested 15-71 and 15-74 lesser inhibition is related to poorer growth, and deficient production of inhibitors, respectively, and greater inhibition by 15-75 is explained by its better growth. Pa10 differences from 15-73 and 15-76 appear a mixture of growth and inhibitor production differences.

**Conclusion:**
SCVs appear to be a heterogeneous group, with a range of inhibitory activity against Af biofilm. Most or all these SCVs would be derived from nonmucoid parents. Some are comparable to or more inhibitory than the potent nonmucoid Pa10 with normal-sized colonies. SCVs must be regarded as important players in the CF microbiome.

MORPHOLOGICAL IDENTIFICATION OF *ASPERGILLUS* SPP. COLONIZING RICE GRAINS

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**Purpose:**
To identify the *Aspergilli* colonizing rice grains.

**Methods:**
Although molecular methods continue to improve and become more rapidly available, microscopy and culture remain commonly used and essential tools for identification of *Aspergillus* spp. In this study we emphasize on morphological methods including; macroscopic and microscopic characteristics for identification of 170 isolates of *Aspergillus* spp. isolated from 1,200 rice grain samples collected from 20 states across the country.

**Results:**
According to morphological characters all these isolates were belonging to *Aspergillus flavus* (84), *A. niger* (66) and *A. ochraceus* (20). Conidia of all isolates were light sparse grey green to pale blue green or parrot green; mycelium fluffy creamy white to dull white color, and exudates were present on surface; reverse uncolored to yellowish or brown and wrinkled mycelial growth; soluble pigments were absent; very few sclerotia were present in wheat brown color.

**Conclusion:**
In our view morphological method using the differential media is the most reliable and sensitive assay to identify important *Aspergillus* species isolated from rice grains or some other sources.
GENETIC VARIABILITY IN AFLATOXIN PRODUCING *ASPERGILLUS FLAVUS* STRAINS OF DISCOLORED RICE IN INDIA

SR Chintala*

*Plant Pathology, Indian Institute of Rice Research, Hyderabad, India*

**Purpose:**
To find out the genetic variability in aflatoxin producing *Aspergillus flavus* strains of discolored rice in India.

**Methods:**
Twenty-two aflatoxin B1 (AFB1) producing *Aspergillus flavus* strains were isolated from 1,200 discolored rice grain samples collected from 20 states across India and tested their potential to produce AFB1 on different agar media. Further these isolates were characterized through randomly amplified polymorphic DNA method.

**Results:**
All the strains of *A. flavus* produced AFB1 on yeast extract sucrose agar media, but none of the strains on *A. flavus* and *A. parasiticus* agar medium. Among the 22 strains, two strains from Tamil Nadu (DRAf 009) and Maharashtra (DRAf 015) produced high amount of AFB1 in all the media tested. To assess the genetic variability in *A. flavus*, the isolates were analyzed by using random amplified polymorphic DNA markers. The isolates showed 17–80% similarity with standard culture of *A. flavus* (MTCC 2799). In our study RAPD helped to confirm the identity of isolates as *A. flavus*.

**Conclusion:**
In this paper we described the potential of AFB1 production by the strains of *A. flavus* and their genetic variability by RAPD-PCR. This is the first comprehensive report on the collection of large number of rice grain samples from across the country, isolation of different strains of *A. flavus*, identification of suitable media for maximizing AFB1 production and studies on genetic variability of *A. flavus* isolates by RAPD.
Purpose:
To investigate the potential of aflatoxin B1 (AFB1) production by five *Aspergillus flavus* strains previously isolated from sorghum grains on cereals (barley, maize, rice, wheat and sorghum), oilseeds (peanuts and sesame) and pulses (greengram and horsegram).

Methods:
Five strains of *A. flavus* were inoculated on all food grains and incubated at 25°C for 7 days; AFB1 was extracted and estimated by enzyme-linked immunosorbent assay.

Results:
All *A. flavus* strains produced AFB1 on all food grains ranging from 245.4 to 15 645.2 μg kg⁻¹. Of the five strains tested, strain Af 003 produced the highest amount of AFB1 on all commodities ranging from 2245.2 to 15 645.2 μg kg⁻¹. Comparatively, the AFB1 accumulation was high on rice grains ranging from 3125.2 to 15 645.2 μg kg⁻¹, followed by peanuts ranging from 2206.2 to 12 466.5 μg kg⁻¹. Less AFB1 accumulation was observed in greengram and sesame seeds ranging from 645.8 to 2245.2 and 245.4 to 2890.6 μg kg⁻¹, respectively.

Conclusion:
Our results showed that all food grains tested are susceptible to *A. flavus* growth and subsequent AFB1 production.
COMPARATIVE MORPHOLOGY AND GENETICS OF BLACK *ASPERGILLI*

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**Purpose:**
Black *Aspergilli* are ubiquitous and commonly found in our environment. Species of this fungal group are very important being notorious animal as well as plant pathogens. The objective of present study was to explore biodiversity of black *Aspergillus* species among the local mycoflora of Pakistan by their isolation, morphological and molecular characterization.

**Methods:**
Complete descriptions based on macro and micro morphological characters were prepared. Genetic characterization of species under investigation was carried out by sequence analysis of the Internal Transcribed Spacer (ITS) region as well as partial calmodulin gene (CAL). The nucleotide sequence of amplified products of both ITS and CAL gene were analyzed for species identification by BLAST as well as for phylogenetic analysis of strains.

**Results:**
Twelve different isolates belonging to five different species of *A. niger* group that are characterized for present study are *A. niger* (FCBP1523), *A. awamorii* (FCBP1500, FCBP1525), *A. tubingensis* (FCBP038, FCBP068, FCBP069, FCBP098, FCBP109, FCBP115), *A. welwitschiae* (FCBP087), *A. neoniger* (FCBP107) and *A. phoenicis* (FCBP067). Pure cultures of fungi were deposited to First Fungal Culture Bank of Pakistan (FCBP) and the nucleotide sequences to GenBank.

**Conclusion:**
Present investigation sheds light on the combine approach of morphological and molecular characterization of *Aspergillus* species. Such kind of studies can be extrapolated to other groups of fungi. Authentic identification of pathogenic species has a direct impact on disease control and management.
PREVALENCE OF AEROALLERGENS IN THE ATMOSPHERE OF KATHMANDU, NEPAL AND CHITTAGONG, BANGLADESH

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Purpose:
The burden of aeroallergens in the atmosphere is increasing so alarmingly in Nepal and Chittagong. Nepal alone has about two million asthmatics patients. It causes approximately 5,000 deaths a year, due inhalation of biologically polluted air most of them are exposed to aeroallergens. The study of aeromycoflora was conducted to determine prevalence of aeroallergens in the atmosphere and compared between Kathmandu, Nepal and Chittagong, Bangladesh.

Methods:
Gravity Slide method and Gravity Plate method were used for the isolation of fungi. Identified the isolated fungi by colony morphology, microscopic method, reference slides and by using standard references. The allergens from Aspergillus fumigatus, A. flavus and Alternaria alternata were extracted and identified the protein bands by Sodium Dodecyl Sulphate-Polyacrelamide Gel Electrophoresis (SDS-PAGE) using standard proteins (Marker). In vivo allergenic immune response was evaluated by intracutaneous inoculation in laboratory animal.

Results:
Total 113 different spore types belonging to 67 genera from Kathmandu, Nepal and 40 types from Chittagong, Bangladesh were identified from the atmosphere. Aspergilli/Penicilli group was reported as the most prevalent spora and constitute the major air spora (81.20%) and (16.32%) from Kathmandu and Chittagong respectively. The allergenic bands 30 to 67 kDa were found common in all three studied species. The most allergenic bands 20-32kDa were more prominent in Aspergillus fumigatus and also reported as the most allergenic to experimental animals with the highest wheal size.

Conclusion:
The fungal spores are the predominant contaminants of air, distributed uniformly during all seasons and areas. They can cause a wide range of allergenic reaction to human beings and the prevalence of aeroallegens increases with the increase of concentration of spores in air.
HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ANALYSIS OF MYCOTOXINS AFLATOXIN IN MAIZE IMPORTED INTO IRAN

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Purpose:
Thirty-three maize samples, from six countries imported into Iran during 2001-2002, were collected from a bulk shipment at ports and analyzed for the presence and concentration of aflatoxins (AFB₁, AFB₂, AFG₁, and AFG₂).

Methods:
Aflatoxin analysis was carried out by High-Performance Liquid Chromatography (HPLC) method.

Results:
Of the 33 samples, 15 (45.5%) were found to contain AFB₁ (4–28 µg/kg). The frequency of AFB₁ found in imported maize samples in decreasing order was Uruguay, Brazil, USA and China (each 50%), followed by Argentina (33.3%) and Canada (25%). The average AFB₁ concentration was 15.73 and 7.2 µg/kg for positive and all samples, respectively. Medians were 14 and 0 µg/kg for positive and all samples, respectively. Only one sample of Brazil showed contamination with all 4 different aflatoxins.

Conclusion:
The results obtained were comparable to results from other studies in maize from various countries. It is thus critical to monitor and control the contamination of food and feed by aflatoxins in both household and imported maize. As large amounts of maize are imported into Iran, this paper stresses the need to develop legislation and enforce standards to ensure trade of maize with the minimal amount of mycotoxins, especially aflatoxins.
IMPACT OF ECHINOCANDIN EXPOSURE ON THE INTERACTOME OF
ASPERGILLUS FUMIGATUS PROTEIN KINASE A

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Introduction:
Echinocandins constitute an important part of the antifungal armamentarium against A. fumigatus, targeting the fungal cell wall. Unfortunately, efficacy of this class is limited by emerging resistance and by tolerance of many fungi to higher doses due to the apparent triggering of poorly-understood compensatory mechanisms. Development of tandem treatments that target such response pathways may be the key to effective clinical re-deployment of echinocandins. However, much remains unknown about the cellular mechanisms that facilitate echinocandin tolerance. Protein kinase A (PKA) has been shown to be an important regulator of growth, development, and virulence of A. fumigatus as well as other pathogenic fungi.

Methods:
Growth of an A. fumigatus strain in which the major catalytic subunit of PKA (PkaC1) was deleted was compared to wild type during exposure to 0.125-2 μg/mL caspofungin. Strains expressing EGFP-tagged forms of PkaC1 and the PKA regulatory subunit (PkaR) were generated and tagged proteins, along with associated interactants, were isolated from whole cell extracts via GFP-Trap affinity purification following growth in the presence or absence of 1 μg/mL caspofungin. Purified proteins were subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify interactants as well as sites of phosphorylation.

Results:
A ΔpkaC1 mutant displayed markedly reduced growth at inhibitory concentrations of caspofungin compared to the wild type. Of 1,103 PKA interactions identified via LC-MS/MS, approximately 7 percent were found to be enhanced during caspofungin exposure, while nearly 20 percent were suppressed. Enhanced interactions involved proteins associated with the TCA cycle, glycolysis, proteolysis, nitrogen metabolism and secondary metabolite production. Suppressed interactions involved protein biogenesis and trafficking, amino acid and fatty acid biosynthesis, cytoskeletal structure, redox signaling and G-protein signaling. Interactions were also identified with a number of regulators of other signaling pathways including calcium signaling (CDPK, CnaA, CnaB), the cell wall integrity pathway (Rho1, PkcA, Mkk2, MpKA) and the high osmolarity glycerol response pathway (SteC, SskB, Pbs2, SakA). Interactions with calcineurin subunits appeared to be suppressed during caspofungin exposure. Phosphoproteomic analysis identified phosphorylation on five residues of PkaC1 in the activation loop and αAB helical subdomain, four of which have not been reported for homologous residues in other fungal or human PKA isoforms. At least three phosphorylated residues were identified on PkaR in the N-terminal targeting domain and hinge region, two of which also appear to be novel.

Discussion:
We report that PkaC1 promotes tolerance of A. fumigatus to the echinocandin caspofungin. Marked shifts observed in the interactomes of PkaC1 and PkaR during caspofungin exposure are suggestive of dramatic metabolic realignments. Our findings also indicate interactions of PKA with key regulators of a number of other major signaling pathways, intimating cross-talk between cAMP signaling and these other sensory channels. Furthermore, novel sites of phosphorylation identified on both PkaC1 and PkaR may represent fungal-specific PKA regulatory mechanisms with potential to be exploited for discrete inhibition of fungal PKA isoforms. This work should provide the foundation for developing a more thorough understanding of the role of PKA in the A. fumigatus antifungal stress response, and potentially for identifying novel stress adaptation mechanisms which may be the focus of future antifungal drug development.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
DISTINCT ROLES OF MYOSINS IN HYPHAL MORPHOLOGY AND VIRULENCE IN ASPERGILLUS FUMIGATUS

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Purpose:
Polarized hyphal growth and septation facilitate invasion of host tissue by A. fumigatus. To better understand these processes, we are studying myosins, a group of motor proteins categorized into classes based on their structure. A. fumigatus contains three myosins encompassing three classes: class I (myoA), class II (myoB), and class V (myoE). These proteins are involved in growth, morphology and septation in other fungi; however, the role of myosins in the growth and virulence of a human pathogen has never been explored. Because of myosins’ involvement in critical cellular processes, an understanding of their precise functions and regulation in A. fumigatus will lead to fundamental knowledge of pathogenesis which may help identify novel drug targets.

Methods:
We generated two myosin single deletion strains (ΔmyoB and ΔmyoE) and a double deletion strain (ΔmyoB ΔmyoE). Attempts to delete the myoA gene were unsuccessful. Radial growth was assessed on solid media daily for five days and conidiation quantified. Transmission electron microscopy was used to visualize septa and hyphal morphology. Conidia viability was assessed using bis-(1,3-Dibarbituric acid)-trimethine oxanol (DiBAC). Virulence was determined using a persistently immunosuppressed murine model of invasive aspergillosis. Phosphorylated residues were determined by GFP-Trap protein purification of MyoE-GFP, TiO2 phosphopeptide enrichment, and LC/MS-MS.

Results:
While the ΔmyoB strain showed no significant difference in radial extension, the ΔmyoE and ΔmyoB ΔmyoE strains resulted in a significant defect (p<0.001 at day 5). Both MyoB and MyoE are required for full conidiation (p<0.0001). Deletion of myoE resulted in hyperbranching and loss of polarity. TEM revealed that the cell walls of both the ΔmyoB and the ΔmyoE strains appear normal but the ΔmyoB strain contains incomplete, thicker septa. Septa in the ΔmyoE strain appear wild-type; however staining revealed that deletion of myoE resulted in hyperseptation (p<0.001). In our murine model, both the ΔmyoB and ΔmyoE strains were hypovirulent (p<0.001 and p<0.01, respectively). Deletion of myoB resulted in a 2-fold increase in inviable conidia (p<0.05), and deletion of myoB or myoE resulted in significantly delayed germination. Because of the significant radial extension and hypovirulence in the ΔmyoE strain, we became interested in MyoE as a drug target and wanted to understand its regulation. We determined that MyoE is phosphorylated at eight residues, encompassing each of its four domains.

Conclusion:
We demonstrated that myosins have distinct roles in hyphal morphology and virulence and that MyoA is likely essential, as in other fungi. MyoB and MyoE are required for conidiation. MyoE has a role in preserving hyphal polarity and/or suppressing new growth foci. MyoB is important for proper septa formation, while MyoE may have a role in septa frequency. MyoE is required for virulence in a murine model. The reduction of virulence in the ΔmyoB strain may be due to lack of conidia viability. We are currently exploring the importance of phosphorylation as a regulation mechanism of MyoE to better understand its role in virulence.
Purpose:
Fungal cell tropisms involve directional changes of growing fungal cells in response to a stimulus. Negative cell tropisms are ubiquitous in filamentous fungi. Two clear examples are: (1) avoidance of adjacent germ tubes during colony initiation; and (2) avoidance of adjacent vegetative hyphae and branches at the colony periphery. Both tropisms have been proposed to be important in reducing the competition of neighbouring germ tubes/hyphae for nutrients. Despite the widespread occurrence of negative tropisms in fungi, little is known about the signalling processes governing the phenomenon. The aim of our work is to understand the mechanistic basis of the negative tropism phenomenon during spore germination and hyphal growth on the human pathogen Aspergillus fumigatus.

Methods:
Confocal microscopy live-cell imaging and quantitative image analysis was employed to image and measure the angles formed between germlings when visualized in both 2D and 3D as the conidial germlings invade the agar substrata of different hardnesses. Mass spectroscopy was used to analyse the volatiles produced by conidial germlings.

Results:
The angles formed between germlings were found to be dependent on the number of germlings in a given group. Our time-lapse imaging data indicated that re-arrangement of the growth axis occurs almost immediately when hyphae approach each other. Furthermore, the avoiding hyphae also exhibited a propensity to invade their agar substratum and this was influenced by its hardness. The ‘avoidance signal’ is probably a volatile compound because the germ tube avoidance response was observed on the surface of cellophane in air. A number of candidate volatile molecules have been identified as hyphal avoidance signals by mass spectrometry. Mutants blocked in secondary metabolism and growth of the wild type in 100% C0₂, or in the presence of a NO scavenger, indicated that the avoidance signal was probably not a secondary metabolite, C0₂ or NO.

Discussion:
The negative tropic response of conidial germ tubes have been quantitatively characterized in living cells but the chemical signal responsible for these negative tropisms has not yet been identified. The avoiding germ tubes have also been found to have a propensity to invade their agar substratum. Our results suggest that both the avoidance and invasive tropic responses may be influenced by gradients in the same volatile molecule. Both the hyphal avoidance and invasive tropisms may play roles during infection to reduce competition for nutrients between spore germlings and to facilitate invasion of the host tissue.

Conclusion:
This study has provided new insights into the mechanistic basis of cell tropisms which may play roles in infection by A. fumigatus.
THE FXDXF MOTIF IN CNAα IS ESSENTIAL FOR PROPER SEPTAL LOCALIZATION AND FUNCTION OF CALCINEURIN IN ASPERGILLUS FUMIGATUS

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Purpose:
The calcineurin complex, comprised of the catalytic subunit (CnaA) and the regulatory subunit (CnaB), localizes at the hyphal tips and the septa to direct proper hyphal growth and septation in Aspergillus fumigatus. We previously showed that septal localization of CnaA is independent of CnaB-binding but the PxIxIT-substrate binding motif residues Asn352, Ile353, and Arg354 (352NIR354) that lie in the substrate recognition β strand of CnaA are required for septal localization. Although the exact mechanism of how calcineurin localizes at the septum remains unknown, identification of functional domains required for septal localization of calcineurin would provide clues towards the modality of its interaction at the hyphal septum and potentially be useful for designing specific inhibitory strategies.

Methods:
We performed extensive mutations in CnaA in the region overlapping the cyclophilin A-cyclosporin A binding domain, CnaB-binding helix and the FK506-FKBP12 binding pocket. The various mutated versions of CnaA were expressed from the CnaA native locus in the A. fumigatus KU80 strain by tagging to EGFP fluorescent protein to visualize the localization of the mutated protein in vivo.

Results:
We identified an FxDxF motif comprising of residues Phe368, Asp370 and Phe372 that are required for proper septal localization of CnaA. Interestingly, mutations in adjacent residues Asn367 (N367D), Trp374 (W374L) and Ser375 (S375T) confer resistance to FK506 but do not influence septal localization of CnaA. While the single mutations of Phe368 or Asp370 to Ala (F368A; D370A) did not alter the septal localization of CnaA, the combined mutation Phe368 Asp370 to Ala (F368A D370A) resulted in aberrant septal localization of CnaA, and the double mutation Phe368 Phe372 to Ala (F368A F372A) completely mislocalized CnaA from the septum. Molecular modeling of the PxIxIT and LxVP-substrate binding motifs of A. fumigatus CnaA confirmed that the FxDxF motif forms a bridge between the two substrate binding motifs and dual mutations in the FxDxF motif likely disrupts substrate interaction.

Conclusion:
Mutation of the FxDxF motif likely disrupts the overall organization of the CnaA α-tower relative to the rest of the globular CnaA domain, which in turn effects interaction with the LxVP and PxIxIT motifs as well as downstream localization to the hyphal septum. Taken together our results suggest the importance of both the PxIxIT and the FxDxF motifs for septal localization and function of CnaA.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
DEGRADATION OF CHEMICAL COMPONENTS OF SHEA BUTTER BY ASPERGILLUS SPECIES: IMPLICATIONS FOR INDUSTRY AND HUMAN HEALTH

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Purpose:
There is high demand for Shea butter in the international market due to its industrial potential and rich chemical constituents (mature kernel contains about 61% edible fat). Shea butter can be used for food, medicinal as well as industrial purposes such as cakes and margarine, in cosmetic and pharmaceutical industries. Despite its wide usage, Shea butter processed in Nigeria is characterized by low quality.

A study of the effect of fungal contamination on the quality of processed Shea butter and identification of the sources of contamination along the different processing stages was carried out with the aim of identifying the fungi associated with processing and to deduce the degradative effect of the associated microbial communities on the butter quality.

Methods:
Shea butter samples were obtained from processing sites in Kwara and Niger States of Nigeria. Microbiological and chemical analyses of the samples were done using standard conventional methods.

Results:
The fungi associated with the local method of processing were Aspergillus niger, A. persii, A. flavus, A. niger (aggregate), A. oryzae and A. fumigatus. The identification of sources of microbial contamination and traceability of fungal organisms to processing stages was analyzed using molecular methods. The results revealed that the fungi contaminated the processing line from the use of unsorted Shea kernels and from the environment. Aspergillus oryzae and A. niger increased the yellow portion of the olein from 16.2Y to 40Y and 20Y, respectively while A. persii, A. fumigatus and A. flavus reduced the yellow fraction of the oil from 16.2Y to 10Y, 7Y and 8Y, respectively. The difference between values for experimental and control for pH and relative density was not significant while that of lipophilic activity of the isolates was highly significant. The presence of the different Aspergillus species affected the olein fraction of the Shea butter such as release of high amounts of free fatty acids and peroxide, thereby reducing butter quality.

Conclusion:
Changes in chemical parameters brought about by Aspergillus presence has implications for health, as well as for pharmaceutical and cosmetic industries.
**Purpose:**

*Aspergillus* species are common and widespread moulds in the nature. They are among the most successful groups of moulds with important roles in natural ecosystems and the human economy.

**Methods:**

They are widely used as cell factories for the production of food ingredients, enzymes and antibiotics. *A. niger* and *A. ochraceus* are used in cortisone production. *A. terreus* produces mevinolin which is able to reduce blood cholesterol. They can be used as an expression system for eukaryotic proteins.

**Results:**

In food industry, *A. oryzae* is used to ferment soybeans to soy sauce. *A. niger* is used in the bread and beer making industries and also is able to decompose plastic. In addition of food industry, fungal biomass of *Aspergillus niger*, is a byproduct of citric acid fermentation that has proven to be a valuable biomaterial that can be both beneficial and practical. From an environmental point of view, it can be a useful bioadsorbent to detoxify and decolorize the wastewater samples.

**Conclusion:**

In this article *Aspergillus* species and their applications will be discussed in food industry.
Purpose:
There has been an alarming rate of fungal sino-nasal infections reported in the last decades from India. With breath as a life line, constant inhalation of humid, dusty and arid spore laden air with compromised sinonasal functioning leads to indolent to invasive fungal rhino sinusitis. Further the burden of *Aspergillus* associated sinonasal fungal infections needs to be documented to discern the exact prevalence in our setting.

Methods:
Epidemiological and mycological findings in sino-nasal fungal infections were reviewed to understand the spectrum of *Aspergillus* species complex encountered during 2004 to 2014 in the tertiary care center at Karnataka State, Southern India. Medical intervention, predisposing factors including the degree of immunosuppression, comorbidities like diabetes, deviated nasal septal (DNS) defects, progression and outcomes in patient groups were monitored.

Results:
The study presents the results of epidemiological and mycological findings on spectrum of *Aspergillus* species complex encountered in sino-nasal fungal infections during 2004 to 2014 in tertiary care center at Karnataka State, Southern India. Medically important *Aspergilli* from section; *Fumigati, Flavi, Nigri, Terrei, Nidulantes* and *Ustus* were encountered from fungal sinusitis. The predisposing factors, progression and outcomes in patient groups will be presented.

Conclusion:
High degree of clinical suspicion, substantial corroborating radiological, histopathological and mycological evidences followed by aggressive therapeutic management coupled with surgical intervention are needed for diagnosis and effective patient care.
PHENOTYPIC MATURATION OF DENDRITIC CELLS IS IMPAIRED BY THE CALCINEURIN INHIBITOR FK506 IN A IN VITRO MODEL OF INVASIVE ASPERGILLOSIS IN LUNG TRANSPLANT RECIPIENTS

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Purpose:
Invasive aspergillosis in lung transplant recipients on immunosuppression is associated with high morbidity and mortality. The calcineurin inhibitor tacrolimus (FK506) inhibits the calcineurin-NFAT axis, which impairs the innate response to fungal infection1. Dendritic cells (DC’s) play a pivotal role in signalling to the adaptive immune system in infection - immature DC’s phagocytose antigen, leading to maturation into DC’s capable of stimulating T-cells. We investigated the effect of FK506 on DC function in invasive aspergillosis by assessing phenotypic maturation of DC’s in response to Aspergillus fumigatus (AF) infection.

Methods:
Healthy volunteer PBMC’s negatively isolated by Ficoll® gradient were differentiated into DC’s with GM-CSF and IL-4. Day 5 cells were matured with IFN-γ. Day 7 cells were treated with FK506 and/or inoculated with swollen conidia of A.fumigatus (MOI 1:1). Cells were then stained with PE-bound anti-CD83 (a late maturation marker) and PerCP-Cy5.5-bound anti-CD-209 (a DC-specific marker) and analysed by the ImageStream® imaging flow cytometer. Statistical analysis was performed with Graphpad Prism v6.0, using unpaired t-tests with Welch correction.

Results:
5000 cells/condition were analysed. DC’s were sub-setted by gating for CD-209 positivity. FK506 was not toxic to cells (similar cell viability between groups).

We demonstrated up-regulation of CD83 (measured by mean fluorescent intensity) with IFN-γ stimulation of DC’s (12534±799.3 vs. 26228±1462, p<0.0001; mean fluorescent units +/-SEM), AF infection of unstimulated DC’s (12534±799.3 vs. 29888±1393, p<0.0001) and for AF infection of IFN-γ-stimulated DC’s (26228±1462 vs. 36778±1356, p<0.0001).

CD83 mean fluorescent intensity was reduced with FK506 treatment of IFN-γ-stimulated DC’s (26228±1462, vs. 20219±846.0, p=0.0004), AF-infected unstimulated DC’s (29888±1393 vs. 24289±1253, p=0.0028), and AF-infected, IFN-γ-stimulated DC’s (36778±1356 vs. 30159±1279, p=0.0004), but unchanged for unstimulated, un-infected DC’s (12534±799.3, vs. 11942±762.5, p=0.5921).

Conclusion:
Both A.fumigatus infection and IFN-γ stimulation promote phenotypic maturation of DC’s in vitro, and treatment with FK506 inhibits maturation in this context. This suggests an inhibitory effect of FK506 on innate antigen presentation to T-cells and may impair the adaptive immune response to invasive aspergillosis in lung transplants recipients.

Reference

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
INFLUENCE OF METEOROLOGICAL PARAMETERS ON ASPERGILLUS SPP. DISPERSION OUTDOOR AND INDOOR DURING A LARGE DEMOLITION WORK AT HOSPITAL

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Purpose:
Demolition can generate fungal spore’s suspension which can be associated with high risk of invasive aspergillosis (IA) in immunocompromised patients. One central block of the 32 blocks of Edouard Herriot University Hospital (France) is being entirely demolished to give way to the construction of a new hospital building. All the care activities continued in the near blocks. Current guidelines to prevent nosocomial infection due to fungal aerocontamination are partly based on adequate environmental monitoring and protective measures. Aspergillus spp. spores load is present in all season and seems actually meteorological parameters (MP) dependent. The aim of this study was to assess the influence of some MP on outdoor and indoor fungal airborne contamination during a period of demolition work.

Methods:
Air sampling was carried out daily between April 2015 and mid-October 2015 in medical wards around demolition site. Environmental survey was realized in 4 intensive care units (ICU), 1 unit of kidney and liver transplantation (KLT) and 3 medical wards. One ICU (G0), the KLT unit (G1) and 3 medical wards (G2, G3 and G4) were located at the north of the demolition site (Figure 1). Air samples were realized outdoor and indoor with an agar impact sampler (Air-Ideal 90 mm, Biomérieux®) onto Sabouraud Chloramphenicol agar (Biomerieux®) and were incubated 48h at 37°C. Daily temperature, relative humidity, and wind direction and speed near the hospital were obtained from Meteociel® site. Aspergillus spp. contamination was expressed qualitatively (presence vs absence). Logistic regression was used to study the effect of meteorological conditions on Aspergillus spp. contamination. P-values <0.05 was considered statistically significant.

Results:
For outdoor contamination (n=204), only wind direction (p=0.01) and relative humidity (p<10-4) had a significant effect on Aspergillus spp. contamination in univariate analysis. In bivariate analysis, effect of both wind direction and relative humidity remained significant with i) Odd Ratio (OR) of Aspergillus spp. contamination = 4.1 (p=0.001) CI 95% [1.7; 10] for south wind direction compared to north direction and 2) OR = 1.5 (p<10-3) for each increase of 10 humidity degrees. Similar results were obtained for Aspergillus spp. indoor contamination (n=384) in the 5 wards located at north of demolition site. In multiple analysis adjusted on ward, OR of Aspergillus spp. inside for south compared to north wind direction decreased OR = 3.0 (p<10-3) 95% CI [1.7;5.4], and OR estimate for relative humidity was unchanged. With medical ward G4 as reference, the OR of Aspergillus spp. inside KLT unit (G1) was of 0.27 (p=0.01) 95% CI [0.12;0.56] and inside G0 of 0.39 (p=0.02) 95%C[0.17;0.88]. The odd of Aspergillus spp. inside G2 and G3 was not significantly different from the odd inside G4 (OR = 0.79 and 1.9, respectively).

Conclusion:
Some MP seemed to impact outdoor and indoor Aspergillus spp. contamination. These results could be helpful to choose the best conditions of hospital construction. The differences of Aspergillus spp. contamination inside the 4 wards also raised the issue of the impact of the impact of filter maintenance of ventilation system on fungal conidia contamination.
Figure 1: Map of Edouard Herriot Hospital, France
EVALUATION OF THE IMPACT OF AIR HANDLING SYSTEM ON FUNGAL COLONIZATION OF THREE MEDICAL WARDS DURING LARGE DEMOLITION WORKS AT HOSPITAL

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Purpose:
A block of Edouard Herriot Hospital composed of 32 blocks (France) is being entirely demolished, while care activities continued in the near blocks. Spores may be suspended during demolition and exposed immunocompromised patients to severe invasive aspergillosis (IA). Construction (demolition, earthmoving, etc.) in hospitals with immunocompromised patients nearby increased spore emission and constitutes a major IA outbreak risk. The aim of the study was to evaluate the impact of ventilation system on fungal conidia colonization of 3 wards of one hospital block during demolition.

Methods:
A daily environmental survey of fungal loads was implemented wards located around demolition site. These environmental survey consisted in sampling air twice a day, indoor and outdoor, by impaction onto Sabouraud Chloramphenicol agar supplemented with antibiotic chloramphenicol plates (Biomerieux®) using an agar impact sampler (Air-Ideal 90 mm). Plates were incubated 48h at 37°C. After incubation Aspergillus spp. were identified. We selected data from one medical block located further at north of the demolition site. Block is composed of the unit of kidney and liver transplantation (W1), one ICU (W2), and the nephrology unit (W3). Characteristics from rooms air handling were collected: W1 has fine particular filter, W2 has the same particular filter and W3 has croase filtering system. Aspergillus spp. contamination was expressed quantitatively in CFU/m³ as mean±SD and qualitatively (presence vs absence) as well. Mean outdoors total fongal load (TFL) near the three units were compared by ANOVA analysis after base 10 logarithm transformation. Logistic regression was used to assess effect of unit and outdoors Aspergillus spp. colonization or outdoors total fongal load (TFL) on indoors Aspergillus spp. contamination. P-values<0.05 was considered statistically significant.

Results:
Indoors Aspergillus spp. load mean was estimated as 0.55±1.6 in W1 (n=44), 1.0±2.9 in W2 (n=32) and 3.8±7.0 in W3 (n=39) CFU/m³. Outdoors Aspergillus spp. load mean and TFL mean ranged from 29 to 35 and from 193 to 229 CFU/m³ respectively and both were not found significantly different across the units (p=0.76 and p=0.53, respectively). In univariate logistic regression, units and outdoors Aspergillus spp. colonization had a significant effect on indoors Aspergillus spp. contamination (p=0.005 and p = 0.003 respectively) whereas outdoors log₁₀ TFL had not any effect (p=0.06). Bivariate logistic regression highlighted a significant interaction of units with outdoors TFL on indoors Aspergillus spp. (p=0.009) and no interaction with outdoors Aspergillus spp. colonization (p=0.56), i.e. additive effects of units (p = 0.002) with outdoors Aspergillus spp. colonization (p = 0.001). With W1 as reference, ORs of indoors Aspergillus spp. colonization were i) of 1.3, 95% CI [0.30; 5.3] (p = 0.75) for W2 and ii) of 6.2, 95% CI [1.9;21] (p=0.003) for W3. In case of outdoors Aspergillus spp. presence, the odd of indoors Aspergillus spp. colonization was multiplied by 6.1 95% CI [1.8;20] (p=0.004).

Conclusion:
The presence of fine particular filters in rooms of W1 and W2 seems to have a significant protective effect against Aspergillus spp. compared to croase filtering of W3. Variation between W1 and W2 could be due to difference of air flow conception in the wards.
EVALUATION A. FUMIGATUS AEROCONTAMINATION DURING LARGE DEMOLITION WORKS AT HOSPITAL? ONGOING ENVIRONMENTAL MONITORING AT EDOUARD HERRIOT UNIVERSITY HOSPITAL

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Purpose:
Without more reliable solution, evaluation of airborne fungal spores is an essential tool to prevent fungal infections prevention and monitoring environmental colonization at hospital. Spores may be suspended during demolition and exposed immunocompromised patients to severe invasive aspergillosis (IA). One central block of the 32 blocks of Edouard Herriot (France) is being entirely demolished, while care activities continued in the near blocks. The aim of this study was to 1) evaluate the influence of meteorological parameters (MP) on airborne A. fumigatus conidia contamination variability indoor and outdoor hospital ward and 2) determine the A. fumigatus diversity and 3) to evaluate the correlation between environmental and clinical data.

Methods:
Since February 2015, daily environmental survey of fungal loads was started in 8 wards located around the demolition site: 4 intensive care units (ICU), 1 unit of kidney and liver transplantation and 3 medical wards. Air sampling was realized indoor and outdoor selected wards with an agar impact sampler (Air-Ideal 90 mm, Biomérieux) by impaction onto Sabouraud Chloramphenicol agar. At each sample location 2 nutritive agars were realized and incubated 48h at 37°C for Aspergillus spp. identification and 5 days at 30°C for total fungal load count (TFL).

Furthermore outdoor was continuously monitored by volumetric sampler model Lanzoni VPPS-2000 (Bologna, Italy, Airtest) with a mean flow rate of 10 L.min⁻¹. This sampler was placed on the roof of a block. Spores were impacted on adhesive tape and identified using a microscope. Aspergillaceae spores identified were expressed by spore/m³/day. Daily temperature, wind direction and speed, relative humidity were obtained from Meteociel® site. Genomic variability of A. fumigatus strains will be genotyping by Multiple–Locus Variable number tandem repeat Analysis (MLV A) technique. Susceptibility to antifungal will be tested using E-test® performed on RPMI buffered agar.

Results:
Until now, about 3200 air samples were realized. By the end of sampling period on December 2015, results of A. fumigatus, Aspergillus spp. and total fungal flora (TFL) collected outdoor with the agar impact sampler will be compared with the volumetric sampler data to see if it can further replace manual sampling. The evaluation of the impact of MP on outdoor and indoor fungal airborne contamination will be used to update recommendations for the preventing of IA during demolition. Genotype of environmental and clinical strains could be interesting to describe diversity of strains and eventually identification of probable transmission. Susceptibility to antifungal will permit us to estimate the proportion of azole resistance in environmental strains.

Conclusion:
The growing impact of fungal infection and the extra cost related to antifungal curative therapy require the evaluation of adequacy of current technique of environmental monitoring in hospital. The first results obtained helped us to improve health protective measures and professional practice.
TARGET SPECIFIC LEAD COMPOUND IDENTIFICATION FROM NATURAL SOURCE AGAINST ASPERGILLUS FUMIGATUS

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Purpose:
Aspergillus fumigatus is an opportunistic fungus responsible for life threatening fungal infections in immunocompromised patients. The pathogenicity and virulence of this fungus is governed by a variety of virulent factors, melanin being one of them. Melanin is a greenish grey pigment present in the outer cell wall of A. fumigatus conidia that protects the pathogen from UV light, ROS, oxidizing agents and harsh environmental factors including host defense system. Adherence of the pathogen to the host tissue is one of the most crucial steps in the initiation of infection. There are a variety of factors that play an important role in host pathogen interaction. Melanin also plays an indirect role in both pathogenesis and assembly of the cell wall layers of resting conidia. The present work aims at identifying compounds from plant sources that have the potential to inhibit fungal melanin synthesis and accumulation in A. fumigatus conidial cell wall there by rendering the pathogen more susceptible to host defense system and antifungal therapy.

Methods:
The hexane extract of Myristica fragrans was investigated for its effect on reducing melanin biosynthesis and accumulation on the conidial cell wall of A. fumigatus. Concentration leading to inhibition of melanin synthesis was calculated using broth micro dilution method. Melanin inhibitory concentration of the extract was used to analyze its effect on reduction in conidia formation, ergosterol content of cell wall, cell surface hydrophobicity of the conidia and cell surface morphology. The extract was also subjected to GC/MS to identify the bioactive components. The restoration of conidiation and melanization was studied using 1mM cAMP and Scytalone respectively. RT-PCR was conducted to understand the expression of alb1 gene responsible for the synthesis of pksP enzyme. Finally, molecular docking studies were undertaken to understand the degree of compound-ligand binding.

Results:
Complete inhibition of melanin was observed at a sub-MIC of 156 mg/ml. Melanin reduction was associated with 50% reduction in conidia formation. Marked decrease in the hydrophobicity of the conidia and Ergosterol content of the cell wall (91%) was also observed. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) was conducted to study the conidial surface morphology, which showed decrease in surface protrusions and melanin content with visibly smoother cell surface. Increase in both conidial number and melanin formation was seen in the restoration experiment. The mRNA transcriptional analysis revealed a 22 fold increase in the expression levels of alb1 gene as compared to the control. Further, GC-MS of the extract was conducted to understand the compounds present in this extract. Results show high concentrations of compounds such as myristicine, elemicin, iso eugenol and cis 9 hexadecanal in the extract. Molecular docking studies revealed minimum binding energy for cis 9 hexadecanal as compared to others.

Conclusion:
The present work may lead to identification of promising compounds targeting melanin synthesis pathway in A. fumigatus, which in combination with presently available drugs in a synergistic approach may increase the efficacy of the present therapy and decreasing the toxicity associated with it. This will pave way and expand the currently available limited antifungal armamentaria.
ALLOIMMUNE-MEDIATED TISSUE IRON OVERLOAD IS A MAJOR DETERMINANT FOR ASPERGILLUS FUMIGATUS ALLOGRAFT INVASION

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Purpose:
One in three lung transplants suffer from Aspergillus-related pulmonary diseases that present as chronic graft rejection, airway anastomotic infections or invasive pulmonary aspergillosis. Using an orthotopic tracheal transplant (OTT) model of A. fumigatus infection, we previously showed that pathogen invasion correlates with rejection-mediated microvascular ischemia. We have extended these findings by studying the mechanisms that underlie the observed association between alloimmunity and fungal invasion, particularly the role of tissue iron overload from microvascular hemorrhage. We posit that alloimmune-mediated microvascular injury in lung transplants increases tissue iron concentration thereby favoring Aspergillus invasion through: a) release of fungal proteases, b) exposure of epithelial adhesion sites from oxidative injury and c) degradation of vasoprotective hypoxia inducible factor (HIF)-1α.

Methods:
We performed allogeneic (BALB/c, donor to B6, recipient) and syngeneic transplants (B6) and studied A. fumigatus invasion by day post transplant. Using an in vivo Doppler flowmetry probe, FITC-lectin perfusion, microbead extravasation and electron microscopy, we studied the microvascular injury and hemorrhage that occurs during acute allograft rejection. Iron was measured histologically and with inductively coupled plasma-mass spectroscopy (ICP-MS). Aspergillus invasion was quantified using a 0-4 histologic scale. We then performed a series of OTT infection experiments, under increasing iron conditions: a) a high iron condition, using a hemochromatosis (HFE) mouse as the donor or artificially with a ‘surgical paint’ containing iron sulfate (FeSO4) applied to the transplant; b) a medium iron condition, using an allotransplant (blank paint); or c) low iron condition, using an allotransplant treated with a iron chelator (deferasirox (DFX)) paint. Expression of A. fumigatus and host iron metabolism genes and Aspergillus proteases were measured using qPCR. Iron-induced oxidative stress was measured with dihydroethidium staining. HIF-1α levels were measured by Western blotting.

Results:
Progressive alloimmune-mediated microvascular ischemia was associated with increased iron deposition and A. fumigatus invasion (P <0.05). Aspergillus was significantly more invasive in allogeneic transplants from HFE donors (P <0.05), a similar trend also was observed in syngeneic transplants from HFE donors (NS). In animals with artificially increased graft iron (FeSO4-surgical paint), both allogeneic and syngeneic transplants demonstrated increased Aspergillus invasion (P <0.05). At day 12 post allotransplant gene expression studies demonstrated a significantly decreased expression of A. fumigatus HapX (0.22-fold), a regulator of siderophore production and increased expression of SreA (2.7-fold), a transcription regulator of A. fumigatus iron acquisition and metabolism. Host gene expression was characterized by an attempt to limit iron to the invading pathogen (increased expression of hepcidin, ferroportin, nRAMP1 and ferritin (P <0.05)). Expression of A. fumigatus protease genes were >5-fold higher in FeSO4 treated animals than in those treated with DFX for 28% (5/18) of proteases examined. Expression of the protease TppA was >6×104-fold higher in the FeSO4-paint treated allotransplants compared to DFX-treated animals (P <0.05). Alloimmune-rejection and Aspergillus infection were associated with tissue damaging reactive oxygen species production. The increased invasion associated with FeSO4-paint was inversely associated with HIF-1α protein levels.

Conclusion:
We now define, for the first time, alloimmune-mediated tissue iron overload as a major determinant for A. fumigatus graft invasion.
MULTIMODAL IMAGING FOR THE NON-INVASIVE ASSESSMENT OF DISEASE DEVELOPMENT AND PROGRESSION OF INVASIVE PULMONARY ASPERGILLOSIS IN MICE

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Purpose:
Aspergillus is a major cause of life threatening lung infections in immunocompromised individuals. Standard techniques used to evaluate experimental infections in small animals are histology and fungal load quantification in tissue samples. These techniques are highly limited by their invasive character and cannot provide us with any insight in dynamic disease processes. Therefore, there is a strong need for non-invasive imaging techniques to investigate dynamic events in the pathogenesis of fungal lung diseases. In this study, we have used magnetic resonance imaging (MRI) to non-invasively investigate disease development and progression in a mouse model of invasive pulmonary aspergillosis. MRI was combined with bioluminescence imaging (BLI) for the in vivo assessment of fungal cell viability.

Methods:
BALB/c mice were immune suppressed by intraperitoneal (IP) injections of cyclophosphamide 4 and 1 days prior to intranasal instillation of 20 µL A. fumigatus strain 2/7/1 (5.10⁵ spores, n=10) or saline (n=5). After infection, the animals were imaged on a daily basis with MRI (9.4 T, Bruker Biospin) and BLI (IVIS spectrum). Multiple lung parameters (signal intensity, lesion volume and total lung volume) were quantified from the MR images to evaluate time-related changes. On day 4 after infection, the lungs were isolated for validation of the imaging results by CFU counts and histology.

(1) The bioluminescent A. fumigatus strain was kindly provided by Dr. Matthias Brock from the Leibniz Institut für Naturstoff-Forschung und Infektionsbiologie, Hans-Knöll-Institut, Jena, Germany.

Results:
Hyper-intense signals were detected within the lungs of the infected animals on the MR images. The signal intensity, lesion volume and total lung volume calculated from the MR images showed a gradual increase over time, which corresponds to progressing lung disease. In addition, a strong increase in bioluminescent signal was detected within the infected lungs over time, which is associated with an increase in the number viable fungal cells. On the contrary, no signals could be detected within the lungs of the control animals.

Conclusion:
We successfully visualized and quantified the development of fungal lesions within the lungs of infected mice with MRI. This non-invasive imaging technique provides valuable insights in the global extent and 3D distribution of developing lesions. Furthermore, BLI allowed for the in vivo detection of viable fungal cells, thereby providing important information on the composition of lesions detected on the MR images. By combining these complementary imaging techniques, an overall longitudinal picture can be provided about the dynamics of fungal lung infections in a completely non-invasive manner in individual animals.
POLY-ICLC CONFRS PROTECTION AGAINST INVASIVE ASPERGILLOSIS CAUSED BY ASPERGILLUS FUMIGATUS AND ASPERGILLUS TANNERI

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Purpose:
Cases of invasive aspergillosis (IA) are typically treated with azoles, caspofungin and amphotericin B. Although Aspergillus fumigatus, the dominant cause of IA, is relatively susceptible to these drugs, prognosis largely depends upon early diagnosis and host immune status. Other pathogenic Aspergilli, such as Aspergillus tanneri, are inherently resistant to antifungals. Type 1 IFNs induced by poly-IC have been reported to be beneficial for mice infected with A. fumigatus. To assess the benefit of type 1 IFNs in mice infected with aspergilli, especially by those unresponsive to antifungals, we tested poly-ICLC, a stable form of poly-IC in a murine model of IA.

Methods:
Mice with chronic granulomatous disease (CGD) were treated with poly-ICLC (control group received PBS) and infected with conidia of either A. fumigatus or A. tanneri. Lung histopathology and fungal DNA load were performed at 3, 15 and 30-days post inoculation (DPI). Levels of IFNa and IFNb in the lungs were analyzed in naïve CGD mice (no fungal infection) treated with poly-ICLC.

Results:
Infection with A. fumigatus: Eighty percent of the control group (PBS-treated) succumbed to IA in 15 days whereas no death was observed in the poly-ICLC treated group during the same period of time. Histology of lungs from the control group showed the expected severity of disease. At 3 DPI, small lesions were visible throughout the lung parenchyma. At 15 DPI the parenchyma was crowded with fully formed granulomas containing numerous fungal hyphae. In stark contrast, the lungs of mice treated with poly-ICLC showed only sporadic lesions at 3 DPI. At 15 DPI, even though the number and size of the lesions increased, only a few granulomas were observed and GMS stained sections revealed no hyphal growth within the granulomas. Fungal DNA loads were substantially higher in the lungs of the control group compared to the poly-ICLC treated mice, suggesting that growth of A. fumigatus in host tissue was hampered by poly-ICLC treatment.

Infection with A. tanneri: While all mice in the control group succumbed to IA in 80 days, 70% of the mice treated with poly-ICLC were still alive at 100 DPI. Histology confirmed the presence of granulomas at 15 and 30 DPI in the lungs of control group, but only small lesions in the poly-ICLC treated mice were observed. Fungal DNA loads were also substantially higher in the lungs of the control group compared to the poly-ICLC treated mice. Levels of IFNa and IFNb in the lungs of naïve CGD mice increased after poly-ICLC treatment.

Conclusion:
Treatment with poly-ICLC significantly improved survival of the CGD mice infected with either A. fumigatus or A. tanneri. Histological findings and fungal burdens, extrapolated by DNA loads, suggested that poly-ICLC hampered fungal growth and invasion. It is likely that the protection conferred by poly-ICLC was due to the induction of type 1 IFNs and its cascading effects. Further studies are underway to evaluate the potential of poly-ICLC as a treatment option for IA, especially when pathogens that are highly resistant to currently marketed antifungals are the etiologic agents.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
**ASPERGILLUS FUMIGATUS TRANSCRIPTOME REVEALS THE REDUCTION OF METAL METABOLISM AND THE INCREASE OF GLIOTOXIN PRODUCTION DURING A DISSEMINATED MURINE INFECTION**

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**Purpose:**
The aim of this study was to analyze the transcriptome of *A. fumigatus* throughout a disseminated infection in order to determine how the fungus deals with the host environment once the infection has been established.

**Methods:**
Three independent murine infections were performed intravenously, being one infected kidney from each day used for RNA isolation. RNA samples were hybridized with a customized *A. fumigatus* expression microarray. Transcriptomic data were compared with a previous study of the germination of this pathogen at 37°C (Sueiro-Olivares et al., 2015) and results were validated by RT-qPCR. Finally, gene expression changes were analyzed and classified according to their biological function.

**Results:**
Statistical analysis of each day of infection using the *A. fumigatus* germination at 37°C as the control condition showed that 4,080, 377, 3,604 and 1,645 genes were differentially expressed on day 1, 2, 3 and 4 of infection, respectively. According to our results, metal metabolism showed down-regulation during the infection. In iron metabolism, different transcription factors involved in siderophore biosynthesis as well as other siderophore production genes (sidA, sidC, sidD) and iron transporters were down-regulated. Something similar was observed in zinc metabolism, in which exporters, importers and even the transcription factor zafA reduced their expression during the infection. On the contrary, gliotoxin biosynthesis pathway stood out as several genes appeared up-regulated throughout the infectious process.

**Conclusion:**
It seems that once *A. fumigatus* infection has established in the host, a reduction of iron and zinc requirements takes place, questioning the choice of these metabolic routes for developing therapeutic and diagnostic strategies, given that they appear to be useful only when the infection is beginning but not once the invasive aspergillosis is established. Nevertheless, gliotoxin biosynthesis seems to be an indispensable pathway for extending the infection, underlining the necessity of increasing the knowledge of this toxin and its use as antifungal and diagnostic target.

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Purpose:
The ability of microbes to thrive in hypoxic environments is important for virulence. In a murine model of invasive pulmonary aspergillosis (IPA), lesions within the lung experience low oxygen tensions (≤1%). Despite the importance of this response for virulence, molecular mechanisms of hypoxia adaption and its mechanistic link to virulence remain elusive. Here we aim to further elucidate underlying mechanisms through serial passaging of a low virulent \textit{A. fumigatus} strain through host-mimicking (hypoxic) conditions.

Methods:
We utilized an \textit{in vitro} microevolution approach to mimic host conditions of low oxygen tension. Strains were collected at early and late stages of serial passaging, and characterized for phenotypes associated with virulence. On going efforts in the laboratory are focused on global gene expression changes in the passed strains to identify candidate pathways involved in metabolic bioenergetics and virulence.

Results:
Serially passaged strains revealed varied phenotypes from the parental strain in respect to increased sensitivity to redox stress and increased fitness in host-mimicking conditions. In a murine corticosteroid model of IPA, passed strains exhibited enhanced virulence compared to the parental strain as measured by murine survival. Evidence points to significant alterations in metabolic bioenergetics in these passage strains that confers enhanced \textit{in vivo} fitness. Within the immunocompetent lung, the passed strain induced higher levels of inflammatory cytokines early after challenge and appears to be cleared from the lungs more quickly than the parental strain. Further characterization of these underlying mechanisms is in progress.

Conclusion:
Adaptation to hypoxic microenvironments during pulmonary infection is essential for the success of \textit{Aspergillus fumigatus}. Preliminary data indicates a role for modulation of metabolic bioenergetics in response to chronic low oxygen environments \textit{in vitro}. These bioenergetic alterations have a role in enhanced fungal virulence \textit{in vivo}. Variation in the virulence of \textit{A. fumigatus} strains is proposed to be correlated with their ability to initiate these metabolic alterations.
PHAGOCYTOSIS IN ABPA

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Purpose:
Aspergillus fumigatus is a ubiquitous environmental fungus. Humans inhale several hundred conidia each day and although the majority of individuals clear \textit{A. fumigatus} without symptoms, some patients with asthma develop allergic bronchopulmonary aspergillosis (ABPA). Patients suffer wheezing, expectoration of brown mucus plugs, have poorly controlled asthma and develop ‘pneumonia’. If untreated, ABPA can result in pulmonary fibrosis and respiratory failure. Of asthmatics seen in hospital referral clinics, 1-8% have ABPA if systematically sought and it has been suggested that the global burden of ABPA with asthma potentially exceeds 4.8 million people. It is unclear why certain asthmatics develop ABPA while the majority remain unaffected by exposure to \textit{A. fumigatus}. There may be an element genetic susceptibility; ABPA can be found in families, and some studies have identified genetic polymorphisms associated with the disease. The immune response to \textit{A. fumigatus} involves many cell types, including macrophages and neutrophils. These are highly phagocytotic cells that phagocytose and kill the fungus, produce a variety of chemotactic and proinflammatory cytokines, and orchestrate an immune response. As macrophages are present in the airways they may be the first innate immune cell to contact the inhaled fungus. Phagocytosis is an important tool in the macrophage armoury. We have previously found that expression of immune genes by monocyte-derived macrophages (MDMs) exposed to \textit{A. fumigatus} is different in MDMs from ABPA subjects compared to those from asthmatic subjects, suggesting that differing macrophage responses may be important in susceptibility to ABPA.

Methods:
In this study, we completed exome-plus sequencing in of a large cohort of 94 ABPA patients and 126 asthmatic controls to identify the genetic factors involved in susceptibility to ABPA. We also investigated the phagocytic ability of MDMs from ABPA patients. Phagocytosis of \textit{A. fumigatus} conidia was tested in cells from subjects with and without the mutations identified by the exome sequencing, as well as in healthy donor cells treated with siRNA against some of the genes identified in the exome sequencing (EEA1, PLD1, VSP4B). This was investigated using stained conidia and confocal microscopy.

Results:
We identified over 7000 mutations to be significantly associated with ABPA. Of these, over 250 are considered to be likely to be high impact (e.g. cause non-functional protein to be produced) and have odds ratios for ABPA of >10. These mutations, and the genes in which they occur fall into only a small number of biological processes, one of which is phagosome generation and/or maturation. We also found that cells from ABPA subjects and healthy controls vary widely in their ability to phagocytose \textit{A. fumigatus} conidia, and preliminary data suggests that siRNA knockdown can affect the phagocytic ability of cells from healthy subjects.

Conclusion:
Identification and functional validation of phagocytosis defects in ABPA patients will revolutionise our understanding of susceptibility to this disease, and is likely to lead to development of robust diagnostic test for the at risk population as well as suggesting novel areas for research into drug targets for treatments. This would be of extreme benefit to patients who are affected by this disease.
ROLE OF THE *ASPERGILLUS FUMIGATUS* ARRESTIN, PALF, IN pH TOLERANCE AND PATHOGENICITY

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Purpose:
In *Aspergillus* species, the fungal arrestin PalF is required to initiate an intracellular response to extracellular alkalinity, which culminates in proteolytic activation of the pH-responsive transcription factor PacC. In the model ascomycete *Aspergillus nidulans*, PalF is post-translationally modified in response to alkaline pH, an event which is critical for normal pH signalling and which requires tethering to a pH-responsive, integral membrane switch called PalH. Since PacC is a critical mediator of mammalian pathogenicity we hypothesised that *Aspergillus fumigatus* PalF would be essential for pathogenicity, provided that a lack of functional redundancy exists at this step in the pH signalling pathway. In order to test this hypothesis we utilised a Tet-responsive promoter (Tet-OFF) to implement the regulatable expression of PalF.

Methods:
A Tet-responsive palF allele was constructed in the ΔakuB KU80 genomic background by insertion of a ptrA-tetOFF module upstream of the palF coding sequence. Transformants were selected for pyrithiamine resistance and correct insertions were verified by PCR. Phenotypic analysis included assessment of radial and hyphal growth rates. Doxycycline concentrations were titrated in pH 5.0 and pH 8.0 minimal media with and without NaCl, to assess the requirement for PalF for pH and salt tolerance *in vitro*. Transwell infection studies were performed using Calu3 monolayers in order to assess epithelial invasion *in vitro*.

Results:
A tet-responsive palF allele was obtained by homologous recombination resulting in the replacement of the native promoter with a tet-responsive (Tet-OFF) module. Titration of doxycycline in the culture medium revealed a slowed growth phenotype, relative to progenitor isolate, on pH 8.0 [250 mM NaCl] medium when doxycycline concentrations were higher than 50 µg/ml. We have shown that doxycycline-mediated repression of palF does not impact radial growth in lower pH but dies impact growth in alkaline and high salt conditions.

Conclusion:
Phenotypic analysis of a tet-OFF PalF allele supports the theory that PalF is a non-redundant arrestin required for PacC-mediated signalling in *A. fumigatus*. Disparity of phenotype between null PalH mutants and the tet-OFF PalF allele is indicative of either leakiness of the tet-OFF module, or prolonged stability of PalF protein followed doxycycline-mediated repression of transcription. Implications of these data for pathogenicity in *A. fumigatus* and validity of PalF as a drug target will be discussed.
DIFFERENCES IN ACCUMULATION OF AFLATOXIN B1 IN INDIAN RICE VARIETIES

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Purpose:
Rice seeds are known to colonize with Aspergillus flavus in the field or under storage and produce aflatoxins in different quantities which have impact on human and animal health. So the studies were made to estimate the differences in accumulation of Aflatoxin B1 in Indian rice varieties which are popular in the country.

Methods:
In this study, we investigated the varietal differences in 30 Indian healthy rice varities for human consumption (15 normal and 15 basmati) for accumulation of aflatoxin B1 (AFB1) after inoculation of aflatoxin producing A. flavus (DRAf 009).

Results:
Significant varietal differences in AFB1 accumulation were observed in normal and basmati rice cultivars. Comparatively, the accumulation of AFB1 is high in normal cultivars ranging from 3.0-628.7 μg/kg than basmati cultivars ranging from 0.2-7.2 μg/kg. This may be due to the differences in phenolic compounds and other nutrients present in various cultivars. The highest accumulation of AFB1 in normal cultivars were observed in PR 106 (628.7 μg/kg) and low accumulation in IR 64 (3.0 μg/kg). In basmati rice cultivars, the highest accumulation of AFB1 were observed in Ranbir basmati (7.2 μg/kg) and lowest in Vasumati (0.2 μg/kg).

Conclusion:
This study may be a basis to develop A. flavus resistant rice cultivars and proper storage structures to produce aflatoxin free rice. This is the first report on evaluation of various rice cultivars for accumulation of AFB1 after inoculation of A. flavus.
INDICATIONS OF POSSIBLE *ASPERGILLUS FUMIGATUS* BIOFILM FORMATION DURING AN INTRANASAL MURINE INFECTION OBTAINED BY TRANSCRIPTOME STUDIES.

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**Purpose:**
*Aspergillus fumigatus* is the most ubiquitous airborne pathogenic mold worldwide, causing invasive aspergillosis (IA) in immunocompromised hosts. Nowadays, to deepen into the biology of this fungus and improve therapeutic and diagnostic methods, fungal virulence factors are studied using animal infection models and transcriptomic technologies. Therefore, our research group performed an intranasal infection model, in order to study the fungal behavior using a customized microarray expression.

**Methods:**
Three independent intranasal experimental infections of immunosuppressed mice with *A. fumigatus* were carried out. Two daily animals were sacrificed and their lungs extracted during the following 3 days. Lung tissue was processed and total RNA was obtained. Fungal RNA expression profiles were studied using the AWAFUGE v.1 expression microarray designed by our research group (Sueiro-Olivares 2015), and results were validated by RT-qPCR. Data analysis was carried out using a modified R code base on Bioconductor and limma libraries. To summarize a linear model was employed in order to compare the different RNA expression levels between days.

**Results:**
In this study, we focused in the comparison of relative expression in LogFC (Fold Change) of fungal genes previously related to virulence. Significant differences were not found between genes and days, but it was observed that several genes related to biofilm formation were up-regulated during all the process with a stable expression. This group includes *RodA* and *RodB* hydrophobins, which have an essential role in immune response evasion and in biofilm formation (Gibbons, 2011). In addition, we studied the synthesis of α1-3 glucan because is an essential compound to hyphae agglutination and, thereby, biofilm formation (Loussert, 2010). The three genes encoding for α,1-3 glucan synthases; *Ags1, Ags2* and *Ags3*, were detected but showing only the last two genes more expression during the process.

**Conclusion:**
In spite of differences not being significant the expression of most of the biofilm formation-related genes detected throughout the infection showed up-regulation or growing expression tendency. These results suggest that the biofilm formation could happen in mice lungs during intranasal infections.

**References**

**Financial support**
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A PH-RESPONSIVE MOLECULAR SWITCH REQUIRED FOR ASPERGILLUS FUMIGATUS PATHOGENICITY

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Purpose:
In fungi, efficient adaption to extracellular pH combines cellular homeostasis and regulated genetic control, the latter exerted via a family of pH-responsive transcription factors PacC/Rim101. Aspergillus species monitor extracellular pH via 7 transmembrane domain (7-TMD) integral membrane proteins called PalH. These putative pH sensors activate PacC-mediated signaling via interaction with a cognate arrestin PalF, potentially acting as pH-dependent molecular switches required for pathogenicity. The aim of this study was to decipher the role of A. fumigatus PalH in PacC-mediated signaling and during mammalian infection.

Methods:
ΔpalH mutants were tested for (i) PacC processing at different pHs (pH 3.5, 5.0 and 8.0) using electrophoretic mobility shift assay (EMSA), (ii) sensitivity to alkaline pH, salt stress and cell wall damaging agents, (iii) damage to epithelial A549 monolayers and (iv) virulence in a leukopenic murine model of pulmonary aspergillosis. To probe the mechanistic basis of PalH-mediated signalling, we utilised a split-ubiquitin Membrane Yeast Two-Hybrid (MYTH) assay to assess protein-protein interactions amongst candidate A. fumigatus signalling proteins of this pathway. To look for novel, membrane-proximal, regulators of PacC, a S. cerevisiae library of recombinant A. fumigatus preys was constructed and screened against a full length PalH bait. A. fumigatus null mutants of putative PalH interactors were constructed and subjected to phenotypic analysis at acidic and alkaline pH.

Results:
EMSA analysis indicated an absolute requirement for PalH for PacC processing, whereby PacC processing was drastically impaired in ΔpalH mutants at all pHs tested. The ΔpalH mutants were sensitive to alkaline pH and to cationic stress, whereby growth was completely abolished at pH 8.0 in the presence of 100 mM NaCl. The mutants were also sensitive to cell wall-perturbing agents such as 0.007% SDS and 20 mg/l congo red and in the presence of the cell wall-active antifungal caspofungin undergo extensive hyphal branching and ballooning compared to the parental and reconstituted strains. In the absence of PalH A. fumigatus-mediated damage to epithelial cells is significantly reduced in vitro. Furthermore, the ΔpalH mutants are drastically attenuated for virulence in a leukopenic murine model of pulmonary aspergillosis. Expression of the recombinant PalH and PalF proteins in Saccharomyces cerevisiae indicated stable interaction between the receptor and its cognate arrestin, and also indicated oligomerisation of the PalH receptor, a hypothesis supported by analysis of differentially-tagged PalH proteins. Iterative screening of the library of recombinant A. fumigatus preys identified several clones expressing putative PalH interactors. Concordant with a role for these gene products in pH-mediated signaling, a variety of pH-sensitive phenotypes were identified amongst the cohort of null mutants.

Conclusion:
Our results indicate that PalH is an oligomerising, non-redundant activator of PacC-mediated signaling for adaptation to extracellular pH. This highly conserved fungal pH sensor is required for A. fumigatus virulence and echinocandin tolerance. Future studies will focus upon the mechanism of PalH-mediated pH sensing.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
BIOSYNTHESIS OF THE SULPHUR CONTAINING AMINO ACIDS IS A VIRULENCE DETERMINANT IN ASPERGILLUS FUMIGATUS

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Purpose:
Despite current treatment options, invasive infections caused by Aspergillus fumigatus continue having a high associated mortality rate. Nutrient supply and metabolic versatility are known to be essential virulence traits, a better understanding of which might derive in the identification of novel suitable molecular targets for the development of more efficient chemotherapy. We had previously demonstrated that regulation of sulphur assimilation is essential for A. fumigatus pathogenicity. Therefore, in this study we aimed to perform an in-depth characterization of the trans-sulfuration pathway to define the relevance of the sulphur containing amino acids for fungal virulence.

Methods:
We have constructed several single, double and conditional mutants eliminating key genes of the trans-sulfuration pathway to create cysteine and methionine auxotrophic strains. Subsequently, we have investigated the nutrient requirements, oxidative resistance and virulence capacities of those strains to determine the relevance of the sulphurated amino acids biosynthesis for A. fumigatus growth in vivo.

Results:
A cysteine auxotrophic strain has reduced virulence capacities, proving that the amount of cysteine in the lungs is limited. This mutant further evidences the robustness of the subordinate glutathione redox system in A. fumigatus. Interestingly, we show that growth of a methionine synthase conditional mutant can only be rescued in the presence of all amino acids, but is avirulent in a murine model of invasive pulmonary aspergillosis. This demonstrates that the amount of readily available amino acids in the lung tissue is rather scarce and, furthermore, that this enzyme is essential for intrapulmonary growth. Taking into account the difference between fungal and mammalian methionine synthases, we propose that it constitutes an ideal metabolic target and will pursue the development of a specific drug to fight A. fumigatus infection.

Conclusion:
Cysteine biosynthesis is important for intrapulmonary growth, whereas the action of the methionine synthase gene product is essential for A. fumigatus virulence.
EXPERIMENTAL ASPERGILLUS NIDULANS AND ASPERGILLUS FUMIGATUS INFECTION IN CHRONIC GRANULOMATOUS DISEASE MICE

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Purpose:
Invasive aspergillosis (IA) is the main cause of premature death in patients with chronic granulomatous disease (CGD). Aspergillus nidulans is the second commonest cause of IA in CGD after A. fumigatus but rarely occurs in other at risk groups, appearing almost unique to CGD. The reasons for this are unclear. Understanding of disease pathogenesis in the CGD host is limited and it is increasingly evident insights from neutropenic models cannot be directly translated to CGD. Additionally, antifungal killing mechanisms and host inflammatory response appear to differ significantly between A. nidulans and A. fumigatus infection. This work aims to unravel the fungal pathogenesis of A. nidulans infection in the CGD host through an experimental murine model of infection and in direct comparison with A. fumigatus infection.

Methods:
CGD (CYBB/C57BL6) and wild-type (C57BL6) mice were infected with either A. fumigatus or A. nidulans by intra-tracheal (IT) instillation of conidia (5 x 10⁴) under anaesthesia. The innate immune response was investigated at set time points following infection using; lung histology and FACS analysis of lung digests to assess leucocyte recruitment; multiplex bead assays to assess cytokine production in BAL and lung homogenates; quantitative culture, PCR and lung histology to assess fungal burden.

Results:
Following infection with A. nidulans, CGD mice demonstrated significantly increased neutrophil recruitment to the lung when compared with wild-type mice at 24 hours (p<0.001), 3 days (p<0.001) and 7 days (p<0.0001). Neutrophil number increased throughout infection with no evidence of resolution in the CGD mice. IL-1β was significantly increased in the lung homogenates of CGD mice by day 3 (p<0.0001). This continued to increase throughout infection (10-fold increase by day 17, p<0.0001). KC was also significantly increased at day 3 (p<0.001) and remained elevated, although non-significantly, throughout infection. TNF-α production was reduced in CGD mice compared with wild-type at day 3 (p<0.0001) but normalized by 7 days. Quantitative culture of lung homogenates demonstrated recoverable A. nidulans from CGD mice at 3 and 7 days following infection. Wild-type samples demonstrated no recoverable fungi at these times.

Conclusion:
A. nidulans infection in CGD mice is characterized by an exaggerated and sustained neutrophilic inflammatory response with significantly increased IL-1β and KC production. This highly pro-inflammatory innate response fails to clear infection and results in significant host damage. These results are in keeping with in vitro work demonstrating increased IL-1β production by CGD phagocytes and excessive neutrophil recruitment in CGD mice during A. fumigatus infection. The failure to resolve inflammatory cytokine production and neutrophil recruitment may explain the ultimately higher mortality associated with A. nidulans infection. Currently, innate immune responses to A. fumigatus infection are being characterized and compared to A. nidulans infection.
REGULATION OF IN VIVO FITNESS AND VIRULENCE THROUGH THE ASPERGILLUS FUMIGATUS TRANSCRIPTION FACTOR CREA

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Purpose:
A. fumigatus encounters a variety of microenvironmental conditions during infection including changes in nutrient availability. To combat these changes fungi use fine tuned regulatory networks to allow use of preferred nutrients before less preferred nutrients. In the case of carbon utilization, this network, in some fungi, is regulated in part by the transcriptional repressor, CreA. Because A. fumigatus and infiltrating host cells metabolize glucose rapidly as a preferred carbon source, we hypothesize that local glucose concentrations in the lung becomes limited quickly, and consequently, the ability to utilize alternative carbon sources in vivo is critical for in vivo growth and virulence.

We have begun to characterize in vivo carbon source utilization, in part, through characterization of the A. fumigatus CreA homologue. The aim of this project is to understand how the activity and regulation of this transcription factor in A. fumigatus allows the fungus to thrive within the host.

Methods:
We generated creA-null mutants in multiple backgrounds, and phenotypically characterized them with liquid and solid plate growth assays on various carbon and nitrogen sources, as well as under drug treatments and environmental stress. We employed UPLC-MS/MS to profile global metabolites in a null mutant and wild type strains in glucose replete conditions. We have also used three immunologically distinct murine models of invasive pulmonary aspergillosis to evaluate in vivo growth, virulence of the creA-null mutant and subsequent host responses.

Results:
Through growth assay characterization and metabolomics profiling of the creA-null mutant, we have identified key pathways that are perturbed in the mutant which directly alter in vivo fitness during infection. Surprisingly, the mutant has a 30 - 50% decrease in radial growth on both repressing and de-repressing carbon sources, as well as on rich and poor nitrogen sources. The exception, however, is growth on proline as the sole nitrogen source, which results in growth comparable to wild type. Furthermore, levels of AMP in the mutant are significantly increased as compared to wild type, as well as the ratio of ADP/ATP, indicating inefficient energy production or usage in this strain. Taken together, with the observation that the creA-null mutant is significantly more sensitive to rapamycin, these data suggest aberrant TOR signaling in this mutant. We hypothesize that loss of CreA not only perturbs carbon metabolism in A. fumigatus, but perturbs nitrogen metabolism as well. In further support of this hypothesis, the levels of several proteinogenic amino acids are significantly decreased in the mutant compared to the wild type. We have also observed that the the creA-null mutant is significantly more sensitive to nitrosative stress, and to a lesser extent, oxidative stress. Finally, we have observed that the creA-null mutant has decreased growth in two immune-suppressed murine models of IPA, as measured by histology and fungal burden. Compared to the wild type, the creA-null mutant causes less damage and vascular leakage within the immune-competent lung, as measured by LDH and albumin, respectively. Furthermore, the creA-null mutant induces lower levels of pro-inflammatory cytokines in this immune-competent model.

Conclusion:
Our data demonstrate that CreA is a master transcriptional regulator of metabolic bioenergetics in A. fumigatus coordinating carbon and nitrogen metabolism. Importantly, loss of this transcription factor results in a significant decrease in in vivo fitness and virulence.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
ASPERGILLUS FUMIGATUS INDUCED AIRWAY WALL REMODELLING IN ASTHMA

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Purpose:
Asthma is a common chronic inflammatory condition which affects over 300 million people worldwide. Thickening of the sub-epithelial layer is a key feature of asthmatic airways and the extent of thickening has been correlated with severity of asthma and increased exacerbations. Recent epidemiological studies have shown a link between fungal sensitisation and exacerbations of asthma leading to increased morbidity and mortality. The major respiratory diseases caused by fungi include allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitisation (SAFS). Airway epithelium acts as an initial defence barrier to inhaled allergens and pathogens and emerging evidence suggests that as well as orchestrating an allergic immune response, it initiates aspects of airway wall remodelling including sub-epithelial thickening. However, induction of a pro-fibrogenic response by the airway epithelium following exposure to inhaled fungi associated with severe asthma, has not been well documented. The aim of this project is to investigate whether exposure to Aspergillus fumigatus contributes to subepithelial fibrosis by stimulating the induction of pro-fibrogenic cytokines from bronchial epithelium.

Methods:
Bronchial epithelial cells were stimulated with Aspergillus fumigatus and expression and production of pro-fibrogenic cytokines including TGF-β1, TGF-β2, IL-6, and endothelin-1, analysed by real time PCR and ELISA, respectively. In order to ascertain whether proteases were important for the production of these profibrotic cytokines protease inhibition studies were performed. Bronchial fibroblasts were stimulated with conditioned media from spore-exposed bronchial epithelial cells to determine their fibrogenic response. Collagen production was investigated by sircol assay and α-SMA production by western blot. Fibroblasts were also exposed directly to spores of Aspergillus fumigatus and expression of procollagen and α-SMA investigated by real time PCR. Using established mouse models of Aspergillus fumigatus antigen and germination of spores we hope to confirm some of our in vitro findings.

Results:
Aspergillus fumigatus induced a statistically significant release of IL-6 and endothelin-1, but not of TGF-β1 or TGF-β2, from bronchial epithelial cells. IL-6 increased significantly between 6 and 12 hours whilst endothelin-1 increased predominantly between 12 and 18 hours. Protease inhibition showed that fungal serine protease activity was involved in the production of endothelin-1. Fibroblasts treated with conditioned media from spore- exposed epithelial cells showed upregulation of procollagen expression but a decrease in overall collagen deposition. Upregulation of procollagen but not α-SMA was also found in fibroblasts directly exposed to Aspergillus fumigatus spores.

Conclusion:
Aspergillus fumigatus exposure caused an upregulation of several key profibrotic cytokines in bronchial epithelial cells. Germination and stage of Aspergillus fumigatus growth influenced the response of epithelial cells. Further studies are required to determine whether fibroblasts are responding to factors produced by the fungus or by the primed epithelial cells.
MODIFICATIONS OF THE COMPOSITION OF THE HYPHAL OUTERLAYER OF \textit{ASPERGILLUS FUMIGATUS} MODULATES HUVEC PROTEINS RELATED TO INFLAMMATORY AND IMMUNE RESPONSE

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Purpose:
\textit{Aspergillus fumigatus} is an angioinvasive fungal pathogen and the main etiologic agent causing invasive aspergillosis. Upon contact with human umbilical vein endothelial cells (HUVECs), this fungus induces cellular injury, inflammatory response and a pro-thrombotic endothelial phenotype. However, the pathogen molecules involved in the endothelial response are still unknown. \textit{A. fumigatus} hyphae have been shown to produce, both \textit{in vivo} and \textit{in vitro}, an extracellular matrix composed of galactomannan, galactosaminogalactan (GAG) and \(\alpha\)-(1,3)-glucan. The deletion of \textit{UDP Galp mutase} gene in \textit{A. fumigatus} (\textit{UGM1}) leads to a mutant \textit{Δugm1} with altered galactomannan free of galactofuranose, and a higher expression of galactosaminogalactan. Our previously results showed that besides a hyperadhesive phenotype to HUVECs, the \textit{Δugm1} strain also induced an increased endothelial inflammatory cytokine release and tissue factor expression. Therefore, we aimed to investigate which pathways were involved in the endothelial response to this mutant.

Methods:
To achieve this goal we performed a label-free proteomic approach using high definition mass spectrometry, comparing four HUVEC experimental conditions: \textit{Δugm1}:HUVEC interaction, WT:HUVEC interaction, GAG:HUVEC interaction and the uninfected HUVEC (control). The protein interactions and pathways were analyzed \textit{in silico} using QIAGEN’s Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity) and the Reactome Pathway Database (Reactome V53, www.reactome.org).

Results:
A quantitative analysis showed that the \textit{Δugm1} strain was able to induce 38\% more differentially regulated protein, in comparison to control, than did the WT strain. In addition, the \textit{Δugm1} mutant of \textit{A. fumigatus} modulated specific endothelial pathways related to the inflammatory and immune response mediated by TNF-\(\alpha\), as well as to stress response. Furthermore, interaction assays of HUVECs with the purified galactosaminogalactan showed that this polysaccharide was able to induce TNF-\(\alpha\) secretion and also modulate some of the pathways identified in the interaction of \textit{Δugm1} with HUVECs. Finally, the TNF-\(\alpha\) was predicted as the major upstream regulator cytokine involved in the HUVEC response to \textit{A. fumigatus} strains and galactosaminogalactan.

Conclusion:
In conclusion, our data indicate that the modifications of the composition of the hyphal outerlayer of \textit{A. fumigatus}, resultant of \textit{UGM1} disruption, modulate HUVEC key proteins and pathways involved in the immune response, inflammation, blood coagulation and response to cell stress. Notwithstanding, it seems that galactosaminogalactan has an important role in the modulation of these pathways and that these processes seem to be dependent on TNF-\(\alpha\) secretion.
DUAL RNA-SEQ OF PATHOGEN AND HOST DURING ASPERGILLUS FUMIGATUS INFECTION

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Purpose:
Aspergillus fumigatus is an opportunistic pathogen involved in a range of human diseases. Clinical manifestations are linked to the host immune response causing fatal invasive aspergillosis (IA) in the immunosuppressed patient. Due to this mutual dependency, a deeper understanding of the pathogenic process requires the simultaneous view of both organisms. To this end, we have applied Dual RNA sequencing (D-RNAseq) which allows analysis of the global transcriptome of host and pathogen from the site of infection in a murine model of aspergillosis.

Methods:
Two independent murine infections were performed. Lungs for RNA isolation were collected at two and four days post infection. Libraries were loaded with a Hi-Seq 2000 instrument (Illumina) obtaining 50-bp paired-end reads. Reads were aligned using TOPHAT against the complete genome sequence of Mus musculus ensemble release 47, and A. fumigatus ensemble CADRE 21. HTSeq package was used to obtain gene counts, differentially expressed genes were detected with DESeq package (adjusted P value < 0.1). A threshold of 1.5-fold change was used to identify over and under expressed genes. Differentially expressed genes were classified according to functional categories. To verify the differential regulation of some gene functions, A. fumigatus gene expression was determined using qRT-PCR and the immune response was assessed by quantification of cytokines by luminex.

Results:
A total of eight datasets from two replicates were created: uninfected and infected mice at two days and four days post infection. As expected the relatively low number of A. fumigatus cells per host cell resulted in an unfavorable fungi:host RNA ratio. We obtained between 94 to 107 million reads mapped to Mus musculus genome (> 95%) and an average of one million of reads mapped to the A. fumigatus reference genome (< 3 %). Comparison of the transcriptome of infected relative to not infected mice at two days post infection reveled a total of 1036 murine genes differentially expressed, of which 223 were induced and 4 were repressed. At a later stage of the infection (four days) we identified 8186 genes that were differentially regulated. Among them 1,094 and 380 genes were over- and under-expressed respectively. We identified numerous enriched pathways involved in immune response such defense response, inflammatory response, response to wounding, chemotaxis and cytokine activity and production. In keeping with the transcriptional data, concentrations of pro-inflammatory cytokines (IL1b, IL6 and TNFα) as well as anti-inflammatory cytokines such as IL10 increased at for days post infection.

Of the 9,898 possible ORFs of A. fumigatus, counts for 4,203 were obtained but less than 1% of the genes were differentially expressed among infected mice between the two time points. According to the enrichment of functional categories for the A. fumigatus up-regulated genes, polysaccharide metabolism, threonine catabolic process, glyoxylate cycle, secondary and fatty acid metabolism were significantly over represented.

Conclusion:
We have seen that D-RNAseq is a powerful tool to quantify transcriptional profiling of mixed species samples. Further characterization of both transcriptomes in vivo will facilitate a better understanding of aspergillosis contributing to design novel strategies against A. fumigatus infections.
THE INTERPLAY BETWEEN \textit{A. Fumigatus} AND AIRWAY MUCINS IN CYSTIC FIBROSIS

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Purpose:
The impact of fungal pathogens such as \textit{A. fumigatus} on cystic fibrosis (CF) airways has until recently been underestimated. The use of more sensitive culture methods has begun to shed light on the prevalence of \textit{A. fumigatus} in CF sputum, however its precise role in the progression of CF lung disease remains unclear. As the first point of contact for \textit{A. fumigatus} following inhalation, the mucus barrier provides an important site for host-conidia interactions. Previous studies have suggested that \textit{A. fumigatus} may alter the composition of the airway mucus barrier by altering mucin gene expression \cite{1}, and in the presence of mucin-based medium, \textit{A. fumigatus} has been shown to upregulate protease expression and secretion \cite{2}. However, many aspects of this interplay are yet to be elucidated. Specifically, the effects of \textit{A. fumigatus} on the major structural components of airway mucus, namely the polymeric gel-forming mucins (MUC5AC and MUC5B), have not been studied in detail. In order to investigate this further we studied the effects of \textit{A. fumigatus} on the structural properties of purified solutions of MUC5AC and MUC5B, and on the physical properties of saliva: a model system of MUC5B-rich mucus.

Methods:
The effects of \textit{A. fumigatus} culture secretions on the size and glycosylation of purified mucins were studied. Micro-rheology studies were also performed to determine whether such changes may be reflected in the rheological properties of mucus. Furthermore, on-going experiments using air-liquid interface culture are studying the effects of \textit{A. fumigatus} culture filtrates on mucin production and mucus gel properties in airway epithelial cells.

Results:
Our data suggest that \textit{A. fumigatus} is able to degrade MUC5AC and MUC5B, by targeting both the protein and carbohydrate portions of the mucin molecules. Preliminary experiments suggest that proteolytic degradation of mucins is most likely mediated by serine protease activity. This degradation is reflected by an apparent loss in the viscosity of saliva following exposure to \textit{A. fumigatus} secretions (3.35 MPa over 1 hour), suggesting that the structural organisation of the mucin network is being altered.

Conclusion:
These effects may highlight important mechanisms of \textit{A. fumigatus} pathogenesis, whereby it degrades mucins either as a nutrient source, or to physically diminish the mucus barrier in order to facilitate airway colonisation.

\textit{NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.}
FUNCTION OF *ASPERGILLUS FUMIGATUS* RIPS PRECURSOR-LIKE GENES IN PH RESPONSE AND PATHOGENICITY

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**Purpose:**
Recently, ribosomal peptide synthesis (RiPS) gene clusters have been identified in *Aspergillus flavus* as a possible new class of secondary metabolic pathway in filamentous fungi. In this pathway, a precursor protein, containing a signal peptide for endoplasmic reticulum trafficking and a highly repeated core peptide sequence, is processed by protease(s) and converted to a peptidyl compound. Although this class of pathway is widely preserved and distributed amongst *Aspergilli*, including *Aspergillus fumigatus*, their biological function is unknown. Since a compound synthesised by this pathway, ustiloxin, has a strong inhibitory activity against tubulin assembly, this pathway or RiPS precursor-like proteins in *A. fumigatus* might negatively impact host cells during fungal infection. To investigate this possibility, several *A. fumigatus* RiPS precursor-like genes were deleted and tested for pathogenicity in epithelial and murine models of disease.

**Methods:**
Based upon amino acid sequence characteristics, RiPS precursor-like genes were computationally detected in the genome sequences of the *A. fumigatus* A1163 and Af293 strains. According to genomic context and expression levels during growth at 24 h under YGMM medium, deletion mutants for two genes were constructed in each of the A1163 and Af293 strains by replacing the gene region with a hygromycin resistance gene by homologous recombination. Transformants were selected for hygromycin resistance and correct insertions were verified by PCR and Sanger sequencing. Phenotypic analyses were conducted to assess growth rates in vitro. Transwell infection studies were performed using Calu3 monolayers in order to assess epithelial invasion in vitro and neutropenic and corticosteroid murine models of invasive aspergillosis were used to assess mammalian pathogenicity.

**Results:**
Around 40 RiPS-precursor-like genes were detected in the genomes of each of the A1163 and Af293 strains. On the basis of transcriptional profiles and genomic context, at least two candidate gene products were deemed as being highly plausible substrates for functional peptidyl compounds. Deletion mutants for these two genes, *rps1a* and *rps2a*, were successfully created in both A1163 and Af293 and showed heavier pigmentation relative to the progenitor isolates, a phenotype which became more potent at high pH.

**Conclusion:**
Amongst ~40 RiPS precursor-like genes identified from each of the *A. fumigatus* A1163 and Af293 genome information, deletion mutants for two, *rps1a* and *rps2a*, were obtained in each of the A1163 and Af293 genetic backgrounds, thereby indicating non-essentiality of gene function. Analysis of mutant growth under different pH and salt conditions revealed a potently pigmented phenotype, particularly at higher pH conditions, as well as reduced alkaline tolerance. These data indicate that the genes are not pseudo genes but functional, and as such the pathogenicity of all null mutants is under investigation in epithelial and murine infection assays. Pathogenicity data will be presented in the poster.
COPPER HOMEOSTASIS IN ASPERGILLUS FUMIGATUS AND IMPACTS ON PATHOGENICITY

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Purpose:
Regulation of metal acquisition and detoxification plays a critical role in survival and virulence of human pathogens. Macrophages phagocytizing Aspergillus fumigatus spores are thought to employ several strategies for killing the pathogen including influx of Cu⁺ ions. Here we explore the role of copper homeostasis and copper regulation in the opportunistic pathogen A. fumigatus in interactions with the host.

Methods:
The genome sequence of A. fumigatus harbors three genes encoding copper fist transcription factors, hypothesized to regulate copper homeostasis in the fungus, that we name aceA, macA, and cufA. All three genes were deleted and deletant strains assessed for virulence and growth on copper deficient and excess media.

Results:
Deletion of aceA impaired resistance to toxic concentrations of copper, most likely through loss of transcriptional activation of the putative copper exporting ATPase CrpA. Consequently, ΔaceA mutants displayed reduced killing rates in a neutropenic mouse model and reduced survival when challenged with murine alveolar macrophages in vitro compared to the wild type. Although, ΔmacA and ΔcufA mutants showed distinct developmental phenotypes regarding extreme copper environments, they did not alter pathogenicity in mice.

Conclusion:
Based on our results, we present a model of how A. fumigatus controls copper homeostasis and compare it to strategies from two other pathogenic fungi, Cryptococcus neoformans and Candida albicans.
**ASPERGILLUS FUMIGATUS ISOLATES FROM AIR AND SURFACES OF THE INTERNATIONAL SPACE STATION**

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**Purpose:**
One mission of the Microbial Observatory Experiments on the International Space Station (ISS) is to examine the molecular phylogeny and radiation resistance of fungal isolates found on the station. Here characterize two *Aspergillus fumigatus* strains isolated from ISS in terms of fungal secondary metabolism, virulence and DNA polymorphisms.

**Methods:**
Two fungal strains, ISSF 21 and IF1SW-F4, were isolated from a HEPA filter and surface of the ISS, respectively. These strains were identified through ITS analysis and the whole genome of ISSF 21 was sequenced. Both strains were assessed for virulence using the zebrafish larval model of invasive aspergillosis (IA).

**Results:**
ITS sequence, toxin profile and whole genome sequence identified the strains as *A. fumigatus*. A comparison of the ISSF 21 genome to that of the reference strain *A. fumigatus* 293 showed 100s of single nucleotide polymorphisms (SNPs); data is now being analyzed to see if any SNPs lie in known *A. fumigatus* virulence genes. A comparison of virulence in the zebrafish IA model indicated that ISSF 21 was more virulent than two clinical strains (Af293 and CEA10). Currently the virulence of IF1SW-F4 is being tested.

**Conclusion:**
At least one of the *A. fumigatus* strains isolated from ISS may be more virulent than two common reference strains. Efforts are under way to determine if this putative increase in virulence is attributed to microgravity.
A FUCOSE SPECIFIC LECTIN (FLEA) FROM \textit{ASPERGILLUS FUMIGATUS} CONIDIA MEDIATES BINDING TO AIRWAY MUCINS AND PHAGOCYTOSIS BY ALVEOLAR MACROPHAGES

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\textbf{Purpose:}
Invasive pulmonary aspergillosis (IPA) infection is initiated primarily with inhalation of airborne conidia; therefore a critical step in establishing fungal ingress is at the primary event of infection. Whereas the importance of host lectins in mediating fungal recognition through attachment to conidial carbohydrates is well known, here we explore the inverse, where a fucose specific lectin (FleA) of \textit{A. fumigatus} conidia may mediate fungal recognition through attachment to host fucose residues.

\textbf{Methods:}
The genomes of two opportunistic \textit{Aspergillus} species, \textit{A. fumigatus} and \textit{A. flavus}, contain \textit{fleA} encoding a fucose binding protein. To assess a possible role of FleA in disease development, deletion strains were made in both species to examine importance in binding. FleA expression was examined by microscopic visualization of \textit{A. fumigatus} FleA::RFP and \textit{A. fumigatus} fleA transcript levels were assessed during fungal development.

\textbf{Results:}
Compared to wild type conidia, ΔfleA conidia bound significantly less to mucins and were poorly phagocytized by macrophages for both \textit{A. fumigatus} and \textit{A. flavus}. Northern analysis revealed that \textit{fleA} expression increases during asexual development and FleA::RFP strains show that FleA is expressed in conidia of \textit{A. fumigatus}. Western analysis shows FleA is also secreted.

\textbf{Conclusion:}
These findings demonstrate that FleA is critical in the mechanisms of mucin binding, mucociliary clearance, macrophage binding, and potentially in immune recognition.
ASPERGILLUS KERATITIS IN CONTACT LENS WEARERS

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Purpose:
Due to the abundance of *Aspergillus* species (e.g. *A. flavus, A. terreus, A. fumigatus* and *A. niger*) in the environment, exposure to their spores is a frequent occurrence. However, disease development due to fungal invasion occurs primarily in individuals with reduced immunity. These spores can get into contact lenses of people thereby contaminating the lenses. Contact lens contamination by *Aspergillus* species has led to a number of ocular disorders such as *Aspergillus* keratitis. The cornea must be in a perfectly transparent state to allow an individual visualize his/her environment. Keratitis, an inflammation of the cornea that frequently arises due to infection, is a threat to corneal transparency. Different species of *Aspergillus* may cause keratitis. *Aspergillus* keratitis needs to be recognized and treated promptly as it is a medical emergency, since the patient frequently presents with pain and loss of vision.

Methods:
A survey of *Aspergillus* keratitis in contact lens wearers reporting to the Optometry Clinic in the University of Benin, Nigeria from February 2012 to April 2013, was done.

Results:
This pain however could be mild or severe depending on the extent of inflammation. Sensitivity to light may also be present and the eye may appear red and watery. This paper, therefore, seeks to highlight the implications of *Aspergillus* keratitis on contact lens wearers.

Conclusion:
Fungal contamination of contact lenses provides evidence that contaminated contact lens cases may be a replenishable source of pathogenic microbes, with high morbidity and blindness.
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Purpose:
Rhinosinusitis caused by the usual Aspergillus spp. is very common. However, rare isolates of Aspergillus causing fungal sinusitis is also on the rise.

Methods:
We hereby report three cases of fungal sinusitis caused by uncommon Aspergillus such as A. versicolor and A. sydowii. Case 1 was a 40-year-old female came with complaints of nasal block and nasal discharge for past one and a half years with history of previous nasal surgery. Her computed tomography (CT) scan of paranasal sinus (PNS) showed bilateral ethmoidal sinusitis. Case 2 was a 43-year-old male known asthmatic presented with complaints of nasal block for last five years, was diagnosed to have bilateral sinonasal polyposis by anterior rhinoscopy. Case 3 was a 17-year-old female known asthmatic presented with headache, nasal discharge and frequent sneezing for last six months. Her CT PNS showed left side deviated nasal septum with left side pan sinusitis along with right frontal sinusitis. All the three patients underwent functional endoscopic sinus surgery (FESS). The material was sent to the microbiology laboratory for fungal culture and potassium hydroxide mount. Speciation by slide culture was not conclusive. Hence, molecular methods were opted for speciation.

Conclusion:
Reporting of these cases will ensure awareness among the microbiologists about the not so common Aspergilli as a cause of fungal sinusitis. The need of molecular methods for speciation has also been emphasized here as it is difficult to speciate these Aspergilli using routine conventional methods.
Invasive aspergillosis (IA) is associated with high mortality and the globally reported commonest causative agent is *A. fumigatus*. It is considered a major threat for hemato-oncological and transplant patients whereas often underestimated in ICUs. This study was conducted to get a complete clinical and mycological picture of IA in medicine ICUs of a tertiary care hospital in Northern India.

Methods:
Two hundred thirty five medicine ICU patients with clinical suspicion of IA were enrolled in the study. The different clinical details were noted with 30-day mortality status. Galactomannan antigen testing was done and ROC curve for appropriate index for this patient group was constructed. Phenotypic identification of the isolates obtained was done by morphology on lactophenol cotton blue mount and growth on different temperatures. Antifungal susceptibility was done to amphotericin B, caspofungin, micafungin, itraconazole, posaconazole and voriconazole by CLSI M38-A2 and EUCAST (E.Def 9.1) methodology. The in-vitro interaction of caspofungin with amphotericin B and voriconazole were also tested (0.015–1.0 µg/ml) on the resistant isolates with a microdilution chequerboard method based on the CLSI M38-A2 reference method and the results were analysed with the fractional inhibitory concentration (FIC) index.

Results:
A total of 34 (14.4%) *Aspergillus* spp. isolates were obtained from 235 patients. The demographic characteristics of the patients were noted highlighting the positive isolation in adult age group and rural geographic location (*P* < 0.0001). The seasonal variation suggested the highest rate of isolation (12 IA/70 cases) and poorest 30-day mortality in the winters (10/12 IA cases). These IA patients had evidence of prolonged hospital stay 3-4 weeks (*P* = 0.002), mechanical ventilation (*P* = <0.004; RR= 2.57), central venous access (*P* = 0.002), chest infiltrates and ground glass mosaic pattern on radiological examination (*P* = 0.004; RR= 3.21), patients examined with BAL (*P* = 0.005; RR= 4.57) and 30-day mortality (*P* = <0.0001) in contrast to those without infection. The galactomannan Ag index of >1 was associated with 82.4% sensitivity and 70.6% specificity on the basis on ROC curve analysis. There were 24 (70.5%) *Aspergillus flavus* followed by 10 (29.4%) *Aspergillus fumigatus* isolated on culture. The MICs were higher for 01 *A.flavus* and 07 *A.fumigatus* to itraconazole (1- >32 µg/ml), for 02 *A.fumigatus* to voriconazole (0.5-1 µg/ml), for all isolates to micafungin and caspofungin (8->32 µg/ml). All other isolates showed lower MICs. The intraclass coefficient for both CLSI and EUCAST methodologies for the drugs tested were 90.1-98.7. A statistically significant difference was observed between FIC indexes of azole resistant isolates by one-way analysis of variance (ANOVA) than the lower MIC isolates, indicating the decrease in synergy in azole-resistant strains.

Conclusion:
This study imparts the focus on relatively underestimated *Aspergillus* infections prevalent in ICUs. The most common species found was *A. flavus*. For a prompt diagnosis galactomannan Ag testing can prove useful, also this work signifies the importance of antifungal susceptibility testing of *Aspergillus* spp. isolated from critically ill patients.
DISTRIBUTION OF PATHOGENIC ASPERGILLUS SPECIES IN HISTORICAL AREAS IN KATHMANDU, NEPAL, AFTER MASSIVE EARTHQUAKE 2015

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Purpose:
Airborne conidia of Aspergillus species are an important source of both pathogens and aeroallergens and associated with high morbidity and mortality due to infection. Infections caused by Aspergillus species range from life-threatening invasive aspergillosis (IA) in immunocompromised patients to chronic pulmonary aspergillosis (CPA) and fungal allergic diseases, including allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitization (SAFS). Furthermore, species of Aspergillus also produce superficial infections i.e. otitis externa, onychomycosis, keratitis and wound infection. In Kathmandu valley, Aspergillus species have been reported as the most abundant airborne pathogenic fungi. We have studied the prevalence of pathogenic Aspergillus species in historical areas of Kathmandu city after a massive earthquake in Nepal.

Methods:
Using a gravity plate method, two sets of duplicate air samples were collected on Sabouraud Dextrose Agar (SDA), with chloramphenicol (50 mg/L), from predetermined sampling sites in the central city of Kathmandu including Basantapur Durbar Square, UNESCO world heritage site, after the massive earthquake on 25 April, 2015 (June-September 2015). The exposed plates were incubated at 28°C and 37°C for up to 3-7 days and examined daily for visible fungal growth. The different types of fungi and their concentrations together with pathogenic Aspergillus species were identified and enumerated by macroscopic and microscopic morphology according to standard methods.

Results:
Of 80 culture plates from the city, 587 isolates of various fungal genera and 293 isolates of pathogenic fungi were recorded. During the study period, more than 22 different spore types belonging to 19 genera were identified from historical areas of Kathmandu, Nepal indicating a wide diversity in airborne fungi. The species of Aspergillus (16.5%), Penicillium (19%) and Fusarium (13%) were the major components of fungi in atmosphere at 28°C. The genus Aspergillus (46%) contributed the most abundant pathogenic fungi of which Aspergillus fumigatus (2.4%), A. flavus (9.2%) and A. niger (34.4%) were recorded and Penicillium (5.5%), Fusarium (2%) and Rhizopus (2.4%) were also predominant at 37°C. The higher number of pathogenic Aspergillus was isolated in Ason area where most of the residential houses over 80 years old had been, most of them damaged or collapsed due to earthquake.

Conclusion:
The spores of pathogenic and allergenic Aspergillus species were more predominant in the historical areas of Kathmandu city after a massive earthquake. The prevalence of pathogenic Aspergillus species was recorded to be 35% during 2011-2012. The increase in number of Aspergillus conidia in the environment could be related to destruction of centuries-old historical monuments and residential houses during earthquake which has indicated threat to the community upon exposure to the spores. Our results suggest the possibility of high burden of Aspergillus infections including ABPA, CPA and SAFS to susceptible host in Kathmandu Valley.

Keywords: Aspergillus, Pathogens, Infections, Kathmandu, Earthquake.
ASPERSGILLOSIS AND HOW THE COMMUNITY RESPONSES IN DEVELOPING COUNTRY

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Purpose:
To know the knowledge, attitude and practice about aspergillosis among healthcare professional in developing country.

Methods:
142 health care professional, nursing and pharmacist was asked to fill the standard questionaires about aspergillosis knowledge, attitude and practice in 2 district western part of Nepal.

Results:
72% of medical professional had the knowledge about the aspergillosis but 31% thought that it is rare case that can not be diagnose correctly in resource poor setting. 13% of nursing personel had not correct knowledge about it. 69% Pharmacist donot have the advance medicine against aspergillosis and the selling the antifungal drugs without any prescription is on practice. Prevention knowledge from aspergillosis is poor (48%).

Conclusion:
Developing country in south asia like India, Nepal having with a high burden of HIV and endemic fungal infection. H. capsulatum and C. neoformans alone are the major causes of HIV related deaths. The main fungal infections in the country are respiratory, gastrointestinal and dermatologic. Among respiratory fungal infections the most prevalent is H. capsulatum, however other endemic fungi infections have been reported.

As sub-optimum hospital care practice, hospital renovation work in the vicinity of immuno-compromised patients, overuse or misuse of steroids and broad-spectrum antibiotics, use of contaminated infusion sets/fluid, packed food and increase in intravenous drug abusers are the predisposing factors for the infection of aspergillosis. Clinicians are aware of good outcome after use of newer drugs like voriconazole/liposomal amphotericin B/caspofungin, but they are forced to use amphotericin B deoxycholate or itraconazole in public-sector hospitals due to economic reasons. Public Health programs are important to spread the knowledge about it.
TWO CASES OF LUNG ABSCESS CAUSED BY *ASPERGILLUS FUMIGATUS* IN CYSTIC FIBROSIS

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Purpose:

Cystic fibrosis (CF) is an inherited multisystem disorder characterized mainly by chronic obstructive lung disease. The lung destruction is caused by a cycle of airway obstruction, infection and chronic colonization with specific bacterial pathogens, especially *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The development of large pulmonary abscess cavities is exceedingly uncommon. Here, we present two rare clinical cases of CF patients who developed lung abscess due to *A. fumigatus* infection which might be characterized as forms of cavitary pulmonary aspergillosis.

Clinical cases:

A 13-year-old male CF patient presented with sore throat and high fever not responding to antibiotic treatment for about 15 days. Clinical examination revealed torticollis and painful swelling of the thyroid gland, while lung auscultation was normal. Laboratory parameters were WBC=17,230 / mm³, CRP=141 mg/l, IgE=15 u/ml. Thyroid ultra sound (US) demonstrated swelling of the isthmus and the left lobe. Patient received meropenem, teicoplanin, amikacin and corticosteroids for the thyroid inflammation and he became afebrile after two days. On day 12, patient presented with fever and the new thyroid US revealed thyroid abscess 4.6x3.9x2.4 cm, while the CXR demonstrated consolidation on the right upper lobe. Drainage of the thyroid abscess was performed and all pus cultures were negative for fungi and non tuberculous mycobacteria. Treatment changed to linezolid, piperacillin/tazobactam and patient became afebrile after two days. On day 18, patient presented again with fever and symptoms from the respiratory system. New CXR revealed a lung abscess with an air-fluid level located in the right upper lobe, while the chest CT revealed two consolidations on the right lung, bronchiectasis and mediastinal lymph nodes. Cultures from pneumocentesis grew *A. fumigatus*, PCR for *A. fumigatus* was positive. Serum *A. fumigatus* antibodies were not detected. Cultures from sputum samples grew *Pseudomonas aeruginosa*, *Candida albicans*, *A. fumigatus*, *Stenotrophomonas maltophilia*, *Candida parapsilosis* and *A. fumigatus*. Meropenem and voriconazole were added to the initial therapy leading to symptoms’ remission and significant improvement in CXR and CT scan.

The second clinical case was a 17-year-old male CF patient who developed fever, cough for 16 days and, finally, pain on the right hemithorax. Patient had been free of *P. aeruginosa* colonization for the last 12 months. Clinical examination was normal and laboratory parameters were WBC=26,640 / mm³, CRP=82 mg/l, specific IgE for *A. fumigatus*=0.11 u/μl. Imaging tests (CXR, CT) demonstrated a lung abscess in the right lobe with an air-fluid level, peripheral bronchiectasis and pleural infusion. Bronchoscopy was performed and cultures from bronchoalveolar lavage grew *Stenotrophomonas maltophilia*, *Candida parapsilosis* and *A. fumigatus*. PCR for *A. fumigatus* was positive, while PCR for *Mycobacterium tuberculosis* was negative. Patient’s initial treatment was changed to cefepime, teicoplanin and voriconazole and his clinical condition, as well as, imaging tests were improved.

Conclusion:

Clinical manifestations of *A. fumigatus* in CF are under intensive research. Although lung abscess as a form of cavitary pulmonary aspergillosis is rarely observed in CF, close monitoring and differential diagnosis for fungal disease should be considered in CF patients who present symptoms of infection. The timely initiation of anti-fungal and anti-bacterial treatment contributed to our patients’ good response.
MYCOLOGICAL PROFILE EVALUATION OF MATE FOR THE PRODUCTION PROCESS AND ESTABLISHMENTS HERBALIST. PARAGUAY 2012

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2Food Analysis Laboratory, INAN, Asunción, Paraguay

Objective:
To assess the mycological profile of Ilex paraguariensis (yerba mate) during the production process and final product.

Material and Methods:
Observational cross sectional descriptive design that included 68 samples collected at different stages of the production process of Ilex paraguariensis (‘yerba mate elaborada’ and ‘yerba mate compuesta’ with the addition of medical herbs): leaves of yerba mate in nature (n: 7), yerba mate after grinding (n: 22), ‘yerba mate elaborada’ (n: 17), and ‘yerba mate compuesta’ (n: 17); during the months of September to December of the year 2011 in 6 selected herbalist establishments within the national territory. Standard values of Paraguayan normative NP 3500193 (1800 CFU/g) for ‘yerba mate elaborada’ and NP 3,500,201 (5000 CFU/g) for ‘yerba mate compuesta’ were used as reference.

Results:
In the ‘Yerba mate elaborada’, 10 out of 22 samples show higher values than of the Paraguayan Normative, 11 out of 17 of the ‘Yerba mate compuesta’ samples do not meet the Paraguayan Normative criteria.

Conclusion:
In the ‘Yerba mate elaborada’ and ‘Yerba mate compuesta’ high levels of yeast and mold counts were found (Aspergillus niger and flavus in sample 1; Aspergillus niger, flavus and ochraceus in sample 2). The consumption of contaminated Ilex paraguariensis (yerba mate) implies a high food related risk, becoming a potential problem for human health.

To demonstrate that the presence of these species of Aspergillus constitutes a risk factor for the emergence of diseases in the respiratory system other studies should be performed.
Fungal and Bacterial Yield from Induced Sputum in Respiratory Outpatients

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Purpose:
Sputum is a key diagnostic sample for those with chronic chest conditions including chronic and allergic Aspergillus-related disease, but often not obtained in clinic. The objective was to evaluate physiotherapeutic interventions to obtain sputum from those not able to spontaneously produce and the subsequent microbiological result.

Methods:
Sputum samples were collected by physiotherapists from patients attending routine outpatient clinics managing their aspergillus-related diseases who were unable to spontaneously produce. Active Cycle of Breathing Techniques (ACBT) was applied first, for 10 minutes. If samples were not procured with this method then, if necessary and safe to do so, this was followed by hypertonic saline induction using a Pari LC plus or Pari Sprint nebuliser. Sputum samples were processed in the laboratory using standard microbiological techniques for bacterial and fungal culture with the addition of Aspergillus real-time Polymerase Chain Reaction (PCR) testing.

Results:
364 patients aged 22-90 years were treated by specialist physiotherapists in clinic. Sputum samples were procured from 353 of 364 (97%) patients, 231 (65%) by ACBT and 119 (34%) with administration of hypertonic saline. Three of 125 (2.4%) patients had significant bronchospasm during sputum induction with hypertonic saline. Sixteen patients’ sputum tested positive for Aspergillus culture, contrasting with 82 whose Aspergillus PCR was positive, 59 with a strong signal. PCR improved detection of Aspergillus by 350%. Sputum from 124 (34%) patients cultured other potentially pathogenic organisms, including Pseudomonas aeruginosa, Stenotrophomonas maltophilia, MRSA and Mycobacteria avium intracellulare, which justified specific therapy.

Conclusion:
Physiotherapeutic interventions safely and effectively procured sputum from patients unable to spontaneously produce. The method for sputum induction was well-tolerated and time-efficient, with important microbiological results.

Table 1: Working clinical diagnoses in 364 patients

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of patients with provisional or confirmed diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic Pulmonary Aspergillosis (CPA)</td>
<td>183</td>
</tr>
<tr>
<td>Allergic Bronchopulmonary Aspergillosis (ABPA)</td>
<td>58</td>
</tr>
<tr>
<td>ABPA and CPA</td>
<td>9</td>
</tr>
<tr>
<td>Aspergillus bronchitis</td>
<td>41</td>
</tr>
<tr>
<td>Single Aspergilloma</td>
<td>5</td>
</tr>
<tr>
<td>Severe Asthma with Fungal Sensitisation</td>
<td>8</td>
</tr>
<tr>
<td>Asthma with fungal sensitisation</td>
<td>3</td>
</tr>
<tr>
<td>Subacute invasive aspergillosis</td>
<td>7</td>
</tr>
<tr>
<td>Aspergillus airway colonisation</td>
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<tr>
<td>Aspergillus pericarditis</td>
<td>1</td>
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<tr>
<td>Aspergillus sinusitis</td>
<td>1</td>
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<tr>
<td>Candida bronchitis</td>
<td>2</td>
</tr>
<tr>
<td>Other</td>
<td>45</td>
</tr>
</tbody>
</table>
SELECTIVE-FUNGAL CULTURE MEDIA ASSOCIATED WITH HIGH PREVALENCE OF ASPERGILLUS IN CYSTIC FIBROSIS

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Purpose:
The presence of Aspergillus isolated from respiratory secretions of cystic fibrosis (CF) patients in the United States (US) has significantly risen to median prevalence of 12%. However, the true prevalence of the Aspergillus infection may be underestimated due to the lack of uniformity in the frequency and methods of fungal surveillance in US CF clinical centers. Utilizing bacterial culture media alone for fungal surveillance may be insufficient. The objective of the study was to accurately describe the prevalence of Aspergillus infection in adults with CF utilizing selective-fungal culture media.

Methods:
A prospective study comparing fungal detection using bacterial culture media and fungal-specific media for expectorated sputum samples of adult CF patients was conducted in the Johns Hopkins clinical microbiology laboratory from July 1, 2014 to June 30, 2015. Consecutive samples were collected from patients in the Johns Hopkins Adult CF clinic regardless of clinical status. Laboratory evaluation involved (1) Standard of care procedures of bacterial culture media (MacConkey, Columbia-CNA, sheep blood, chocolate, chocolate with bacitracin, Burkholderia cepacia selective, CHROMagar) and Sabouraud agar and incubation at 37° Celsius for 3 days and (2) Research protocol involving fungal-specific media, including inhibitory mold agar (IMA), Sabouraud with gentamicin, and brain-heart infusion (BHI) agar and incubation at 30° Celsius for 14 days. Detection and identification of Aspergillus spp were performed and compared between standard evaluation and research protocol using Fisher’s exact test. Clinical data during the 12-month study period was collected.

Results:
The study included 211 unique subjects with a mean of 2.28 samples (sd 1.3) per subject. Baseline characteristics of subjects are included in Table 1. A total of 487 expectorated sputum samples were collected and analyzed. Among these, 264 (54.2) specimens did not grow any fungi. Of the 223 fungi-positive samples, 144 (64.6%) samples positive for Aspergillus species. Bacterial culture media detected Aspergillus in 46 of 144 (31.9%) Aspergillus-positive samples; failing to detect 68.1% of Aspergillus-positive samples. When compared to bacterial culture, detection of Aspergillus was superior in IMA (58.3%, p<0.001), Sabouraud with gentamicin at 30 degrees Celsius (60.4%, p=0.01), Sabouraud with gentamicin at 37 degrees Celsius (56.9%, p<0.001) and BHI (57.6%, p=0.006). Of the 211 subjects, prevalence of Aspergillus was 40.8%. The prevalence of persistent Aspergillus infection was 17.1%. Forced expiratory volume in one second percent predicted was no different in subjects with Aspergillus compared to those without Aspergillus (58.0 vs 62.2, p=0.22).

Conclusion:
The prevalence of Aspergillus spp was 40.8% in an adult cohort in the United States. Bacterial culture media alone are insufficient in detection of Aspergillus in the respiratory secretions of individuals with CF.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
Purpose:
Invasive aspergillosis (IA) is a severe complication among immunocompromised patients. We describe here the epidemiology of IA from a four-year single center observational study.

Methods:
Medical charts of patients with positive cultures for Aspergillus spp. collected at Duke University (Durham, NC, USA) between 2009-2013 were retrospectively screened. Patients with proven/probable mold infections (EORTC-MSG definitions) were included in the analysis. Patient characteristics, microbiology data, antifungal therapy and IA outcomes were analyzed.

Results:
A total of 51 episodes of IA were identified over the study period (34 proven, 17 probable). 19 (37%) patients had hematological malignancies and 17 (33%) were solid-organ transplant recipients. A. fumigatus was the predominant causal agent (52%), followed by A. flavus (22%), A. terreus (12%), A. niger (6%), A. ustus (6%) and A. versicolor (2%). Except for A. ustus isolates and one A. fumigatus complex strain, all isolates exhibited voriconazole MIC ≤2 µg/ml. Lung was the origin of infection in 73% cases (followed by nose/sinus in 10% of cases). A disseminated infection (i.e. evidence of ≥2 affected organs) was observed in 43% cases. Antifungal prophylaxis was ongoing at onset of disease in 18% cases. Voriconazole was used as first line therapy in 63% cases. Mortality was 20% at 4 weeks and 31% at 12 weeks. A trend towards higher mortality rate was found among patients with disseminated infections (p=0.07). IA episodes were compared to 48 proven/probable episodes of non-Aspergillus invasive infections (23 mucormycosis, 14 fusariosis, 7 scedosporiosis, 3 Paecilomyces and 1 Scopulariopsis infections) occurring during the same period. Compared to other mold infections, IA tended to be more frequently observed in non-neutropenic patients (p=0.08) and had a better survival at 4 weeks (p =0.05).

Conclusion:
IA was a severe complication in immunocompromised patients with an increasing proportion of cases reported among non-hematologic cancer patients and an important proportion (near 50%) of cases due to Aspergillus spp. other than A. fumigatus including emerging and notoriously azole-resistant A. ustus. Disseminated infection was frequently observed and mortality was high (20% at 4 weeks). In comparison, non-Aspergillus mold infections were more frequently observed in neutropenic cancer patients with higher mortality rates.
**Purpose:**
Fungi have been identified as emerging cause of rhinosinusitis. Both acute and chronic rhinosinusitis are caused by a variety of fungal agents, most common being *Aspergillus* species worldwide. In the present study of clinical, radiological and histopathological evaluation was done to determine the type of fungal rhinosinusitis. Antifungal susceptibility testing of all isolates was done, to aid as a guide to determine the prognosis and the most effective line of management.

**Methods:**
This prospective observational study was conducted among patients of acute and chronic rhinosinusitis undergoing endoscopic guided sinus surgery. Sinonasal biopsies were processed by standard mycological methods. Further, histopathological examination on H&E, PAS and GMS was done and categorization done. Total serum IgE and absolute eosinophil count were measured to establish a possible correlation, which was determined by ELISA. The antifungal susceptibility testing was done using CLSI M38-A2 protocol.

**Results:**
A total of 106 sinonasal biopsies were received and 51 showed growth of *Aspergillus* species on culture. Out of total 51, 32 were positive on KOH wet mount (28 had septate, 2 aseptate and 2 had both septate and aseptate hyphae) and 19 were negative. On culture, 46 showed single *Aspergillus* species and 5 showed mixed infection with two concomitant infecting species. Out of cultures showing single fungal species, 30 had *Aspergillus flavus*, 6 had *Aspergillus fumigatus*, 6 had *Aspergillus niger* and 3 had *Aspergillus* species. In mixed infections, pairs of fungal species identified were *A. flavus + Rhizopus arrhizus*, *A. niger + Rhizopus arrhizus*, *A.niger + Penicillium* species, *A. flavus + A. niger* and *A. niger + Candida tropicalis*. On histopathology, out of 45 cases, 31 had AFRS, 5 had EFRS, 4 had fungal ball, 2 had granulomatous invasive FRS, 2 had acute invasive FRS and 1 had both acute and chronic invasive FRS. Total serum IgE was raised in all cases of AFRS, ranging from 225.50 IU/ml to 1675.10 IU/ml and all had allergic mucin on histopathology. In EFRS, total serum IgE ranged from 13.88 to 178.32 IU/ml and none had allergic mucin on histopathology. In 4 cases of fungal ball, raised IgE was seen in *Aspergillus fumigatus* (2 cases), *A. flavus* (1 case) and *Aspergillus* species (1 case). Total serum IgE was also raised in both cases of granulomatous invasive FRS (884 and 823 IU/ml) caused by *Aspergillus flavus*. Of the 52 *Aspergillus* isolates, 86.53% were susceptible to amphotericin B, 90.38% to itraconazole, 98.07% to voriconazole and 96.15% to caspofungin. All 52 *Aspergillus* isolates were susceptible to posaconazole, anidulafungin and micafungin; resistant to fluconazole and 5-flucytosine.

**Conclusion:**
Fungal rhinosinusitis is emerging sino-nasal disease that requires an increased awareness of clinicians, radiologists, microbiologist and pathologists. This prevents FRS to cause pressure/invasive effects that can be managed only by surgical removal of fungal mass and conservative treatment. In addition, total serum IgE and absolute eosinophil count are strong indicators towards FRS caused by *Aspergillus* species due to its profound allergenic effect. Fungal sinusitis should be kept as one of differential diagnosis in such type of presentation so that detection can be made in time.
Purpose:
Invasive aspergillosis (IA) is an important cause of morbidity and mortality in immunocompromised patients with hematological malignancies. However, Greek epidemiological data are lacking. The aim of this study was to prospectively investigate the epidemiology and outcome of IA in a Greek institution.

Methods:
This is a prospective, registry study that collected microbiology, histopathology and clinical data and outcome in patients with proven, probable and possible IA, according to the EORTC definitions, between 1 January 2014 and 31 May 2015, at the Laiko University Hospital, Athens, Greece. The hospital is a 350-bed, regional centre for hematological malignancies and autologous stem cell transplantation.

Results:
During the study period we identified 68 cases of IA among 2,130 patients with hematological malignancies. The median age of the patients was 57 years, ranging from 17 to 87 years old; 42 (62%) were male. Proven IA had 3 (4,4%) patients, probable IA 28 (41%), while the remaining 37 (54%) patients were considered as having possible IA. The total cumulative incidence of IA was calculated and was found to be 3,2 cases per 100 patients with hematological malignancies. No monthly incidence variation was observed. The median ECOG score was 1 and the median Glasgow Coma Score 15. The average stay of these patients in the hospital was found to be 89 days. Regarding the underlying malignancy 36 (53%) of the patients had AML, 4 (6%) MDS, 5 (7%) ALL, and 21 (30%) had lymphoma. Only 29 patients (43%) received prophylactic therapy, with 34,5% receiving posaconazole, 20,6% micafungin and 27,6% fluconazole. Voriconazole was given as secondary prophylaxis in 6,8% of the patients following a previous IA. Neutropenia and steroid use are considered to be two of the most important predisposing factors of IA, as 73% of the patients were neutropenic (54% with neutrophil count <100) and 44,1% have been treated with steroids before the diagnosis of IA. Regarding treatment, 66,1% received antifungal monotherapy with liposomal amphotericin B or voriconazole and 5,8% received combination therapy. The overall clinical response rate was 50% within 14 days of treatment. The crude mortality rate was 26% during their 28-day follow-up, either due to their underlying malignancy or due to multiple infections.

Conclusion:
This is the first epidemiological data on IA in patients with hematological malignancies in Greece. Understanding the epidemiologic trends and burden of IFIs may lead to improved management strategies and study design.
Background:
Cutaneous and fatal fungal infections have been studied extensively in Japan, and latterly chronic pulmonary aspergillosis (CPA). However no estimate of the nationwide incidence and prevalence of fungal infections has been attempted. Here we estimate the burden of serious fungal infections in Japan.

Methods:
We searched for existing data and estimated the incidence and prevalence of fungal diseases based on the populations at risk and available epidemiological data. Data were derived from the World Health Organization (WHO), The Joint United Nations Programme on HIV/AIDS (UNAIDS) and national and regional published reports. When no data existed, risk populations were used to estimate frequencies of fungal infections, using previously described methodology by LIFE.

Results:
The population of Japan is ~127 million; 13% are children, and 30% are women >65 years. Recurrent vulvovaginal candidiasis (>4 episodes/year) is estimated to occur in 1,525/100,000 females. ABPA and SAFS were estimated in 20.8/100,000 and 27.5/100,000 respectively, in ~1 million adult asthmatics. An estimated 3,957 have CPA after pulmonary tuberculosis (19,615 survivors in 2012) 50% of the total burden. HIV affects 1,546 people, with 17 deaths, so few fungal infections. Using a rate of 5/100,000, an estimated 6,350 patients develop candidemia, 40% of the total of invasive candidiasis (15,875), including an estimated 476 with intra-abdominal candidiasis. Invasive aspergillosis is estimated to affect 1,308, mostly in acute leukemia and after allogeneic stem cell transplantation. Mucormycosis may affect 254 patients annually. There are no incidence data on tinea capitis or fungal keratitis, but probably both uncommon or rare.

Conclusion:
The present study indicates that around to 1.9% (2,370,300) of the population is affected by a serious fungal infection, predominantly recurrent VVC in women. Further epidemiological studies are needed to validate and extend these estimates.
<table>
<thead>
<tr>
<th>Infection</th>
<th>Number of infections per underlying disorder per year</th>
<th>Total burden</th>
<th>Rate/100K</th>
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<tr>
<td><strong>Total burden estimated</strong></td>
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<td><strong>2,370,314</strong></td>
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RELATION BETWEEN GENE MUTATION IN CYSTIC FIBROSIS (CF) AND ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA) AND SEVERE ASTHMA WITH FUNGAL SENSITIZATION (SAFS)

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Purpose:
The delta – F508 CFTR mutation is the most common defect in the gene encoding the CFTR protein among patients of Northern European population with cystic fibrosis (CF). The delta – F508 CFTR gene occurs in approximately 3.5% of the Caucasian population of Northern Europe. However, the relationship between delta – F508 CFTR and ABPA has been investigated by only small studies.

The heterozygous state is associated with an increased risk of development of pulmonary diseases. In comparison to the general population, patients who are heterozygous for the delta – F508 gene have an increased risk for asthma, reduced performance on pulmonary function tests, and a possibly increased risk of chronic obstructive pulmonary disease.

Methods:
The following data were collected from each medical record: diagnosis of ABPA, SAFS or CPA; age; gender; pulmonary co-morbidities (severity of bronchiectasis; severity of asthma; concomitant COPD); Mannose Binding Lectin (MBL); anti-Aspergillus IgG, anti-Aspergillus IgE and total IgE antibodies. For our genetic studies, we used a commercial kit, plus sequencing if a single mutation found and extensive bronchiectasis was present.

The frequency of delta-F508 CFTR gene in the ABPA or SAFS population of the National Aspergillosis Centre has been compared with the estimated frequency of delta-F508 CFTR gene in the general population.

Results:
In a total of 238 ABPA or SAFS patients who were genetically screened for CF, 20 were found to have delta-508 CFTR mutation and 7 other CF – related genetic mutations. Genetic frequency of those with delta-508 CFTR mutation (8.4%) does substantially exceed that of the general population. In those 27 patients with positive genetic testing mean age was 60ys (25 – 77ys) and 14 (52%) were males. Among these patients, 13 (48%) had bronchiectasis, and two (7%) chronic pulmonary aspergillosis (CPA). Ten patients (37%) had Mannose Binding Lectin (MBL) deficiency (< 1 mg/L); three patients had hypergammaglobulinemia (IgG >16 g/L), whereas none demonstrated hypogammaglobulinemia. Twelve had elevated Aspergillus IgG antibodies (range 49-303 mg/L), 20 (74%) had raised total IgE (range 120 – 22,000 KIU/L), and 19 (70%) had elevated Aspergillus IgE titers (range 0.5 – 48 kAU/L).

Conclusion:
While it is known there are various genetic alterations in the CFR gene, the dominant change in our ABPA population is delta – F508. We conclude that the delta-508F CFTR gene is over-represented in the ABPA /SAFS population of the United Kingdom. We also have identified a population of ABPA /SAFS with delta-508F and other alterations of the CFTR gene. Those who are heterozygous for the delta – F508 gene have more advanced and progressive pulmonary disease. More than one-third of the study population demonstrated MBL deficiency; this factor is associated with a pre-disposition to autoimmune diseases and infection. Our preliminary results are encouraging enough to merit further investigation among patients with ABPA /SAFS with a case-controlled study allowing us to explore ultimately the contribution of delta – F508 CFTR mutation in ABPA population, and further understand the clinical outcome of ABPA /SAFS disease.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
TRIAL OF POSACONAZOLE THERAPY FOR CHRONIC PULMONARY ASPERGILLOSIS

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Introduction:
The first line therapies for chronic pulmonary aspergillosis (CPA) are itraconazole or voriconazole. However therapy with these agents is often curtailed due to toxicity or the development of drug resistance. Posaconazole is a potential alternative for these patients, but is a high cost medication. The National Aspergillosis Centre receives approximately 110 new referrals annually and cares for >380 patients with chronic pulmonary aspergillosis. All modern healthcare systems must decide on how to allocate resources in an equitable manner to maximise patient outcomes. The NHS Highly Specialised National Commissioners agreed to fund the use of posaconazole on an ‘n of 1’ trial basis to maximise clinical benefit for patients with long term continuation based on meeting targets at four and six months.

Methods:
Patients who had previously failed therapy with itraconazole or voriconazole or whose isolates were resistant to these agents were considered for a trial of posaconazole. Only those who could clinically improve were selected. Quality of life was assessed with the St George’s Respiratory Questionnaire (SGRQ). Patients were required to gain 2Kg in weight and/or 8 points on the SGRQ at 4 months and 3Kg and/or 12 points on the SGRQ at 6 months. All patients were informed that posaconazole therapy would be withdrawn if they failed to meet these targets. Response, adverse events and posaconazole levels were monitored throughout.

Results:
78 patients were commenced on a trial of posaconazole. 26 (33%) patients achieved the target(s) for continuation of therapy and have been maintained on posaconazole. Six patients (8%) failed to achieve targets, including one who was non-compliant. 23 (29%) had adverse events and six patients died (multi-factorial, not thought to be drug-related). 17 patients were pending outcomes. Of the 23 patients who could not continue due to side effects, 16 (70%) were required to stop within the first 4 months and 7 (30%) before reaching the 6 month follow-up. Side effects included shortness of breath, fatigue, neuropathy, hair loss and one patient had suicidal thoughts. Successful trials of therapy were associated with major improvement in symptoms, including increased energy, less dyspnoea, improved appetite, feeling stronger and managing day to day tasks better.

Conclusion:
Putting patients on a trial of posaconazole had numerous benefits both for the patients and the centre. High cost drugs for chronic diseases pose a significant financial challenge. Establishing criteria for therapeutic success offered a clear, equitable and sustainable method of identifying patients who benefit from additional therapy, and minimised continuation of ineffective therapy in those who did not.
**ASPERGILLUS SPP. INFECTIONS IN STAT 3 DEFICIENT PATIENTS: A NATIONALWIDE STUDY IN FRANCE**

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7Centre d’étude des déficits immunitaires (CEDI), Hôpital Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris, France
8CEREDIH, Centre de Référence des Déficits Immunitaires Héréditaires, Hôpital Universitaire Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris, France
9Service d’hématologie, Hôpital Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris, France

**Background:**
Autosomal dominant loss of function mutation of signal transducer and activator of transcription 3 (STAT3) gene (STAT3-deficiency) predisposes to recurrent bacterial pneumonia that are complicated in 67% of patients with bronchiectasis, cavitations and *Aspergillus* infection or colonization in 22% of patients1. We aimed to report the prevalence, describe clinical, mycological, pathological and radiological presentation and both medical and surgical treatment of mold infections in the National French cohort.

**Methods:**
Referent physicians of STAT3-deficient patients (n= 74 patients) were contacted to know if patients had evidence of colonization or infection with molds. Clinical and mycological information were collected and imaging was centralized. An expert committee reviewed all charts and classified the cases (EC, SP, CT, AD, FL, OL, MOC).

**Results:**
Eighteen episodes of filamentous fungal infection in ten (13.5%) STAT3-deficient patients were identified. The median age at first episode was 12 years (IQR 10.2-25). Ninety percent of patients had underlying pulmonary disease, bronchiectasis and cavitations, usually multiple. Mold infections were classified as follow: three aspergillomas, six Chronic Pulmonary Aspergillosis (CPA), five Allergic BronchoPulmonary Aspergillosis (ABPA), two mixed forms ABPA and CPA, one Chronic Allergic Sinus Aspergillosis and one *Rasamsonia* invasive pulmonary infection. According to EORTC/MSG definitions, no cases of invasive aspergillosis were reported. *Aspergillus fumigatus* was isolated in 13 cases, *Rasamsonia argillocea* in one case. *Aspergillus* precipitins were detectable in 86 % of cases (12/14).

Two-thirds of fungal episodes (12/18) were breakthrough infections (itraconazole prophylaxis in most cases), and half of the cases (9/18) occurred while on immunoglobulin substitutive therapy. First line antifungal therapy was voriconazole only (8/18) or amphotericin B alone or in association (6/18). Five patients required surgery (4 CPA, 1 aspergilloma). One patient died from respiratory failure at 11 years old.
**Conclusion:**
Mold infections occurred in 13.5% of STAT3-deficient patients from the French cohort, mostly on anatomical modification of the lung. Notably, patients developed aspergilloma, ABPA or CPA, but no invasive aspergillosis. Despite prolonged antifungal treatment and/or surgery half of the patients (5/10) relapsed.

**Reference**
ONSET OF CHRONIC PULMONARY ASPERGILLOSIS (CPA) MAY OCCUR DURING ACTIVE PULMONARY TUBERCULOSIS

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Purpose:
CPA is estimated to affect 3 million people globally. The most common underlying condition in CPA is treated pulmonary tuberculosis. Our recent cross-sectional survey identified CPA in 8.2% of 400 Ugandan adults with previously treated pulmonary tuberculosis (Union Lung Conference 2015, abstract PC-957-05).

CPA diagnosis is often made years after completing tuberculosis treatment, but no prospective surveys have defined the natural history. Co-infection with atypical mycobacteria frequently occurs in CPA. Co-infection with Mycobacterium tuberculosis and Aspergillus has been described in several case reports. Subacute invasive aspergillosis (SAIA) has been documented in 3-11% of autopsies of patients who died of AIDS, in the absence of pulmonary tuberculosis. This raises the possibility that onset of CPA may occur during active pulmonary tuberculosis, especially in patients with AIDS. We aimed to estimate the prevalence of aspergillosis in patients with active pulmonary tuberculosis.

Methods:
Stored sera were available from 57 adult patients admitted to Mulago Hospital, Kampala between March 2010 and March 2011. All patients had between 2 weeks and 6 months cough and were diagnosed with pulmonary tuberculosis based on culture or GeneXpert PCR testing of sputum and/or broncho-alveolar fluid. We measured Aspergillus-specific IgG in these samples using the Siemens Immulite assay, which has a specificity of 98% and sensitivity of 96% for the diagnosis of chronic pulmonary aspergillosis (Journal of Infection, in press).

Results:
46 (81%) patients and 2% controls were HIV positive. Mean CD4 count in patients with HIV was 99 cells/mL (range 2 - 581 cells/mL). 35 (61%) patients had CD4 count <100 cells/mL and 24 (42%) patients had CD4 count <50 cells/mL.

Mean Aspergillus-specific IgG levels were 5 mg/L in healthy controls and 11mg/L in pulmonary tuberculosis cases (p > 0.000). Raised levels were found in 2 (2%) of healthy controls and 27 (47%) pulmonary tuberculosis patients (p > 0.000). There was no significant difference in 2-month mortality in tuberculosis patients with normal or raised levels of Aspergillus-specific IgG.

Conclusion:
These results cannot differentiate Aspergillus colonization from active disease. This is a select group of tuberculosis patients requiring emergency hospital admission and results may not be generalisable to other patients with tuberculosis. However, given the diagnostic accuracy of the Siemens Immulite assay active Aspergillus co-infection is probably present in many of those with positive results. This possibility should be considered in patients who fail to improve or clinically relapse in spite of appropriate tuberculosis therapy.

The frequency of raised Aspergillus-specific IgG levels is four times higher in these patients with active pulmonary tuberculosis than the rate of CPA found in patients with treated tuberculosis in our earlier survey. It is possible that many patients develop Aspergillus colonization during active pulmonary tuberculosis, which then resolves as the tuberculosis is cured. Alternatively, our earlier survey may have failed to identify those who died of CPA soon after completing tuberculosis treatment and so underestimated the frequency of CPA complicating pulmonary tuberculosis. Prospective studies are now needed to record the outcome of patients with pulmonary tuberculosis and raised Aspergillus-specific IgG and define the natural history of CPA secondary to pulmonary tuberculosis.
COMPARATIVE EFFICACY OF SIX *ASPERGILLUS*-SPECIFIC IgG ASSAYS FOR THE DIAGNOSIS OF ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA)

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2Manchester Academic Health Sciences Centre, UK
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Purpose:
Recent ISHAM guidance includes raised *Aspergillus*-specific IgG as an alternative to precipitins testing as one of the “other criteria” for the diagnosis of allergic bronchopulmonary aspergillosis (ABPA). Many commercial *Aspergillus*-specific IgG kits exist, but their diagnostic efficacy for ABPA is poorly described. The studies upon which manufacturers’ recommended diagnostic cut-offs are based are either tiny or unpublished. We aimed to define the optimal diagnostic cut offs for *Aspergillus*-specific IgG in ABPA and to describe the diagnostic efficacy of different methods in this context.

Methods:
We acquired stored sera from the following groups; 1 – 80 patients with known ABPA, not complicated by chronic pulmonary aspergillosis (CPA) who were not on antifungal treatment at the time of sampling, 2 – 100 healthy controls, 3 – 100 diseased controls with asthma, but not ABPA, 4 - 242 samples from patients with CPA.

*Aspergillus*-specific IgG was measured by ImmunoCAP, Siemens Immulite (Germany), Serion (Germany), Omega (UK) and Dynamiker (China) assays, plus precipitins by counterimmunoelectrophoresis using Microgen *Aspergillus* antigens (UK).

Results:
Receiver operating characteristic curve results are shown in the table. Diagnostic cut-offs with a specificity of ≥95% against severe asthmatic controls were identified. The Immulite cut-off (40 mg/L) has 50% sensitivity. Dynamiker cut-off (500 AU/ml) has 20% sensitivity. Serion cut-off (100 AU/ml) has 27% sensitivity. Genesis cut-off (20 U/ml) has 38% sensitivity. Precipitins testing produced specificity of 97% and sensitivity of 4%.

Alternative diagnostic cut-offs with a specificity of 98% against healthy controls were also assessed. The Immulite cut-off (10 mg/L) has 81% sensitivity. ImmunoCAP cut-off (20mg/L) has 77% sensitivity. Dynamiker cut-off (65 AU/ml) has 66% sensitivity. Serion cut-off (35 AU/ml) has 62% sensitivity. Genesis cut-off (15 U/ml) has 46% sensitivity.

We considered whether *Aspergillus*-specific IgG could be used to identify cases of CPA complicating ABPA. ImmunoCAP levels of over 150 mg/L had 95% specificity and 37% sensitivity and Immulite levels of over 125 mg/L had 95% specificity and 65% sensitivity. Positive precipitins had 96% specificity and 59% sensitivity. Other assays performed poorly

Conclusion:
All the ELISAs performed substantially better than precipitins for the diagnosis of ABPA. The asthmatic controls used in this retrospective study are severe asthmatics attending a tertiary referral clinic. These patients were not specifically screened for aspergillosis and so may include undiagnosed cases of CPA, ABPA or *Aspergillus* bronchitis resulting raised levels of *Aspergillus*-specific IgG. Cut-offs defined in relation to this group may not be optimal for use in other asthmatics.

An alternative option is to use a cut off with high specificity against healthy controls. Such a method does not allow *Aspergillus*-specific IgG to be used as a single test to differentiate ABPA from asthma, but *Aspergillus*-specific IgG has never been used in this manner in ABPA diagnosis. Using healthy controls allows the identification of patients with ‘abnormally’ high levels of *Aspergillus*-specific IgG, to be used as a single aspect of a composite diagnostic criteria. These cut-offs are also optimal for use in the diagnosis of CPA and other forms of aspergillosis and probably represent the best substitute for the role precipitins has traditionally played in ABPA diagnosis.
IDENTIFICATION OF CLINICALLY RELEVANT *Aspergillus* SPECIES BASED ON THE ANALYSIS OF THEIR MELTING CURVES ON DIFFERENT BLOOD PANELS

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**Purpose:**
During the past few years the High Resolution Melting (HRM) analysis has established itself as a versatile and reliable application to identify specific double stranded DNA targets in different clinical samples based on their sequence diversity. The purpose of this work was to estimate the diagnostic accuracy of our (HRM) assays identifying *Aspergillus fumigatus*, *A. lentulus*, *A. terreus* and *A. flavus* on whole blood, serum and plasma panels.

**Methods:**
We designed PCR primers for the specific identification of relevant *Aspergillus* species involved in invasive aspergillosis based on the representative melting curves and peaks of their amplicons in the presence of a single color saturating dye; LightCycler®480 ResoLight (Roche Diagnostics) and appropriate standard internal controls. To estimate the diagnostic accuracy of our HRM assays we introduced them to certain *Aspergillus* isolates from clinical specimens; *Aspergillus fumigatus* (8 India isolates), *Aspergillus lentulus* (2 India and 1 Hungarian isolates), *Aspergillus terreus* (7 India isolates) and *Aspergillus flavus* (8 India isolates). To estimate the diagnostic performance, dynamic ranges of our HRM assays have been estimated by analysing whole blood, serum and plasma panels spiked with different *Aspergillus* gDNA and conidia of seven orders (10⁶-1 GE and CFU respectively) of magnitude.

**Results:**
We managed to design species specific HRM assays for the reliable identification of certain clinical isolates of *Aspergillus fumigatus*, *A. lentulus*, *A. terreus* and *A. flavus* species in different blood samples. The HRM curves and peaks proved to be discriminative when testing different *Aspergillus* species. Beside the diagnostic accuracy we also estimated the robustness and the throughput of our (HRM) assays on large number *Aspergillus* conidia and gDNA containing blood panels in MagNa Pure LC 2.0 robotic system.

**Conclusion:**
This study introduces cost-efficient, optimized HRM assays to detect four relevant *Aspergillus* species based on the representative melting plots of their amplicons.
RADIOLOGICAL AND MICROBIOLOGICAL FEATURES IN NEUTROPENIC PATIENTS WITH PERSISTENT FEVER FOLLOWING POSACONAZOLE OR OTHER ANTIFUNGAL PROPHYLAXIS: A SINGLE CENTER RETROSPECTIVE STUDY

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Purpose:
Invasive fungal infections (IFIs), in particular invasive aspergillosis (IA), are a life-threatening complication in immune-compromised patients, especially in those treated with cytotoxic agents for hematologic malignancies. In this single center retrospective study, we reviewed microbiological and radiological features in patients with neutropenia and persistent fever, then we compared the results of IFI diagnostic work-up of the patients in prophylaxis with posaconazole versus those who received other type of antifungal prophylaxis.

Methods:
Patients hospitalized in the Hematology department of Federico II University from 2008 to 2012 for chemotherapy treatments of hematological malignancies, were investigated. We selected cases who developed neutropenic persistent fever unresponsive to broad spectrum antibiotics thus requiring systemic antifungal therapy. All cases suspected for IA were classified according to the EORTC 2008 criteria. For each case we reported several aspects including the results of microbiological and radiological diagnostic work-up, in particular blood cultures, Galactomannan (GM) and (1-3)-β-D-glucan (BDG) values, chest computed tomography (CT) and CT-guided histological procedures.

Results:
During the study period, 100 patients (53 M/47 F) were analyzed. Patients characteristics are listed in the Table. Before the infection, 43% had severe neutropenia for a median time of 19.5 days (range 2-95). Antifungal prophylaxis was done in all patients: 45% received posaconazole while the remaining 55% different antifungals (23/55 fluconazole, 7/55 itraconazole, 7/55 amphotericin B, 6/55 voriconazole, 1/55 caspofungin, 11/55 nistatine). Regarding the microbiological results, median values of serial GM obtained from the two groups were analyzed; the median result was 0.5 (range 0.1-0.8) in the posaconazole group and 0.8 (range 0.1-2) in the different prophylaxis group \((p<0.0001)\) Figure. Furthermore the median value of BDG tested in 18 patients among those who had a negative GM test in the posaconazole group was 187 pg/mol (range 17-352 pg/mol). CT scans most frequent results for the posaconazole group were clusters of micro-nodules in 44% (20/45) of the cases, reversed halo sign in 18% (8/45) and central hypodensity in 18% (8/45). In the different prophylaxis group, CT scans frequent findings were: 50% (28/55) of macro-nodules, 27% (15/55) of halo sign and 16% (9/55) of cavities. Overall, proven/probable IA were documented in the 11% (5/45) of the posaconazole group and in the 45% (25/55) of the different prophylaxis group. However if we consider the new category of neutropenic fever plus one (NF+1), in the 40% (18/45) of the posaconazole group and in the 20% (11/55) of the different prophylaxis group \((p=0.007)\) IA is suspected.

Conclusion:
This single center retrospective study showed that for the 40% of patients in the posaconazole group (NF+1 category), pulmonary infiltrates and GM results did not fulfill EORTC IA diagnostic criteria. These results suggest the possibility of revising EORTC criteria by extending the suspicion of IA to less specific chest CT scan and/or a GM borderline value in patients with posaconazole prophylaxis, in order to act a pre-emptive therapeutic approach in an early stage of IFI. In addition, BDG test resulted a good diagnostic tool for patients in posaconazole prophylaxis who are borderline cases following 2008 EORTC criteria.
Table. Clinical characteristics of 100 patients with febrile neutropenia unresponsive to broad-spectrum antibiotics thus requiring systemic antifungal therapy

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Posaconazole Group (n=45)</th>
<th>Different Prophylaxis group (n=55)</th>
<th>Total cases (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, n male (%)</td>
<td>23 (51)</td>
<td>30 (54)</td>
<td>53</td>
</tr>
<tr>
<td>Age, median years (range)</td>
<td>48</td>
<td>51 (18-71)</td>
<td></td>
</tr>
<tr>
<td>Type of hematologic disease, n cases (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>27 (60)</td>
<td>37 (67)</td>
<td>64</td>
</tr>
<tr>
<td>ALL</td>
<td>9 (20)</td>
<td>4 (7)</td>
<td>13</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>7 (16)</td>
<td>6 (11)</td>
<td>13</td>
</tr>
<tr>
<td>Myeloma</td>
<td>1 (2)</td>
<td>4 (7)</td>
<td>5</td>
</tr>
<tr>
<td>CLL</td>
<td>1 (2)</td>
<td>3 (6)</td>
<td>4</td>
</tr>
<tr>
<td>CML</td>
<td>-</td>
<td>1 (2)</td>
<td>1</td>
</tr>
<tr>
<td>Severe neutropenia, n cases (%)</td>
<td>26 (57)</td>
<td>17 (30)</td>
<td>43</td>
</tr>
<tr>
<td>Radiological findings, n cases (%)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EORTC-MSG specific findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macronodules</td>
<td>5 (11)</td>
<td>28 (50)</td>
<td>33</td>
</tr>
<tr>
<td>Halo sign</td>
<td>2 (4)</td>
<td>15 (27)</td>
<td>17</td>
</tr>
<tr>
<td>Cavity or air crescent sign</td>
<td>2 (4)</td>
<td>9 (16)</td>
<td>11</td>
</tr>
<tr>
<td>Aspecific findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cluster of micronodules</td>
<td>20 (44)</td>
<td>3 (5)</td>
<td>23</td>
</tr>
<tr>
<td>Reversed Halo</td>
<td>8 (17)</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Central hypodensity</td>
<td>8 (17)</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Consolidation</td>
<td>7 (15)</td>
<td>3 (5)</td>
<td>10</td>
</tr>
<tr>
<td>Pericardial effusion</td>
<td>2 (4)</td>
<td>3 (5)</td>
<td>5</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>3 (6)</td>
<td>1 (2)</td>
<td>4</td>
</tr>
<tr>
<td>Mycological criteria, median value (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM from serum</td>
<td>0.5 (0.1-0.8)</td>
<td>0.8 (0.1-2)</td>
<td></td>
</tr>
<tr>
<td>BDG from serum**</td>
<td>187 (17-352)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Proven/Probable IA, n cases (%)</td>
<td>5 (11)</td>
<td>25 (45)</td>
<td>30</td>
</tr>
<tr>
<td>NF+1, n cases (%)</td>
<td>18 (40)</td>
<td>11 (20)</td>
<td>29</td>
</tr>
</tbody>
</table>


*In each patient more than one radiological finding has been observed at computed tomography examination.

**BDG was tested in the posaconazole group patients whose GM test resulted negative.
Figure. Comparison between median values of GM determination in posaconazole and different prophylaxis groups. Each point represents a median of the GM determinations for each patient in the study. Four data points are not shown in the figure because they are outside the axis limits.
MYCOLOGICAL AND HISTOPATHOLOGICAL CLASSIFICATION OF ASPERGILLUS SINUSITIS IN SAUDI ARABIA

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Purpose:
Fungal rhinosinusitis (FRS) is known for decades and is increasingly reported. Only recently, further defined classification and characterization of the pattern of the disease were reported. Aspergillus species is the most common etiologic agent. Aspergillus sinusitis has several distinct clinical manifestations with variable clinical significance. The aim of this study was the characterization and classification of the disease pattern of Aspergillus sinusitis using a retrospective review of paranasal sinus biopsies.

Methods:
Culture results, the histopathological reports and slides for 85 patients with Aspergillus sinusitis were reviewed. Based on histopathological findings, cases were categorized into non-invasive and invasive. Non-invasive included fungus ball (FB) and allergic fungal rhinosinusitis (AFRS). Invasive form included acute invasive fungal rhinosinusitis (AIFRS), chronic invasive fungal rhinosinusitis (CIFRS), and chronic invasive granulomatous fungal rhinosinusitis (CGFRS).

Results:
85 patients with Aspergillus sinusitis were included in the study. The mean age of the patients was 28.1 years (SD ±7.1) with range from 13 to 58 years. There were 36 (42.4%) male and 49 (57.6%) female patients with male to female ratio of 1: 1.4. Based on histopathologic features, 95.3% of patients had non-invasive disease (including 55.3% AFRS, 27.1% FB, and 12.9% mixed AFRS and FB) and 4.7% of patients had invasive disease (all with AIFRS). Aspergillus flavus was the most common species isolated (87.1%) followed by A. niger (7.1%) and A. terreus (3.5%).

Conclusion:
In this study, most of Aspergillus sinusitis cases were non-invasive with allergic aspergillus sinusitis was the most common presentation. Aspergillus flavus was the most common species.
DEVELOPMENT AND VALIDATION OF A HIGH RESOLUTION MELTING ASSAY TO DETECT AZOLE RESISTANCE IN ASPERGILLUS FUMIGATUS

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2European Program for Public Health Microbiology Training (EUPHEM), European Centre for Disease Prevention and Control, Stockholm, Sweden

Purpose:
In the last years, there has been an emergence of azole-resistant Aspergillus fumigatus strains reported worldwide, representing a growing public health concern. The prompt identification of these resistances for the selection of the proper antifungal treatment is critical for the management of the A. fumigatus infected patients. A rapid molecular –based assay for the detection of the most frequent mutations related to azole resistance in A. fumigatus was designed and validated in this study.

Methods:
Five PCR reactions targeting the G54, L98, M220, G448 point mutations in cyp51A and, the three tandem repeats described in the promoter region of this gene (TR34, TR46 and TR53) were designed with similar cycling conditions to run simultaneously in a LC 480 Roche thermocycler. After amplification, a High Resolution Melting (HRM) assay for the identification of the amplicons according to the temperature of the melting curves was performed. For the standardization of this technique, 33 A. fumigatus strains with previously known mutations (4 susceptible and 29 resistant) were used. In addition, 80 A. fumigatus strains, including susceptible and azole resistance strains were blind tested for validation.

Results:
The method was able to specifically differentiate the mutations M220V, M220I, M220T, L98H, G448S and the three tandem repeats. The mutations G54E, G54W, G54V and G54R could not be distinguished, although they were clearly differentiated from the wild type and therefore detected as resistant. Regarding the validation, the success rate of this HRM blind study was 98.75%. Only one strain containing the mutation M220K was not properly identified.

Conclusions:
i) A single HRM assay to detect the most frequent mutations related to azole resistance in A. fumigatus was designed, standardized and validated.
ii) All resistant strains were clearly differentiated from the wild type by HRM, except one strain with the mutation M220K.
iii) This methodology is a reliable diagnostic tool for the rapid identification of azole resistant in A. fumigatus strains.
ISOLATION AND IDENTIFICATION OF AIRBORNE FUNGI AND THEIR RELATION TO ALLERGIC DISEASE IN SULAIMANI CITY, IRAQ

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2Biology Department, College of Science, University of Sulaimani, Iraq

Purpose:
Identify the fungi in the air of the Sulaimani city of and their relationship to the allergies. Fungi are essential components of ecosystems and widely distributed in nature. Fungal spore may be easily dispersed into indoor environments associated with a number of adverse health effect. This study was designed to investigate airborne fungi and their relation to allergic disease in Sulaimani city.

Methods:
The airborne fungi were isolated by settle plate method in different areas of Sulaimani city during two seasons; (Autumn October 2014) and (Spring April 2015), in which Sabouraud dextrose agar, containing plates chloramphenicol were opened and exposed to air for 1 hour. Standard fungal allergens of *Alternaria*, *Cladosporium*, *Penicillium* and *Aspergillus* were used in skin prick test for individuals consulted health center of asthma and allergy in Sulaimani city suffering from symptom allergy their ages ranged between (1-59) years old. Total IgE concentration and percentage eosinophiles were measured in patients whose skin prick tests were positive to at least one of allergens under study, and in control person.

Results:
These results were obtained from current study: a total of $2.409 \times 10^2$ CFU belonging to twenty genera with a group of yeasts and twenty four species, and the percentage of most predominant isolated fungi from the environment at different locations of Sulaimani city were *Penicillium* spp. 28.1%, *Aspergillus* spp. 20.25%, yeast 13.33%, *Cladosporium* spp.12.1%, and *Alternaria* spp. 6.72%. The highest number of fungi was isolated during spring $1.492 \times 10^2$ CFU compared to $9.17 \times 10^2$ CFU in autumn, *A.niger* and *A.flavus* were the predominant species of *Aspergillus* isolated, while the most common *Penicillium* species were *P. chrysogenum* and *P. spinulosum*.

Conclusion:
The highest number of fungi were isolated from dietary factories $6.37 \times 10^2$ CFU followed by houses $4.71 \times 10^2$, factories $4.21 \times 102$ CFU, dormitories $3.96 \times 10^2$, schools $3.32 \times 10^2$, and hospitals $1.52 \times 10^2$. 

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AUTOPHAGY IN *ASPERGILLUS FUMIGATUS* INFECTION AND IMMUNITY

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Purpose:

*Aspergillus fumigatus* is the predominant airborne fungal pathogen in immunocompromised patients. It causes a wide spectrum of diseases in humans and it is the main causal agent of invasive aspergillosis (IA). Among the immunological mechanisms that are important for resistance to infection, autophagy constitutes an essential mechanism for cellular homeostasis in response to various stresses and shows the ability to sanitize the cellular interior by killing intracellular pathogens. Recent studies have demonstrated the importance of LC3II in immunity to *A. fumigatus* although the molecular mechanisms of autophagy induction as well as its contribution in resistance against fungi remains unknown. We focused our attention on the death-associated protein kinase 1 (DAPK1) that is an IFN-γ-induced protein involved, beyond autophagy, in cell survival and apoptosis.

Methods:

In this study, we have highlighted the impact of DAPK1 in antifungal immunity in mice and humans. To this purpose, we monitored DAPK1 expression in vitro in RAW264.7 cells exposed to *A. fumigatus* conidia and in vivo in C57BL/6 mice intranasally infected with *A. fumigatus*. The genetic study includes 277 patients undergoing allogeneic hematopoietic stem-cell transplantation (HSCT) at the Hospital Santa Maria della Misericordia (Perugia, Italy) and their respective donors. Five SNPs were selected from a literature review and genotyping was performed using KASPar assays.

Results:

We demonstrated that DAPK1 participates in the immune responses against *A. fumigatus* and that the swelling of conidia was a prerequisite for DAPK1 activation. The IFN-γ-ATF6-C/EBP-β-DAPK1 axis not only protected against *A. fumigatus* through an autophagic pathway potentiated by IFN-g, but also regulated pathogenic inflammasome activation during infection. Moreover, among SNPs analyzed, we found a loss of function for DAPK1 that increased the susceptibility of HSCT patients to aspergillosis.

Conclusion:

Recognizing specific autophagy hallmarks triggered during *A. fumigatus* infection and determining these signals in the lung may provide important insights into how autophagy and antifungal immunity are integrated and reciprocally regulated to provide the optimal response to the fungus.

*This work is supported by the Specific Targeted Research Project FUNMETA (ERC-2011-AdG-293714).*

**NOTE:** THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
POLYMORPHONUCLEAR NEUTROPHILS DEPLETION ABLATES HEAT KILLED SACCAROMYCES INDUCED RESISTANCE TO ASPERGILLOSIS

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²Infectious Diseases and Geographic Medicine, Stanford University, CA, USA

Purpose:
We have shown that heat-killed Saccharomyces cerevisiae (HKY) vaccination protection against aspergillosis is not affected by lack of T cells, B cells, antibody (Ab), C’, or NK cells. To further investigate the mechanism of HKY-induced protection, we depleted granulocytes (PMNs) using anti-Gr-1 antibody (Ab), after vaccination and before infection.

Methods:
Male CD-1 mice were divided into 6 groups of 10 mice: PBS, HKY, HKY treated with anti-Gr-1 Ab, HKY treated with an irrelevant (control) Ab, anti-Gr-1, or control Ab. HKY at 2.5 mg/dose or PBS was given s.c., 3 weekly doses beginning 28 days prior to infection with Aspergillus fumigatus conidia i.v. Anti-Gr-1 or control Ab (25 µg/dose i.v.; both rat IgG) were given on day -1 of infection and days 1, 3, 5, and 7 postinfection. Survival and CFU in the target organs (brain, kidney) was determined on day 14.

Results:
The initial two doses of anti-Gr-1 reduced WBC by 9.5-fold for HKY-vaccinated and 7-fold for PBS controls. Unvaccinated mice given anti-Gr-1 died sooner than PBS controls or those given control Ab (P <0.0001). HKY-vaccinated and HKY given control Ab had equivalent survival, and both better than all other groups (P = 0.004 to <0.0001). Anti-Gr-1 in HKY-vaccinated ablated resistance almost entirely (inferior to HKY alone, P < 0.0001) though some HKY protection remained (superior to anti-Gr-1/no HKY, P = 0.004). CFU recovery showed all mice were infected in both organs. HKY or HKY + control Ab had fewer CFU than HKY + anti-Gr-1 (P < 0.04, both organs). Control Ab had fewer CFU than anti-Gr-1 in kidney (P < 0.02).

Conclusion:
Overall, these results demonstrate PMNs are the primary cells conferring resistance after HKY vaccination. Anti-Gr-1 Ab did not entirely ablate HKY protection, suggesting other cell types also contribute to the host protective response. These studies suggest HKY may be most useful in the persons with intact, or acquired or congenitally depressed cellular or humoral immunity, but not in those lacking functional PMNs.
THE IMMUNOREGULATORY ROLE OF MICROBIOTA TRYPTOPHAN METABOLITES IN FUNGAL ALLERGY.

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Purpose:
Human-associated microbes represent the source of many bioactive microbial products. These proteins and metabolites play key functions both in human host pathways as well as in microbe-microbe interactions. Recently, it has been found that highly adaptive lactobacilli of the gut, in particular Lactobacillus reuteri, switching from sugar to tryptophan as an energy source were expanded and produce the AhR ligand - Indole-3-aldehyde (3-I Ald) active on innate lymphoid cells that 3-I Ald contributes to mucosal resistance against the opportunistic pathogen Candida albicans. Interestingly recent studies have shown that gut microbiota metabolism influence allergic airway disease, therefore we investigated whether 3-I Ald could impact on allergic response in Allergic Bronchopulmonary Aspergillosis (ABPA).

Methods:
Mice were anesthetized by inhalation of 3% isoflurane (Forane Abbot) in oxygen prior intranasal injection of 20 µg of A. fumigatus culture filtrate extract (CCFA) dissolved in incomplete Freund’s adjuvant (Sigma-Aldrich). The first administration is followed by two consecutive intranasal injections (a week apart) of the same amount of CCFA. Seven days after the third intranasal challenge, mice received 3-I Ald aerosol intranasally followed by three daily intranasal injections of 1x10⁹ Aspergillus resting conidia. Mice were sacrificed a week later. Broncho Alveolar Lavage were performed on mice lungs using 1.0 ml aliquots of pyrogen-free saline through a 22-gauge bead-tipped feeding needle introduced into the trachea. The levels of cytokines in lung homogenates were determined by mouse ELISAs (R&D Systems). Real-time RT-PCR was performed using the Stratagene Mx3000P QPCR System and SYBR Green chemistry (Stratagene).

Results:
Interestingly, parameters typically characterizing ABPA resulted particularly reduced upon treatment with aerosolized indole derivatives in mice.

Conclusion:
Accumulating evidence points to a critical role for the gut microbiome in regulating normal functioning of immune response. In particular, it is becoming clear that the microbial influence on tryptophan metabolism and on mucosal response against pathogen may be an important node in such regulation. At this purpose our study shows that tryptophan metabolites, produced by gut microbiota, could impact also on allergic immune reaction in a distal site. This study reveals how tryptophan metabolites could be important in host physiology and lays the foundation for future studies.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
CLEARANCE OF PTX3 PRE-OPSONIZED CONIDIA OF _ASPERGILLUS FUMIGATUS_ FROM THE LUNG OF RATS IMMUNOSUPPRESSED BY CORTISONE ACETATE

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\(^3\)Biotechnology, Sigma-tau SpA, Rome, Italy

**Purpose:**
PTX3 is an innate immunity multimeric glycoprotein that plays a no redundant protective role in the infection caused by _A. fumigatus_. The protein binds to the fungus and promotes phagocytosis and killing of conidia by innate immunity cells, a process defined as opsonization.

In order to provide proof of concept that direct administration of PTX3 to the respiratory mucosa would preserve protein mediated clearance of _A. fumigatus_ from the lung of rats immunosuppressed by cortisone acetate, PTX3 pre-opsonized conidia were intra-tracheal administered in these animals.

**Methods:**
Pre-opsonization was performed by incubating conidia with several concentrations of PTX3 for 1 hour at 24°C in saline. Then conidia were washed and bound PTX3 was evaluated by densitometry on PTX3 western blotting of conidia lysates. Three PTX3 concentrations were selected for the in vivo study corresponding to saturation, intermediate and low binding of PTX3 to conidia. Twenty four hours after intra-tracheal administration with 5x10\(^7\) pre-opsonized conidia, rats were sacrificed and fungal burden evaluated in lung and blood by CFU and galactomannan (GMI) testing, respectively.

**Results:**
Bound PTX3 progressively increased with protein concentration with a maximum binding capacity at about 2 pmol/millions of conidia. The lung of rats challenged with PTX3-saturated conidia, showed similar CFU and GMI compared to lung challenged with PTX3-free conidia. A progressive reduction of lung CFU was observed in rats infected with intermediate and low PTX3 concentrations suggesting a pro-zone effect in the mechanisms of opsonization.

**Conclusion:**
Present results are consistent with PTX3 opsonic activity and suggest that local administration of PTX3 on the respiratory mucosa, for instance by aerosol, could be useful but dose selection should be carefully identified.
CORTICOSTEROIDS IMPAIR NEUTROPHILS BUT NOT OTHER CD11B+ MYELOID CELLS TO CONTROL PULMONARY ASPERGILLUS FUMIGATUS INFECTION

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Introduction:
Despite improved antifungal agents, invasive Aspergillus fumigatus lung infections cause high morbidity and mortality in immunocompromised patients, for instance, in patients with therapy related neutropenia. In these dismal situations granulocyte transfusions have been tested as an alternative therapy for the management of high-risk neutropenic patients with invasive fungal infections. To increase the granulocyte yield for transfusion, donors are treated with corticosteroids. Yet, the efficacy of granulocyte yield for transfusion and functional defense mechanisms of granulocytes collected from corticosteroid treated donors remain elusive.

Methods:
We have used a combination of in vivo murine models and in vitro experiments to determine the efficacy granulocytes collected from corticosteroid treated donors to control invasive A. fumigatus infections.

Results and Conclusion:
Here we show that transfusion of granulocytes from corticosteroid treated mice did not protect immunosuppressed mice against lethal A. fumigatus infection in contrast to granulocytes from untreated donors. To study effects of corticosteroids on granulocyte antifungal defense functions we employed a corticosteroid treated A. fumigatus infection model. Upon infection increased levels of inflammatory cytokines helped to recruit neutrophilic granulocytes to the lungs of corticosteroid treated mice. Yet, after corticosteroid treatment, neutrophils failed to form neutrophil extracellular traps (NETs) against A. fumigatus. Corticosteroids impaired ROS production against A. fumigatus, which is also important for NET formation. Importantly, corticosteroids impaired the β-glucan receptor Dectin-1 (CLEC7A) on neutrophils to efficiently recognize and phagocytize A. fumigatus, which markedly impaired fungal killing. Collectively, our data indicate that corticosteroid treatment of granulocyte donors for increasing neutrophil yields could result in deleterious effects on neutrophil antifungal functions, thereby limiting the benefit of neutrophil transfusion therapies against invasive fungal infections. However, we also demonstrate that transfusion with CD11b+ myeloid cells collected from corticosteroid treated donors protect immunosuppressed mice against A. fumigatus infection.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
ASPERGILLUS FUMIGATUS STRAIN-SPECIFIC HOST RESPONSE IS REGULATED BY GLUCOSE SENSING AND GLIOTOXIN PRODUCTION

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Purpose:
Our knowledge of how Aspergillus fumigatus growth is controlled in the respiratory tract is developing, but still enigmatic. Recruitment of neutrophils and inflammatory monocytes within the respiratory tract is critical to control fungal germination and prevent mortality. It has recently been shown that the clinical strains of A. fumigatus, CEA10 and Af293, induce unique inflammatory leukocyte responses. Here, we aim to understand why these different A. fumigatus strains induce different host immune responses, and how this ultimately affects fungal-induced immunopathology and disease outcome.

Methods:
We used an immunocompetent model of invasive pulmonary aspergillosis (IPA) in mice to evaluate the immune response induced by CEA10 and Af293. Fungal genetic null mutants were used to assess the role of CreA and gliotoxin in development of fungal-induced immunopathology and invasive disease. We analyzed bronchoalveolar lavage fluid (BALF) and lung homogenate (LH) to assess which cytokines and chemokines are needed for leukocyte recruitment, as well as histological slides to determine in vivo growth of each fungal strain.

Results:
We observed that CEA10 undergoes significantly greater germination than Af293 within the respiratory tract of immunocompetent animals. Moreover, CEA10 induced greater lung damage, vascular leakage, and inflammation compared to Af293. Neutrophil recruitment following challenge with CEA10 was dependent on IL-1alpha, whereas after Af293 challenge, neutrophil recruitment was IL-1beta dependent. The enhanced germination by CEA10 was regulated by its ability to sense glucose through CreA-mediated signaling, which correspondingly regulated lung damage, vascular leakage, and inflammation. Furthermore, upon germ tube formation the secondary metabolite gliotoxin is produced. Interestingly, there was a marked increase in lung damage induced in the absence of gliotoxin, which corresponded with decreased inflammatory cell recruitment and increased fungal growth.

Conclusion:
Overall, these findings suggest the inflammatory response to A. fumigatus might be regulated in a stepwise manner in response to the threat posed by the specific A. fumigatus strain. Specifically, our data demonstrate that the CEA10 is able to germinate efficiently within the lung in part due to glucose sensing through CreA-mediated signaling, which ultimately leads to necrotic host cell death, IL-1alpha release, and subsequent IL-1alpha dependent neutrophil recruitment. In contrast, Af293, which swells but fails to germinate in immunocompetent animals, activates the inflammasome leading to inflammasome-dependent neutrophil recruitment. By understanding why different A. fumigatus isolates induce different cell death pathways and immune pathology, we can reveal novel therapeutic targets for the treatment of IPA, on both the host and pathogen side. Clinically, our data support the idea that A. fumigatus strain phenotypic variation may significantly contribute to disease outcomes.
INNATE ANTIFUNGAL EFFECTOR MECHANISMS OF CYSTIC FIBROSIS PHAGOCYTES

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Introduction and Purpose:
Cystic fibrosis (CF) is the most common lethal inherited disease among Caucasians. Morbidity and mortality of CF patients is predominantly due to chronic suppurative lung disease leading to progressive respiratory insufficiency. CF is caused by mutations in the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR). The effect of CFTR mutations in epithelial cells has been well studied but its consequence on the function of CF phagocytes is less known. We investigated the role of CFTR in innate antifungal defence mechanisms by CF phagocytes against Aspergillus fumigatus, a fungus known to infect up to 50% of adult CF patients leading to various disease entities, although its role in progressive lung disease is not clear.

Methods:
Peripheral blood mononuclear cells (PBMC), polymorphonuclear cells (PMN) and monocytes (MNC) were isolated from blood of healthy volunteers and CF patients upon informed consent. PBMC, PMN and MNC were stimulated with live A. fumigatus conidia for various incubation periods. Differences in fungicidal activity were assessed by measuring the fungal metabolic activity using the XTT-assay after an 18-hour incubation period. Differences in reactive oxygen species (ROS) production against A. fumigatus by the various cell populations were evaluated during a 2-hour period by lumino-enhanced chemiluminescence by using a Luminescence plate reader. Protein expression of Dectin-1 was examined on fixed non-stimulated healthy and CF PMN and MNC by flowcytometry. To assess the role of Dectin-1 and TLR4 recognition, pre-incubation of the phagocytes with either glucan phosphate (Dectin-1 inhibition) or the TLR4 antagonist LPS-RS was performed. To investigate the importance of ROS production for fungicidal activity, NADPH oxidase activity was blocked by pre-incubating the phagocytes with Diphenyleneiodonium (DPI).

Results:
Fungicidal activity of CF PMN or MNC was not impaired. In contrast, CF PBMC displayed increased killing of A. fumigatus compared to cells from healthy volunteers (p=0.002). Blocking of TLR4, but not Dectin-1, significantly reduced fungal killing by healthy PBMC (p=0.03) and a similar trend was observed for CF PBMC. Strikingly, ROS production against A. fumigatus conidia was increased 2-4 fold by CF PBMC, PMN and MNC (p<0.05). Stimulation of ROS production was independent of Dectin-1 or TLR4 recognition. Blocking of NADPH-oxidase resulted in decreased fungicidal activity by CF phagocytes.

Discussion and Conclusion:
The fungicidal activity of CF phagocytes isolated from peripheral blood is not impaired. The exaggerated increase in ROS production by CF phagocytes may explain the detrimental effects of A. fumigatus infection of the CF lung. Both Dectin-1 and TLR4 seem not to be involved in the increased ROS production. Our current research is now focussing on whether the increased ROS in CF phagocytes is associated with defective autophagy.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
COMPARTMENT-SPECIFIC ACTIVATION OF THE INFLAMMASOME BY
ASPERGILLUS FUMIGATUS

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Purpose:
Host resistance to invasive pulmonary aspergillosis (IPA) is critically dependent on the timely recruitment and activation of neutrophils and inflammatory monocytes to the respiratory tract. The primary etiological agent of IPA is Aspergillus fumigatus. IPA is medically important because it is associated with unacceptably high mortality rates with limited treatment options. One line of research that holds promise for improving IPA patient outcomes is to understand the host immune response; in doing so we aim to identify key inflammatory pathways necessary for control of fungal infection that could be therapeutically targeted to augment existing anti-fungal drug regimens.

Methods:
We utilized an in vivo immunocompetent animal model to determine the role of IL-1 cytokine family members and inflammasome components following intratracheal challenge with A. fumigatus conidia. We analyzed bronchoalveolar lavage fluid (BALF) and lung homogenate (LH) to assess which cytokines and chemokines are needed for leukocyte recruitment, as well as histological slides to determine in vivo growth of each fungal isolate.

Results:
A. fumigatus conidia undergo swelling within 4 hours after inhalation and under appropriate conditions can begin to form germ tubes around 6-8 hours after inhalation. A. fumigatus isolates which undergo swelling induce inflammasome activation resulting in IL-1β and IL-18 secretion, while isolates that initiate germ tube formation also drive robust cell death and tissue pathology resulting in IL-1α secretion. IL-18 expression drives robust systemic complications such as hypothermia and hypercoagulation. Interestingly, we now describe opposing roles for inflammasome activation within radio-sensitive and radio-resistant compartments, which leads to either enhanced or diminished neutrophil accumulation in the lung airways, respectively. Inflammasome activity within the radio-sensitive population was responsible for IL-1β production, while within the radio-resistant population inflammasome activity regulated IL-18 secretion. Furthermore, the absence of Il18 and Pycard within the radio-resistant compartment resulted in increased inflammation and neutrophil accumulation, which correlated with increased CXCL1/KC and CXCL2/MIP-2 production.

Conclusion:
Overall, these findings provide a deeper understanding of how neutrophil recruitment to and activation in the lungs following A. fumigatus challenge is regulated by the IL-1 cytokine family. Specifically, IL-1α and IL-1β work together to enhance neutrophil recruitment to the airways through a CXCR2-dependent pathway. In contrast, IL-18 expression negatively regulates neutrophil recruitment through CXCR2-dependent pathways. Moreover, IL-18 contributes to systemic disease parameters following pulmonary A. fumigatus challenge. Through gaining a better understanding of how the IL-1 cytokine family regulates the local and systemic inflammatory response to A. fumigatus, we can identify much needed novel immunotherapeutic interventions for the treatment of IPA.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
HOST-PATHOGEN INTERACTIONS BETWEEN NATURAL KILLER CELLS AND ASPERGILLUS FUMIGATUS

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Purpose:
Aspergillus fumigatus (Af) is an opportunistic filamentous fungus that is currently one of the most frequent causes of invasive fungal disease in immunosuppressed individuals. NK cells have demonstrated to have a key role in tumour surveillance and viral clearance but NK cell responses against fungal infections, particularly against Af, are still poorly characterized. I aim to provide further understanding of the role of NK cells in invasive aspergillosis and to characterise the cross-talk between NK cells and Af.

Methods:
Human natural killer cells were isolated from healthy donors by negative selection and were incubated overnight with GFP Af in the presence of IL-2. NK cell response to Af was assessed by FACS, confocal microscopy and ELISA. Statistical analysis was performed using GraphPad Prism software. Paired Student t-test was used and P < 0.05 was considered statistically significant.

Results:
Analysis of NK cell degranulation on FACS, using a degranulation marker (CD107a), showed a significant percentage (P = 0.0004) of cells responding to Af, with an average of 2.5% of the cells responding via cytotoxic pathway. However no significant increase on perforin or granzyme B levels was detected by ELISA assay. Interaction with Af also results in high reduction on CD56 expression on NK cell surface. FACS analysis showed that Af does not elicit an IFN-γ and TNF-α cytokine response. ELISA and Luminex multiplex assays confirmed that NK cells do not produce cytokines in response to Af, but showed a significant production of MIP-1α, MIP-1β and RANTES chemokines.

It was also observed that up to 50% of the NK cells were attached to the fungi suggesting that FACS analysis may have underestimated the interaction of NK cells with the fungus.

Analysing NK cell interaction with Af by confocal microscopy, a polarization of perforin and granulysin’ granules towards the fungus was observed and propidium iodide (PI) staining showed evidence of fungal damage. Quantification of fungal gDNA by qPCR in the supernatant of cultures with Af and in the supernatant of co-cultures of Af-NK cells, showed a significant increase (P < 0.0001) on fungal gDNA release from Af when NK cells are present.

Conclusion:
NK cells interact with Af resulting in cell attachment to the fungus, granule polarization towards the fungus, increase in CD107a expression and damage of the fungi. In contrast to previous reported, up-regulation of pro-inflammatory cytokines was not observed, although exposure does induce chemokine secretion. Interaction with Af also results in high reduction on CD56 expression on NK cell surface. Reduction on CD56 expression on NK cells, has already been described in HIV-1 and HCV patients and is associated with loss of capacity to degranulate and to produce IFN-γ in response to K562 or PMA, but with normal ability to produce chemokines. The function of CD56 glycoprotein on NK cells remains unclear, but it is known to be involved in both homophilic and heterophilic adhesion. Taken together, these results may suggest that CD56 downregulation may be a mechanism developed by the pathogen to impair NK cell response.
DECTIN-2 IS A RECEPTOR FOR GALACTOMANNAN

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**Purpose:**
Innate immune responses to 	extit{Aspergillus fumigatus} are key host defenses yet incompletely understood. Since the fungal cell wall is composed of most carbohydrates, we sought to identify mammalian receptors that serve to recognize and activate macrophages. Galactomannan is a carbohydrate found on the surface of the cell wall of 	extit{A. fumigatus} and other related fungal organisms. We sought to determine the mammalian receptor for this carbohydrate.

**Methods:**
The use of synthetic fungal like particles composed of size-matched polystyrene beads covalently attached to highly-purified fungal derived carbohydrates has been a useful discovery platform to identify cellular receptors and relevant signaling pathways.

**Results:**
Using fungal like particles decorated with galactomannan from 	extit{A. fumigatus}, we determined that TNF-\(\alpha\) production required the presence of Dectin-2. Expression of Dectin-1, any TLRs, Mincle and DC-SIGN in the absence of Dectin-2 failed to stimulate TNF-\(\alpha\) production. Macrophages expressing either mEmerald-Dectin-2 or mCherry-Dectin-2 show uptake of galactomannan beads into phagosomes with robust recruitment of the fusion protein suggesting specific recognition.

**Conclusion:**
These data indicate that Dectin-2 is a specific receptor for 	extit{A. fumigatus} galactomannan.

**NOTE:** THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
EXPOSURE TO ASPERGILLUS SPORES IN A LABORATORY IS ASSOCIATED WITH ELEVATED SPECIFIC T-CELL FREQUENCIES IN LABORATORY PERSONNEL

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Purpose:
Mould specific T-helper cells have been described as a novel diagnostic tool in invasive fungal diseases. Recently, we showed that healthy donors with frequent mould exposure in their working or residential environment harbour elevated frequencies of Aspergillus fumigatus specific T-cells. This study sought to assess whether handling of Aspergillus cultures in a laboratory leads to increased frequencies of specific T-helper cells detectable by flow cytometry.

Methods:
Five scientists and technicians experienced in processing fungal cultures harvested A. fumigatus (ATTC 46645) spores from mature colonies grown on beer wort plates to prepare a spore solution according to the laboratory’s standard operating procedures. The harvest procedure (duration of exposure) took 10 – 20 minutes per scientist and was conducted under a level 2 bio safety cabinet. Beer wort plates at different places in the laboratory were used to identify and quantify fungal spores in the room air prior, during, and after fungal harvest. Blood samples were collected from the scientists up to 7 days prior to and exactly 7 days after exposure to quantify CD154+ A. fumigatus specific T-helper cells according to the previously published protocol using a commercially available mycelial lysate for cell stimulation.

Results:
The mean frequency of A. fumigatus specific T-cells in our cohort of scientists not exposed to fungal cultures during a period of at least 4 weeks was 0.028 %. 7 days after A. fumigatus harvest, the mean frequency of these cells inclined to 0.099 % (p < 0.01). The increase of T-cell frequencies in individual subjects ranged from 0.038 % to 0.127 % (Figure A). No colonies were found on sedimentation plates placed under the sterile workbench in close proximity to the plates processed by the scientists, indicative for a clean harvesting procedure and proper function of the safety cabinet. Few colonies of A. fumigatus were however identified on a plate placed in front of the safety cabinet next to the scientists during the harvest procedure. This may reflect slight spore contamination of the gloves or lab coat whirled up by disruption of the cabinet’s laminar flow. The observation of significantly elevated specific T-cells after processing A. fumigatus cultures was validated by retrospective analysis of the values from three scientists regularly donating blood for assay quality control. In all three subjects, significantly higher frequencies of specific T-helper cells were observed, if the respective scientist processed A. fumigatus cultures within 4 weeks of blood collection (Figure B).

Conclusion:
Our findings suggest that exposure to A. fumigatus spores in the laboratory setting is associated with significantly elevated specific T-cell frequencies. Analysis of cytokine patterns in culture supernatants from the subjects’ PBMCs stimulated with an A. fumigatus lysate will help to explore whether frequent mould exposure may contribute to a protective Th1 response. Moreover, the data underline previous publications showing that the CD154-based T-cell assay highly sensitively detects specific immune response to mould fungi. Apart from diagnosing invasive fungal infections, the assay may also be considered as a supportive test (biological effect monitoring) in the environmental-medical context.
SELECTION OF ANTIFUNGALS FOR INITIAL MAINTENANCE THERAPY OF CHRONIC PULMONARY ASPERGILLOSIS: A LONGITUDINAL ANALYSIS

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Purpose:
There is still no evidence which of oral anti-Aspergillus agent is suitable for initial maintenance therapy of chronic pulmonary aspergillosis (CPA). Aim of this study is to clarify which of oral itraconazole and voriconazole is appropriate for initial maintenance therapy of CPA by longitudinal analysis.

Methods:
This is a retrospective follow-up observational study in CPA patient enrolled to past two randomized, multicenter, open-labeled trials, one of which compared efficacy of intravenous micafungin and intravenous voriconazole, and another one of which compared efficacy of liposomal amphotericin B and intravenous voriconazole. In those studies, patients received intravenous antifungal treatment for at least 2 weeks with a maximum duration of 4 weeks. After the end of those acute phase treatment, each primary physician decided on courses of maintenance therapy and followed up patients. The median observational period was 730 days (5%-95%: 267–1407) for patients who were alive at the end of observation.

Results:
Of the 273 CPA patients, 59 patients started maintenance therapy by oral itraconazole and 101 patients started by oral voriconazole just after the end of acute intravenous therapy. Percentage of patient with improvement was seemed to be higher in voriconazole group than itraconazole group (40.0% vs 18.2%); however, if patients with stable status were added to improved patients, no statistical difference was seen (52.6% vs 50.9%). Patients who were administered itraconazole group were more likely to readmit in hospital and switch to other antifungal agent than that of voriconazole group (P = 0.020, P <0.001, respectively). Multivariable Cox regression analysis showed no significant influence of choice of initial maintenance treatment (itraconazole or voriconazole) in not only overall mortality but also CPA associated mortality. Instead, presence of chronic obstructive pulmonary diseases showed high HR 4.2 (95% CI, 1.4–12.6) in death associated with CPA. Higher efficacy rate of antifungals showed low HR 0.65 (95% CI, 0.44–0.97) in death associated with CPA. The factors associated with hospital readmission and switching rate to other antifungals were evaluated using multivariable logistic regression. It showed the facts that selection of itraconazole for initial maintenance therapy was independent risk factor for hospital readmission and switching to other antifungal agents (OR 3.3, 95% CI 1.3–8.0; OR 5.6, 95% CI 2.1–15.1, respectively). Higher efficacy rate of antifungals reduced risk of hospital readmission (OR 0.7, 95% CI 0.5–0.9).

Conclusion:
There was no difference in prognosis of CPA patients between initial maintenance therapy of itraconazole and voriconazole. However, patients who were started with oral itraconazole treatment were more likely to readmit to the hospital and switch to other agents than patients started by oral voriconazole treatment.
119 INVASIVE ASPERGILLOSIS IN HEMATOLOGICAL MALIGNANCY PATIENTS: RISK FACTORS, GALACTOMANNAN ANTIGEN DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE AND ANTIFUNGAL SUSCEPTIBILITY PATTERNS WITH MUTATIONS IN CYP51A FOR ASPERGILLUS FUMIGATUS

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Purpose:
Aspergillus sp. leads to asymptomatic colonization of sinuses, cavities; allergic syndrome to invasion of tissues depending on the host’s immune status. In our country India, Aspergillus flavus is most frequently isolated Aspergillus sp. Antifungal prophylaxis has resulted in the increasing occurrence of resistant species. Invasive aspergillosis (IA) is generally associated with poor outcome. This study was conducted to know the significant cut-off of galactomannan Ag in the prompt diagnosis and the prognostic value, if any, in our patients. The antifungal susceptibility testing was done to know the prevalent resistant rate.

Methods:
Six hundred malignancy patients with clinical suspicion of IA were enrolled in the study and phenotypic identification was done as per conventional mycological procedures. Antifungal susceptibility was done to amphotericin B, caspofungin, micafungin, itraconazole, posaconazole and voriconazole by CLSI M38-A2 and EUCAST (E.Def 9.1) methodology. From the proven (03), probable (431) and possible (163) cases of IA (EORTC/MSG guidelines) sera were subjected to galactomannan Ag assay. For the resistant A.fumigatus isolates PCR followed by DNA sequencing was done to look for the hot-spot mutations (L98H, M220 and TR).

Results:
A total of 40 (6.6%) Aspergillus spp. isolates were obtained from 600 hematological malignancy patients. These IA patients had evidence of neutropenia (P = 0.004), consolidation and ground glass mosaic pattern on radiological examination (P = 0.002; RR= 4.31), and 30-day mortality (P =<0.0001) in contrast to those without infection. There were 26 (65%) Aspergillus flavus followed by 14 (35%) Aspergillus fumigatus isolated on culture. The MICs were higher for 04 A. fumigatus to itraconazole (4+ >32 µg/ml), for 02 A. fumigatus to voriconazole (1 µg/ml), for all isolates to micafungin and caspofungin (8->32 µg/ml). All other isolates showed lower MICs to the antifungals tested. The mutations in the cyp51A gene were found in the 4 resistant A. fumigatus isolates. The galactomannan Ag index of >1 was associated with 99.1% sensitivity and 74.1% specificity on the basis on ROC curve analysis. The galactomannan Ag appeared in 234/597 patients (39.1%) sera before the appearance of any radiological signs. The second sample for galactomannan Ag was tested in 319 patients (53.4%) and the decrease in index was noted in 207/319 patients (64.8%). The decrease was noted in <7 days with voriconazole and 7-14 days with amphotericin B.

Conclusion:
The incidence of invasive aspergillosis was found to be associated with high mortality. Patients with high (>1) galactomannan Ag index value had greater severity of disease, but with drug voriconazole the decrease in galactomannan Ag titre and improvement in the mortality rate was noted. This imparts the need of detection of early markers in our diagnostic settings and as A. fumigatus was found to be resistant, appropriate antifungal therapy based on the susceptibility profiles should be done for the better management of the patients.
TARGETING INFECTION SITE HYPOXIA AS AN ADJUNCTIVE TREATMENT OF INVASIVE ASPERGILLOSIS

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Purpose:
Aspergillus fumigatus is a filamentous fungus with an important environmental role of nutrient recycling. However, it is also the leading cause of invasive aspergillosis (IA) in immunocompromised patients. Even though breakthroughs have been achieved in understanding molecular mechanisms by which Aspergillus fumigatus causes disease, patient outcomes associated with invasive aspergillosis remain sub-optimal. Hypoxia at the IA infection site microenvironment is a major challenge in improving disease outcomes. Hypoxia promotes fungal virulence and increases host tissue damage which can potentially affect in vivo antifungal drug efficacy. Consequently, we hypothesize that increased oxygen levels at the site of infection will improve IA outcomes. To address this hypothesis, we are investigating the role of hyperbaric oxygen to relieve infection site hypoxia, increase angiogenesis and alter the fungal virulence genetic network to improve anti-fungal treatment outcomes.

Methods:
Hyperbaric oxygen (100% Oxygen at 2.5 or 3.5 ATA absolute) was used in vitro and in murine models of IA. To decipher the pathways targeted by HBO, we compared the transcriptome of Aspergillus fumigatus in normoxia and HBO conditions. Fungal burden and murine survival analysis was done as described previously (Grahl et al., 2011).

Results:
To date, we report 4 major results from our studies: (1) HBO significantly inhibited both conidia mediated and biofilm growth of A. fumigatus (2) Effects of HBO on fungal proliferation in vivo were directly correlated with the absolute pressure of oxygen i.e. murine survival was increased using 3.5ATA absolute as compared to 2.5 ATA absolute. (3) HBO increases Aspergillus fumigatus’ susceptibility to voriconazole in vitro. (4) Major re-wiring of the A. fumigatus transcriptome in response to HBO treatments was observed.

Conclusion:
Our current data suggests that hyperbaric oxygen reduces fungal proliferation in vivo and enhances survival in a chemotherapy murine model of IA. We are analyzing our transcriptome data to gain insights into the pathways regulated by oxygen in A. fumigatus. Future studies will explore the potential synergistic effect of HBO on antifungal drug efficacy in vivo and identify mechanisms of action that could be targeted through other approaches.
In vivo efficacy of intranasally dosed PC945, a novel antifungal agent in Aspergillus fumigatus infection in immunocompromised mice

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Background:
PC945 is a novel antifungal agent developed as an inhalation therapy for the treatment of invasive pulmonary aspergillosis. In this study, we examined the effects of intranasally dosed PC945 on biomarkers and survival rates in Aspergillus fumigatus infected immunocompromised mice.

Methods:
A/J mice (males, 5 weeks old) were dosed with hydrocortisone (125 mg/kg, sc,) on days 3, 2 and 1 before infection, and with cyclophosphamide (250 mg/kg, ip) 2 days before infection to induce temporary neutropenia. On day 0, animals were infected intranasally with 35 µL of the spore suspension of Aspergillus fumigatus (ATCC 13073) at a concentration of $1.67 \times 10^8$ spores mL$^{-1}$ of physiological saline. PC945, posaconazole or voriconazole were given intranasally on days 0, 1, 2 and 3 (early intervention) or days 1, 2 and 3 (late intervention) and animals were culled 6 hours after the final treatment on day 3. Bronchoalveolar lavage fluid (BALF) and serum were collected for biomarker analysis. Alternatively, the survival of animals was observed for 7 days. Furthermore, the effects of extended prophylaxis treatment (treatment daily on days -7 to +3 or days -7 to 0) were investigated and compared with those of the other treatment regimens.

Results:
Early and late interventions with PC945 (0.08, 0.4, 2, 10 mg/ml isotonic saline suspension) were found to inhibit fungal load in the lung, and to decrease galactomannan concentrations in both BALF and serum in a dose-dependent manner. PC945 also inhibited inflammatory cell accumulation into BALF, IFNγ, IL-17 and malondialdehyde (MDA, an oxidative stress marker) levels in BALF, and TNFα and IL-6 levels in serum, all of which were elevated by Aspergillus fumigatus infection. Intranasal dosing with either posaconazole or voriconazole also inhibited infection, but neither compound was as potent as PC945. Either PC945 or posaconazole (0.4 mg/mL) was delivered intranasally once/daily from days 1 to 7 post infection, and “survival” was determined by death or ≥20% body weight reduction from day 1 post infection. 75% of mice treated PC945 were survived although posaconazole showed only 25% survival rate. Furthermore, the effects of extended prophylactic treatment with PC945 were evaluated. Extended prophylaxis with PC945 (0.016mg/ml, a 25 fold lower dose than used in biomarker study above) was found to inhibit fungal load in the lung, the galactomannan concentrations in both BALF and serum and cytokines more potently than treatment on days -1 to +3. Similarly, when evaluated on day 3 post infection, extended prophylactic treatment on days -7 to 0 generated superior anti-fungal effects than resulted from treatment on just days -1 and 0.

Conclusion:
Intranasally dosed PC945 showed more potent inhibitory effects of fungal load, galactomannan concentrations and A. fumigatus-dependent inflammation than intranasal treatment with posaconazole or voriconazole. The data suggest that the anti-fungal effects in the lung of PC945 accumulate repeat dosing and that these effects are persistent. Thus, PC945 has the potential to be a novel inhaled therapy for the treatment of Aspergillus fumigatus infection in humans.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
IN VITRO AND IN VIVO ANTI-FUNGAL ACTIVITY OF PC945, A NOVEL AZOLE, ON AZOLE SENSITIVE AND RESISTANT ASPERGILLUS FUMIGATUS STRAINS AS A TOPICAL MONOTHERAPY OR IN COMBINATION WITH ORAL POSACONAZOLE

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Background:
PC945 is a novel antifungal agent being developed as an inhalation therapy for the treatment of aspergillosis. This study examined the activity of PC945 on azole sensitive and resistant Aspergillus fumigatus (A. fumigatus) strains as a topical monotherapy or combined with posaconazole in vitro using a model of human alveoli and in vivo using A. fumigatus-infected immunocompromised mice.

Methods:
For the in vitro study, a model of human alveoli was constructed in transwells consisting of a bilayer of human alveolar epithelial and endothelial cells. PC945 (0.1 μg/mL azole sensitive strain; 1 μg/mL azole resistant strain) was added as monotherapy once/daily on days 0, 1 and 2 to the epithelial compartment or was combined with posaconazole (0.01 μg/mL azole sensitive strain; 0.1 μg/mL azole resistant strain) added to the endothelial compartment. Transwells were infected with azole sensitive (NCPF2010) or resistant (TR34/L98H Paris) A. fumigatus at a concentration of 1 x 10^4 spores mL^-1, one hour after treatment on day 0. Subsequently, levels of galactomannan in the endothelial compartment were quantified by ELISA. For the in vivo study, A/J mice (males, 5 weeks old) were dosed with hydrocortisone (125 mg/kg, sc) on days 3, 2 and 1 before infection, and with cyclophosphamide (250 mg/kg, ip) 2 days before infection and 1 day after infection. On day 0, animals were infected intranasally with 35 μL of the spore suspension of A. fumigatus (ATCC 13073) at a concentration of 1.67 x 10^8 spores mL^-1 of physiological saline. PC945 (0.4 mg/mL suspended in isotonic saline) was given intranasally and posaconazole (1 mg/kg, po, in 20% PEG400) was given orally once/daily on days 1, 2, 3, 4, 5 and 6 after infection, and body weight and survival were monitored daily up to day 7.

Results:
Using the in vitro model of human alveoli, posaconazole (0.01 μg/mL; endothelial compartment mimicking oral treatment) or PC945 (0.1 μg/mL; epithelial compartment mimicking inhalation treatment) alone had only mild inhibitory effects on azole sensitive A. fumigatus invasion whereas a combination of PC945 and posaconazole achieved marked inhibition of fungal invasion. A similar pattern was observed for the azole resistant strain TR34-L98H, with posaconazole (0.1 μg/mL) or PC945 (1 μg/mL) monotherapy weakly inhibiting fungus invasion. In contrast, treatment with a combination of PC945 and posaconazole provided much greater protection. Furthermore, in vivo monotherapy with PC945 (0.4mg/ml) or posaconazole (1.0mg/kg) showed limited benefit, however a combination of PC945 and posaconazole showed marked effects on survival with 83% mice surviving to day 7 in Aspergillus fumigatus infected immunocompromised mice.

Conclusion:
In this study, combination therapy of PC945 and posaconazole was shown to inhibit azole-sensitive and azole-resistant A. fumigatus growth to a greater extent than either compound as monotherapy. PC945 therefore has the potential to be used in combination with established antifungal drugs for the treatment of azole sensitive and resistant A. fumigatus infection in humans.
Background:
PC945 is a novel antifungal agent designed for inhalation treatment, and in clinical development for the treatment of aspergillosis. In this study, the in vitro activity of PC945 was investigated versus *Aspergillus fumigatus* (*A. fumigatus*) and a range of pathogenic yeasts and moulds.

Methods:
CYP51A and CYP51B binding affinity and enzyme inhibitory activity were determined using recombinant *A. fumigatus* CYP51A/B. Ergosterol levels in extracts of *A. fumigatus* with alcoholic potassium hydroxide was also measured using HPLC or GC/MS. Anti-fungal potency was evaluated using the modified EUCAST broth microdilution method by visual inspection and using optical density (OD) measurements to quantify growth. The inhibitory effects and persistence of action of PC945 were also investigated in *A. fumigatus* infected bronchial epithelial cell line (BEAS2B), by measuring galactomannan using an ELISA. Anti-fungal potency against a wide range of fungi were evaluated using CLSI broth microdilution assays (Eurofins-Panlabs).

Results:
PC945 has shown to be a strong tight binding inhibitor of CYP51A/B enzyme activity. PC945 also showed the depletion of ergosterol content in *A. fumigatus* membranes with the characteristic accumulation of 14-methylated sterols (lanosterol and eburicol). In broth microdilution assays, PC945 was found to be a potent and highly effective inhibitor of growth of *A. fumigatus*-itraconazole susceptible strains (NCPF2010, AF293 and AF294), with MIC₉₀ values (OD-based) of 0.010 µg/mL, 0.0041 µg/mL and 0.043 µg/mL, respectively. PC945 also demonstrated potent inhibition of growth of the *A. fumigatus*-itraconazole resistant strains (AF91 (M220V) and AF72 (G54E): MIC₉₀ values of 0.060 µg/mL, 0.0029 µg/mL, respectively). For TR34-L98H strain (Paris), where voriconazole showed no activity up to 1 µg/mL, PC945 showed a very potent MIC₅₀ value (0.033 µg/mL) although the maximum inhibitory effect did not reach to 90%. In *A. fumigatus*-infected BEAS2B cells, one hour contact with PC945 followed by a 24 hour washout resulted in only a 11.5-fold loss of potency compared with cells where there was no washout period, suggesting that short contact with PC945 would lead to a long duration of action subsequently in bronchial epithelial cells. In a panel with a wide range of fungi, PC945 was found to be a potent inhibitor on *Rhizopus oryzae*, *Cryptococcus neoformans*, *Rhizocladosporium globosum*, *Penicillium chrysogenum* and *Trichophyton rubrum* as well as *Candida* spp.

Conclusion:
In this study, PC945 was found to be a potent *A. fumigatus* CYP51 inhibitor and demonstrated potent inhibitory activity against several strains of *A. fumigatus*, including some with well characterised CYP51A mutations, and some effects versus several yeast and filamentous fungi. PC945 demonstrated a long duration action versus *A. fumigatus*. PC945 therefore has the potential to be a novel therapy for the treatment of *A. fumigatus* infection in humans.
ACTIVITY OF PC945, A NOVEL AND LONG ACTING AZOLE, AGAINST CLINICAL ASPERGILLUS FUMIGATUS ISOLATES FROM FRANCE AND THE UNITED KINGDOM

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Background:
PC945 is a novel antifungal agent developed as an inhalation therapy for the treatment of pulmonary aspergillosis. In this study the antifungal activity of PC945 was assessed by performing susceptibility testing against clinical isolates of Aspergillus fumigatus (A. fumigatus) in France and United Kingdom.

Methods:
Anti-fungal potency was evaluated using the EUCAST broth microdilution method by visual inspection and using optical density (OD) measurements to quantify growth. Clinical A. fumigatus strains were collected from the St Louis Hospital in Paris over the last 5 years and by the North West England Mycology Reference Centre.

Results:
PC945 was found to be a potent and highly effective inhibitor of growth of A. fumigatus isolated at the St Louis Hospital (50 isolates). The median MIC of PC945 was 0.063 µg/mL (Quarter range: 0.063 – 0.125), lower than that of voriconazole (median 0.5 µg/mL (Quarter range 0.25 – 0.5)) and similar to that of posaconazole (median 0.032 µg/mL (Quarter range 0.032 – 0.032)). Regarding clinical isolates from North West England, 13 out of 46 strains of A. fumigatus were posaconazole resistant based on the EUCAST ECOFF. In these isolates, although PC945 did not inhibit growth completely when used at concentrations up to 8 µg/mL in 5 strains out of 46, the median MIC of PC945 against the entire set was 0.25 µg/mL (Quartile range: 0.125 – 0.5), which was similar to that of posaconazole (median 0.25 µg/mL (Quarter range 0.25 – 0.5)). In addition, the median MIC50 of PC945 (median 0.06 µg/mL (Quartile range 0.03 – 0.125)) was lower than posaconazole (median 0.125 µg/mL (Quartile range 0.06 – 0.125)) in all strains used.

Conclusion:
In this study, PC945 demonstrated potent activity against clinical isolates of A. fumigatus, being more potent than voriconazole and similar in potency to posaconazole. PC945 therefore has the potential to be a novel therapy for the treatment of A. fumigatus infection in humans.
ANTI-FUNGAL ACTIVITY OF PC1244, A NOVEL AZOLE, ON AZOLE SUSCEPTIBLE AND RESISTANT *ASPERGILLUS FUMIGATUS* STRAINS AND OTHER FUNGI

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Background:
PC1244 is a novel antifungal agent designed for inhalation treatment of invasive pulmonary aspergillosis or difficult fungi infection. In this study, in vitro profile of PC1244 was investigated against *Aspergillus fumigatus* (*A. fumigatus*) and a range of yeasts and moulds.

Methods:
CYP51A and CYP51B binding affinity and enzyme inhibition were determined using recombinant *A. fumigatus* CYP51A/B. Anti-fungal potency was evaluated with using the EUCAST broth microdilution method by visual inspection and using optical density (OD) measurements to quantify growth. Anti-fungal potency against an extended range of fungus were evaluated using CLSI broth microdilution in Eurofins-Panlabs.

Results:
PC1244 has high affinity for both *A. fumigatus* CYP51A and CYP51B proteins, and was a strong tight binding inhibitor of CYP51A/B enzyme activity. PC1244 also showed the depletion of ergosterol content in *A. fumigatus* membranes with the characteristic accumulation of 14-methylated sterols (lanosterol and eburicol). In broth microdilution assays, PC1244 was a potent and highly effective inhibitor of growth of *A. fumigatus*-itraconazole susceptible strains (NCPF2010 and AF293) with MIC\(_{90}\) (90% inhibition of growth determined by OD) of 0.0022 µg/mL and 0.012 µg/mL, respectively. PC1244 also showed more potent inhibition of growth of *A. fumigatus*-itraconazole resistant strains (AF91 (M220V), AF72 (G54E), TR34-L98H (Paris) and TR46-Y121/T289A (India)) than voriconazole or posaconazole, with MIC\(_{90}\) of 0.024 µg/mL, 0.026 µg/mL, 0.024 µg/mL, 0.17 µg/mL, respectively. Against clinical strains, PC1244 also demonstrated potent inhibition of growth of 58 *A. fumigatus* isolates from the St Louis hospital including 8 TR34 L98H isolates (median visual MIC 0.016 µg/mL (Quartile range 0.008 – 0.25) and 46 clinical isolates from North West England Mycology centre including 13 posaconazole resistant isolates based on EUCAST epidemiological cut-off (median visual MIC 0.25 µg/mL (0.125 – 2 µg/mL). In a panel against an extended range of fungi, PC1244 was found to be a potent inhibitor on other *Aspergillus* spp. (*flavus, carbonarius*), *Rhizopus oryzae*, *Cryptococcus neoformans*, *Rhizocladosporium globosum*, *Cladosporium argillaceum*, *Penicillium chrysogenum/citrinum*, *Fusarium graminerarum* and *Trichophyton rubrum* as well as *Candida* spp. (MIC range: 0.0031 – 1 µg/mL)

Conclusion:
In this study, PC1244 was shown to be a potent *A. fumigatus* CYP51 inhibitor and demonstrated more potent activity against several strains of *A. fumigatus*, including those with well characterised cyp51A mutations, and clinical isolates. We also found beneficial effects of PC1244 on several yeast and filamentous fungi. PC1244 therefore has the potential to be a novel therapy for the treatment of *A. fumigatus* and other difficult fungi infections in humans.
COMBINATION THERAPY WITH ISAVUCONAZOLE AND MICAFUNGIN FOR TREATMENT OF EXPERIMENTAL INVASIVE PULMONARY ASPERGILLOSIS: PRELIMINARY REPORT

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Purpose:
Invasive pulmonary aspergillosis (IPA) is an important cause of morbidity and mortality in patients with cancer, hematopoietic stem cell transplantation, solid organ transplantation, and other immunodeficiencies. Despite the use of single agents such as amphotericin B, its lipid formulations, antifungal triazoles, and echinocandins, mortality associated with IPA remains high. We hypothesize that simultaneous inhibition of the biosynthesis of key components of the fungal cell membrane and cell wall, respectively, by an antifungal triazole and echinocandin combination may result in a synergistic interaction in vivo. We, therefore, studied combination treatment with the third-generation broad-spectrum triazole isavuconazole and echinocandin micafungin for experimental IPA in persistently neutropenic rabbits.

Methods:
A well established persistently neutropenic New Zealand White rabbit model of experimental invasive pulmonary aspergillosis was used for this study. Treatment groups included rabbits receiving orally administered prodrug isavuconazonium sulfate (BAL8557) equivalent to active moiety isavuconazole (BAL4815, ISA) at 20 (ISA20), 40 (ISA40), and 60 (ISA60) mg/kg/day, micafungin at 2 mg/kg/day (MFG2), or combination of (ISA20+MFG2), (ISA40+MFG2), (ISA60+MFG2), or untreated rabbits (UC). Treatment started 24 h after endotracheal administration of A. fumigatus inoculum and continued administered once daily for up to 12 days. Blood samples for galactomannan (GMI) antigenemia and serum (1→3)-β-D-glucan (BG) levels were obtain every other day. A panel of therapeutic outcome variables were evaluated at the completion of all experiments.

Results:
There was a significant reduction of residual fungal burden (CFU/g) in ISA20-, ISA40-, ISA60-, ISA20+MFG2-, ISA40+MFG2-, and ISA60+MFG2-treated rabbits vs that of MFG2-treated or UC (p<0.01). As measures of organism-mediated pulmonary injury, lung weights and pulmonary infarct score were significantly lower in ISA60-, ISA20+MFG2-, ISA40+MFG2-, and ISA60+MFG2-treated rabbits in comparison to that of MFG2-treated and UC (p<0.05). Rabbits treated with ISA20+MFG2, ISA40+MFG2, and ISA60+MFG2 significantly prolonged survival in comparison to that of UC (p<0.01). In addition, the combination group of ISA40+MFG2 demonstrated significantly lower lung weights and pulmonary infarct scores, and prolonged survival in comparison to that of single therapy of ISA40. These outcome variables data correlated directly with a significant decline of GMI antigenemia and serum BD levels during therapy in comparison to progressive GMI and BG levels of UC (p<0.01).

Conclusion:
In summary, rabbits treated with ISA20+MFG2, ISA40+MFG2, and ISA60+MFG2 demonstrated significant dose-dependent reduction of CFU/g, decreased pulmonary injury, prolonged survival, lower GMI, and lower serum BD levels in comparison to that of untreated controls. The data suggest faster elimination of A. fumigatus in combination treatment groups in comparison to that of single therapy, thereby preventing organism-mediated pulmonary injury.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
IMPACT OF SHORT-COURSES OF LIPOSOMAL AMPHOTERICIN B THERAPY ON CHRONIC PULMONARY ASPERGILLOSIS

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Purpose:
Intravenous amphotericin B therapy is a recognised therapy for patients with chronic pulmonary aspergillosis (CPA). Limited data is available on the clinical benefit associated with liposomal amphotericin (LAmB) therapy in CPA patients and whether repeated treatment courses are associated with a deterioration in renal function.

Methods:
We retrospectively audited the clinical response and renal function observed in CPA patients who received one or more short-courses (< 6 weeks duration) of LAmB therapy (Gilead Sciences) at the National Aspergillosis Centre in Manchester before July 2013. A patient case-note review was undertaken using a standardised proforma. Data collected included patient demographics, indication for LAmB treatment, renal function (estimated glomerular filtration rate (eGFR) at baseline, during treatment and up to 6 to 8 months post treatment) and whether they developed an increased risk of or an acute kidney injury (AKI; RIFLE criteria) with treatment, the dose and duration of LAmB therapy and the clinical response to treatment. Patients who received <3 doses of LAmB or started intermittent long-term LAmB treatment within one month of completing a short-course of therapy were not evaluated for a clinical response (N=6). Changes in renal function were not assessed in patients who received < 1 full dose of LAmB (N=2) or in one patient who received intermittent long-term LAmB therapy between his short-courses of LAmB therapy.

Results:
71 CPA patients (41 male) were identified aged 28 – 86 years (median 64) when treated. Median duration of prior azole therapy was 12 months (range 0 - 112). Primary indications for LAmB therapy were respiratory symptoms (N=33; 50.7%), constitutional symptoms (N=2; 2.8%) or both (N=36; 50.7%). LAmB doses ranged between 2.5 – 5 mg/Kg/day. The duration of therapy ranged between 4 to 36 days (median 20.5 days). 48 patients (73.8%) had a clinical response (respiratory and / or constitutional symptoms) to their first LAmB course and quality of life (QOL) improvements were noted in 37 (92.5%) of 40 patients with pre- and post- treatment QOL data available.

20 patients received at least two short-courses of LAmB therapy. Clinical response rates for repeated short-courses of LAmB were 76.6% and QOL improvements were seen in 91.7% of treatment courses.

34 (50%) and 17 (25%) patients respectively developed an increased risk of AKI or AKI with their first LAmB course. 24 (35.3%) patients had at least one other contributing factor to their deteriorating renal function; 17 (25%) had a contrast scan and 9 (13.2%) patients received concomitant medications with associated nephrotoxicity risks. The adjusted geometric mean eGFR significantly fell during treatment and did improve post treatment but did not return to pre-treatment levels by 6 to 8 months follow-up (p<0.001). A similar pattern was observed in patients receiving a second treatment course.

Conclusion:
Around 74% of CPA patients receiving a short course of LAmB therapy experienced a clinical response. A deterioration in renal function was observed in the majority of patients with 50% of individuals developing an increased risk of AKI and 25% experiencing an AKI with their first treatment course. Whilst CPA is responsive to LAmB, caution should be exercised with repeated courses of treatment, especially if other treatment options are available.
Primed IL-17-expressing neutrophils mediate protection against Aspergillus fumigatus lethal second-challenge

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Purpose:
Phagocytic leukocytes restrict germination of Aspergillus fumigatus conidia preventing establishment of invasive pulmonary aspergillosis in immunocompetent mice. Due to the essential contribution of innate immune cells in preventing the infection, we addressed the question whether mice can be primed and consequently protected against aspergillosis?

Results:
Using bioluminescence imaging, flow cytometry and different knock out mice strains, we observed that immunocompetent mice that had recovered from primary A. fumigatus infection are protected against a second lethal dose of conidia. Protection was associated with enhanced CXCR2 and Dectin-1 expression on bone marrow-phagocytes and IL-17+ GR-1+ CD11b+ myeloid cells that are rapidly recruited to the site of infection. These cells mediate an early pro-inflammatory response. Furthermore, in protected mice rapidly recruited neutrophils released increased ROS and MPO levels associated with efficient phagocytosis and killing of conidia. Both processes are impaired in susceptible infected CXCR2 deficient mice. By using RAGc, RORgT, and IL-17RA-/ mice, we show that protection is independent of T and B lymphocytes, but requires a myeloid cell dependent IL-17 signaling cascade.

Conclusion:
Our model of invasive pulmonary aspergillosis supports the hypothesis that trained immunity following a first encounter with a non-lethal dose of A. fumigatus provides an efficient protection from a lethal inoculum.
MOLECULAR BASIS OF INVASIVE GROWTH DURING MOULD INFECTION OF THE LUNG

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Purpose:
Aspergillus fumigatus is the major cause of human aspergilloses accounting for >90% of all cases of disease. Inhaled conidia interact with lung epithelial cells, and germinate into hyphae that penetrate and damage underlying tissues. It has been shown that epithelial cells respond actively to the inhaled fungus in addition to posing an anatomical barrier but the exact mechanism of response, and whether or not these mechanisms are protective or detrimental to the host is largely unknown. The aim of this study is to generate a global map of epithelial responses to A. fumigatus over time and characterise the role of these mechanisms in eventual epithelial cell destruction.

Methods:
A549 epithelial cell line was co-incubated with live A. fumigatus and culture filtrate and induction of the following mechanisms in the epithelial cells over time were assayed (i) activation of signalling pathways using the Luminex multiplex assay, (ii) transcriptional responses using the TransAM ELISA assay for DNA binding activity and (iii) profile of cytokine expression using human XL cytokine profiler and Luminex method. The amount of damage induced by both live fungi and secreted products independently in A549 monolayer was measured using the LDH assay.

Results:
Live A. fumigatus induced epithelial cell damage as measured by LDH after 12 h of infection, whereas CF induced cell detachment in addition to damage as early as 30 min post exposure. Live A. fumigatus induced early and sustained NF-κB activation and late JNK activation, whereas CF activated JNK, p38 and ERK1/2 at 30 min post exposure. Live A. fumigatus increased the DNA binding activity of the transcription factors c-Fos and members of the canonical NF-κB pathway (p50 and p65), while decreasing MEF-2, Jun D and Rel B activities. On the other hand, CF exposure increased the DNA binding activity of the non-canonical NF-κB pathway (Rel B and p52), while decreasing the binding activity of MEF-2 and c-Myc. Live A. fumigatus induced significant production of GM-CSF and IL-8 but not IL-6 and G-CSF, while CF significantly reduced the levels of IL-8, IL-6 and G-CSF.

Conclusion:
Together, this data suggests that A549 cells recognize and respond differently to live A. fumigatus and its secreted products. Current studies focus on elucidating the specific host and fungal properties driving these responses.
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