6th ADVANCES AGAINST ASPERGILLOSIS

Madrid, Spain

27 February - 1 March 2014

Meliá Castilla Conference & Convention Centre

www.AAA2014.org
Dear Advances Against Aspergillosis Colleague

This 6th Advances Against Aspergillosis conference is the 10th anniversary of the first meeting. The previous five international 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, and the fifth meeting in 2012 (Istanbul) with 375 attendees from 39 countries. This conference has now established itself as the premier forum for dedicated discussion of all aspects of Aspergillus infection and research, and published proceedings from the five prior conferences have been well-received.

The Aspergillus field is in more active state of rapid advancement, including the publication of numerous post-genomic papers and substantial advances in translational and diagnostic research. The global burden of aspergillosis has been modelled and studied for the first time, with major national and international epidemiological studies ongoing with significant implications for global health. We anticipate the launch of another oral anti-Aspergillus antifungal in the next 18 months and anticipated clinical trials of at least one other agent. Antifungal resistance has emerged in many countries, newer molecular diagnostic tools are available and better studied, and combination therapy has been tested in the first large clinical trial. The incidence of invasive aspergillosis is increasing, especially in less common clinical groups, and patient mortality remains high. The world’s aspergillosis experts are here in one venue. A fundamental tenet of this research colloquium continues to be to engender collaborative relationships amongst clinicians, scientists, and industry to further advance the field.

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

We also thank all the speakers and poster presenters, and every one of you, for contributing to the success of this effort. We hope you will enjoy the meeting, the conference hotel, and the exciting city of Madrid. Please also join us at the welcome reception, the Astellas 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 6th meeting will once again be published in a special journal supplement, creating what we hope will be highlights of the newer insights from the many disciplines that encompass Aspergillus research and care. Our plan is to continue this conference every other year, and you will notice that there is a special open planning session for the next conference at the end of this meeting. We invite you to come and offer any suggestions for new sessions or topics or locations you would like to see in the future.

Yours sincerely,

William J. Steinbach
Co-Chairman

David W. Denning
Co-Chairman

David A. Stevens
Co-Chairman
ACKNOWLEDGEMENTS

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

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27 February - 1 March 2014 - Madrid, Spain

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SCHOLARSHIP AWARDS

Tonia Akoumianaki, Greece
Ulrike Binder, Austria
Anuradha Chowdhary, India
Heather Clark, USA
Blandine Denis, France
Lalit Kumar Dubey, Switzerland
Katie Dunne, Ireland
Jose A. G. Ferreira, Brazil
Martin Hoenigl, Austria
Sara Gago Prieto, Spain
Anupam Jhinghan, USA
Ushana Shrestha Khwakhali, Nepal
Alexander Kuko, Russia
Frederic Lamoth, Switzerland
Mark Lee, Canada
David Lowes, UK
Saeid Malek Zadeh, Iran
Olga Mashedi, Kenya
Melinda Paholcske, Hungary
John Penner, USA
Benjamin Ralph, Canada
Marwa Fathi Saad, Egypt
Raquel Sabino, Portugal
Cheshta Sharma, India
Christopher Sirivoranankul, USA
Timothy Smith, USA
Hector Toledo, Spain
Clara Valero, Spain
Isabel Valsecchi, France
Jose Vargas-Muniz, Puerto Rico
Cesar Velasco Munoz, Spain
Carla Viegas, Portugal

Vasily M. Leschenko Memorial Scholarship
Igor A. Riabinin, Russia
SCHOLARSHIP AWARDS

Advances Against Aspergillosis gratefully thanks the invited speakers, who forego honoraria to make funding for scholarships available.

Advances Against Aspergillosis gratefully thanks the following donors, who made scholarships possible, by augmenting funds from the conference itself for this purpose:

ALLFUN, Italy

European Confederation of Medical Mycology

International Society for Human and Animal Mycology

Astellas

Gilead Sciences

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Fungal Infection Trust

Foundation for Research in Infectious Diseases
PROGRAMME

TUESDAY 25 FEBRUARY
Hidalgo Room

Pre-Advances Against Aspergillosis Workshop:
*Aspergillus* Speciation in the 21st Century -
Implications for Laboratory and Clinical Practice

*Supported by an unrestricted educational grant from Gilead Sciences*

**Organisers:**
Scientific Committee of Advances Against Aspergillosis
Instituto de Salud Carlos III (Centro Nacional de Microbiologia)

**Workshop Coordinator:**
Manuel Cuenca-Estrella, MD PhD
Mycology Department of Spanish National Center for Microbiology
Instituto de Salud Carlos III, Majadahonda, Spain

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| 15.00 - 15.15 | **Opening**
|           | Manuel Cuenca-Estrella, MD PhD                                           |
| 15.15 - 16.00 | **Current classification of the clinically relevant*Aspergillus* species complexes**
|           | Cornelia Lass-Flörl, MD PhD                                              |
| 16.00 - 16.45 | **Methods for identification: conventional, DNA sequencing, MALDI-TOF**
|           | Mohammad T. Hedayati, PhD                                                |
| 16.45 - 17.30 | **Diagnostic methods to detect*Aspergillus* species in clinical samples using conventional and non-culture techniques**
|           | Jesús V. Guinea, PhD                                                      |
| 17.30 - 18.00 | **Discussion**                                                            |
| 18.00     | **Close**                                                                |
WEDNESDAY 26 FEBRUARY
Hidalgo Room

Pre-Advances Against Aspergillosis Workshop:
*Aspergillus* Speciation in the 21st Century -
Implications for Laboratory and Clinical Practice

Supported by an unrestricted educational grant from Gilead Sciences

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| 09.00 - 09.45 | Epidemiology of infections by cryptic species of *Aspergillus*: TRANSNET, SCARE multicenter study, FILPOP survey  
Jan W.M. van der Linden, MD |
| 09.45 - 10.30 | *In vitro* antifungal susceptibility of cryptic *Aspergillus* species  
Ana Alastruey-Izquierdo, PhD |
| 10.30 - 11.15 | Clinical profile of cryptic *Aspergillus* species  
David W. Denning, FMedSci |
| 11.15 - 11.45 | Break |
| 11.45 - 12.30 | Treatment of infections by cryptic *Aspergillus* species  
Alessandro Pasqualotto, MD PhD |
| 12.30 - 13.15 | Visual session of unusual *Aspergillus* species: images and plates  
Janos Varga, PhD |
| 13.15 - 14.30 | Lunch |
| 14.30 - 18.15 | Case studies (clinical and microbiological)  
Chairman: José M. Aguado, MD PhD  
- Diagnosis and treatment of aspergillosis (mixed infection with cryptic species)  
  Patricia Muñoz, MD PhD  
- Multi-resistant aspergillosis due to cryptic species  
  Susan J. Howard, PhD  
- Study of antifungal resistance mechanisms in cryptic species  
  Emilia Mellado, MD PhD  
- *In vivo* response in a mixed infection model of azole susceptible and resistant *Aspergillus* spp. strains  
  Laura Alcazar-Fuoli, PhD  
- Recommendations for a routine daily practice  
  Manuel Cuenca-Estrella, MD PhD |
| 18.15 | Close |

EARLY REGISTRATION FOR
6TH ADVANCES AGAINST ASPERGILLOSIS

17.00 - 19.00 Upper Hall
THURSDAY 27 FEBRUARY

All sessions will take place in the Auditorium unless otherwise stated

08.00 - 09.00 Meet the Professor Session
PK/PD of anti-Aspergillus triazoles
William Hope, BMBS FRACP FRCPA PhD

08.00 - 09.00 Meet the Professor Session
Aspergillus cell wall and biofilm
Anne Beauvais, PhD

09.00 - 09.10 Opening Remarks
David W. Denning, FMedSc

Session 1: Factors affecting Aspergillus Pathogenesis
Moderators: Jean-Paul Latgé, PhD & David S. Askew, PhD

09.10 - 09.35 Hyphal growth and septation
Praveen Rao Juvvadi, PhD

09.35 - 10.00 Zinc homeostasis and its significance for Aspergillus fumigatus virulence
José Antonio Calera, PhD

10.00 - 10.15 Selected Abstract Presentation:
Modification of exopolysaccharide composition can increase the virulence of non-pathogenic Aspergillus species
Mark J. Lee, MSc

10.15 - 10.30 Selected Abstract Presentation:
The ZrfC alkaline zinc transporter is required for Aspergillus fumigatus virulence and its growth in the presence of the Zn/Mn-chelating protein calprotectin
Jorge Amich, PhD

10.30 - 10.55 Mycotoxin synthesis control
Jin Woo Bok, PhD

10.55 - 11.05 Discussion

11.05 - 11.35 Coffee Break

Castilla
Session 2: New Anti-Aspergillus Drug Targets
Moderators: William J. Steinbach, MD & José Maria Aguado, MD PhD

11.35 - 12.00 New agents for aspergillosis: development pathways and economic pull
John H. Rex, MD

12.00 - 12.15 Selected Abstract Presentation:
Study of the MtfA transcription factor in the opportunistic fungal pathogen
*Aspergillus fumigatus*
Timothy Smith

12.15 - 12.30 Selected Abstract Presentation:
*In vivo* manipulation of lung oxygen content to reduce fungal growth
during murine invasive pulmonary aspergillosis
Robert A. Cramer, PhD

12.30 - 13.00 Lunch

Session 3: Difficult Clinical Cases

13.00 - 14.45 Poster Session 1

13.45 - 14.45 Difficult clinical cases
José Maria Aguado, MD PhD & Dimitrios P. Kontoyiannis, MD

Session 4: Antifungal Resistance
Moderators: Cornelia Lass-Flörl, MD PhD & Manuel Cuenca-Estrella, MD PhD

14.45 - 15.10 *Aspergillus* virulence profiles and azole resistance in *Aspergillus fumigatus* - clinical isolate screening, culture selection and genetics
Liliana Losada, PhD

15.10 - 15.25 Selected Abstract Presentation:
A novel transcriptional regulator AtrR of *Aspergillus fumigatus* is required for azole resistance, hypoxia growth, and expression of ABC transporter gene *cdr1B*
Daisuke Hagiwara, PhD

15.25 - 15.40 Selected Abstract Presentation:
Voriconazole resistant *Aspergillus fumigatus* carrying TR$_{46}$/Y121F/T289A mutation from the Indian environment
Cheshta Sharma

15.40 - 16.00 Agricultural triazole fungicides should be withdrawn – PRO
Katrien Lagrou, PharmD PhD

16.00 - 16.20 Agricultural triazole fungicides should be withdrawn – CON
Bart Fraaije, PhD

16.20 - 16.35 Discussion from the floor and vote

16.35 - 17.05 Coffee Break
Session 5: Therapeutics
Moderators: Dimitrios P. Kontoyiannis, MD & Alessandro Pasqualotto, MD PhD

17.05 - 17.30  Combination therapy update
Johan Maertens, MD PhD

17.30 - 17.55  Posaconazole breakthroughs
Jutta Auberger, MD

17.55 - 18.10  Selected Abstract Presentation:
Identification of Aspergillus fumigatus wall compounds that affect activity and function of human platelets
Cornelia Speth, PhD

18.10 - 18.25  Selected Abstract Presentation:
Effect of ghrelin for prevention in murine invasive pulmonary aspergillosis model
Kazuaki Takeda, MD

18.25 - 18.50  Second line therapy for allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitization (SAFS) - how far have we gone?
Livingstone Chishimba, MB ChB MRCP

19.00 - 19.30  Welcome Reception

Astellas Satellite Symposium:
Advancing Outcomes and Overcoming Adversities in Invasive Aspergillosis

19.30 - 19.40  Welcome and introductions
Clinical perspectives
David W. Denning, FMedSci

19.40 - 20.00  Treating invasive aspergillosis: strengths and limitations
Johan Maertens, MD PhD

20.00 - 20.20  Ongoing challenges: drug interactions and tolerability
Russell Lewis, PharmD FCCP BCPS

20.20 - 20.30  Q&A followed by closing comments
David W. Denning, FMedSci

20.30 - 21.30  Drinks & Desserts

Astellas Pharma Europe Ltd are not supporting or funding the social aspects of the congress programme but will be funding the Welcome Reception alongside their symposium.
FRIDAY 28 FEBRUARY
All sessions will take place in the Auditorium unless otherwise stated

08.00 - 09.00 Meet the Professor Session
Aspergillus immunology update
Luigina Romani, MD PhD

08.00 - 09.00 Meet the Professor Session
Aspergillus, sex and recombination
Janos Varga, PhD

09.00 - 09.10 Announcement of Scholarship Winners
David A. Stevens, MD

Session 6: Immunopathology
Moderators: Luigina Romani, MD PhD & David A. Stevens, MD

09.10 - 09.35 Fungal role in pulmonary microbiome: Pseudomonas-Aspergillus interaction
Richard B. Moss, MD

09.35 - 10.00 Targeting Aspergillus fumigatus virulence factors in a mouse model of corneal infection
Eric Pearlman, PhD

10.00 - 10.25 Immunoevasive Aspergillus virulence factors
Sanjay H. Chotirmall, MD PhD

10.25 - 10.40 Selected Abstract Presentation:
Noncanonical autophagy is a target of fungal cell wall melanin
Tonia Akoumianaki, PhD

10.40 - 10.55 Selected Abstract Presentation:
Aspergillus fumigatus CalA binds to β1 integrins and mediates host cell invasion and virulence
Hong Liu, PhD

10.55 - 11.05 Discussion

11.05 - 11.35 Coffee Break
## Session 7: Diagnostics

Moderators: Juan Luis Rodriquez Tuldela, MD PhD & Ritesh Agarwal, MD DM

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<td>Culture and sensitization in asthma, CF and COPD - what does it tell us?</td>
<td>Catherine Pashley, PhD</td>
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<td>12.00 - 12.25</td>
<td>MALDI-TOF - any use for <em>Aspergilli</em>?</td>
<td>Maurizio Sanguinetti, MD</td>
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<td>12.25 - 12.40</td>
<td>Selected Abstract Presentation: Diagnosis of invasive aspergillosis (IA) in HIV infected patients</td>
<td>Blandine Denis, MD</td>
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<td>12.40 - 12.55</td>
<td>Selected Abstract Presentation: Direct detection of an <em>Aspergillus fumigatus</em>-specific breath volatile organic metabolite profile for the diagnosis of invasive aspergillosis</td>
<td>Sophia Koo, MD</td>
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<td>12.55 - 13.20</td>
<td>Recombinant Abs in IA diagnostics</td>
<td>Michael D. Gunn, MD</td>
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<td>13.20 - 13.30</td>
<td>Discussion</td>
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<td>13.30 - 14.30</td>
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<td><em>Tour and Palace Visit, followed by Dinner</em></td>
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SUNDAY 2 MARCH
All sessions will be held in the Auditorium unless otherwise stated.

08.00 - 09.00  Meet the Professor Session
Protein degradation and virulence in *Aspergillus fumigatus*
Gerhard H. Braus, PhD

09.00 - 09.10  NIAID resources and the global research community
Dennis M. Dixon, PhD

Session 8: *Aspergillus* Biology
Moderators: Josep Guarro, PhD & Donald Sheppard, MD

09.10 - 09.35  Ras pathway and polarized growth
Jarrod R. Fortwendel, PhD

09.35 - 10.00  Cell wall and galactosaminogalactan and immunosuppression
Frank van de Veerdonk, MD PhD

10.00 - 10.15  Selected Abstract Presentation:
*Aspergillus fumigatus* – deciphering the role of the chitin synthase families I and II by multiple gene targeting deletion
Vishu K. Aimanianda

10.15 - 10.30  Selected Abstract Presentation:
Molecular detection of *Aspergillus fumigatus* and section *Fumigati* by multiplex real time PCR using ITS and β-tubuline sequences
Jimena V. Fernandez Molina

10.30 - 10.55  Imaging and analysing intracellular calcium dynamics in *Aspergillus fumigatus* in response to environmental stress
Nick D. Read, PhD

10.55 - 11.05  Discussion

11.05 - 11.35  Coffee Break

Castilla
Session 9: Chronic and Allergic Aspergillosis
Moderators: Richard B. Moss, MD & Koichi Izumikawa, MD PhD

11.35 - 12.00  Chronic pulmonary aspergillosis (CPA) secondary to tuberculosis and HIV
Iain Page, MB ChB

12.00 - 12.15  Selected Abstract Presentation:
The temporal sequence of the transition from asthma through allergic bronchopulmonary aspergillosis to chronic pulmonary aspergillosis
David Lowes

12.15 - 12.30  Selected Abstract Presentation:
*Aspergillus* lipoxygenase: a novel instigator of asthma
Greg Fischer, BS

12.30 - 12.55  The burden and distinctive character of ABPA in India
Ritesh Agarwal, MD DM

12.55 - 13.05  Discussion

13.05 - 14.20  Lunch & Poster Session 2

Session 10: Immunogenetic Associations
Moderators: Karl V. Clemons, PhD & Emilia Mellado, MD PhD

14.20 - 14.45  Genome-wide scan using the Immunochip to identify susceptibility loci for IA
Juan Sainz Pérez, PhD

14.45 - 15.10  Functional aspects of IA-linked genetic variants
Agostinho Carvalho, PhD

15.10 - 15.25  Selected Abstract Presentation:
Differential regulation of the host response to *Aspergillus fumigatus* by NOD receptors
Mark Gresnigt

15.25 - 15.40  Selected Abstract Presentation:
Induction of MUC5AC expression to *Aspergillus fumigatus* in airway epithelial cells and suppressive effects brought about macrolide antibacterial agents
Katsuji Hirano, MD

15.40 - 16.05  Genetic factors underlying ABPA
Paul Bowyer, PhD
Session 11: Clinical and Scientific Future of Early Anti-Aspergillus Agents
Moderators: David W. Denning, FMedSci & John H. Rex, MD

16.05 - 16.30 Early clinical studies of novel antifungal agents
Haran Schlamm, MD

16.30 - 17.00 Roundtable discussion

17.00 Awards for best submitted abstract
Closing remarks
William J. Steinbach, MD

17.15 Farewell Drinks and Discussion about AAA 2016
Hidalgo
ABSTRACTS
OF
INVITED
FACULTY
PK/PD OF ANTI-ASPERGILLUS TRIAZOLES

William Hope, BMBS FRACP FRCPA PhD

University of Liverpool - United Kingdom

THURSDAY 27 FEBRUARY 2014 (08.00 - 09.00)

The currently licensed members of the triazole class of antifungal agents with activity against Aspergillus spp. include itraconazole, voriconazole and posaconazole. Isavuconazole is an additional agent that is currently being investigated in clinical trials. The triazoles have a common mechanism of action. Structural differences in the side account for idiosyncrasies in toxicity, pharmacokinetics, and potency. An improved understanding of pharmacokinetics and pharmacodynamics (PK/PD) of the triazoles has been important for a better understanding of the clinical use of the agents for treatment of infections caused by Aspergillus spp. In particular PK/PD has helped in the appropriate dosages for treatment of wild type and mutant isolates, the development of antifungal susceptibility breakpoints and for the development of drug exposure targets that are safe and effective for individual patients.
6th ADVANCES AGAINST ASPERGILLOSIS
27 February - 1 March 2014 - Madrid, Spain

ASPERGILLUS CELL WALL AND BIOFILM

Anne Beauvais, PhD
Institut Pasteur - France

THURSDAY 27 FEBRUARY 2014 (08.00 - 09.00)

A major characteristic of the fungal cell is to be surrounded by a cell wall. In *A. fumigatus*, like in other pathogenic fungi, the cell wall acts as a sieve and a reservoir for effector molecules such as antigens and enzymes that play an active role during infection. The cell wall is essential for fungal growth as well as for resisting environmental stresses such as phagocytic killing. In addition, cell wall biosynthetic enzymes represent unique drug targets, as validated by echinocandin drugs inhibiting β(1,3)-glucan synthesis.

The cell wall is almost exclusively composed of polysaccharides. The fibrillar core of the *A. fumigatus* cell wall is composed of a branched β-(1,3)-glucan (4% of β-(1,6)-branch points) to which chitin/chitosan, β-(1,3)-/β-(1,4)-glucan and β-(1,5)-galacto-α-(1,2)/α-(1,6)-mannan are covalently bound. The alkali-soluble amorphous fraction is mainly composed of linear chains of α-(1,3)-glucan with intra-chain α-(1,4)-linked glucose units for every hundred α-(1,3)-linked glucose units.

In the case of *A. fumigatus*, the air borne conidia are the morphotype that comes first in contact with the host and is responsible for establishing the disease. Our studies have shown that the cell wall of the conidia and mycelium is different. If the same polysaccharides are found in the cell wall of both morphotypes, their concentration and localization differ between them. In addition, 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 (in vivo as in vitro), melanin and rodlets disappeared, exposing α-(1,3)-glucans at the surface. The *A. fumigatus* mycelium grows as a network of agglutinated and hydrophobic hyphae. The surface of the network revealed the presence of an extracellular matrix (ECM) rich in polysaccharides: galactomannan, galactosamino-galactan (GAG) and α-(1,3)-glucans. This ECM also contains hydrophobins and we recently discovered they are important for the survival of the conidia in air. We also recently discovered that galactosamino-galactan (GAG) and α-(1,3)-glucans have an essential role during infection and especially can promote or inhibit protective immunity:

1. Mutants devoid of α-(1,3)-glucans (Δags) were less virulent than the parental strain in experimental model of murine aspergillosis. Only non-germinated conidia were observed in the lung of neutropenic mice infected with the Δags conidia and the lack of vegetative growth was associated with low inflammation. The conidia of the Δags mutants were more efficiently phagocytosed and killed by mouse alveolar macrophages than conidia of the parental strain. The low resistance of the Δags conidia in vivo was associated to structural modifications of the conidial cell wall. In contrast to parental conidia, the conidial surface of the Δags mutants was amorphous and hydrophilic due to the coverage of the rodlets by a layer of glycoproteins, which induced an immediate host immune response. In addition, in contrast to parental strain, β-(1,3)-glucan and chitin PAMPs are exposed at the surface of the germinating conidia of the Δags mutants stimulating the antifungal response.

2. Murine experiments have shown that GAG favors fungal growth in vivo. No inflammatory pathology was seen in the lung where actual fungal growth was observed in GAG-treated mice and the cytokine pattern showed that GAG inhibited protective Th1/Treg cells and promoted Th2 responses. GAG reduces neutrophil infiltrates in the lung during infection and induces neutrophil apoptosis and the production of IL1Ra. This study points to a new virulence mechanism developed by a fungal pathogen to escape to the innate immune system.

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 polysaccharide cell wall biosynthesis. A major difficulty is the fact that the composition and structural organization of the cell wall is not immutable set and is constantly reshuffled. Even though the same polysaccharides are present, their amount and localization vary with the growth conditions and morphotypes indicating 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.
HYPHAL GROWTH AND SEPTATION

Praveen Rao Juvvadi, PhD

Duke University Medical Center - USA

THURSDAY 27 FEBRUARY 2014 (09.10 - 09.35)

Calcineurin plays diverse roles in fungi in regulating stress responses, morphogenesis and pathogenesis. Fungal pathogens have adapted the calcineurin pathway to effectively survive the host environment and cause life-threatening infections (1). The immunosuppressive calcineurin inhibitors (FK506 and cyclosporine A) are active against fungi, yet cross-reactive with human calcineurin in their current formulations, making targeting calcineurin a promising antifungal drug development strategy. Our novel findings on the requirement of calcineurin activity and calcineurin localization at the hyphal tips and septa, the active regions of cell wall synthesis, confirmed the importance of calcineurin for hyphal growth and septum formation in *Aspergillus fumigatus* through regulation of other proteins involved in cell wall biosynthesis, septation and growth (2, 3). Our recent efforts to understand fungal-specific attributes of calcineurin and develop novel antifungal therapeutic approaches have not only revealed key functional domains required for localization and function of calcineurin at the hyphal septum, but also uncovered an evolutionarily conserved novel mode of calcineurin regulation by phosphorylation in filamentous fungi that is responsible for virulence of *Aspergillus fumigatus* (4). This phosphorylation occurs on a cluster of four serine residues located in a unique serine-proline rich domain of calcineurin that is completely absent in humans. This finding of a new fungal-specific mechanism controlling hyphal growth and virulence represents a new potential target for antifungal drug therapy. With our large collection of calcineurin domain mutants we are now exploring ways to identify calcineurin interactants/calcineurin phosphorylation-dephosphorylation mechanisms. This will ultimately translate fungal biology into tangible clinical benefit by identifying targets that specifically inhibit fungal calcineurin, resulting in fungal killing without suppressing the immune system of the host.

References


ZINC HOMEOSTASIS AND ITS SIGNIFICANCE FOR ASPERGILLUS FUMIGATUS
VIRULENCE

José Antonio Calera, PhD

Universidad de Salamanca - Spain

THURSDAY 27 FEBRUARY 2014 (09.35 - 10.00)

Zinc is an essential micronutrient required for the growth of all microorganisms. To grow within the lungs of a susceptible patient Aspergillus fumigatus has to obtain zinc from the surrounding tissues. Most zinc in living tissues is tightly bound to proteins and, consequently, the concentration of free zinc ions in them is much lower than that required for optimal fungal growth in vitro. In addition, the zinc bioavailability during infection can be reduced further in fungal abscesses by calprotectin released by neutrophils as a host defence mechanism to reduce the fungal growth capacity. Then, how does A. fumigatus manage to grow during infection in a zinc-limiting environment as is the lung tissue? A. fumigatus is equipped with several zinc transporters (ZrfA, ZrfB and ZrfC) that enable it to uptake zinc efficiently under the extreme zinc-limiting conditions provided by a susceptible host. The ZafA transcriptional regulator induces the expression of all these transporters, which is modulated further by ambient pH through the PacC regulator. The ZrfA and ZrfB transporters are expressed at a larger extent and play a major role in acidic zinc-limiting media. In contrast, the ZrfC is expressed and required for fungal growth just under alkaline zinc-limiting conditions. Accordingly, both a ΔzafA single mutant and a ΔzrfAΔzrfBΔzrfC triple mutant are non-virulent in a mouse model of IPA. Nevertheless, these zinc transporters contribute differentially to fungal virulence. Thus, the acidic ZrfA and ZrfB transporters are dispensable for fungal virulence in the presence of the alkaline ZrfC transporter. Indeed, the contribution of the acidic transporters to virulence is very low compared to that of the alkaline transporter ZrfC. This is largely due to the unusually long N-terminus only present in ZrfC, which enables it to scavenge zinc efficiently from alkaline zinc-limiting media. In addition, ZrfC also enables A. fumigatus to overcome the inhibitory effect of calprotectin on fungal growth in non-leucopenic mice. These results indicate that regulation of zinc homeostasis and uptake could be promising targets for the discovery and development of new compounds that could be used to treat IPA.
MYCOTOXIN SYNTHESIS CONTROL

Jin Woo Bok, PhD

University of Wisconsin-Madison - USA

THURSDAY 27 FEBRUARY 2014 (10.30 - 10.55)

Aspergillus fumigatus displays many attributes contributing to pathogenicity including the production of dozens of developmentally regulated toxic secondary metabolites, often referred to as mycotoxins. These toxins are largely under the regulation of the global regulator of secondary metabolism (SM), LaeA and its nuclear partner VeA. The first toxin to garner considerable attention was the LaeA regulated metabolite gliotoxin (1). Since then, we have identified several additional virulence factors regulated by LaeA, including but not limited to additional toxins. These SMs include endocrocin (packaged in spores and an inhibitor of neutrophil recruitment, 2, 3), hexadehydroastechrome (exacerbating invasive aspergillosis possibly through complexing with iron, 4), the SM supercluster composed of fumagillin, pseurotin and fumitremorogin (5), and spore specific fumiquinazolines (6). Here we will describe the known and hypothesized mechanisms of LaeA regulation of these and additional toxin clusters in A. fumigatus.

References

NEW AGENTS FOR ASPERGILLOSIS: DEVELOPMENT PATHWAYS AND ECONOMIC PULL

John H. Rex, MD

AstraZeneca - USA

THURSDAY 27 FEBRUARY 2014 (11.35 - 12.00)

The need for new agents. New antifungal agents are needed: resistance has emerged to current agents, there remain fungi for which current therapies are as yet inadequate, and options to address safety or drug-drug interaction issues are needed. Relevant to the focus of this meeting, azole resistance has emerged in Aspergillus in recent years, most notably in Europe. The current drug pipeline consists of 3 CYP inhibitors, 2 glucan synthesis inhibitors, 1 chitin synthesis inhibitor, and ~5 agents with a variety of MOAs both known and unknown. The most advanced agent is a CYP inhibitor which recently reported Phase 3 results for invasive aspergillosis.

Development is changing. In 2012, antifungal agents were specifically recognized in the United States as eligible for Qualified Infectious Disease Pathogen (QIDP) status and the development incentives of the GAIN Act. In addition, changes recently made to support development of new antibacterial agents appear to have direct application to development of newer antifungal agents. In particular, PK-PD-based dose selection now appears possible for the mycoses and likely to become important in a fashion similar to its role in antibacterial development. Building from a strong PK-PD base providing clear insight into the key PK-PD driver, it should increasingly be possible to register based on achieving adequate PK in patients and single Phase 3 trials. Pharmacometric analyses of may further strengthen PK-PD-based insight. Finally, new diagnostic tools will enable clear patient selection and hence increase trial efficiency. As a first example of this, detection of galactamannan appears to be accepted for patient qualification for studies of invasive aspergillosis.

Changing economics. In addition, the economic value of novel antimicrobial agents is increasingly recognized. For antibacterial agents, a recent analysis has shown that a narrow spectrum agent can have a cost per quality adjusted life year saved of as little as $3K/year even when the agent is priced at $10K/course of therapy. An initial version of a parallel analysis for Aspergillus suggests a similar cost/life year saved even up to $25K/course of therapy. A refined version of the model will be presented at the meeting.

Summary: There is a meaningful unmet need for new antifungal agents. The current limited development pipeline does not address all gaps. Advances in PK-PD, diagnostics, the regulatory landscape, and drug valuation should facilitate and encourage development of new agents.
DIFFICULT CLINICAL CASES

José Maria Aguado, MD PhD
Hospital Universitario 12 de Octubre - Spain

Dimitrios P. Kontoyiannis, MD
University of Texas MD Anderson Cancer Center - USA

THURSDAY 27 FEBRUARY 2014 (13.45 - 14.45)
Recently, Sugui et al described *Aspergillus tanneri*, a novel, highly virulent, aggressively invasive, and drug resistant IA pathogen. In a mouse model of invasive aspergillosis (IA) and in a non-vertebrate insect model, distinct virulence profiles for *A. tanneri* relative to *Aspergillus fumigatus* and *Aspergillus flavus* were observed. Comparative genomics showed that *A. tanneri* has a larger genome than other aspergilli, encoding nearly 1900 more genes than *A. fumigatus*. *A. tanneri* genes have numerous orthologs in the other two genomes, however, an abundance of genes are unique to *A. tanneri*. Among the unique genes were multiple gene clusters that are responsible for the biosynthesis of secondary metabolites, suggesting that *A. tanneri* produces novel secondary metabolites that may play a role in its pathogenic profile. Analysis of genes commonly associated with drug resistance showed that *A. tanneri* carried mutations in CTP51A which result in or contribute to azole resistance.

An important issue featured in the *A. tanneri* fatal cases and in clinical management of IA is the general limitation in treatment options. Drugs used to treat fungal infections target only two factors that differ between human and fungal cells: the presence of ergosterol in fungal cell membranes and of glucans in their cell walls. There remains an urgent need to understand the broad range of genes present in the genomes of fungal pathogens that may participate in the resistance to the clinically therapeutic antifungals. To identify novel mechanisms that mediate azole resistance in *A. fumigatus*, we used whole genome sequencing of clinical isolates and of *in vitro* selected azole-resistant strains. To further refine the most significant mechanisms required for resistance, we collaborated with June Kwon Chung and employed a classical genetic system that enables the analysis of complex traits in *A. fumigatus*. In parallel, Paul Bowyer employed a Tn-seq approach to address the same issue. These approaches identified mutations in the target protein, CYP51A, and in an additional co-target HMG CoA reductase as well as other genes. The results from this study identify novel drug targets in *A. fumigatus* and also show that next-generation sequencing coupled with Tn-seq and classical genetics experiments is a powerful approach to identify genes involved in complex traits.
Invasive aspergillosis due to pan-azole-resistant *Aspergillus fumigatus* isolates was first described in 2007 in a series of Dutch patients. A new resistance mechanism was discovered in these isolates which consists of a tandem repeat of 34 bases (TR34) in the promoter of the CYP51A gene combined with an amino acid substitution (L98H). Since then, TR34/L98H *A. fumigatus* isolates were detected both in the environment and patients of many European countries but also outside Europe for instance in India, China and Iran. Molecular epidemiology of TR34/L98H strains indicates that this resistance mechanism developed locally from a common ancestor that arose after the introduction of agricultural triazole fungicides with a comparable molecule structure to medical triazoles. Most likely isolates harboring this trait subsequently migrated across the borders of European countries. There is accumulating evidence that the geographical spread of TR34/L98H strains is associated with the widespread use of agricultural fungicides. Alarming is the fact that while TR34/L98H is spreading, a new environmental *CYP51A*-mediated resistance mechanism (TR46/Y121F/T289A) rapidly migrates across Dutch hospitals and is associated with voriconazole therapy failure. Strains with this new resistance mechanism were also detected in the neighboring country Belgium both in patients and in the environment. The European Center for Disease Prevention and Control (ECDC) published a report integrating all evidence for a causal role of fungicides in resistance in *A. fumigatus* in February 2013. This emphasizes the fact that azole resistance in *Aspergillus* is taken seriously and considered as a public health concern. Indeed, triazoles are the mainstay of the treatment of *Aspergillus* infections. Case series consistently show high failure rates of patients with azole-resistant *Aspergillus* diseases to azole therapy. As the majority of azole-resistant *A. fumigatus* isolates are multi-azole-resistant, only polyenes and echinocandins remain as treatment alternatives. Unfortunately both drug classes are only available in intravenous form and clinical evidence that these agents are efficacious in azole-resistant disease is lacking. Azole resistance is particularly problematic in patients with azole-resistant central nervous system aspergillosis as an alternative drug with comparable efficacy to voriconazole is not available. In patients with chronic *Aspergillus* diseases that require long-term antifungal therapy, oral treatment is the only feasible treatment option; unfortunately this is not possible in case of azole resistance. The fact that in about half of the patients with invasive aspergillosis no isolate is available for susceptibility testing complicates the situation even more.

In summary, *A. fumigatus* disease caused by environmental resistant strains is a growing public health concern with global dimensions. This concern motives the withdrawal of agricultural triazole fungicides that bind to the same active site of medical triazoles.
AGRICULTURAL TRIAZOLE FUNGICIDES SHOULD BE WITHDRAWN – CON

Bart Fraaije, PhD

_Rothamsted Research - United Kingdom_

**THURSDAY 27 FEBRUARY 2014 (16.00 - 16.20)**

Azole resistance in the opportunistic human pathogenic fungus _Aspergillus fumigatus_ can develop in patients and lower the likelihood of successful treatment. Azole resistance evolves during therapy as shown by the recovery of isogenic strains without resistance conferring mutations before the start or during azole therapy. However, azole resistance is becoming an emerging public health problem as increasing numbers of azole insensitive strains belonging to novel CYP51A variants (e.g. TR34/L98H and TR46/Y121F/T289A) have recently been found in treatment-naive patients (before receiving azole therapy). Furthermore, genetically related strains carrying the same CYP51A variants have also been found in the environment. Further spread of these strains has been linked with agricultural use of triazoles and measures to ban certain triazole fungicides have been suggested. However, the origin of resistance is not clear. Resistant strains seem to spread from the Netherlands to surrounding countries, and no data is available to show that agricultural use of fungicides has triggered resistance development and/or is further selecting for resistance in the environment. Banning azole applications would have a worldwide impact on human and animal health, the environment, material preservation and food security. Recent risk assessment studies show that the risk for resistance selection is highest in human medicine, veterinary applications, material preservation and a few agricultural applications. More research is needed to identify and investigate high risk areas for azole resistance development in the environment. Confirmation of potential risk areas will provide a basis for precautionary measures, such as limiting the use of azoles in particular fungicide applications, as well as sanitary measures aimed at reducing the selection and spread of azole resistant isolates in the environment.
COMBINATION THERAPY UPDATE

Johan Maertens, MD PhD

University Hospitals Leuven - Belgium

THURSDAY 27 FEBRUARY 2014 (17.05 - 17.30)
POSACONAZOLE BREAKTHROUGHS

Jutta Auberger, MD
Paracelsus Medical University Salzburg - Austria

THURSDAY 27 FEBRUARY 2014 (17.30 - 17.55)

Posaconazole (PCZ) is a relatively new oral triazole agent with an enhanced spectrum of \textit{in vitro} activity against a wide range of medically important fungi, including \textit{Candida} spp., \textit{Aspergillus} spp., Mucormycetes, and other filamentous fungi [1]. In two multicenter, double-blinded trials involving either allogeneic hematopoietic stem cell transplantation (HSCT) patients with graft-versus-host disease (GVHD) or nontransplantation patients with hematologic malignancies and prolonged neutropenia, posaconazole was superior to fluconazole in preventing invasive aspergillosis and death from invasive fungal infection [2,3].

However, the efficacy of PCZ might be limited by poor absorption [4]. A broad line-up of co-medications, an inadequate dietary intake and abnormal gastric pH levels are common in critically ill hematological patients i.e. suffering from mucositis following chemotherapy or from GVHD following HSCT which may result in unpredictable bioavailability and subtherapeutic plasma concentrations [5,6]. Thus, therapeutic drug monitoring (TDM) is mandatory in patients receiving PCZ. Concerns also rise over the emergence of resistant strains and a shift in epidemiology towards rare fungal pathogens. The incidence of breakthrough infections (bIFIs) during PCZ prophylaxis in our retrospective single center observational study was 12% exclusively caused by non-\textit{Aspergillus} molds and yeasts. Furthermore, fungal colonization during PCZ prophylaxis shifted towards rare fungal pathogens. Remarkably and important, susceptibility for liposomal Amphotericin B was maintained in all pathogens causing bIFIs. Summarized, antifungal prophylaxis has to be chosen wisely in consideration of individual risk profiles of patients.

References

SECOND LINE THERAPY FOR ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA) AND SEVERE ASTHMA WITH FUNGAL SENSITIZATION (SAFS) - HOW FAR HAVE WE GONE?

Livingstone Chishimba, MB ChB MRCP

University of Manchester - United Kingdom

THURSDAY 27 FEBRUARY 2014 (18.25 - 18.50)

The precise treatment for Allergic Bronchopulmonary Aspergillosis (ABPA) and severe asthma with fungal sensitization (SAFS) is uncertain. Traditionally, treatment for allergic fungal disease has involved the use of systemic oral corticosteroids (OCS) to suppress the inflammation and immunological activity [1]. In the last decade, there has been a move towards the use of azole antifungal agents. Randomized controlled trials (RCTs) show that treatment with oral Itraconazole offers therapeutic benefit to approximately 60% of ABPA patients [2, 3]. Similarly, a marked beneficial effect on quality of life (QOL) and other end points have been demonstrated among patients with SAFS treated with itraconazole [4]. Recent epidemiological data suggest that treatment with Voriconazole or Posaconazole improves asthma severity control and quality of life (QOL) in both SAFS and ABPA and leads to resolution of pulmonary consolidation and cavitation in ABPA patients [5]. More recently, prospective audit suggests that the use of Nebulised Amphotericin B offers benefit to about 14.3% patients. Despite good efficacy, the use of these agents is associated with a high frequency of adverse events and drug interruptions, hence the need for development of safer and more potent agents.

References
ASPERGILLUS IMMUNOLOGY UPDATE

Luigina Romani, MD PhD

University of Perugia - Italy

FRIDAY 28 FEBRUARY 2014 (08.00 - 09.00)

Aspergillosis and other Aspergillus-mediated diseases may occur in some patients when infected with Aspergillus fumigatus. A central tenet of host-fungus interaction is that, at the individual level, clinical outcome depends on the net result of two forces: resistance to damage caused by the pathogen, and resistance to damage caused by the immune response invoked to eliminate the pathogen. Differences in host gender, age, genetics, and nutritional status can explain some, but not all, differences in susceptibility to and pathophysiological consequences of these fungal infections and diseases. The control of inflammation leading to tolerance, the molecular bases of immune regulation and dysregulation, and the way in which Aspergillus spp can switch from a ‘friendly’ relationship to a pathological relationship, by evading or subverting host inflammation, have become challenging issues in fungal pathogenesis and therapy. Indeed, as tolerance mechanisms are not expected to have the same selective pressure on fungi, new drugs that target tolerance will provide therapies to which fungi will not develop resistance. Thus, the greatest challenge is to pave the way for newer classes of drugs and immune-modulating therapies targeting anti-inflammatory pathways in fungal diseases. I will discuss recent developments in this field highlighting the nature and relative contribution of additional, host–dependent and –independent, factors that contribute to differential inflammatory states associated with different clinical outcome at the individual level.

Supported by the Specific Targeted Research Project FUNMETA (ERC-2011-AdG-293714).
**ASPERGILLUS: SEX AND RECOMBINATION**

Janos Varga, PhD  
*University of Szeged - Hungary*

**FRIDAY 28 FEBRUARY 2014 (08.00 - 09.00)**

The genus *Aspergillus* is one of the most widespread groups of fungi on Earth, comprising about 300-350 species assigned to various subgenera and sections. The lifestyle of different species of the genus is very diverse. Most species are known to produce asexual propagula (conidia) on conidial heads. By contrast, a sexual cycle is unknown in the majority of species. Where sexual reproduction is present, species exhibit either homothallic (self fertile) or heterothallic (obligate outcrossing) breeding systems. A parasexual cycle has also been described in some species. The sexual stages of the *Aspergillus* have traditionally been assigned to different genera such as *Eurotium*, *Neosartorya* or *Emericella*. However, according to the new rules of the Melbourne Code adopted by the 18th International Botanical Congress in 2011, only one name can be used for one fungus, and the International Commission on *Penicillium* and *Aspergillus* (ICPA) decided to use the name *Aspergillus* in 2012. As in other fungi, sexual reproduction is governed by ‘mating-type’ (MAT) genes which determine sexual identity and are involved with regulation of later stages of sexual development.

Previous studies using population genetic analyses indicated that some supposedly “asexual” *Aspergillus* have a recombining population structure indicating that they might have a cryptic sexual cycle. Genome analysis has also revealed the presence of mating-type and other genes needed for sexual reproduction in several “asexual” *Aspergillus* species, again consistent with latent sexuality. As a result, experimental work has been undertaken, drawing on knowledge of MAT gene presence, and major breakthroughs have been made with the discovery that sexual reproduction can be induced in several species previously thought to be unable to undergo sexual reproduction. Ongoing work assessing sex and recombination in *Aspergillus* species will be described, and the medical implications of this research considered.

In section *Fumigati*, sexual stages have been discovered in the opportunistic human pathogens *A. fumigatus* and *A. lentulus*. This has implications for the ability of these species to evolve in response to selection from antifungal drugs. Research is being performed to develop super-mater strains, to assess the extent of genetic fertility within populations, and to assess whether gene flow is possible between species. *Aspergillus* section *Nigri* also includes species capable of causing opportunistic infections of humans. Genome sequencing of *A. niger* revealed the presence of a MAT1-1 mating-type gene. We therefore screened a population of more than 200 black *Aspergillus* isolates from different origins for the presence of MAT genes. In the case of putative *A. niger* and *A. tubingensis*, near 1:1 ratios of MAT1-1: MAT1-2 isolates were detected, but in *A. welwitschiae* collected from *Welwitschia* seeds, onions, indoor air or otitis cases we detected a 6:1 ratio of MAT1-1: MAT1-2 isolates, indicating a clonal population structure. Indeed, VCG tests indicated that the *A. welwitschiae* strains isolated from *Welwitschia* seeds all belong to the same VCG forming a clonal lineage. Consistent with these findings, a sexual cycle has recently been induced in *A. tubingensis*. In section *Flavi*, which also includes species capable of causing opportunistic infections, only two homothallic sexual species have traditionally been known (*A. alliaceus*, *A. albertainensis*). However, it has recently been discovered that other species, including *A. flavus*, *A. parasiticus* and *A. nomius*, are also able to reproduce by sexual means involving heterothallic breeding systems. These findings may have consequences both for clinical practice and in the use of nontoxicigenic isolates to lower aflatoxin levels in different crops. Regarding the population structure of *A. flavus*, comparison of the distribution of MAT genes in environmental, indoor and clinical isolates revealed that clinical isolates have a skewed distribution of MAT genes with MAT1-1 dominating. The reasons for this observation are still unclear. Recently, ascospore production has also been induced in another medically important fungus, *A. terreus*. The clinical relevance of these findings will be discussed.

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FUNGAL ROLE IN THE PULMONARY MICROBIOME: PSEUDOMONAS-ASPERGILLUS INTERACTION

Richard B. Moss, MD

Stanford University - USA

FRIDAY 28 FEBRUARY 2014 (09.10 - 09.35)

Aspergillus fumigatus [Af] and Pseudomonas aeruginosa [Pa] play important roles in respiratory disease, and particularly in the setting of cystic fibrosis [CF] appear to interact in clinically significant fashion. Whereas the role of Pa (the major CF bacterial pathogen) in accelerating disease progression has been intensively studied and is relatively well understood [1], the role of Af, the major filamentous fungus colonizing people with CF, is less well established, with most studies suggesting a pathogenic impact [2-7], independent of its causal role in allergic bronchopulmonary aspergillosis. The interaction of Pa and Af in CF is a new field of research and interest [8]. This interaction in turn occurs in the context of a complex pulmonary microbiome which is altered in a characteristic way in CF and contains a rich diversity of bacterial and fungal taxa that is only now being elucidated [9-10]. Both Pa and Af are suspected as important agents in promoting mucus plug formation in the airways, and both are known to form biofilms in vitro and in vivo, sometimes as part of a polymicrobial biofilm. The attribution of a role in mucus plugging and biofilms stems from the extracellular production of glycan polymers by Af and alginate by Pa and utilization of extracellular DNA by Af in biofilm formation [11]. Af secondary metabolites such as gliotoxin and verriculogen are known epithelial respiratory cell toxins and have immunosuppressive actions on the host response. Pa-derived pyocyanin, phenazines, and homoserine lactones can inhibit the development of Af biofilm in vitro arising from conidia [12]. There are also differences in the ability of Pa isolates (and their spent culture media) to inhibit established Af biofilms in vitro: Pa isolates from CF patients are more inhibitory than isolates from non-CF sources, and nonmucoid Pa isolates from CF patients are more inhibitory than mucoid isolates [12-14]. These in vitro findings call attention to the possible interactions between these pathogens in CF airways in vivo. They stress the importance of studying chronic clinical infections: in contrast to in vitro results, a recent clinical study found anti-Pa antibiotic therapy in CF reduced the frequency of both Pa and Af in CF sputum, suggesting Pa facilitates the persistence of Af in CF airways [15]. This supports prior epidemiologic evidence that early Pa infection predisposes to Af infection and vice versa [16]. Of potential relevance, Af is able to utilize Pa phenazines to its adaptive advantage [17]. While CF patients chronically infected with either Pa or Af have significantly accelerated loss of lung function compared to non-infected CF patients, the greatest rate of decline occurs in co-infected patients [3]. Overall, current data suggest an important pathogenic interaction between Af and Pa in CF requiring further study for better understanding of mechanisms and therapeutic implications.

References
TARGETING ASPERGILLUS FUMIGATUS VIRULENCE FACTORS IN A MOUSE MODEL OF CORNEAL INFECTION

Eric Pearlman, PhD

Case Western Reserve University - USA

FRIDAY 28 FEBRUARY 2014 (09.35 - 10.00)

Fungal pathogens, in addition to causing life-threatening systemic disease, can also invade the cornea and cause blindness and severe visual impairment. We use mouse models of *Aspergillus* corneal infections in addition to *in vitro* fungal killing assays with mouse and human neutrophils to identify genes that regulate fungal survival *in vivo*. These include: 1. **Antioxidant activity**: our studies implicated the Yap1 reactive oxygen species sensing transcription factor, superoxide dismutases, and the Yap1-regulated thioredoxin antioxidant pathway as having essential roles in hyphae survival in infected corneas and against oxidation by neutrophils (1); 2. **Iron scavenging**: we identified an essential role for *A. fumigatus* SidA, SidF and HapX genes in survival in corneal infections and when incubated with human neutrophils (2), indicating an important role for extracellular siderophores. 3) **Zinc scavenging**: the *A. fumigatus* ZafA zinc-sensing transcription factor and the ZrfC zinc importer, are required for survival in corneal infections, and also in low zinc conditions in the presence of neutrophils, due to neutrophil S100A8/A9 (calprotectin) competition for available zinc; 4) **Blocking host cell recognition**: we demonstrated that hydrophobin ΔrodA conidia are more rapidly killed in infected corneas than the parent G10 strain (3), thereby identifying the hydrophobin RodA as an important virulence factor in fungal keratitis, primarily by masking recognition by c-type lectins. We also show that therapeutic targeting of specific pathways facilitates fungal killing and disease resolution in *A. fumigatus* corneal infections.
IMMUNOEVASIVE *ASPERGILLUS* VIRULENCE FACTORS

Sanjay H. Chotirmall, MD PhD

*Royal College of Surgeons in Ireland - Ireland*

**FRIDAY 28 FEBRUARY 2014 (10.00 - 10.25)**

*Aspergillus* moulds are ubiquitous and as spores are inhaled in large numbers daily. Removed by intact anatomical barriers and an effective immune response, disease occurrence is dictated by the state of the host immune system and virulence of the infecting fungal strain. Interactions between *Aspergillus* species and the host immune system are bi-directional. The fungus elicits an immune response that may result in its clearance while concurrently it produces virulence factors that evade immune recognition. The major components of the immune system involved in recognition and removal of the fungus include phagocytosis, antimicrobial peptide production, and recognition by pattern recognition receptors. Cytokine responses remain critical in facilitating cell-to-cell communication, links to adaptive immunity and promoting the initiation, maintenance, and resolution of the host response to invasion (1). *Aspergillus* virulence however is multifactorial and under polygenetic control. Strategies are multi-faceted and include fungal structure, capacity for growth, adaptation to stressful conditions, the ability to damage host cells and evade immune-recognition. This talk addresses virulence factors associated with *Aspergillus* that evade immune recognition and facilitate *Aspergillus*-associated disease including its rodlet layer, 1,8-dihydroxynaphthalene (DHN)-conidial melanin and detoxifying systems against reactive oxygen species (ROS) such as catalases and superoxide dismutases (SOD). Gliotoxin, a potent immune-evasive mycotoxin will also be discussed. Its immunosuppressive roles include the inhibition of macrophage and monocyte phagocytosis, mitogen-activated T cell proliferation, mast cell activation and cytotoxic T-cell responses. Additionally, it inhibits superoxide production and impairs neutrophil phagocytic capacity, reduces ciliary movement of epithelial cells leading to their damage and dysfunction. In the context of *Aspergillus* colonization in cystic fibrosis (CF), our group has shown that the Vitamin D receptor (VDR), a key component of an important immune-modulating pathway is down-regulated by gliotoxin. Treatment with itraconazole decreases bronchoalveolar lavage (BAL) gliotoxin concentrations, restoring VDR expression, diminishing systemic Th2 cytokines IL-5 and IL-13 with concomitant improvement in clinical and radiological patient parameters (2). Understanding the ability of *Aspergillus* to evade protective immune strategies mechanistically is critical to the development of future therapeutic approaches for *Aspergillus*-associated disease states such as that in CF.

References


CULTURE AND SENSITIZATION IN ASTHMA, CF AND COPD – WHAT DOES IT TELL US?

Catherine Pashley, PhD

University of Leicester - United Kingdom

FRIDAY 28 FEBRUARY 2014 (11.35 - 12.00)

Collectively asthma, COPD (chronic obstructive pulmonary disorder) and cystic fibrosis (CF) are very common, important causes of disease and ill health. Filamentous fungal colonisation of the airways can occur in all three disease groups, although the clinical relevance is unclear. Allergic bronchopulmonary aspergillosis (ABPA) is a well-recognised severe complication of airway colonisation associated primarily but not exclusively with *Aspergillus fumigatus*.

Fungal colonisation may have a deleterious effect without fulfilling all the diagnostic criteria of ABPA; however, a lack of standardisation in processing respiratory samples hampers comparisons. Whilst mycology laboratory accreditation programs are common, most countries including the USA, Canada and Australia, have no national standard guidelines for processing respiratory samples. In the UK most clinical microbiology laboratories follow the national standard method set out in BSOP57 [1]. However, recent studies suggest this method is insensitive for detecting fungi and should be reviewed. Studies in both COPD [2] and aspergillosis patients [3] have directly compared the BSOP57 protocol to in-house protocols using higher concentrations of sputa. Both found significantly higher recovery of yeast and fungi with in-house protocols. In CF a number of alternative methods are routinely employed and a multi-centre study looking at prevalence of fungi using different culture protocols highlighted the need for adopting a standardised approach [4].

In CF incidence of fungal recovery can be around 40% and one of the strongest risk factors associated with isolation of fungi is decreased lung function, even after exclusion of patients diagnosed with ABPA [5]. A study of people with moderate to severe asthma isolated filamentous fungi from the sputum of 54% of patients and they had significantly lower lung function than those who were culture negative [6]. In a study in COPD culture rates were 49%; however, there was no association with lung function [7]. It is unclear whether fungal isolation contributes to lower lung function or is a marker of more severe lung disease and aggressive therapy.

Fungal sensitisation may contribute to the persistence of active symptoms; however, the exact prevalence is unclear. Sensitisation to *A. fumigatus* has been associated with reduced lung function in asthma [8] and COPD [7]. Skin prick tests (SPT) and specific serum IgE tests are used to determine sensitisation. The SPT is a simple diagnostic tool useful for screening, but is not without limitations. The majority of SPT positive individuals are also positive by specific IgE; however a significant proportion of individuals with positive IgE tests are SPT negative [9]. *In vitro* measurement of specific IgE antibodies are more costly than SPTs. It has suggested that both SPTs and specific IgE measurement by the ImmunoCAP system should be used in diagnoses of allergy, due to discordance in test results in around a quarter of severe asthma patients [10]. There is currently no widely adopted consensus as to which fungi to test for, although recommendations exist.

References

MALDI-TOF – ANY USE FOR *ASPERGILLI*?

Maurizio Sanguinetti, MD

*Catholic University of Sacred Heart - Italy*

**FRIDAY 28 FEBRUARY 2014 (12.00 - 12.25)**
RECOMBINANT ABS IN IA DIAGNOSTICS

Michael D. Gunn, MD

Duke University Medical Center - USA

FRIDAY 28 FEBRUARY 2014 (12.55 - 13.20)

Despite the rising incidence and high mortality of Invasive Aspergillosis (IA), timely diagnosis of this disease remains suboptimal. The widely used Platelia assay, a sandwich ELISA based on a rat anti-galactomannan antibody (Ab), has been reported to generate a significant number of false positive results. Reported cross-reacting substances include certain foods, *Penicillium* spp., and piperacillin-tazobactam. Similarly, assays for another fungal cell wall component, (1,3)-β-glucan, also display poor specificity. Thus, developing an improved assay for the diagnosis of IA would significantly improve outcomes for patients with this disease.

Our goal has been to develop an IA diagnostic assay based on the detection of *Aspergillus* antigens in the blood of patients. To this end, we have generated a highly specific anti-*Aspergillus* Ab using single-chain Ab phage display technology. The term single-chain Ab comes from the fact that they are constructed by cloning the \( V_H \) and \( V_L \) regions of Abs, which normally reside on different polypeptides, into a construct that places them on a single polypeptide chain. In practice, millions \( V_H \) and \( V_L \) regions are subcloned simultaneously and expressed as single-chain Ab fusion proteins on the tips of bacteriophage, providing vast libraries of Abs that can be screened as phage based on their binding properties. One advantage this technology provides, as compared with the more commonly used monoclonal Abs, is the ability to select against binding to specific antigens.

To identify phage clones that bind to *Aspergillus* antigens, we performed 7 selections/screens using three different human antibody phage display libraries. We selected against *Penicillium*, *Fusarium*, and *Rhizopus* preparations to eliminate binding to antigens known to interfere with the Platelia assay. Among 67 clones that bind *Aspergillus*, we identified one; E2G9 that reacted strongly with *A. fumigatus* excreted antigens, had moderate reactivity with an extract of mixed *Aspergillus*, but displayed no cross reactivity with *Penicillium*, *Fusarium*, *Rhizopus*. When converted to a full length IgG, E2G9 continued to bind *Aspergillus* extracts and showed no reactivity toward *Candida albicans*, *Scedosporium prolificans*, *Paecilomyces variotti* or piperacillin-tazobactam (Zosyn). E2G9 was subjected to *in vitro* affinity maturation, resulting in a 10-fold increased in the limit of detection for this Ab. When used as both capture and detection Abs in a sandwich ELISA assay, E2G9 detects *Aspergillus* antigen at a dilution of 1:1,200,000 from conditioned medium and displays a sensitivity of 100% in detecting *Aspergillus* infection when tested against serum from infected and uninfected guinea pigs. We conclude that E2G9, a high affinity recombinant Ab reactive to an *Aspergillus* antigen present in blood during infection, displays markedly greater specificity than existing IA diagnostic Abs and may form the basis of an improved assay for the diagnosis of IA.
PROTEIN DEGRADATION AND VIRULENCE IN *ASPERGILLUS FUMIGATUS*

Gerhard H. Braus, PhD

*Georg August University - Germany*

**SATURDAY 1 MARCH 2014 (08.00 - 09.00)**

Rapid adaptation to a versatile host represents a challenge for the opportunistic human pathogen *Aspergillus fumigatus* for successful infection. F-box proteins are the adaptor subunits of E3 SCF (Skp1-cullin-1 F-box protein) ubiquitin ligases. They recognize target proteins, which are marked by the SCF complex for degradation in the 26 S proteasome. We have identified Fbx15 as an F-box protein, which links *A. fumigatus* virulence to protein degradation. *A. fumigatus* deletion strains which have lost fbx15 are unable to infect immunocompromised mice in a murine model of invasive aspergillosis. Fbx15 is required for growth during stress including increased temperature, oxidative stress and amino acid starvation. Fbx15 is also required for controlling the synthesis of the antiphagocytic gliotoxin. Fbx15 interacts in the nucleus with the linker protein Skp1/SkpA suggesting that SCF<sup>Fbx15</sup> primarily targets nuclear proteins. Fbx15 represents a molecular link between protein degradation, stress response and virulence.
NIAID RESOURCES AND THE GLOBAL RESEARCH COMMUNITY

Dennis M. Dixon, PhD

National Institute of Allergy and Infectious Diseases - USA

SATURDAY 1 MARCH 2014 (09.00 - 09.10)

The NIH/NIAID supports basic, translational, and clinical research in medical mycology. The goal of this presentation is to make the community aware of some of the newer opportunities available to the international research community, with an emphasis on aspergillosis. Traditional funding opportunities will be referenced, and newer preclinical and clinical resources to facilitate drug, vaccine, and medical diagnostics will be delineated. The majority of the newer product development resources for this research are based in the Division of Microbiology and Infectious Disease (DMID) in the NIAID. A key web page linking to the available resources can be found at:

http://www.niaid.nih.gov/LABSANDRESOURCES/RESOURCES/DMID/Pages/default.aspx

or by searching the Internet using the following key words: DMID Resources. Gap-filling preclinical resources include in vitro and in vivo microbial screens, animal models for product testing, toxicology tests, help with pilot manufacturing of drug and vaccine candidates, and other key steps in the product development pathway. Capability also exists for early phase clinical testing. Contact information for details about how to access the funding opportunities are listed at the above links by type of service.
RAS PATHWAY AND POLARIZED GROWTH

Jarrod R. Fortwendel, PhD

University of South Alabama - USA

SATURDAY 1 MARCH 2014 (09.10 - 09.35)

Invasive pulmonary aspergillosis (IPA) is propagated by inhalation of *A. fumigatus* conidia that germinate and invade the lung tissue in search of nutrients. We have shown that the *A. fumigatus* RasA GTPase protein is necessary for germination, hyphal morphogenesis, cell wall integrity, and pathogenesis of IPA. Our recent studies, focusing on highly conserved protein domains, have revealed that spatiotemporal regulation of RasA is crucial for regulation of *A. fumigatus* growth and virulence. Although these data highlight the functional conservation of Ras protein domains between humans and fungi, the importance of fungal-specific Ras protein domains to Ras function in fungal morphogenetic signaling and virulence remains largely unexplored. To address this knowledge gap, we identified fungal-specific Ras protein domains by comparing RasA-homolog sequences to their human counterpart, H-ras. Our alignments revealed a novel area of significant divergence with H-ras, which we have termed the Invariant Arginine Domain (IRD). This novel protein domain is: i) located at the extreme N-terminus of the Ras protein; ii) conserved in the RasA homologs of every available fungal genome, but not present in H-ras; and iii) characterized by an invariant arginine residue located immediately upstream of the core Ras G-domain. Alanine scanning mutations of the IRD, especially of the invariant arginine (RasAR7A), caused stunted growth, altered hyphal morphology and reduced conidiation. Although growth analyses of the IRD mutants suggested that RasA pathway function was aberrant, the RasAR7A mutation did not affect activity or localization of the RasA protein. However, co-immunoprecipitation analyses revealed altered interaction profiles of the RasAR7A mutant with the morphogenetic signaling proteins, ModA and RacA. Taken together, our data support the hypothesis that the fungal-specific IRD is important for RasA effector interactions in a manner that is not dependent on regulation of Ras protein activity or localization. Further characterization of this fungal-specific domain, and of the protein interactions to which it contributes, may reveal a novel signaling paradigm for fungal Ras pathways.
CELL WALL AND GALACTOSAMINOGALACTAN AND IMMUNOSUPPRESSION

Frank van de Veerdonk, MD PhD

The Radboud University Nijmegen Medical Centre - The Netherlands

SATURDAY 1 MARCH 2014 (09.35 - 10.00)

Galactosaminogalactan (GAG) has potent anti-inflammatory effects. Recent work has shown that GAG can inhibit proinflammatory T-helper (Th)1 and Th17 cytokine production in human cells, which is dependent on the capability of GAG to induce IL 1 receptor antagonist (IL-1Ra), a potent anti-inflammatory cytokine that blocks IL 1 signalling. Moreover, in a mouse model of invasive aspergillosis GAG induces IL-1Ra in vivo, and the increased susceptibility to invasive aspergillosis in the presence of GAG in wild type mice is not observed in mice deficient for IL-1Ra. A striking observation is that IL-1Ra knockout mice are completely protected against invasive pulmonary aspergillosis, which opens new treatment strategies that target IL-1Ra in the setting of fatal invasive fungal infections. Due to the potent anti-inflammatory effects of GAG, GAG has also been explored as a therapeutic agent in (auto)inflammatory diseases. GAG can protect mice from DSS-induced colitis, which makes GAG or a derivative structure of GAG a potential treatment compound for IL 1 mediated diseases.
IMAGING AND ANALYSING INTRACELLULAR CALCIUM DYNAMICS IN 
*ASPERGILLUS FUMIGATUS* IN RESPONSE TO ENVIRONMENTAL STRESS

Nick D. Read, PhD

*University of Manchester - United Kingdom*

**SATURDAY 1 MARCH 2014 (10.30 - 10.55)**

Calcium signalling and homeostasis are essential for the growth, differentiation and virulence of filamentous fungi. During infection, *Aspergillus fumigatus* must balance concomitant demands to: (1) withstand toxic levels of exogenous calcium (3-5 mM) in the host environment which can be >100,000x that of the fungal cytosolic free calcium ([Ca$^{2+}$]) concentration; (2) appropriately integrate homeostatic and stress-responsive adaptations; and (3) undergo normal calcium signalling. There is evidence for calcium signalling regulating numerous processes including spore germination and hyphal tip growth. The low resting level of [Ca$^{2+}$]$_c$ (50-100 nM) is maintained by Ca$^{2+}$-pumps and -antiporters, and cytoplasmic Ca$^{2+}$-buffering. However, [Ca$^{2+}$]$_c$ becomes an intracellular signal when its concentration is transiently increased. We have developed two methods for routinely measuring and imaging [Ca$^{2+}$]$_c$ in *A. fumigatus*: (1) 96-well plate luminometry using the genetically encoded, bioluminescent aequorin; and (2) fluorescence microscopy using the genetically encoded calcium-sensitive, fluorescent protein G-CaMP5G. Aequorin is ideally suited for quantitative measurements of calcium signatures in cell populations whereas fluorescence imaging of the G-CaMP5G is good for single cell and subcellular measurements of [Ca$^{2+}$]$_c$ dynamics. Using the aequorin methodology we have found that transient increases in [Ca$^{2+}$]$_c$ with specific, reproducible calcium signatures in *A. fumigatus* arise from exposure to stresses such as high external calcium, alkaline or oxidative stress. Imaging calcium dynamics with GCaMP5G is providing extraordinary insights into the temporal and spatial dynamics of [Ca$^{2+}$]$_c$. Pulses in [Ca$^{2+}$]$_c$ occur within actively growing hyphal tips. Exposure of conidial germlings to high external calcium induces dramatic and very dynamic changes in [Ca$^{2+}$]$_c$, with the generation of localized [Ca$^{2+}$]$_c$ transients and waves. Furthermore, there is considerable heterogeneity in the [Ca$^{2+}$]$_c$ responses of different germlings within the cell population. Calcium imaging and measurement using genetically encoded probes, particularly when combined with pharmacological and genetic analyses, is initiating a revolution in our understanding of calcium signalling in filamentous fungi.
CHRONIC PULMONARY ASPERGILLOSIS (CPA) SECONDARY TO TUBERCULOSIS AND HIV

Iain Page, MB ChB

University of Manchester - United Kingdom

SATURDAY 1 MARCH 2014 (11.35 - 12.00)

Between 87 and 100% of African patients with CPA have treated tuberculosis (TB) as an underlying lung disease. Prior TB was also the main risk factor for CPA in 75% of cases in India, 71% of cases in China and 93% of cases in Korea. As TB is common in Africa CPA may be much more common in Africa than in Europe or America.

The risk of developing CPA after TB was described in a survey of 544 patients who were treated for TB in the UK in the 1968-70. 34% of all patients had developed antibodies to *Aspergillus* and aspergilloma and 63% of patients with antibodies developed an aspergilloma within three years. 42% of those with aspergilloma had haemoptysis (1). TB and CPA are reported in several countries. In Japan 20% of treated TB patients had antibodies to *Aspergillus* (2). In India *Aspergillus* antibodies are present in 27% of patients with TB at one centre (3) and 23% of patients with “chronic lung diseases”, of whom 96% had prior TB, at another centre (4). In Brazil, 21% of in-patients with TB at a tertiary chest clinic with *Aspergillus* antibodies had aspergilloma (5). The total global prevalence of CPA secondary to TB has estimated at between 0.8 and 1.37 million cases with 43 cases per 100,000 population in Congo and Nigeria (6).

The UK survey was performed prior to the HIV pandemic. In Africa, however 31% of new cases of TB are associated with HIV. CPA has been described in association with HIV in case studies in the USA, with a higher rate of progression than in HIV negative controls. Autopsy studies in India and Italy show pulmonary aspergillosis in 2-3% of all AIDS related deaths, with only around 10% of these infections diagnosed during life. A recent study in Uganda showed that 7% of patients with AIDS and sub-acute respiratory disease grew *Aspergillus* on cultures of broncho-alveolar lavage (BAL) fluid.

Pulmonary aspergillosis is probably common in areas of high TB and HIV prevalence, both as sub-acute invasive pulmonary disease secondary to AIDS and as chronic pulmonary aspergillosis complicating TB. Our group is performing a cross-sectional survey of patients with treated TB in Gulu to define the prevalence of CPA in this population. We are also testing serum samples from the Kampala AIDS cohort for *Aspergillus* antigens to provide further evidence of pulmonary aspergillosis in the group.

It should be possible to treat pulmonary aspergillosis in resource poor settings with generic drugs such as amphotericin B and itraconazole. The main barrier to treatment programs is diagnosis. Many commercial *Aspergillus* IgG exist, but there is little comparative data on their efficacy in chronic or sub-acute aspergillosis. Many assays are inappropriate for use in resource poor settings due to the need for expensive and complex equipment. The ELITech haemagglutination *Aspergillus* IgG assay and OLM medical *Aspergillus* antigen lateral flow device may be ideal for use in resource-poor settings, but their efficacy in this setting has not yet been described.

References

THE BURDEN AND DISTINCTIVE CHARACTER OF ABPA IN INDIA

Ritesh Agarwal, MD DM

Postgraduate Institute of Medical Education and Research - India

SATURDAY 1 MARCH 2014 (12.30 - 12.55)

Allergic bronchopulmonary aspergillosis (ABPA) is an immunological pulmonary disorder caused by hypersensitivity reactions against *Aspergillus fumigatus*. It usually complicates the course of patients with bronchial asthma and cystic fibrosis. The clinical manifestations include uncontrolled asthma, recurrent pulmonary infiltrates, and bronchiectasis. The interest in this entity stems from the fact that early diagnosis and treatment can prevent the occurrence of bronchiectasis, a marker of irreversible lung damage. There is a tremendous burden of this disorder in India. In fact, in the last decade, majority of the case series have been reported from India.

In 2011, the asthma prevalence in Indian adults was estimated at about 17-30 million with burden of ABPA calculated to be as low as 0.12 million to as high as 6.09 million (best estimate, 0.86-1.52 million). More than six decades have elapsed since its first description, still the disorder continues to be underrecognized in India with almost one-third of the patients still misdiagnosed as pulmonary tuberculosis. The disease was considered to be uncommon in the Southern States of India, however systematic screening of asthmatic patients has shown that the disorder is widely prevalent across the country. The presentation of ABPA in India is different from other accounts. As compared to the developed world, most patients still present late in the course of the disease with extensive bronchiectasis. Unlike descriptions from other geographic locales, a large number of patients with ABPA from India are detected with high-attenuation mucus. There are other distinct problems in the Indian scenario with many patients not being able to afford anti-fungal azoles due to the high cost. Finally, widespread awareness and advocacy for screening all asthmatic patients (irrespective of severity or control) for ABPA is likely to decrease the morbidity associated with this chronic disorder.
GENOME-WIDE SCAN USING THE IMMUNOCHIP TO IDENTIFY SUSCEPTIBILITY LOCI FOR IA

Juan Sainz Pérez, PhD

University of Granada - Spain

SATURDAY 1 MARCH 2014 (14.20 - 14.45)

Invasive Aspergillosis (IA) is a life-threatening infection that frequently affects acute myelogenous leukemia (AML) and hematopoietic stem cell transplantation (HSCT) patients [1]. Despite the recent improvements in the prophylaxis and treatment for this opportunistic infection, its incidence and morbidity and mortality rates are unacceptably high even among those patients who lack of traditional risk factors (neutropenia, blood or bone marrow transplant, graft versus host disease, corticosteroid therapy, and AIDS) [2, 3]. Although both innate and adaptive immune responses against *Aspergillus* ssp. have been extensively characterized [4], it still remains unclear why some patients develop this invasive fungal infection while others with similar clinical conditions do not. In this regard, a growing number of genetic association studies have suggested that common genetic variation in specific antifungal defense genes (such as Dectin-1, DC-SIGN, TLR5, TLR3, IL10, TNFR2, CXCL10, etc.) may contribute to determine the host susceptibility to IA infection [4-6]. However, so far only a limited number of susceptibility markers have been undoubtedly identified and independently replicated [7]. In order to identify new loci associated with IA infection through a more efficient, unbiased and non-hypothesis driven approach, we are conducting a genome-wide association study including 544 hematological patients at high-risk of IA infection ascertained from 3 European populations through a two-phase study desing. In phase 1, three hundred and fifty hematological patients at high-risk of IA infection were recruited by the three clinical partners of the aspBIOmics consortium (www.aspbiomics.eu) between February 2010 and November 2012. In phase 2, 194 hematological patients from the PCRAGA clinical trial (EU clinical trial number: 2010-019406-17) were subsequently recruited. Of those 2 groups of patients, a total of 95 patients were diagnosed with proven or probable IA according to the revised criteria of the EORTC Study Group [8]. All participants gave their written informed consent to participate in the study, which was approved by the ethical review of participant institutions. Demographic information and clinical data were obtained by detailed review of hospital records. The genotyping is being carried out using the Immunochip® (Illumina), a custom genotyping array that features 195.806 immune-related SNPs and target abnormalities associated with both autoimmune and non-autoimmune traits. This genotyping array offers boosted coverage in pericentromeric regions, subtelomeric regions, and sex chromosomes and it contains high-value targets such as 140.000 SNPs for fine mapping (156 immune loci), 30.000 SNPs associated with autoimmune diseases, 6400 SNPs from the HLA region and 34.000 SNPs for non-autoimmune traits. We expect that the culmination of this project will facilitate the identification of genetic markers associated with the risk of IA infection and, therefore, the development of novel risk-adapted prophylaxis and even treatment strategies.

References
FUNCTIONAL ASPECTS OF IA-LINKED GENETIC VARIANTS

Agostinho Carvalho, PhD

University of Perugia - Italy

SATURDAY 1 MARCH 2014 (14.45 - 15.10)

Invasive aspergillosis (IA) is a major complication associated with hematopoietic stem cell transplantation. Despite significant progress attained in the management of IA, its prevention, diagnosis, and therapy remain extremely difficult, rendering it a leading cause of death among immunocompromised patients. Concerns over antifungal prescription and the remarkably high healthcare costs have been diverting clinicians from universal prophylaxis to risk stratification and preemptive approaches. This has inspired the search for novel individual prognostic factors, particularly genetic, to apply in the categorization of those patients most vulnerable to infection. In this context, several genetic variants in immune genes have already been disclosed as determinants of the immune response to \textit{Aspergillus} and some are nowadays regarded as promising targets to exploit towards improved diagnosis and therapy of IA. However, our existing knowledge of the genetic bases of susceptibility to IA mostly derives from studies screening single variants in candidate genes using small patient cohorts. The lack of validation in larger, independent cohorts or via biological studies of disease mechanisms are additional common limitations. Altogether, understanding the functional impact of the genetic variability of the immune system may contribute to the discovery and interpretation of specific immunogenetic signatures and immune profiles that may be used to discriminate the efficiency of antifungal responses, eventually helping to guide clinicians in the use of antifungal prophylaxis and therapy in high-risk patients.
GENETIC FACTORS UNDERLYING ABPA

Paul Bowyer, PhD

University of Manchester - United Kingdom

SATURDAY 1 MARCH 2014 (15.40 - 16.05)

Allergic Bronchopulmonary Aspergillosis (ABPA) is one of the most common fungal diseases affecting 2-5% of asthmatics worldwide and causing severe disease in affected individuals. Disease takes the form of an apparent colonisation of the airways without invasion of tissue and symptoms arise from blockage and narrowing of air passages with additional allergic symptoms. Recent genomic sequencing of many *A. fumigatus* isolates provides no evidence that specific fungal genotypes are associated with different forms of fungal disease therefore it is most likely that the 2-5% of asthmatics suffering ABPA carry a “fungus – susceptible” genotype. Here we present evidence from both large scale *A. fumigatus* genome sequencing, human DC and macrophage transcriptomics and exome sequencing of a large ABPA and control atopic asthma cohort to provide support for this hypothesis.
EARLY CLINICAL STUDIES OF NOVEL ANTIFUNGAL AGENTS

Haran Schlamm, MD

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SATURDAY 1 MARCH 2014 (16.05 - 16.30)

Despite the development of new antifungals and new treatment strategies, the mortality associated with invasive aspergillosis in some patient groups is substantial. This observation suggests that there is still a need for new and more effective antifungals to treat this infection.

In order to develop a new antifungal for treatment of aspergillosis, the unmet medical need must be clearly established. Is a new antifungal needed or can we use the currently available antifungals better, either in combination or at an earlier stage of infection, or both? Will a more effective antifungal be sufficient to address the issue of unsatisfactory response to treatment, or will better control of the underlying disease be the key to improved outcome? Is the unmet medical need related to the development of resistance to currently available antifungals?

The FDA has recently provided guidance for the development of new antibiotics\(^1\) that will potentially allow for more streamlined and targeted development programs. Much of this guidance can theoretically be applied to the development of new antifungals for aspergillosis, particularly those that are targeted to treat unmet medical needs, e.g. patients who have failed standard treatment, patients with infections caused by azole (or polyene) resistant *Aspergillus*, patients with high fungal burdens, or patients with CNS infection.

The preclinical program for a new antifungal should include *in vitro* studies that demonstrate activity against *Aspergillus*, and there should be evidence that the agent is able to reach crucial body sites, including the lungs and brain. Animal models of infection should demonstrate good efficacy at these clinically relevant sites. To be appropriate for treatment of patients with azole-resistant *Aspergillus* infections, the new antifungal should have a novel mechanism of action, an added inhibitor that neutralizes the mechanism of resistance, or an altered structure that is no longer susceptible to the resistance mechanism.

According to the FDA guidance, the clinical development program for an anti-infective drug that addresses an unmet medical need can be potentially streamlined. If the unmet medical need is urgent, it may no longer be necessary to demonstrate efficacy in a traditional phase II “proof of concept” study in patients with non-life threatening forms of infection. The clinical program can be initiated directly in patients with the unmet medical need, e.g. patients with aspergillosis who have failed standard treatment.

In their guidance document, the FDA provides examples of several potential study designs. The first option would be to conduct a prospective, comparative study in which the new antifungal is compared to the “best available” treatment, in a patient population “enriched” for the unmet medical need. This approach would be appropriate for the development of a new antifungal for treatment of patients with azole-resistant *Aspergillus* infection, for example, where the susceptibility would not typically be available for patients at study entry.

In this scenario, the FDA is willing to accept innovative study designs and analysis plans including an “adaptive” study design in which the sample size is adjusted based on the observed rate of infection with azole-resistant *Aspergillus* at various time points in the study. A variant of this approach is a nested, actively controlled non-inferiority/superiority study that allows for entry of all patients with aspergillosis, but tests for superiority only in the subgroup with documented azole-resistant *Aspergillus* infection.
The FDA will consider an open-label non-comparative study with historical controls, especially of the standard of care is considered to be suboptimal with resulting high mortality. This approach would be appropriate for patients with aspergillosis who have failed standard treatment, for example. However, it would be preferable to try to incorporate a small comparative group in the study, and incorporate Bayesian or Frequentist (logistic regression) models to merge the data from these patients with that of historical controls.

Another alternative would be to conduct a relatively small, non-controlled study in patients with the unmet medical need, supported by another larger comparative study in patients without the unmet need, with the objective of demonstrating non-inferiority to standard treatment. This approach would be appropriate for a new antifungal for patients with aspergillosis with relatively high fungal burdens, based on serum galactomannan levels. In this context, the FDA is willing to consider reduction in a surrogate marker, e.g. serum galactomannan, as the primary endpoint for the study, as long this outcome has been adequately established to correlate with clinical benefit. In this case, it would be necessary to verify the clinical benefit in post-marketing studies.

At the time of filing, the FDA is willing to consider a safety database with as few as 300 patients as long as they received the new antifungal at the proposed dose and duration of treatment. This safety database can include patients with the unmet medical need as well as patients without that need.

Reference
1. FDA Guidance for Industry: Antibacterial Therapies for Patients With Unmet Medical Need for the Treatment of Serious Bacterial Diseases, July 2013
POSTER ABSTRACTS
THE CLINICAL RELEVANCE OF *ASPERGILLUS* ISOLATION FROM RESPIRATORY TRACT SAMPLES & DETECTION OF *ASPERGILLUS* GALACTOMANNAN ANTIGEN IN SERUM OF PATIENTS WITH ACUTE EXACERBATION OF COPD

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**Purpose:**
This study aimed at finding an approximate incidence of IPA in patients with acute exacerbation of COPD and whether a combination of two tests, serological detection and mycological culture yielded a more specific diagnosis of IPA.

**Methods:**
This study included 30 COPD patients with the criteria of acute exacerbation according to the GOLD guidelines who were admitted at the Chest Department, Ain Shams University Hospital.

All patients were subjected to the following: full history taking, thorough clinical examination, chest-X-ray, arterial blood gases, ECG, routine laboratory investigations, fibroptic bronchoscopy to obtain samples of bronchial lavage, blood samples to obtain serum samples.

‘Probable’ *Aspergillus* infection of the lower respiratory tract was diagnosed in the COPD patient, who had severe disease (stage III or IV) according to GOLD criteria, with recent exacerbation of dyspnea resistant to appropriate treatment (including antibiotics), accompanied by one of the following: i) positive culture for *Aspergillus* from LRT, ii) positive serum antibody test for *Aspergillus fumigatus*, iii) positive serum Galactomannan (GM) antigen test (Gao et al., 2010). Colonization with *Aspergillus* was diagnosed if culture was positive for *Aspergillus* with no other evidence of fungal infection. Culture was performed on Sabouraud Dextrose agar, incubated for 7 days at both 25°C and 37°C. Colonies were identified according to McClenny, 2005.

Galactomannan (GM) antigen of *Aspergillus* was detected in serum specimens by Platelia *Aspergillus* Ag Enzyme immunoassay (EIA) kit supplied by Bio-Rad, Marnes la Coquette, France.

**Results:**
Of the 30 specimens, 7 (24%) yielded a positive culture of *Aspergillus fumigatus*. 23 (76%) serum specimens were positive for *Aspergillus* antigen by EIA testing. The 7 positive culture specimens were also positive for *Aspergillus* antigen, giving a diagnosis of ‘Probable’ *Aspergillus* infection of the lower respiratory tract with 2 criteria in 24% of patients, whereas the remaining 16 patients (54%) were diagnosed as ‘Probable’ *Aspergillus* infection with only 1 criterion.

**Conclusions:**
A highly significant statistical correlation between the severity of COPD and the presence of *Aspergillus* galactomannan antigen in serum. We propose that IPA should be thought of in all patients with severe exacerbations of COPD, especially those refractory to antibiotic therapy. The sensitivity and specificity of the GM antigen test obtained in this study, warrants its use, in combination with culture from BAL, as an early indicator of probable IPA. The only drawback of the antigen detection test is its cost, which could be a limitation to its use in facilities with limited resources.
A TEN-YEAR SURVEY OF GENERAL HOSPITAL CORRIDORS FUNGAL AERO-CONTAMINATION: RELIABLE SENTINEL TO PREDICT FUNGAL EXPOSURE RISK?

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Purpose:
Invasive mould infections (IMI) represent a threat for high-risk patients hospitalized in hematology units. Fungal aero-contamination controls are performed quarterly, as recommended by the French guidelines. Since 2002 Besançon university hospital planned several new buildings. Consequently environmental controls were reinforced weekly.

The aim of our study was to assess retrospectively the contribution of the fungal aero-contamination measurement in hematology corridors (HC) and main hospital corridors (MC) as sentinel to predict fungal exposure and IMI risk.

Methods:
2706 air sampling were carried out by impaction every week in the same places in HC and MC over a ten year period. All fungal species were identified. Hematology departments were alerted each time that a peak of opportunistic species was detected and corrective action were consequently planned. Since 2007, each invasive aspergillosis (IA) (based on EORTC/MSG criteria) (n=81) was signaled to the French health authority. Statistical analysis used Cuzick test, Mann-Kendall trend test, autocorrelation and Spearmann correlation-rank test.

Results:
In ten years of surveillance, 12 peaks of *Aspergillus fumigatus* (>40 CFU/m³) were observed in MC and *A. fumigatus* contamination was detected up to six times per year in HC. Constant interactions with the medical staff led to the control of ventilation systems and heating, to an increase of bio-cleaning and establishment of instructions to prevent fungal exposure.

Conclusions:
No significant link was observed between *A. fumigatus* detection and IA diagnosis. Weekly survey helped to improve the vigilance of the medical teams. Nevertheless 81 cases of IA arose in 69 months.
A SYSTEMATIC COMPARISON OF PRE-TREATMENT PROCEDURES AND DNA EXTRACTION FOR THE STANDARDIZATION OF ASPERGILLOSIS REAL-TIME PCR DIAGNOSTIC

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Purpose:
Standardization of molecular diagnosis of invasive aspergillosis is still a problem in clinical routine practice. Critical steps in real-time PCR analysis are fungal cell wall disruption and DNA extraction. The aim of this study was to investigate enzymatic, chemical and physical cell wall disruption procedures onto efficiency and applicability as a routine diagnostic technique and since, numerous DNA extraction kits have been tested before, yet under different conditions and pre-treatment methods. We searched on a panel of twelve different extraction methods which are commonly used in routine diagnostic and tested them under the same conditions.

Then we used promising DNA extraction kits revealing best results as well as the automated method with spiked whole blood samples from healthy volunteers to mimic the in vivo situation.

Methods:
A total number of ten different pre-treatment methods for fungal cell wall disruption and twelve DNA extraction kits including automated DNA extraction with the MagNA Pure LC instrument were tested for efficiency and specificity for conidia suspensions at three different concentrations (10^3, 10^5 and 10^7 conidia/ml of Aspergillus fumigatus).

One pre-treatment method and the five promising DNA extraction kits revealing best results plus automated method were further used with spiked (10^3, 10^5, 10^7 conidia/ml) whole blood samples. Real-time PCR targeting A. fumigatus 18S rRNA gene was used. Samples without A. fumigatus served as negative controls.

Results:
Bead beating with Precellys® glass beads (diameter 0.5 mm) was identified as the best cell wall disruption method giving highest Aspergillus DNA amounts.

Five DNA purification kits revealed statistically higher DNA amount at 10^3 conidia /ml than the other kits. Two out of these five kits showed also statistically significant higher efficiency for the concentration of 10^5 and three extraction kits showed statistically significant higher efficiency at 10^7 conidia/ml.

For spiked whole blood samples, the automated DNA extraction method showed statistically significant higher amounts of DNA than the five other tested kits in all three concentrations and statistically significant lowest results in negative controls.

Conclusion:
The pre-treatment method revealing best results was bead beating with Precellys® glass beads. The comparison of the different bead materials identified glass as material with the lowest binding capacity for nucleic acids as it is approved from data on RNA extraction from filamentous fungi.
A parameter which is important to gain useful results with PCR, is the ability to efficiently isolate fungal DNA. Several studies on fungal DNA extraction kits have been performed and published yet under different conditions and different pre-treatment methods which make comparison difficult.

In this study the automated DNA extraction method showed best results for spiked blood samples negative controls. The clear advantage of the MagNA Pure LC instrument is the fully automated system. It is possible to time efficiently isolate many samples in a standardized manner with low contamination risk.

It is suggested that the fungal burden in the bloodstream of patients is low. Therefore, a high degree of analytical sensitivity is needed to detect these small amounts of DNA to avoid false negative samples.
DETERMINATION OF THE IDEAL SPECIMEN FOR THE DETECTION OF ASPERGILLUS DURING INVASIVE ASPERGILLOSIS

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Purpose:
Invasive aspergillosis (IA) is increasingly recognised in immunocompromised patients and still associated with a high mortality rate. The diagnosis of IA is still problematic. Often, different blood specimens are used for PCR diagnosis including serum, plasma or EDTA blood. However, there is no consensus on the blood sample type that should be used for the detection of Aspergillus spp.

The aim of this study was to systematically investigate the suitability of different blood specimens for diagnostic analysis. Therefore, we spiked blood samples from healthy donors with Aspergillus fumigatus conidia and hyphae at various concentrations. Then we examined fungal growth in the different samples by solid- and fluid culture, fluorescence- and light-microscopy.

Methods:
Native, EDTA and citrated blood samples were taken from healthy volunteers. The samples were spiked with A. fumigatus (10², 10³, 10⁴, 10⁵ and 10⁶ conidia/ml), gently mixed and incubated for 30 min at 37°C followed by selective centrifugation to gain different blood compartments namely EDTA whole blood, citrated blood pellet, platelet rich plasma (PRP), serum, serum blood, solid and lysed clot.

Same experiments were performed with hyphae. Lysis of the clot was performed with tissue lysis buffer and proteinase K incubation for 60 min at 55°C. Then 10 µl and 100 µl of each specimen were plated on solid sabouraud agar and poured into liquid Sabouraud and incubated for 24h, 48h and 72h at 37°C. Fungal growth was determined by counting colony forming units (cfu) and monitoring the confluency.

Untreated A. fumigatus served as positive control. Additionally, calcofluor-white fluorescence- and light- microscopy out of the specimens were performed to detect conidia or hyphae in the compartments.

Results:
Solid growth culture experiments at 10⁶ cfu/ml showed fungal growth in EDTA (42%), PRP (30%), citrated blood (50%) and the clot (50%) after 24h of incubation, whereas serum samples only showed 7% growth. After 72h all specimens except serum (40%) showed 100% fungal growth.

At 10² cfu/ml no growth for serum and PRP could be detected after 72h, whereas the clot (78%), EDTA (60%), citrate blood (75%) and serum blood (49%) showed growth. Same results were found with hyphae. With light and fluorescence microscopy more conidial or hyphal structures could be found in the clot in comparison to all other specimens.

Conclusion:
In routine fungal diagnostic different blood specimens are used for Aspergillus identification. At low fungal concentration we found strongest fungal growth in the clot and citrated blood and no growth in serum samples. That absence or low detection of conidia or hyphae in serum is consistent with the pelleting of fungal elements probably resulting from centrifugation.
The increased incidence of fungal conidia and hyphal structures found by microscopy and growth experiments might be an indication that highest fungal load is found in the clot also at low fungal concentrations. A reason for this finding could be that platelets interact with *Aspergillus* species by adhering and covering conidia. These finding has to be proven with real-time PCR and might help to gain better results and improve standardization for the diagnosis of IA.
FIRST DIAGNOSTIC OF INVASIVE ASPERGILLOSIS IN ALGERIA BY GALACTOMANNAN TEST

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Purpose:
The invasive aspergillose affects particularly patients with malignant pathologies in hematology yards. Our hospital is an anti-cancer center located in Blida which is 50 km far away from Algiers. During 3 years, 208 patients with severe neutropenia were included in our study.

Methods:
208 patients were included in our study, the average age is 33.60 years, standard deviation is 14.68 years, age between 16 and 72 ends, with a median of 30 years and mode 17. We conducted various samples: sputum, samples from the mouth, nose, ears and finally sera for research galactomannan. We used galactomannan test in serum as recommanded by EORTC/MSG twice a week.

Thus, we conducted 94 sputum samples and 1039 swabs of the mouth, nose and ears. We obtained 669 serum with an average of 3.91 and a standard deviation of 2.41, the maximum number of samples per patient was 9 and the minimum is 1 (the patient died). On the other hand we explored the surrounding flora of these patients by withdrawal of objects (1160 samples) and air (237 prélèvements). In total we obtained 3699 samples from patients, their environment and air.

Results:
58 patients involved in the study showed a positive serology galactomannan. According to the criteria of the EORTC / MSG 16 patients among the 58 have probably developed a probable invasive aspergillosis and 21 had false-positive serology.

Of all the different swabs of the mouth, nose and ears, carried out on 208 patients collected the genus \textit{Aspergillus}, and is ranked second after the yeast-like fungi. \textit{Aspergillus niger} is ranked first, followed by \textit{Aspergillus flavus}, \textit{Aspergillus fumigatus} is then third. According to the results of our study of the environement \textit{Aspergillus niger} is in the first position followed by \textit{Aspergillus flavus}, \textit{Aspergillus terreus}, \textit{Aspergillus fumigatus} and \textit{Aspergillus clavatus}.

Conclusion:
Monitoring the serology is essential \textit{Aspergillus} galactomannan in neutropenic patient hematology. It must go through a full diagnosis involving radiological data including CT scan results of \textit{Aspergillus} serology and hoping that in the near future guided transmural biopsy.

The \textit{Aspergillus} galactomannan technology now available in our country, make it easier to identify invasive pulmonary aspergillosis cases dreaded disease in neutropenic leukemia. This delicate technique requires for its interpretation of close collaboration between the mycologist and hematologist. All data concerning the clinic, laboratory tests, treatment, are important, the information sheet would be the key element of this interpretation.

In our study we found that 7.59\% of patients in the Hematology CAC BLIDA suffer from probable invasive aspergillosis. For the first time in our hospital, cases of invasive aspergillosis are described the galactomannan test is very helpful in a developing country, where the molecular tests are so expensive and not disponible.
ANTIRADICAL AND ANTIFUNGAL ACTIVITIES OF THE ESSENTIAL OILS OF MONODORA MYRISTICA, XYLOPIA AETHIOPICA AND XYLOPIA PARVIFLORA (ANNONACEAE)

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Purpose:
Excessive production of free radicals causes direct damage to biological molecules, so causing a decrease of the immune system, an increase of the permeability of cell membranes, which results in the vulnerability of the body to attack by opportunistic pathogens including fungi of the genus Aspergillus. Aspergilli are important as human and animal pathogens, spoilage agents of food, producers of toxic metabolites are the second generally isolated from opportunistic mycoses after Candida. They are the cause of invasive infections associated with increased morbidity and mortality especially in immunocompromised patients despite antifungal chemotherapy which face the development of fungal resistance. In order to find more efficient component from plant extracts which could alleviate this situation, we investigated the chemical composition with antiradical and antifungal activities of essential oils from fruits of Monodora myristica, Xylopia aethiopica and Xylopia parviflora (Annonaceae).

Methods:
The essential oils extracted with Clevenger type apparatus were analyzed by gas chromatography and gas chromatography–mass spectrometry (GC/MS). The ability of the oil to reduce diphenylpicrylhydrazyl (DPPH .) was used to evaluate the antiradical activity and Agar dilution method was used to assess the antifungal activity against A. fumigatus, A. flavus and A. niger from Centre Pasteur of Cameroon.

Results:
Major components of the essential oils were:
- α-phellandrene (57.8%) and p-cymene (12.7%) for M. myristica,
- β-pinene (46.9%) and α-pinene (16.9%) for X. parviflora ,
- β-pinene (25.8%) and α-pinene (11.5%) for X. aethiopica’s sample. In regard to antiradical activity, essential oils exhibited low antiradical power in comparison with BHT. X. aethiopica was the most active free radical scavenger with the highest antiradical power (AP= (2.28 ± 0.00)10^{-5}) compared to M. myristica (AP = (1.89 ± 0.01)10^{-5}) and X. parviflora (AP = (1.42 ± 0.00)10^{-5}). X. parviflora showed the best antifungal activities (4mg/ml≤ MIC ≤5.2mg/ml) followed by X. Aethiopica (5mg/ml≤ MIC≤7.5mg/ml) and M. myristica (9mg/ml≤MIC≤10.2mg/ml).

A. fumigatus was the most sensitive isolate and A. flavus was the most resistant. With MFC/MIC ratio < 4, all the essential oils exhibited fungicidal activity during twelve days of observation.

Conclusion:
The results show that essential oils of these fruits may either be used as a dietary supplement for patients suffering of oxidative stress related diseases through their radical scavenging properties but also as fungicides in the prevention of fungal contaminants of the genus Aspergillus.

Keys words:
Essential oils, antiradical, antifungal, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger
DIAGNOSING INVASIVE PULMONARY ASPERGILLOSIS IN ICU PATIENTS: THE ROLE OF BRONCHOALVEOLAR LAVAGE LATERAL-FLOW DEVICE TEST

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Purpose:
Invasive pulmonary aspergillosis (IPA) represents an increasing problem in patients requiring intensified medical care. Due to the crude mortality of 80-90% in absence of adequate treatment, timely diagnosis and early start of antifungal therapy are key factors in successful treatment of IPA. The Lateral-Flow Device (LFD) test is a single sample point-of-care test for native BAL testing that is based on the detection of an Aspergillus extracellular glycoprotein antigen by monoclonal antibody JF5. This study evaluates the LFD test by using bronchoalveolar lavage (BAL) samples.

Methods:
A total of 91 BAL samples from 87 ICU patients (63 samples from Graz, 24 from Innsbruck) were included between 2011 and 2013 at the two Austrian University Hospitals of Graz (East of Austria) and Innsbruck (West of Austria). 12 had probable or proven IPA (8 Graz, 4 Innsbruck). Diagnostic accuracy of LFD for probable/proven IPA was evaluated. For IPA grading fungal cultures as well as BAL GM (cut-off 1.0) were used.

Results:
Sensitivity and specificity of LFD test for probable/proven IPA was 67% (Graz 75%, Innsbruck 50%) and 92.4% (Graz 90%, Innsbruck 95%), respectively. PPV was 57%, NPV 95%. BAL GM levels were available in 66 samples and significantly lower in patients with negative than in those with positive LFD result (P<0.001). LFD resulted negative in 4 patients with probable IPA. BAL GM was tested in two of those patients and revealed levels of 3.4 and 0.2. In a total of three patients (including the latter with a BAL GM below the cut-off) BAL culture grew Aspergillus fumigatus.

Conclusion:
The LFD test of BAL specimens is performed easily in ICU patients and may provide rapidly available decision support whether to initiate antifungal therapy.
8 FACTORS ASSOCIATED WITH LOW POSACONAZOLE PLASMA CONCENTRATIONS AND IMPACT OF STRUCTURED PATIENT EDUCATION

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Purpose:
The objectives of the study were to analyze factors associated with low posaconazole plasma concentrations among patients with hematological malignancies receiving the suspension prophylactically and to evaluate the impact of a structured patient education on low levels.

Methods:
The study was conducted from July 1st 2012 to June 1st 2013 at the Division of Hematology, Medical University of Graz, Austria. Steady state PPCs measured on day 7 or later in patients with hematological malignancies and posaconazole prophylaxis were included. Concentrations above the target of 0.5 mg/L were defined as satisfactory and those below the target as low PPCs. In patients with low drug levels a structured personal on-site education concerning the intake of posaconazole was performed.

Results:
258 trough PPCs were measured in 65 patients (23 GVHD phase after haematopoietic stem cell transplantation, 42 neutropenia after induction therapy; median PPC 0.59 mg/L, IQR 0.25-0.92). 141/258 (55%) of PPCs were satisfactory, while 54 (21%) were below 0.2 mg/L. Diarrhea at the time the PPC was obtained remained an independent predictor of low PPCs in multivariable analysis (OR 0.14; 95% CI 0.03-0.67). Higher BMI, receipt of systemic corticosteroids and T-cell suppressant were predictors for satisfactory PPC’s. Initial steady state PPCs were sufficient in 29 (45%) and low in 36 (55%) patients. In 28/36 patients with low PPCs a personal on site education was performed. In 12/28 (42.9%) patients the personal on-site education led to sufficient levels, while in 16 (57.1%) the PPCs stayed below the target, although increasing from below 0.2 to above 0.3 in 6 of those patients.

Conclusion:
Low PPCs were frequently observed and diarrhea remained the strongest predictor of low PPCs in multivariable analysis. A structured personal on site education led to sufficient levels in more than 40% of patients and may therefore be a promising tool to increase low PPCs.
Efficacy of Antifungal Therapy with Inhaled Itraconazole Against Murine Invasive Pulmonary Aspergillosis with Azole-Low Susceptible Aspergillus fumigatus


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Background:
Fungal infection is one of the most serious problems in immunocompromised patients during the medication of anticancer and immunosuppressant drugs, and among HIV/AIDS patients. Invasive pulmonary aspergillosis (IPA) results in significant mortality in severely immunocompromised patients. We found the case of itraconazole (ITCZ) resistant Aspergillus fumigatus (Af) strains isolated. The tolerance to azole antifungal drug has become a serious concern recently. Targeted intrapulmonary delivery of antifungals has the potential to reduce systemic toxicity and improve treatment efficacy as well as prophylaxis. Therefore, we investigated inhalation of ITCZ treatment against IPA model with ITCZ-low susceptible A. fumigatus.

Objective and Method:
A. fumigatus MF367 and MF469 were clinically obtained from a patient admitted to the Nagasaki University Hospital. The MIC of MF367 was 0.5mg/L and that of MF469 was 8.0mg/L which were determined by Clinical and Laboratory Standards Institute (CLSI) M38-A2 broth microdilution method. Six-week-old female ICR mice (Charles River Breeding Laboratories, Shiga, Japan) were immunosuppressed and then challenged on day 0 with 5×10^4 conidia of A. fumigatus MF367 or MF469 intratracheally for monitoring of survival for 14 days. Mice were immunosuppressed by intraperitoneal injection of cortisone acetate (Sigma, Tokyo, Japan) at 250 mg/kg of body weight and cyclophosphamide (Sigma, Tokyo, Japan) at 200 mg/kg on days -2 and 0 for the survival study. Mice were assigned into the following groups: control mice challenged with MF367 or MF469 without treatment, the mice challenged with MF367 or MF469 receiving ITCZ oral administration (20mg/kg, 40mg/kg, 80mg/kg), and the mice challenged with MF367 or MF469 receiving ITCZ inhalation (5ml mixture solution of ITCZ and saline of 20%, 40%, 80% and 100%).

Result:
The survival rate of MF-367-infected mice treated with nebulized ITCZ (40%, 80%, and 100%) were 63%, 71%, and 80%, respectively, and that of ITCZ (80mg/kg) oral administration was 62%. The survival rate of MF-469-infected mice treated with nebulized ITCZ (80% and 100%) were 60%, and 71%, respectively, and that of ITCZ (80mg/kg) oral administration was 50%. The survival rates of the group of mice receiving nebulized ITCZ were significantly higher than those of the group of mice receiving oral ITCZ administration. Additionally, MF-367 (ITCZ-susceptible strain)-infected mice were more sensitive to nebulized ITCZ compared to MF-469 (ITCZ-low susceptible strain)-infected mice.

Conclusion:
Our result indicated the possibility of inhalation of ITCZ administration is effective to IPA with ITCZ-low susceptible A. fumigatus strain.
MAXILLARY SINUS MYCETOMA DUE TO ASPERGILLOSIS: CASE REPORT

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Purpose:
Paranasal sinus aspergilloma is a rare disease of sinus which is sometimes considered as mycetoma. In this condition, there is gathering of fungal mycelium in sinus cavity without any bone or vessel invasion. It presents as a chronic sinusitis with headache, nasal discharge and obstruction. Diagnosis of aspergillosis infection is often dependent on clinical judgment combined with radiologic evaluation and demonstration of the fungus in tissue specimens obtained from the presumed site of infection.

Case Report:
A 53-year-old man with uncontrolled insulin dependent diabetes mellitus presented with a six month history of headache. He had used various drugs prescribed by different physicians in the past six months without any effect. Because of progressive headache, he was admitted in the department of otolaryngology and surgical sinus debridement was done. Histopathological examination was suggestive of maxillary sinusitis with fungal ball of aspergilloma. The patient was treated with itraconazole and after 6 months, he felt better with no clinical and paraclinical signs.

Conclusion:
Since fungal sinusitis presentation is similar to chronic sinusitis and in the other hand, the incidence of aspergillosis is increasing, the early diagnosis and treatment is important especially when the clinical and paraclinical signs are suggestive.
INSULATION ASPERGILLUM SPP. WITH VEGETARIAN FOOD

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Purpose:
Various species of the genus Aspergillums are responsible for causing opportunistic infection in both humans and a wide range of animals. Early diagnosis is critical for effective treatment and for avoiding inappropriate administration of costly toxic antifungal therapy. The diagnosis bee mainly based on traditional culture methods. Fruits and vegetables are rich in vegetable care in protein, vitamins, and rich in carbohydrate, ferment and electrolytes and are not surprising that there is deterioration and increase the appearance of various types of mold, fungi and so on. The aim of this study was to examine various types of fruits and vegetables in preparation for feeding insulation Aspergillums species.

Methods:
We used the first singles various types of fruits and vegetables, which are after fragment with mixer and underwent maceration in solution electrolyte, pasteurizing performed at 56/60°C for 12-18h. As electrolyte, mixture been used: Sodium chloride, Cupric sulfate, Magnesium sulfate, Ferric ammonium citrate and Potassium metabisulfite. After pasteurization content, we spat as a tea (no filter), add 20g/l agar heated to boiling and poured into Petri dishes. We decided on the type of fruit and vegetables that have shown a good port Aspergillums species, especially if they are shown and selective activity and bee not allowed to increase Gram positive and negative types. We decided on a few well-chosen types of fruits and vegetables, which are supplemented agar made feeding the isolation of Aspergillums species from clinical samples. In parallel, we used the classical method of isolation using Sabouraud agar. Samples been incubated at 24-37°C for 18-24h.

Results:
The results were very successful and have shown that the tendency on vegetable and fruit can bee used as a medium in the isolation of Aspergillums species. Medium was without necessity add antibiotics to achieve selectivity. Fruit gives a much better isolation of Aspergillums species of vegetables. Increase and thus insulating Aspergillums species is much faster and better than on Sabouraud agar.

Conclusion:
Vegetables and fruits in particular offer great opportunities isolation of fungi, especially those that are sensitive to antibiotics and selective additions that today use in Sabouraud agar. Arrive faster to the results of what is important in the treatment of infections caused by fungal diseases. Manufacturer dehydrated feeding in microbiology should pay attention to the production of feeding from fruits and vegetables.
DIAGNOSIS OF INVASIVE ASPERGILLOSIS (IA) IN HIV INFECTED PATIENTS

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Purpose:
A revised EORTC/MSG (EORTC) classification defining Invasive Aspergillosis (IA) has been proposed in 2008 based on host factors, clinical and mycological criteria. Major host factors are prolonged neutropenia, immunosuppressive treatments, allogeneic stem cell transplant patients, inherited severe immunodeficiency, but HIV status is not considered as a host factor. A review has reported that ≤50% of IA cases in HIV infected patients occurred with a neutrophil count <500/mm3 (Khoo and Denning, CID 1994). In HIV infected patients, IA was previously described as a life-threatening complication of advanced AIDS, with a median of survival of 2 months after diagnosis. As the EORTC classification was mainly developed for patients from hematology/oncology, we investigated its relevance in the context of HIV infection.

Methods:
Medical records of all HIV-infected patients followed up in the French Hospital Database on HIV (FHDH-ANRS CO4) cohort between 1992 and 2011 were screened for aspergillosis according to the ICD-10 codes (B440-B441-B442-B447-B448-B449) and reviewed by 2 experts. Each criterion for EORTC classification was checked. When another pathogen, such as M. tuberculosis and avium, S. pneumoniae, Histoplasma capsulatum, Pneumocystis jirovecii, that could be responsible for the clinical signs, was also identified with the Aspergillus specie, we excluded an IA diagnosis. We also excluded patients without clear radiologic involvement, incomplete charts, aspergillomas, allergic bronchopulmonary aspergillosis, Aspergillus colonization, or acute viral diseases. When validated cases of IA did not meet EORTC criteria, IA were defined as HIV-related IA based on previously published recommendations (Lortholary O and al, Am J Med 1993). Three periods were analyzed: before combined antiretroviral therapy (<1996), before voriconazole availability (1996-2001), and after 2001.

Results:
Diagnosis of IA was rejected for 102 of the 347 medical records which were reviewed. Out of 245 validated IA diagnosis, 72 (29%) cases occurred before 1996, 82 (34%) in 1996-2001 and 91(37%) in 2002-2011. IA diagnosis was assessed according to all EORTC criteria for 124 (51%) patients (7 possible, 65 probable and 52 proven) while HIV related IA were noted in 121 (49%) patients (83 probable and 38 possible). Over the three periods, the proportion of IA defined by EORTC criteria was unchanged with 50%, 48%, and 54%, respectively. Out of the 56 patients with hematologic diseases (23 Non Hodgkin Lymphomas, 8 Hodgkin diseases, 25 other (mainly myelodysplasias)), 8 (14%) did not fulfill EORTC criteria. Immunodepression was observed in the majority of patients with IA diagnosis. In the last period, 2002-2011, median CD4 at IA diagnosis were 37/mm3 (IQR: [9-300]) and 115/mm3 (IQR:[20-327]) for EORTC and HIV-related cases, respectively (p=0.11). Mortality at 3 months did not differ between EORTC and HIV-related cases (27% and 33%, p=0.5).
Conclusion:
EORTC criteria that have been proposed for patients from hematology/oncology applied to only half of IA diagnosed in HIV-infected patients. These criteria relied mainly on polynuclear cell count and could be misleading in HIV-infected patients. As IA remained an important threat with a 3-months mortality rate of 30% in the most recent period, we need to better identify specific HIV risk factors in order to improve diagnosis and early treatment.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
ENVIRONMENTAL STUDY OF AZOLE-RESISTANT ASPERGILLUS FUMIGATUS WITH TR34/L98H MUTATIONS IN THE CYP51A GENE IN IRAN

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Purpose:
Triazole antifungal drugs such as itraconazole, posaconazole and voriconazole are the mainstay of therapy in the management and prophylaxis of aspergillosis. However, in recent years, triazole-resistant clinical isolates of Aspergillus fumigatus have emerged in Europe (the Netherlands, Denmark, Spain, UK, Belgium, Germany and France) and Asia (China, India) resulting in several cases of therapeutic failure caused by triazole-resistant A. fumigatus. Azole resistance may develop in patients who are treated with long-term azole therapy or may develop in the environment through the exposure of the fungus to the azole fungicides used in agriculture.

Methods:
In the present study, we investigated the presence of azole resistance in environmental A. fumigatus isolates from hospital surroundings in Sari and Tehran.

Results:
The TR34/L98H mutation was the only resistance mechanism. Overall 3.3% of the A. fumigatus isolates from hospital surroundings in Sari and Tehran had the same TR34/L98H STRAf genotype and was related to some resistant clinical and environmental TR34/L98H isolates from the Netherlands and India. It is emphasized that routine resistance surveillance studies focusing on environmental and clinical samples are warranted to yield the true prevalence of azole resistance in A. fumigatus in Iran.

Conclusion:
The widespread application of triazole fungicides in the environment in Iran could have contributed to the emergence of environmental azole-resistant A. fumigatus. Given the emergence of azole resistance in environmental strains, continued surveillance of resistance in clinical A. fumigatus strains in Iran is desirable for successful therapy of aspergillosis. Furthermore, the need for enhanced understanding of the evolution of azole resistance and measures to prevent the emergence of multiple-azole-resistant A. fumigatus strains in countries using fungicides can hardly be over-emphasised.

Key words:
Aspergillus fumigatus, cyp51A, TR34/L98H, azole resistance, Iran, microsatellite typing
ANTI-FUNGAL ACTIVITY OF *U*VARIODENDRON *CALOPHYLLUM* FRACTIONS

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Purpose:
Aspergillosis is a spectrum of diseases of humans and animals caused by members of the genus *Aspergillus*. *Aspergillus* sp. organisms are capable of living both a saprophytic and parasitic way of life and susceptible hosts have numerous opportunities to contact this agent. Aspergillosis and *Candida* species are opportunistic pathogens that cause superficial and systemic candidiasis particularly in immunocompromised patients. Resistance to many clinically used antifungal agents has created a need to identify new compounds and develop drugs for therapeutic use. Therefore, the main objective was to carry out bioguided fractionation of the ethanolic and aqueous extracts of the leaves, twigs, stem, stem bark and trunk of *Uvariodendron calophyllum*.

Methods:
The leaves were macerated in ethanol and water, their yields of extraction were calculated. The broth micro dilution and agar dilution method was used for antifungal susceptibility testing of yeasts (*Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Cryptococcus neoformans* isolates and four *candida albicans* strains ATCC L26, ATCC 12C, ATCC P37039, ATCC P37037) and moulds (*Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus flavus*). Phytochemical screening on the crude extracts was done. Then, bioguided fractionation was done on the crude extracts using Hexane, Methylene Chloride and Methanol. Column chromatography was done on the methylene chloride fraction of the leaf ethanolic extract using different solvent systems.

Results:
The yields of extraction ranged from 1.125% to 6.77% for the aqueous extract and from 3.57% to 10.67% for the ethanol extracts. The phytochemical screening showed the presence of phenols, tannins, flavonoids and glycosides in both extracts. The MIC ranged from 0.63±0.27 to 2.19±1.43 mg/mL for the ethanol extracts, from 1.88±0.00 to 7.50±0.00 mg/mL for the aqueous extract on the Yeasts microorganisms. The various MIC ranged from 22 -36 mg/ml on *A. Flavus*, 23-35mg/ml on *A. niger* and 23-36mg/ml on *A. fumigatus*. The most active was obtained for *U. calophyllum* twig extract (22 mg/ml) on *A. flavus* strain. The MIC of amphotericine B and griseofulvin were greater than 2mg/ml. 35 fractions were obtained after partition and their MIC results ranged from 1.25±0.00 to ≥10±0.00 mg/mL on the tested microorganisms. Nineteen fractions were obtained and labeled fraction A to fraction S with MIC varying from 0.002 to ≥2mg/mL with fraction S having the best activity.

Conclusion:
The crude ethanol, aqueous extracts and fractions of *Uvariodendron calophyllum* could be potential sources of compounds with anti-fungal activity.

Key words:
Bioguided fractionation, Anti-fungal activity, *Uvariodendron calophyllum*, phytochemicals
ANTIFUNGAL ACTIVITY OF ALOE VERA EXTRACT AGAINST PATHOGENIC SPECIES OF ASPERGILLUS ISOLATED FROM KATHMANDU, NEPAL

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Purpose:
*Aspergillus* species, a human pathogenic fungus, causes a life threatening pulmonary infections and invasive & allergenic aspergillosis in immune suppressed individuals. Invasive aspergillosis is mainly caused by *Aspergillus fumigatus* and *A. flavus* is the second leading cause of aspergillosis. *A. niger* also cause invasive infections to a lesser extent in immunocompromised patients. *A. fumigatus* represents a major cause of morbidity and mortality in the patients of Allergic Bronchopulmonary Aspergillosis (ABPA). Global burden of azole resistance in *Aspergillus* has dragged an attention towards the use of herbal medicine. *Aloe vera* is one of the most important traditional folk and alternative medicine all over the world for treatment of infectious diseases with lesser side effects. In addition to antibacterial, antiviral and antifungal activity, it has been shown to possess anti-inflammatory, immuno stimulatory and cell growth stimulatory activity. In present study, *in vitro* antifungal activity of *Aloe vera* extracts (aqueous, ethanol and acetone) was investigated against pathogenic *Aspergillus* species as these are predominant in atmosphere of Kathmandu, Nepal.

Methods:
A total of 94 isolates of pathogenic *Aspergillus*- 23 *A. fumigatus*, 35 *A. flavus* and 36 *A. niger* from atmosphere of Kathmandu Valley were included in this study. *Aloe vera* extracts in various solvents (aqueous, ethanol and acetone) were prepared by using soxhlet apparatus [Alade and Irobi (1993), Ibrahim et al. (2011)] and stored for further studies. Antifungal activity of all extracts of *Aloe vera* against pathogenic *Aspergillus* was determined by disc diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines and using Sabouraud Dextrose agar (SDA) in triplicates for each test [Ibrahim et al. (2011), Al-Wathiqi et al. (2013)]. The results were read after 24 to 72 h of incubation and by measuring diameter of zone of inhibition (millimeters) at outermost point of marked decrease in fungal density. Phytochemical analysis of aqueous, ethanol and acetone extracts of *Aloe vera* was carried out qualitatively [Harborne (1973), Trease and Evans (1989), Sofowora (1993)].

Results:
*Aloe vera* extracts (aqueous, ethanol and acetone) showed antifungal activity against pathogenic *Aspergillus* species investigated. Maximum antifungal activities were observed in acetone extract than ethanol and aqueous extracts against all pathogenic *Aspergillus* species. Acetone extract showed highest antifungal activity against *A. fumigatus* (15mm) and *A. flavus* (16mm) and moderate activity against *A. niger* (10mm) while ethanol extract showed low antifungal activity than acetone extract against *A. fumigatus* (11mm), *A. flavus* (11mm) and *A. niger* (10mm). Antifungal activity of aqueous extract against *A. fumigatus* (8mm) and *A. niger* (8mm) was less significant and *A. flavus* showed no zone of inhibition. Phytochemical analysis of all extracts of *Aloe vera* showed the presence of metabolites- aminoacids, carbohydrates, alkaloids, flavonoids, glycosides, phenols, anthraquinones, tannins and saponins.

Conclusion:
The acetone extract of *Aloe vera* possess significant antifungal activity against pathogenic *A. fumigatus*, *A. flavus* and *A. niger* than ethanol and aqueous extracts. Phytochemical analysis revealed that all extracts of *Aloe vera* contain secondary metabolites with antifungal properties. Extracts of *Aloe vera* could be exploited as an effective antifungal agent against pathogenic *Aspergillus* species.
THE FUNGI OF THE GENUS *ASPERGILLUS* IN LUNG CAVITIES OF TUBERCULOSIS PATIENTS: A SPECIES SPECTRUM AND SUSCEPTIBILITY TO ANTIMYCOtICS

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**Purpose:**
To identify species of *Aspergillus* fungi found in lung cavities of pulmonary tuberculosis (TB) patients and analyze strains resistance to widely-used antifungal agents.

**Methods:**
Microscopy and inoculation (onto Sabouraud medium with chloramphenicol) of samples from lung cavities (resection samples, punctured tissue samples, aspirates); species identification of isolated strains of mycelial fungi (conventional methods, identification media: Czapek-Dox and potato dextrose agar); determination of antimycotic susceptibility of isolated strains of the genus *Aspergillus* by the broth microdilution method with determination of MIC (μg/ml) (the «Sensititre» system).

**Results:**
During 2002-2013 we studied samples from lung cavities (caverns, tuberculomas, cysts, aspergillomas) from 458 pulmonary TB patients (cavernous TB, fibrocavernous TB, tuberculoma). Fungi of the genus *Aspergillus* were found in lung cavities of 49 patients: *A. fumigatus* – in 29, *A. terreus* – in 6, *A. niger* – in 5, *A. flavus* – in 4, *A. restrictus* – in 2, *A. versicolor* – in 2, *A. glaucus* – in one patient. We determined drug resistance as follows: 1) to amphotericin B: for strains *A. fumigatus* (n=29) MIC range was 0.5-2 μg/ml, *A. terreus* (n=6) – 4-8 μg/ml, *A. niger* (n=5) – 1-2 μg/ml, *A. flavus* (n=4) – 2-8 μg/ml, *A. versicolor* (n=2) – 2-4 μg/ml, *A. glaucus* (n=1) – 0.5 μg/ml; 2) to voriconazole: *A. fumigatus* (n=29) – 0.06 – 0.5 μg/ml, *A. terreus* (n=6) – 0.12 – 0.25 μg/ml, *A. niger* (n=5) – 0.25 – 1 μg/ml, *A. flavus* (n=4) – 0.12 – 0.5 μg/ml, *A. versicolor* (n=2) – 0.25 – 0.5 μg/ml, *A. glaucus* (n=1) – 0.25 μg/ml; 3) to itraconazole: *A. fumigatus* (n=29) – 0.12 – 1 μg/ml, *A. terreus* (n=6) – 0.12 – 0.25 μg/ml, *A. niger* (n=5) – 0.5 – 1 μg/ml, *A. flavus* (n=4) – 0.06 – 0.5 μg/ml, *A. versicolor* (n=2) – 0.5 μg/ml, *A. glaucus* (n=1) – 0.06 μg/ml; 4) to posaconazole: *A. fumigatus* (n=29) – 0.03 – 0.25 μg/ml, *A. terreus* (n=6) – 0.12 – 0.25 μg/ml, *A. niger* (n=5) – 0.12 – 0.5 μg/ml, *A. flavus* (n=4) – 0.06 – 0.5 μg/ml, *A. versicolor* (n=2) – 0.12 – 0.5 μg/ml, *A. glaucus* (n=1) – 0.06 μg/ml.

**Conclusion:**
The fungus *A. fumigatus* remains the most common causative agent (59.2% of cases) of secondary aspergillosis in TB patients with lung cavities (aspergilloma, other forms of aspergillosis). In more than 10% of cases (12.3%) the fungus *A. terreus* resistant to amphotericin B was isolated from patients’ cavities. It was shown that MIC ranges of amphotericin B vary for different *Aspergillus* species.
MULTI-AZOLE RESISTANT ASPERGILLOSIS DUE TO TR_{34}/L98H
ASPERGILLUS FUMIGATUS IN A TERTIARY CARE HOSPITAL IN DELHI,
INDIA, 2009-2012

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Purpose:
Aspergillus fumigatus is the commonest etiologic agent of invasive and chronic pulmonary
aspergillosis (CPA). The recent increase in itraconazole resistance has been linked to a single allele
of cyp51A, termed ‘TR_{34}/L98H’ in many European countries. However, non-cyp51A mediated
mutations are also increasingly reported especially from Manchester, United Kingdom. Apparently,
TR_{34}/L98H mutation is now spreading, since over the past years this mutation has been reported to
occur world-wide in patients as well as in the environment. Azole resistance in A. fumigatus isolates
impacts the management of aspergillosis, since the azoles are primary agents used for prophylaxis
and therapy. The present study investigated the prevalence of azole resistance in clinical A. fumigatus
isolates and determined the effects of resistance on patient management during a 3-year retrospective
study from Delhi, India.

Methods:
A total of 260 A. fumigatus strains from 225 patients admitted to V. P. Chest Institute, Delhi, India,
during 2009-2012 were screened for azole resistance on Sabouraud dextrose agar (SDA) plates
supplemented with 4 mg/L itraconazole and 1 mg/L voriconazole. The patients underlying conditions,
diagnosis and demographic data were collected. The identification of the resistant isolates was
confirmed by calmodulin and β tubulin gene sequencing. They were investigated for susceptibility
to azoles such as itraconazole, voriconazole, posaconazole and isavuconazole using CLSI M38-A2
broth microdilution method. For detection of mutations leading to triazole resistance, all of the
resistant A. fumigatus isolates were subjected to mixed-format real-time PCR assay. Genotyping
of the resistant isolates was performed with a panel of nine short tandem repeat (STRs) and for
phylogenetic analysis Indian isolates were compared with Chinese, Dutch, German and French
isolates of A. fumigatus containing the TR{sub 34}/L98H genotype.

Results:
Seven A. fumigatus strains originating from 7 patients showed growth on voriconazole (VRC+) and
itraconazole (ITC+) SDA plates. Barring one all of the 7 seven patients were azole naïve. Of the
7 patients, 3 had invasive aspergillosis (IA), 3 allergic bronchopulmonary aspergillosis and one had
CPA. Of the 3 IA patients, biopsy and repeated BAL specimens yielded triazole resistant A. fumigatus
in two invasive pulmonary aspergillosis patients and in one patient with invasive sinusitis the
resected sinus tissue grew resistant A. fumigatus. Of the 4 patients with IA and CPA, 3 (75%) had
a fatal outcome which included 2 of IA and a solitary case of CPA. All of the resistant A. fumigatus
isolates had high MIC values of itraconazole (>16 mg/L), voriconazole (2 mg/L), posaconazole
(2 mg/L) and isavuconazole (8 mg/L) and exhibited a specific TR34/L98H mutations in cyp51A
gene. Phylogenetic analysis of the Indian TR34/L98H isolates showed identical STR genotypes but
were distinct from the TR34/L98H strains of Chinese, Dutch, French and German origin.

Conclusion:
The increasing rate of azole resistant A. fumigatus from 1.9% in 2005-2009 to 2.6% during
2009-2013 of TR_{34}/L98H in clinical setting in India is alarming. TR_{34}/L98H is the only resistant
mechanism prevalent and is consistent with a route of resistance development through exposure to
azole compounds in the environment.
VORICONAZOLE RESISTANT ASPERGILLUS FUMIGATUS CARRYING TR_{46}/Y121F/T289A MUTATION FROM THE INDIAN ENVIRONMENT

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Purpose:
The emergence of a new resistance mechanism, TR_{46}/Y121F/T289A in the cyp51A gene responsible for voriconazole resistance in Aspergillus fumigatus, has been recently reported in patients and from their environment in the Netherlands. Molecular studies of well-studied pan azole resistant A. fumigatus carrying TR_{34}/L98H mutation from Europe and India suggest that clinical azole resistant isolates originated from environmental or fungicide driven route of resistance development. The present study reports the occurrence of TR_{46}/Y121F/T289A in A. fumigatus strains from environmental sources in three cities of India.

Methods:
A total of 117 environmental soil samples from the agricultural fields of Yamuna bank, Delhi (n=63), Varanasi, Uttar Pradesh (n=42) and soil from Ganga river basin and nearby forest (n=12) area from Himachal Pradesh, were investigated during October 2012 to June 2013. The samples were inoculated on Sabouraud dextrose agar (SDA) plates and A. fumigatus colonies were subcultured on SDA plates supplemented with 4µg/mL itraconazole (ITC) and 1µg/mL voriconazole (VRC). Identification of ITC+ and VRC+ A. fumigatus strains was confirmed by ITS, β-tubulin and calmodulin gene. All the ITC+ and VRC+ A. fumigatus were subjected to a mixed-format real-time PCR assay for detection of mutations and genotyped with microsatellite analysis. In vitro activity for all the standard azole antifungals and the 10 azole fungicides (bromuconazole, cyproconazole, difenoconazole epoxiconazole, hexaconazole, metaconazole, penconazole, tebuconazole, triadimefon and tricyclazole) most commonly used in India were investigated using CLSI M38-A2 broth microdilution.

Results:
Of 117 soil samples, 47 (40%) samples (Delhi, n=27; Varanasi, n=10; Himachal Pradesh, n=10) yielded 198 A. fumigatus strains, including 88 from Delhi, 38 from Varanasi and 72 from Himachal Pradesh, on SDA plates. A total of sixteen isolates were resistant, of which eight each (6.8%) grew on 1 mg/L voriconazole (6 from Varanasi and 2 from Himachal Pradesh) and 4 mg/L itraconazole (4 each from Delhi and Varanasi). TR_{46}/Y121F/T289A originated from potato and Trigonella foenum-graecum (common name fenugreek) fields of Varanasi and Ganga river basin soil from Himachal Pradesh. The geometric mean MICs of 8 VRC+ A. fumigatus isolates showed reduced susceptibility of voriconazole (11.31µg/mL) and isavuconazole (8µg/mL). These isolates exhibited low itraconazole MICs (1.78µg/mL) but showed cross- resistance to all the fungicides tested. All eight VRC+ A. fumigatus isolates exhibited the TR_{46}/Y121F/T289A mutation.

Conclusion:
This is the first environmental survey outside Europe revealing presence of the TR_{46}/Y121F/T289A mutation in A. fumigatus strains with reduced susceptibility to voriconazole and cross resistance to commonly used fungicides. Genotypically the Indian environmental isolates were similar to Dutch clinical isolates. The widespread application of triazole fungicides and its persistence in the environment could have contributed to spread of azole resistant A. fumigatus. This resistance mechanism in A. fumigatus strains poses a threat to patients limiting the use of voriconazole, which is a drug of choice for invasive aspergillosis.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.

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Objectives:
Invasive aspergillosis (IA) is a major life-threatening disease in immunocompromised patients, with mortality rates from 40% up to 90% in high-risk populations. The most common species causing aspergillosis is Aspergillus (A.) fumigatus, accounting for approximately 90% of infections. Depending on regional distinctions, A. flavus and A. terreus are frequently reported. During infection, fungal pathogens must adapt to microenvironmental stresses, including hypoxia as well as high CO2 levels. Such oxystress conditions are usually not taken into account in current in vitro models of infection, the assessment of antifungal sensitivities or the release of biomarkers used for diagnosis.

Methods:
Using Etest strips, the in vitro activity of amphotericin B (amB), different azoles and echinocandins in hypoxic conditions (1% O2, 5% CO2) to their activity in normoxic conditions against aspergilli. For evaluation of biomarker release, the amount of β-1,3 glucan (BG) and galactomannan (GM) in Aspergillus supernatants was determined by commercially available detection kits (Platelia/Fungitell).

Results:
On surface cultures, we found a reduction of the minimal inhibitory concentration (MIC) for amB for all aspergilli in hypoxic conditions. Similarly, a significant reduction in the MIC for all tested azoles was demonstrated for A. terreus isolates, while for A. fumigatus isolates differences were less pronounced. For echinocandins, little or no change in the MEC (minimal effective concentration) was detected between hypoxic and normoxic conditions for all aspergilli. Most interestingly, A. terreus strains, that are resistant to amB in normoxia, exhibited sensitivity to amB in hypoxic conditions, defining a breakpoint of > 2 µg/ml. Notably, for none of the strains tested, MIC/MEC values increased in hypoxia. Our results so far indicate, that there is no significant difference in the amount of ergosterol whether mycelia is grown in hypoxia or normoxia.

The detection of circulating fungal antigens in serum for Aspergillus galactomannan or β-D-glucan has become an accepted diagnostic strategy. However, sensitivity and specificity vary extremely and the reasons are only partially clear; therefore, we are currently checking whether hypoxia influences the physiological kinetics of GM and β-glucan release.

Conclusion:
ECOFFs in hypoxia differ from those in normoxia for antifungal drugs targeting ergosterol or its biosynthesis. Supplementation of test media with blood or ergosterol abrogated the reduction of the MIC, but there is no direct link between ergosterol content and increased susceptibility to amB and azoles, but further analysis of sterol intermediates needs to be done in more detail.
FITNESS OF *A. FUMIGATUS* CYP51A TRIAZOLE RESISTANT STRAINS

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**Purpose:**
The emergence of *A. fumigatus* triazole resistant strains makes the antifungal triazole treatment obsolete for patients colonized by resistant strains. There are three known triazole resistance mechanisms in *A. fumigatus*: Cyp51A aminoacid substitutions due to point mutations; Cyp51A overexpression due to a tandem repeats in their promoter, and overexpression of efflux pumps that expel azoles consuming energy or exchanging H⁺. In order to understand the low environmental dispersion of some of the *A. fumigatus* triazole resistant strains, we decided to analyze the fitness *in vivo* and *in vitro* of *A. fumigatus* strains carrying the mutations M220K or G54W.

**Methods:**
M220K or G54W Cyp51A mutants were constructed with a barcode of 40 specific nucleotides long, which allowed us to design primers in order to difference and quantify them by qPCR. To restore the wild type (wt) phenotype, complemented M220K and G54W Cyp51A strains were also constructed. Sequencing and testing for itraconazole sensitivity were carried out for all strains.

**Results:**
M220K or G54W Cyp51A mutants became itraconazole resistant with a MIC ≥ 30 µg/ml after transformation. No significant differences in ct values between wt and mutant strains were found, *in vitro* or in lungs of immune-suppressed mice. The complemented strains restored the phenotype as wt, MICs (≤ 0.25 µg/ml),

**Conclusion:**
Wt and mutants strains grew comparably *in vitro* and *in vivo* assays. Reversion to wt suggested that the triazole resistance was due to only the presence of these point mutations.
ISOLATION AND IDENTIFICATION OF *Aspergillus Spp*, OTHER MOULD AND YEAST OF CONTAGIOUS SKIN NECROSIS IN CAMELS (Camelus dromedarius) IN GADARIF STATE, SUDAN

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**Purpose:**
To determine the ability of fungi to be one of the causative agent of contagious skin necrosis skin disease of camel.

**Methods:**
A total of 40 pus samples from suspected contagious skin necrosis lesions were collected with sterile absorbent swabs and were labelled with date of collection, sex, age and location. Samples were collected from Gadarif state during winter and summer. Swab from contagious skin necrosis lesion was cultured by dipping it into brain heart infusion broth and incubated for 1-2 days at 37°C. The isolate was sub cultured on Sabouraud’s dextrose agar and incubated at 37°C for 1-2 days. Identification of mould was done by rate of growth, texture and the pigmentation of cultures was used, (Laura, *et al*, 1998).

**Results:**
From forty swabs of camel infected with contagious skin necrosis, eleven mould isolates of three genera were recovered from forty samples on Sabouraud’s dextrose agar, *Aspergillus niger* (4 isolates), *Aspergillus flavus* (2 isolates), *Aspergillus terreus* (1 isolate), *Penicillium spp* (1 isolate) and *Scopulariopsis brevicaulis* (3 isolates). Three *Candida spp* were isolated and were identified as *Candida parapsillosis*, *Candida gulliermondii* and *Candida zeylanoides*.

**Conclusion:**
*Aspergillus spp* can be causative agent of contagious skin necrosis skin disease of camel.
DIAGNOSIS AND TREATMENT COMBINATION OF INVASIVE ASPERGILLOSIS AND MUCORMYCOSIS IN HEMATOLOGICAL PATIENTS IN SAINT-PETERSBURG, RUSSIA

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Objectives:
To analyze demographic parameters, underlying diseases, etiology, treatment and survival rate of hematological patients with combination of invasive aspergillosis (IA) and mucormycosis in St. Petersburg, Russia.

Methods:
The prospective study was conducted during the period 2007-2013 y.y. Diagnosis of IA and mucormycosis was made according to EORTC/MSG criteria (2008). Species identification of Aspergillus and mycormycetes was confirmed by sequencing of beta-tubulin and ITS/D1-D2 fragments of fungal ribosomal DNA, respectively.

Results:
We observed 15 hematological patients with IA and mucormycosis. The mean age of patients in this cohort was 25 years (range 5-56), male and female ratio 1:1, adults were 60%. Main underlying conditions in hematological patients with IA and mucormycosis were: acute myeloid leukemia – 33%, acute lymphoblastic leukemia – 33%, acute leukemia – 7%, chronic myeloid leukemia – 7%, chronic lymphoblastic leukemia – 7%, non-Hodgkin’s lymphoma - 7% and Hodgkin’s lymphoma – 7%.

Aspergillus spp. were isolated from 67% of patients. The main agent of IA was: A. fumigatus - 50%, A. niger - 20%, A. nidulans - 10%, A. flavus - 10%, Aspergillus spp. - 10%. Test «Platelia Aspergillus EIA» (Bio-Rad) was positive in 60% of patients.

Diagnosis of mucormycosis was confirmed by histology and direct microscopy of biopsy samples in all patients. Culture of clinical materials was positive in 73% cases: Rhizopus spp. (36%), Rhizomucor spp. (27%), Rhizomucor pusillus (18%), Lichtheimia corymbifera (9%) and Rhizopus oryzae (9%). The main sites of infection were lungs (67%), sinuses (33%), gastrointestinal system (7%), more then two organs were affected in 40% patients.

Antifungal therapy was performed all of patients: voriconazole - 60%, posaconazole – 33%, amphotericin B deoxycholate – 33%, caspofungin – 33%, amphotericin B lipid complex – 28%. Combination therapy was used for 33% of patients. Surgical treatment was used in 40% of patients. Duration of antifungal therapy was 3 - 180 days (median - 65). Overall survival at 12 weeks was 20%.

Conclusion:
The main underlying diseases in hematological patients with IA and mucormycosis - acute leukemia (73%). The main aetiology agents were A. fumigatus (50%) and Rhizopus spp. (45%). Twelve weeks overall survival was 20%.

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**Background:**
Chronic pulmonary aspergillosis (CPA) is a chronic, usually progressive infection in non-immunocompromised patients. Many underlying pulmonary diseases are associated with CPA. Contemporary series suggest a 75-85% 5 year mortality.

**Methods:**
Data from 392 patients treated for CPA at the UK’s National Aspergillosis Centre (NAC) prior to June 2012 were retrospectively analysed. The impact of age, sex, previous pulmonary conditions, dyspnea score, quality of life score and radiological appearances was assessed using Kaplan-Meier curves, Log-Rank tests and the Cox proportional hazards modelling. A sample of patient notes were used to examine the medical history and estimate the onset of CPA and the time taken from onset to referral to the NAC. Cause of death was recorded were possible. Statistical analysis was performed using IBM SPSS statistics package version 20. The project was registered with the hospital trust’s clinical audit department.

**Results:**
The mean age at referral was 59.4 years (range 18 to 86 years), 59.4% were males. Survival of patients with CPA at the NAC was 86% at one year from referral, and 57% at five years. Sub-groups with worse prognosis included those with a history of non-tuberculous Mycobacterium infection (p=<0.000). Age at referral was a strong predictor of mortality (Hazard ratio 1.058 per year, p=<0.000). The median time from estimated onset of CPA to referral to the NAC was 8 months (range 0 to 21 years).

**Discussion:**
A number of factors associated with increased risk of mortality from CPA were identified. The survival of CPA patients treated at NAC was better than reported previously by others. The long-term antifungal therapy approach undertaken at the NAC may contribute to this. Survival of patients may be under-estimated due to some patients presenting many years after the probable onset of disease.
DNA BASED ASPERGILLOSIS DIAGNOSTICS IN HUNGARY; STATE OF THE ART AND FURTHER CHALLENGES

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Purpose:
Despite the rapid development in antifungal therapy during the past decade invasive aspergillosis is the main cause of infection-associated mortality in patients being treated with haematological malignancies and is an emerging disease in solid organ transplant recipients. Mayor causative agents of this highly devastating systematic mycoses are mainly the opportunistic filamentous fungi of the Aspergillus genus. The saprophytic Aspergillus species are ubiquitous in our environment. The exposure to Aspergillus spores or conidia is therefore almost constant. Due to the immunocompromised state of these individuals aspergillosis can become invasive and cause systemic infections. In spite of the fact that in the status of the primary disease improvement may appear, the secondarily evolved infections lead to death. The only means of the survival is the specific antifungal therapy initiated early enough. For an improved patient outcome, early, sensitive and reliable diagnosis is mandatory. The most reliable microbiological and histopathological methods are time consuming, signs and symptoms of systemic diseases caused by Aspergillus species are non-specific and patients are often unable to undergo invasive diagnostic procedures. Real-time PCR technique is a highly sensitive method detecting small amount of fungal DNAs in biological samples and supporting diagnosis.

Methods:
We have developed species specific, sensitive quantitative real-time PCR diagnostic assays for detecting and identifying Aspergillus fumigatus and A. terreus, two of the most frequent species causing aspergillosis, in whole blood and serum respectively. Depending on the fungal orthologs of the Streptomyces facC gene present in Aspergillus fumigatus and A. terreus genomes (two copy and one copy respectively) our assays are dual-copy and single-copy assays. Recently we also consider that direct-PCR could be a new approach in the field, being well reproducible among different centers while enhancing the impact of species based nucleic acid detection methods.

Results:
The LoD values for our assays range between 5-10 GE in biological samples both in manual and in automated DNA extraction systems. We are performing routine aspergillosis tests in parallel with GM analyses both on whole blood and serum samples in Debrecen. Fungal infections were categorized according to the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group, National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria. Indication for sample taking is the neutropenic fever. DOR was 72. PPV and NPV were 12% and 83% respectively. The Kappa-value was 0,63 which corresponds to a fair agreement.

Conclusion:
Due to the fact that out of the human pathogen strains that could potentially exist in blood our biological markers, the facC orthologs are present exclusively in Aspergillus fumigatus and A. terreus strains. Therefore the high rate of false positive results which are given from the presence of other invasive pathogen species theoretically will be reduced to zero. We have also successfully tested our assays on different Aspergillus fumigatus and A. terreus clinical isolates as well as on many other pathogen species without facC orthologous markers and we did not experienced cross false-positives. Further clinical testings are still in progress.
Introduction:

Aspergillus fumigatus is an opportunistic pathogen known to cause a spectrum of diseases including Allergic Bronchopulmonary Aspergillosis (ABPA) and life-threatening angioinvasive pulmonary disease. A. fumigatus is the most common agent causing fungal infections in chronic lung disease such as Cystic Fibrosis (CF), most often presenting as ABPA. There are a number of antifungal agents available for the treatment of A. fumigatus infections but resistance to triazole antifungals in A. fumigatus isolates from CF patients has been reported. In this study the epidemiology of A. fumigatus in an Irish patient population that included CF patients pre and/or post itraconazole treatment in a major CF Centre (Hospital 1) and a non-CF patient population from a University Teaching Hospital (Hospital 2) was investigated and the antifungal drug susceptibility of all isolates collected was determined.

Methods:

A. fumigatus isolates from colonized adult CF patients (n=19) and from non-CF patients were collected (n=37). All isolates from the study were confirmed as A. fumigatus by PCR and sequencing of the ITS region. Isolates were genotyped using the Short Tandem Repeat assay for A. fumigatus (STRAf assay). Minimum Inhibitory Concentrations (MICs) of all A. fumigatus isolates to nine antifungal drugs were tested using the Sensititre Plate system (TREK Diagnostic Systems).

Results:

Three distinct A. fumigatus colonization patterns were observed in the CF cohort, (1) persistent colonization over time with the same genotype (≥2 consecutive samples with indistinguishable genotypes), (2) non-persistent colonization with distinguishable genotypes over time and (3) patients sharing an indistinguishable genotype suggesting the possibility of a common source of acquisition. No shared genotypes between the two hospitals were found. These colonization patterns were observed in both CF and non-CF patients. No antifungal drug resistance was observed from any study isolate, even for isolates collected following exposure to itraconazole for 6 weeks. Twelve of 56 A. fumigatus isolates had MICs of 2µg/ml for amphotericin B and further investigation is required here.

Conclusion:

No A. fumigatus genotype was linked with any one colonisation pattern. This suggests that A. fumigatus infection of CF patients may be a host trait. Some patients shared indistinguishable genotypes suggesting a common source. No triazole antifungal drug resistant strains of A. fumigatus were detected during this study.
MONITORING OF ASPERGILLUS FUMIGATUS DEVELOPMENT BY QUANTITATIVE PCR AND GALACTOMANNAN ENZYME IMMUNOASSAY FROM LUNG TISSUES OF INTRA-TRACHEALLY INFECTED TURKIES (MELEAGRIS GALLOPavo)

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Purpose:
Aspergillus fumigatus remains a major respiratory pathogen in birds, but diagnosis is still difficult. In poultry, aspergillosis may induce significant economic losses particularly in turkey production. The development of experimental models of infection is an essential prerequisite for a better understanding of the disease pathophysiology through characterization of the early stages of the fungal development in the air sacs and lungs and evaluation of relevant biological markers.

Methods:
We developed an infection model by intra-tracheal nebulisation of conidia via a stainless steel gauge atomizer (MicroSprayer®) in turkeys. We tested different inoculum doses (10^5, 10^6, 10^7, and 10^8 spores of Aspergillus fumigatus per bird) and assessed the quantity of fungal burden in lungs by numeration of colony forming units, quantitative PCR and galactomannan enzyme immunoassay from lung tissues. The histologic examination of lung confirmed the diagnosis of aspergillosis.

Results:
The nebulisation of spores in the trachea of one-week-old turkeys readily induced respiratory symptoms, typical macroscopic and microscopic lesions and inflammatory responses, whose intensity increased with the concentration of the inoculum. A significant mortality occurring in the first 96 hours after inoculation was only observed with the highest inoculum dose. Culture counts, GM index and qPCR results on one hand and inoculum size on the other hand appeared to be clearly correlated.

Conclusion:
An intratracheal nebulisation of 10^8 conidia per bird is necessary to reproduce an acute aspergillosis in young turkeys. The new model and the markers will be used for the evaluation of the efficacy of antifungal treatments that could be applied in poultry farms.
COMPARISON OF ASPERGILLUS PRECIPITIN WITH QUANTITATIVE ASPERGILLUS IGG ASSAY

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Purpose:
Detection of IgG antibody to *Aspergillus fumigatus* is important for diagnosing chronic pulmonary aspergillosis. Immunodiffusion assay has been widely used for detecting *Aspergillus* precipitin, however, it is seems to be time consuming and lack of sensitivity. Recently, quantitative measurement of IgG to *Aspergillus* by using fluorescent immunoenzyme assay (ImmunoCap™) is commercially available. In this study, we examined performance of ImmunoCap™ for detecting *Aspergillus* precipitin.

Methods:
From January 2007 to August 2013, we determined serum *Aspergillus* precipitin for 353 consecutive patients who suspected chronic pulmonary aspergillosis. We also measured specific IgG to *Aspergillus* for these collected sera by ImmunoCap™ (Phadia, Uppsala). The patients with history of antifungal treatment were excluded.

Results:
There were 249 male and 104 female. Median age was 72.6 years. One hundred and twenty-six patients (35.7%) were positive for *Aspergillus* precipitin. *Aspergillus* IgG level was significantly higher in the precipitin positive group (148.2 mgA/L) than in the precipitin negative group (39.8 mgA/L) (p <0.01). ROC analysis revealed that cut off value of ImmunoCap™ for detecting *Aspergillus* precipitin was 50 mgA/L. (AUC 0.83, sensitivity 0.78, specificity 0.76).

Conclusion:
The sensitivity and specificity of ImmunoCap™ for detecting *Aspergillus* precipitin was acceptable. Detecting circulating *Aspergillus* antibody by ImmunoCap™ in the early stage of disease may result in relatively high false positive rate (0.36). Thus, ImmunoCap™ may alternate with conventional precipitin test for diagnosing chronic pulmonary aspergillosis. In addition to it, the quantitation of IgG value might be useful for monitoring marker of antifungal treatment response.
MOLECULAR EPIDEMIOLOGY OF \textit{Aspergillus} Nosocomial Infections: A Novel Restriction Enzyme

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\textbf{Purpose:}
The aim of this study was searching the frequent \textit{Aspergillus} species causing hospital acquired infections and the environmental sources for \textit{Aspergillus} infections in hospital indoors.

\textbf{Methods:}
The subject of our study included bronchial fluids and sputum were collected from the hospitalized patients with acute respiratory symptoms. For the environmental study, some specimens were collected from air and environment surfaces. A morphological study was firstly performed including; growth characteristics and microscopic features of \textit{Aspergillus} species on mycological media. For the confirmation of \textit{Aspergillus} isolates which similarly found in clinical and environmental sources, PCR-restriction fragment length polymorphism using a novel restriction enzyme \textit{Mwo I} and the molecular technique of random amplified polymorphic DNA (RAPD) were carried out for searching the hospital aspergillus sources.

\textbf{Results:}
Totally of 102 fungal isolates, including \textit{Candida} species 82 (80%), \textit{Aspergillus} spp.20 (19.6%) and the other fungi 2 (0.4%). Among the clinical isolates, \textit{Aspergillus flavus} (8.4%), \textit{Aspergillus fumigatus} (4.2%) and \textit{Aspergillus niger} (2.8%) were identified, as well as environmental \textit{Aspergillus} isolates \textit{Aspergillus flavus} (19.4%), \textit{Aspergillus niger} (6.5%) and \textit{Aspergillus fumigatus} (6.5%).

\textbf{Conclusion:}
Comparing the clinical and environmental findings, 2 of 11 clinical \textit{Aspergillus} isolates were matched with environmental isolates from ventilation systems and wall surfaces, using RAPD but the other environmental sources were not confirmed.
TWO CASES OF SUCCESSFUL TREATMENT OF HISTOLOGICALLY PROVED CHRONIC NECROTIZING PULMONARY ASPERGILLOSIS

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Introduction:
Chronic necrotizing pulmonary aspergillosis (CNPA) usually occurs in patients with chronic lung
diseases (tuberculosis, sarcoidosis, chronic obstructive pulmonary disease (COPD), etc.). The
treatment efficacy of CNPA is not well investigated.

Objective:
We report two cases of successful treatment CNPA in non-immunocompromised patients.

Materials and methods:
Diagnosis criteria included clinical and radiological findings, serological and mycological tests.
Bronchoalveolar lavage (BAL) and sputum samples were examined (direct microscopy and culture
for fungal infection, specific test for M. tuberculosis). The diagnosis CNPA was confirmed by a
histological demonstration of tissue invasion by Aspergillus spp.

Results:
Case 1: A 48-year-old smoker man was admitted to hospital on February 2012 with a 3-month
history of cough, chest pain, fever and weakness. CT-scan results were non-specific and included
air cysts, fibrosis and traction bronchiectasis in the left upper lobe. Aspergillus precipitation test
was positive. Bronchoalveolar lavage (BAL) and sputum cultures (fungal and mycobacterial) were
negative. Diagnostic thoracotomy with biopsy was performed. Tuberculosis and sarcoidosis were
excluded. Histological examination of the biopsy specimens showed septate branching hyphae in
lung tissue and chronic inflammatory process with necrosis areas. The patient was treated with
itraconazole 200 mg bid. After three months of treatment, the patient was asymptomatic with
regression of lesions on CT. Overall duration of itraconazole treatment for 11 months. There was no
relapse in follow up period.

Case 2: A 59-year-old non-smoker man was admitted on May 2012 with long-term cough,
episodically bloody sputum and weakness. He had a medical history of pulmonary tuberculosis
and resection of the right upper lobe on 1977. On 2009 chest CT scan showed at right lung cavities
with a fungus balls. In 2011 CT scans revealed increasing in the number and size of lung cavities.
The IgG for Aspergillus was positive. Right pneumonectomy was performed on September 2012.
Histological examination detected a cavitary lesion with necrotic lung tissue invaded by septate
branching hyphae. The final diagnosis was CNPA and treatment with itraconazole (200 mg bid)
was started for 8 months. In follow-up period the patient’s condition stable, he had insignificantly
reduction of lung function, and normal range of vital capacity. CT signs of the disease were absent.

Conclusion:
Combination of surgical and antifungal therapy needed for successful treatment of chronic necrotizing
pulmonary aspergillosis. Itraconazole may be effective in the treatment of this disease.
PULMONARY COLONIZATION BY *ASPERGILLUS FUMIGATUS* AND SERUM SPECIFIC IgG AGAINST THIS FUNGUS IN PATIENTS WITH TUBERCULOSIS

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**Purpose:**

*Aspergillus* is a ubiquitous fungus, which causes a wide spectrum of infections including invasive pulmonary aspergillosis (IPA), depending on the patient’s immune status and underlying lung disease. Among the *Aspergillus* spp, *A. fumigatus* remains the predominant agent of IPA. In patients with a preexisting lung cavity from a variety of causes, such as pulmonary tuberculosis (TB) *Aspergillus* can colonize and grow into the cavity to form a pulmonary aspergilloma. Therefore, we assessed TB patients for pulmonary colonization with *A. fumigatus* and specific IgG against this species.

**Material and Methods:**

During one year, we studied 124 patients with TB at Massih Daneshvari hospital from Tehran, Iran. Sputum specimens were analyzed by direct microscopic examination (DME) with 20% potassium hydroxide. These samples were also processed for fungal culture. The clinical and radiological features or CT-scan report of all patients were recorded. All patients were screened for serum specific IgG against *A. fumigatus*, using *Aspergillus* IgG ELISA Kit (Genesis Diagnostics Ltd, Cambridgeshire, UK). The results are expressed in U/mL. IgG >12 U/mL was considered as positive result based on kit manufacturer instruction.

**Results:**

Out of 124 patients with tuberculosis (66 male, 58 female, age range: 10-91 years), 54 had abnormal chest radiographic findings. Chest X-ray findings showed that 48 patients (38.7%) exhibited residual cavities (31 cases in right lobe, 10 in left lobe and 7 in both lung). Round shaped mass lesion was detected only in 6 patients (6.8%). DME of sputum was positive in 10 patients for septate fungal hyphae. *A. fumigatus* was grown from 14 samples of TB patients. Out of 124 TB patients, 55(44.3%) cases were positive for specific serum IgG against *A. fumigatus*. There was a significant relationship between positive culture, DME and serum IgG profile level (*P* < 0.05). Totally, three patients (2.4%) met criteria for aspergilloma.

**Conclusion:**

Aspergilloma is a rare presentation of pulmonary aspergillosis and is usually incidentally found in immunocompetent patients with underlying lung disease such as TB. Colonization with *Aspergillus* in preexisting lung cavity produced by TB should be considered as a risk factor for aspergilloma.
DETECTION OF BISMETHYLGLIOTOXIN IN SERUM FROM PATIENTS WITH PROBABLE ASPERGILLOSA: VALIDATION OF THE BIOMARKER AND DEVELOPMENT OF AN ELISA TEST

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Purpose:
Invasive aspergillosis (IA) is the most common nosocomial opportunistic infection caused by pathogens of the genus Aspergillus (mainly by Aspergillus fumigatus) that occurs in patients who are severely immunosuppressed and carries a high mortality. This is due in part to the absence of optimal diagnostic modalities, which hamper early specific disease detection. We have previously shown that the secondary metabolite bis(methylthio)gliotoxin (bmGT) is detected in serum from patients with possible and probable aspergillosis. It is the aim of this study to monitor the presence of bmGT in sequential series of serum with possible/probable and proven IA and develop an ELISA kit to monitor the presence of this biomarker in serum from patients at risk of IA.

Methods:
A mAb against bmGT has been produced in mice and the conditions for its use in ELISA have been optimised. The presence of bmGT in sequential series of serum from patients at risk of IA (retrospective and prospective samples) or with proven aspergillosis (retrospective samples) is simultaneously analysed by chromatography and ELISA test and compared to the GMN index.

Results:
We have established a fast and sensitive ELISA test for bmGT detection in human serum. The specificity of this test has been validated in serum samples as well as in cell cultures of different Aspergillus strains including those that do not produce bmGT. In most cases we found a good correlation between bmGT and GMN values indicating that bmGT is a useful biomarker to confirm GMN positive values. However, in some samples either GMN or bmGT is negative. Preliminary analyses suggest that in some cases, in which sporadic GMN positives are found in patients, bmGT detection could be very useful to avoid false positives. Of note bmGT is detected earlier than GMN in some cases suggesting that it may be an early diagnosis biomarker. In addition, we have found that bmGT concentration drops faster than GMN index when therapeutic concentrations of voriconazole are found in serum. Finally the ELISA test is able to differentiate patients containing bmGT and GMN in serum from those that do not present biochemical evidences of infection.

Conclusion:
Detection bmGT in serum for patients at risk of IA would complement GMN index in order to improve diagnosis of IA. Moreover, our data suggest that by including determination of bmGT in routine management of patients at risk of IA we could improve the rate of false negative and false positive cases due to GMN determination. Our data provide the basis for the implementation of the bmGT ELISA test as routine Aspergillus diagnostics in clinical laboratories.
EFFECT OF GHRELIN FOR PREVENTION IN MURINE INVASIVE PULMONARY ASPERGILLOSIS MODEL

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Background:
Invasive pulmonary aspergillosis (IPA) occurs in immunocompromised patients and its mortality rate is quite high. Antifungal prophylaxis is commonly used, however, its efficacy and tolerability are insufficient.

Ghrelin is a peptide hormone that is produced mainly from stomach. It increases appetite and stimulates food intake in humans. It has also been shown to have an effect on inflammation and the immune system. In this study, we examined the effect of ghrelin in a mouse model of pulmonary aspergillosis.

Methods:
Seven-weeks-old female ICR mice were given 200 mg/kg cortisone acetate for immunosuppression at day 1 to 3. Mice were challenged with Aspergillus fumigatus conidia intratracheally at day 2. Ghrelin administration was initiated at day 0, and continued daily for 9 days. Survival rate were monitored throughout the study. In addition, mice were sacrificed on day 6 for fungal burden analysis, histopathological examination and cytokine measurement.

Results:
Survival rate was significantly improved in ghrelin-treated group (63%) compared with untreated group (0%). Body weight of ghrelin-treated group was significantly heavier than that of untreated group. Recovered fungal cell numbers from lung tissue were not significantly different in both groups.

Conclusions:
Ghrelin showed preventive effect in the IPA mouse model. Since ghrelin didn’t show anti-fungal activity, further studies for the mechanism of action of ghrelin is needed.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
COMBINATION OF INVASIVE ASPERGILLOSIS AND PNEUMOCYSTIS JIROVECII PNEUMONIA IN HEMATOLOGICAL PATIENTS

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Objectives:
To analyze demographic parameters, underlying diseases, etiology, treatment and survival rate of hematological patients with combination of invasive aspergillosis (IA) and P. jirovecii pneumonia in St. Petersburg, Russia.

Methods:
The prospective study was conducted for 5 years (2008-2013 y.y.) Diagnosis of IA and P. jirovecii pneumonia was made according to EORTC/MSG criteria (2008).

Results:
4 hematological patients had been observed with IA and P. jirovecii pneumonia from 2008 up to 2013. The mean age was 25 years (range 18-34), males - 100%, adults - 75%. Main underlying conditions were: acute myeloid leukemia – 50%, acute lymphoblastic leukemia – 25% and Hodgkin’s lymphoma – 25%.

The diagnosis of IA was confirmed by mycological investigation. At direct microscopy of bronchoalveolar lavage (BAL) septate hyphae branching at acute angle were found in one patient (25%). *Aspergillus fumigatus* was isolated from BAL of one patient (25%). Galactomannan test in BAL ("Platelia Aspergillus", BioRad) was positive in 100% of patients.

The diagnosis of *P. jirovecii* pneumonia was confirmed by «Monofluo» (Bio-Rad) test (75%) and cytology in BAL (25%).

Antifungal therapy of was performed for all patients: voriconazole (100%), caspofungin (50%), amphotericin B deoxycholate (25%). Therapy of *P. jirovecii* pneumonia by cotrimaksozole was performed for all patients. Duration of antifungal therapy was 30 - 157 days. Overall survival at 12 weeks was 75%.

Conclusions:
This study confirms possibility of combination of invasive pulmonary aspergillosis and *P. jirovecii* pneumonia in patients with the oncohematological pathology. The early diagnosis and appropriate treatment are required.
LECTINS OF HUMAN PROBIOTIC BACTERIA INFLUENCE BIORHYTHMIC LANDSCAPES OF HUMAN BIOFILMS “CANDIDA – ASPERGILLUS/PENICILLIUM”: RESISTANT IN TIME LYSIS REGIONS POSSESSING SYNERGISTIC BROADENED ANTIMICROBIAL SPECTRUM

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Purpose:
Probiotic bacterial lectins (PBL) are members of new class of antimicrobials, synergistic metabolic imitators of probiotics, perspective ingredients of synbiotics. The aim is to study influence of PBL towards heterobiofilms involving human Ascomycetes.

Methods:
PBL from industrial strains of probiotic strains of lactobacilli and bifidobacteria of human origin (acidic and basic PBL as four preparations), freshly obtained and identified fungal isolates from patients, prolonged growth of isolates on agar in Sabouraud medium in the presence of discs-PBL in stress conditions at low temperatures, analysis of computer photos.

Results:
1. Mosaic distribution of discs-PBL influenced forming and distribution of final “Yeast like Candida – filamentous fungi” massives as assymetric landscapes of “Lacunas - Hills” type. Aspergillus or Penicillium coisolates protected residual Candida biofilm at both steps of colonia forming and further growth as upper layer resulting in decreasing summary Lacunas regions in plates.

2. Mycobiota biorhythm of changing PBL-initiated symbiotic landscapes was observed. Aspergillus colony forming was localized along borders between Lacunas and Candida residual biofilms. Primary Aspergillus brown pigment population (later as black one) was spread Candida sensor thick border along gradient of increasing Candida mass in direction of formal centers of local communicative bodies. Similarly to Aspergillus (but later), Penicillium formed independent compact homogenous massives (of age depended on varying green color for separately placed massives) on free exposed residual Candida biofilms. Aspergillus or Penicillium layers protected Candida biofilms from further lysis.

3. Lacunas were completely free of microbiota for a long time and served as reservoirs of antimicrobials broadening in spectrum upon prolongation. Three months later, the only seldom spots (1-3 of 5-8 mm in diameter, per plate) of one type non-pigmented conidial fungus were registered. 4. Disc’s surface and closed to discs events were non-significantly depended on PBL type.

Conclusions:
Results further develop our conception of communicative bodies for cases of Ascomycetes communities similar to “Yeast like fungi – Filamentous fungi” heterofungal biofilms. PBL may serve as regulators of earlier mycobiota biofilm forming as well as destructors of Candida based biofilms involving later Aspergillus or Penicillium. Anti-candidosis agents and factors may be effective against aspergillosis. PBL-initiated primary and secondary contents of Lacunas can serve as regions of directed, controlled, diagnostic, prognostic and constructed antimicrobial events, natural locally different sources of synergistic suitable panels and mixtures of antimicrobials depending on mosaics of surrounding discs contributors. “Lacunas – Biofilms” can serve as important informative model of functioning relatively pathogenic fungal communities in the presence of constructed symbiotic non-cellular ingredients and alternatives. New antifungal strategies (for example, against aspergillosis) are proposed, and new mechanisms of antimicrobial actions (for example, antymycotic ones) of symbiotic biotope compartment in human are predicted.
CLINICAL RISK FACTORS AND BRONCHOSCOPIC FEATURES OF INVASIVE ASPERGILLOSIS IN INTENSIVE CARE UNIT PATIENTS

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Purpose:
Invasive aspergillosis (IA) is an important cause of morbidity and mortality in immunocompromised patients. During recent years, a rising incidence of IA in ICU patients has been reported. The patterns of IA related infection may differ according to the type of underlying disease. Unfortunately little is known about the characteristics of IA in ICU patients. In the present study we assessed IA related clinical and bronchoscopy findings in ICU patients.

Methods:
This study was performed at the ICU units in Sari, Mazandaran from August 2010 through September 2011. We retrospectively analysed 43 ICU patients with underlying predisposing conditions. Bronchoalveolar lavage (BAL) samples were collected by bronchoscope twice a weekly. The samples were analyzed by culture and non-culture based diagnostic methods for the occurrence of IA. Patients were assigned a probable or possible diagnosis of IA according to the consensus definition of the EORTC/MSG.

Results:
Out of 43 suspected patients to IA, 13 (36.1%) cases showed IA. According to criteria presented by EORTC/MSG, they were categorized as: 4 cases (30.8%) of possible IA and 9 (69.2%) of probable IA. The observed mortality was 69.2%. The main underlying predisposing conditions were neutropenia (53.8%), COPD (30.8%) and hematologic malignancy (15.4%). The macroscopic finding in bronchoscopy included of Prulent secretion (46.6%), Mucosal bleeding (33.3%), Mucosal erythema (26.6%), Trachobronchomalasia (13.3%).

Conclusion:
The diagnosis of IA in patients with critical illness in ICU is even more difficult. The clinical diagnostic process is often dependent on indirect circumstantial data enhancing the probability of IA. Bronchoscopy with inspection of the tracheobronchial tree, sampling of deep airway secretions and BAL can be helpful.
IN VITRO COMBINATION THERAPY OF ISAVUCONAZOLE AGAINST MEDICALLY IMPORTANT ASPERGILLUS SPP

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Purpose:
Isavuconazole is an extended-spectrum antifungal triazole with in vitro, in vivo, and clinical activity against Aspergillus spp. and invasive pulmonary aspergillosis. The purpose of our study was to assess whether isavuconazole has synergistic activity in combination with an echinocandin or amphotericin B.

Methods:
Clinical isolates of three Aspergillus species (A. fumigatus, A. flavus and A. terreus) were studied. Minimum inhibitory concentrations (MICs) were determined by broth microdilution methods according to the reference procedure of antifungal susceptibility testing of filamentous fungi (CLSI-M38-A2). In vitro interactions between isavuconazole and micafungin or amphotericin B were studied using a two-dimensional checkerboard microdilution method in 96-well flat-bottom plates. Each isolate was tested three times on different days. The choice of the appropriate range of drug concentrations was based on the MICs of the individual drug and isolate. The combined effects of antifungal agents were quantified after 48 h of incubation at 37ºC by the metabolic reduction XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]2H-tetrazolium-5-carboxanilide) assay. The interactions between antifungal agents were analyzed using the fractional inhibitory concentration index (FICI) and the Bliss independence model.

Results:
For the isavuconazole-amphotericin B interactions, the median FIC indices of all isolates ranged from 1 to 1.5 indicating an additive or indifferent effect. No value of FICI>4, which is indicative of antagonism, was observed. However, according to the Bliss model, the combination of isavuconazole and amphotericin B resulted in antagonistic interactions in A. fumigatus and A. flavus. Treatment of A. terreus with isavuconazole (0.030 to 16 mg/liter) and amphotericin B (0.125 to 8 mg/liter) showed antagonistic interactions at high amphotericin B (1 to 8 mg/liter) and isavuconazole (1 to 16 mg/liter) concentrations, while at low amphotericin B (0.125 to 0.5 mg/liter) and isavuconazole (0.250 to 2 mg/liter) the interaction was synergistic. However, the magnitude of these interactions (related to the ΔE value which were 0.04% and 1.35% for the antagonistic and synergistic effect, respectively) was not considered to be pharmcologically significant.

For isavuconazole-micafungin interactions, median FICI of all strains ranged from 0.28 to 1.06, which were compatible with synergy. The combination of isavuconazole and micafungin resulted in synergistic interaction in all strains when analyzed by Bliss modeling. The degree of synergy was ranged significantly from 19.3% to 65.59% (mean ΔE value) with the highest was noted in A. flavus.

Conclusion:
The combination of isavuconazole with micafungin demonstrates strong in vitro synergy against A. fumigatus, A. flavus, and A. terreus. In vivo studies are required to validate this synergy as a potentially effective strategy in treatment of invasive aspergillosis.
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**Purpose:**
New *Aspergillus* species have been described as emergent pathogens. Variable antifungal susceptibility profiles among species have also been reported and antifungal resistance in *Aspergillus* strains has been increasing.

After environmental screening for *Aspergillus*, a large number of isolates from *Aspergillus ochraceus* (*Circumdati*) complex, especially found in hospital environment, necessitated performing more detailed studies of those isolates.

**Methods:**
A polyphasic approach was therefore applied in the identification of cryptic species among this complex. Morphological features and molecular analysis (β-tubulin and calmodulin sequencing) were performed.

**Results:**
A high prevalence of cryptic species within that complex was detected and from the 16 isolates analyzed, at least five different cryptic species were identified (*A. ochraceus*, *A. westerdijkiae*, *A. insulicola*, *A. ostianus*, *A. sclerotium*), most of them growing at 37°C.

Antifungal susceptibility patterns of those isolates revealed high minimal inhibitory concentrations (MIC) to amphotericin B (geometric mean = 6.17) and itraconazole (geometric mean = 3.67).

**Conclusion:**
Regarding the high prevalence of isolates from *Circumdati* complex in hospital environment, their reduced susceptibility to several antifungals, ability to grow at 37°C and their potential for producing mycotoxins that could aid tissue invasion, this complex is probably underestimated as etiological agent of infection.

This work aims to contribute to a deeper knowledge of this complex, raising the attention towards possible infections caused by these species and the implications of their presence in hospital environment.
IDENTIFICATION OF *ASPERRILUS FUMIGATUS* WALL COMPOUNDS THAT AFFECT ACTIVITY AND FUNCTION OF HUMAN PLATELETS

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**Purpose:**
*Aspergillus fumigatus* is able to interact with platelets and to trigger their activation. As putative consequences, platelets might start an efficient antifungal immune reaction, but also contribute to some hallmarks of invasive aspergillosis such as inflammation and thrombosis. Since these processes might profoundly influence the course of infection, we aimed to identify those surface structures on conidia and hyphae that mediate the interaction with thrombocytes.

**Methods:**
Human platelets derived from concentrates were incubated with *A. fumigatus* conidia, hyphae, melanin ghosts or isolated cell wall components. Activation of the platelets was quantified by measuring CD62P, annexin binding, or phagocytic capacity by FACS analysis. *A. fumigatus* mutants lacking defined surface structures were also used for some experiments.

**Results:**
Conidia of *A. fumigatus* were potent triggers of thrombocyte activation and CD62P exposition, with only minor differences between various fungal isolates of this species. The activatory capacity of the conidia is at least partly due to melanin, since purified melanin ghosts were also able to bind platelets and to induce thrombocyte stimulation. In addition, an *A. fumigatus* mutant lacking melanin pigmentation showed significantly reduced potency to activate platelets. In contrast, the conidial hydrophobin layer is masking relevant structures for thrombocyte stimulation, since incubation of thrombocytes with an *A. fumigatus* mutant lacking the hydrophobic RodA protein induced a higher CD62P signal than the referring wild-type conidia.

Not only the *A. fumigatus* conidia, but also the hyphae contain surface compounds that interact with platelets; a crude preparation of hyphal cell wall components was capable to stimulate the release of both alpha and dense granules by platelets. This effect was caused neither by galactomannan, nor by chitin or β-glucan, since the isolated compounds had no effect on the thrombocytes. In contrast, the newly identified fungal polysaccharide galactosaminogalactan potently triggered platelet activation.

**Conclusion:**
Melanin and hydrophobin on *Aspergillus fumigatus* conidia as well as galactosaminogalactan on the hyphae represent important virulence factors that modulate thrombocyte activity in patients with disseminated infection. A putative consequence could be a platelet-driven antimicrobial response and inflammation. On the other hand, this process might end up in thrombosis, tissue damage, and phagocyte-driven thrombocyte loss.

**NOTE:** THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
FUNGUS-DRIVEN OPSONIZATION OF PLATELETS MIGHT INFLUENCE THE PATHOLOGY OF INVASIVE ASPERGILLOSIS

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Purpose:
Thrombocytes and the complement system are known to be part of the innate immune defence in fungal infections and affect the pathogenesis of invasive aspergillosis. Previous results showed that conidia, hyphae and culture supernatants of Aspergillus can activate platelets. Based on these facts we investigated if this leads to platelet opsonization by complement with subsequent consequences for other immune cells.

Methods:
Human platelets were available as concentrates; serum from a pool of healthy donors was used as complement source. Aspergillus (A.) fumigatus was grown in RPMI medium; the culture supernatant with secreted fungal factors was obtained by filtration. Complement deposition and presence of membrane-bound complement regulators as well as cell activation were investigated by flow cytometry (FACS). Measurement of mitochondrial activity (MTS assay) and a live-dead staining were used to assess cell viability. Interaction between granulocytes and thrombocytes was studied by lifetime microscopy.

Results:
Induction of platelet activation by A. fumigatus supernatant (SN) also leads to subsequent deposition of complement proteins on platelets, which was detected by quantification of complement factor C3 on their surface. We could show that this stimulation of the complement cascade is at least partially triggered by secreted fungal compounds that degrade or mask membrane-bound complement regulators on the platelets.

Fungus-induced opsonization of thrombocytes might result in their lysis by formation of the membrane attack complex (MAC), enhanced binding to and/or activation of neutrophils, and clearance by phagocytosis.

A significant decrease of platelet mitochondrial activity could be shown by MTS assay. Decreased viability and membrane damage was also detected by live-dead staining using a dye that binds to intracellular amines; massive presence of dead thrombocytes in the presence of fungal SN and complement further confirmed these findings.

The activity of neutrophil granulocytes was not markedly modulated by contact to opsonized platelets. Quite in contrast to this finding, the uptake of thrombocytes by neutrophils was significantly increased when they were pretreated with fungal supernatant and serum.

Conclusion:
Secreted fungal factors can induce both activation of platelets and deposition of complement on their surface. This opsonization reduces their viability and increases their clearance by phagocytes. These effects of platelet activation and opsonization may be important principles for frequently occurring thrombosis and thrombocytopenia in invasive aspergillosis.
40 STUDY OF THE MTFA TRANSCRIPTION FACTOR IN THE OPPORTUNISTIC FUNGAL PATHOGEN ASPERGILLUS FUMIGATUS

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Purpose:
Aspergillus fumigatus is the leading causative agent of invasive aspergillosis. The number of cases are on the rise with mortality rates being as high as 90%. This study covers a novel regulatory gene in A. fumigatus that could potentially be used to design novel anti-fungal therapies. In this work we investigated the role of the transcription factor gene mtfA in A. fumigatus. Our recent study of the mtfA ortholog in the model fungus Aspergillus nidulans revealed that mtfA is a master genetic regulator that governs sexual and asexual development as well as the production of various secondary metabolites. In the present study we show that mtfA in A. fumigatus controls veA and laeA expression and plays a role in growth, development gliotoxin production, protease activity and virulence.

Methods:
In order to characterize mtfA in A. fumigatus deletion mtfA, complementation, and over-expression strains were generated. Colony growth was determined by point inoculating the strains on Czapek-Dox medium and allowing them to grow for five days. Conidiation data was collected from top-agar inoculated cultures at 48 and 72 hrs. For all gene expression data, strains were inoculated in liquid stationary cultures and samples collected at 48 h and 72 h. Gene expression was measured by qRT-PCR. Gliotoxin production was analyzed on culture filtrates at 3 d and 5 d by HPLC. Protease activity was measured using an Azo-Casein Assay. Virulence studies were done using the Galleria mellonella infection model.

Results:
Deletion and over-expression of mtfA in A. fumigatus resulted in a decrease in colony growth (deletion of mtfA resulted in a 18% decrease in colony diameter while over expression resulted in a 24% decrease). Complementation of the mtfA deletion strain with the wt-type allele was able to partially restore wild-type colony growth. Along with a decrease in colony growth, both deletion and over-expression of mtfA resulted in a decrease in conidiation. Our studies also showed that mtfA is necessary for proper levels of veA and of laeA expression. laeA expression was most affected (over-expression of mtfA in A. fumigatus resulted in a two fold increase in laeA expression levels at both 48 h and 72 h after inoculation). Additionally, protease activity levels in the deletion mtfA mutant were decreased 50% with respect to the wild-type strain, while mtfA over-expression resulted in an increase in protease activity up to 116%. With respect to gliotoxin, both deletion and over-expression of mtfA resulted in an increase in the production of this compound at 72 h and 120 h. At 72 h the mtfA deletion strain showed a 5.7 fold increase in gliotoxin with respect to wild-type and over-expression showed a 1.8 fold increase. At 120 h deletion mtfA showed a 6.2 fold increase in gliotoxin production and over-expression levels were 8.7 fold greater. Although an increase in gliotoxin was observed, in the Galleria infection model deletion of mtfA resulted in a decrease in virulence. Thirty percent of the larvae infected with the deletion mutant were still alive at the end of the study whereas only 6% of those infected with the isogenic wild-type strain remained living. Over-expression showed no statistical difference in survival rate with respect to wild type in this study.

Conclusion:
Our studies have revealed that mtfA plays a role in growth and conidiation in the opportunist pathogen Aspergillus fumigatus. Interestingly, mtfA regulates the expression levels of the global regulators veA and laeA, known to control development and secondary metabolism in many fungal species. Proper levels of mtfA are required for wild-type levels of protease activity. Gliotoxin production in A. fumigatus is also affected by mtA. Importantly, deletion of mtfA resulted in decreased virulence in a Galleria infection model.

NOTE: THIS ABSTRACT AS BEEN SELECTED FOR ORAL PRESENTATION.
41 THE BIOINFORMATICS AND MOLECULAR APPROACHES FOR IDENTIFICATION OF NOVEL DRUG TARGETS AGAINST THE HUMAN FUNGAL PATHOGEN ASPERGILLUS FUMIGATUS

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Purpose:
Aspergillus fumigatus is currently the most significant air-born fungal pathogen and the leading cause of invasive aspergillosis (~85% of cases). Despite the introduction of new effective antifungals, the mortality rate of invasive aspergillosis still exceeds 40%. Moreover, the emergence of drug resistance adds more complications to the treatment process. Hence, new antifungal agents are urgently required. So finding novel targets against Aspergillus fumigatus was our purpose.

Methods:
First of all, Bioinformatics tools have been used in this study to compare whole proteomes of two organisms, A. fumigatus as a human pathogen with yeast Saccharomyces cerevisiae. Afterwards, the RBP gene was discriminated for knocking down and evaluating by RNAi and Homologous recombination methods. Finally, MIC test has applied for comparing mutant and wild strains.

Results:
Based on the LAST alignment of whole proteomes (9,630 proteins), 474 unique proteins were recognized for A. fumigatus as the homologous of these proteins did not exist in S. cerevisiae. All of the 474 proteins were considered by the following databases: KEGG, NCBI, EXPASY and EBI. Functions of 161 proteins have been explained and their accession numbers are available. Finally, 50 proteins were selected from 161 proteins, these proteins have been annotated, their functions exist and also only belong to fungi. AfuRbp deletion fragment was used for transformation of A. fumigatus (akuAKU80 pyrG-) protoplasts. Primary screening of transformants using PCR demonstrated that 3 out of 10 colonies were deletants. RT-PCR analysis on these deletion strains confirmed that AfuRbp does not express in these mutants (data not shown). One of these deletion mutants was selected and subjected to further analysis. The growth phenotype of this mutant was examined in various media containing different carbon and nitrogen sources and the results showed no significant difference between this strain and the parental strain (data not shown). This indicates that AfuRbp is not essential for growth of A. fumigatus under tested condition. However, MIC tests with Juglone, Phenylglyoxal, Cyclosporine A (CYC) and FK506 have shown significant sensivity in mutant strain.

Conclusion:
Our alignment has demonstrated the group of genes that are specific and may be essential in A. fumigatus. In this study, we evaluated RBP protein by bioinformatics approach as a sample from fifty proteins that were obtained by comparative alignment. The RBP protein was annotated as a PPIs enzyme family. So this was seemed a proper target. For analyzing the RBP three inhibitors selected and synthesized and MIC tests were done with this inhibitors and wild together with mutant strains. Mutant strains that were used in this study did not have the RBP protein with RNAi and Homology recombination methods. According to gained results of MIC tests, evaluating the RBP protein was successful but it was not essential for A. fumigatus. Maybe it can be deduced that the RNA Binding Protein plays mediate role in a significant pathway with other gene or genes. Thus, as for the obtained results, the RBP possibly not proper target for drug discovery. Moreover, other remains forty nine proteins can suitable target for future studies.
Purpose:
The purpose of this work was to establish if Caspofungin induced an oxidative stress in *Aspergillus fumigatus*

Methods:
Proteomic analysis was performed to identify proteins altered in expression following exposure of *A. fumigatus* to caspofungin. The activity of a variety of enzymes was analysed.

Result:
Exposure of *A. fumigatus* to 0.1 or 1.0 µg/ml caspofungin for 4 hours resulted in protein leakage from hyphae and the increased expression of a variety of proteins involved in the oxidative stress response (e.g. catalase, antibiotic response protein), virulence (e.g. Asp F3, 18kDa antigen) and homeostasis (e.g. glyceraldehyde 3-phosphate, translation elongation factor, ATP synthase F1). In addition there was an increase in the activity of catalase, glutathione reductase and superoxide dismutase in hyphae exposed to 0.1 µg/ml caspofungin for 1 hour.

Conclusion:
The results indicate that when non-growing cultures of *A. fumigatus* were exposed to caspofungin there was an increase in the expression of a number of proteins associated with virulence and the oxidative stress response, and the activity of detoxifying enzymes possibly in an attempt by the fungus to circumvent the intracellular effects of the antifungal agent.
SULPHUR METABOLISM AFFECTS ASPERGILLUS FUMIGATUS PATHOGENESIS

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Purpose:
Sulphur is an essential macro-element for all living organisms, as S-containing metabolites are involved in a multitude of fundamental cellular processes. Consequently, all pathogens have to absorb sulphur compounds from the infected tissue to propagate inside the infected host. Despite of the importance of sulphur assimilation, its relevance in fungal virulence is largely unexplored. Here, we describe our efforts to investigate sulphur assimilation in *Aspergillus fumigatus*, aiming to broaden the knowledge on fungal virulence and to identify novel targets for antifungal therapy.

Methods:
In a pioneering approach, the *A. fumigatus* transcription factor MetR was targeted in order to characterise the *in vitro* phenotype, gene expression patterns, and virulence capacity of the corresponding deletion strain. After proving the importance of sulphur assimilation and to interrogate this pathway further, focus was set on three putative sulphur-containing compounds - sulphate, cysteine and methionine - that may serve as S-source during invasive pulmonary aspergillosis. Accordingly, selected genes were targeted to construct deletants strains that allow assessing any relevance of the specific S-containing compound for intrapulmonary growth of the fungal pathogen.

Results:
We demonstrate that the MetR transcription factor is essential for growth on a variety of sulphur sources and fundamental for assimilation of inorganic S-sources, but dispensable for utilization of methionine as it supports expression of genes related to inorganic sulphur assimilation. MetR action is further required for proper regulation of iron homeostasis, which demonstrates an unprecedented regulatory crosstalk of sulphur metabolism with this *A. fumigatus* virulence determinant. Moreover, MetR is essential for progression of invasive aspergillosis (IA) in leukopenic mice, showing the importance of sulphur assimilation for virulence. Elimination of the sulphate transporter-encoding gene *sB* completely prevents growth on sulphate as sole S-source but does not affect fungal virulence, demonstrating that uptake of sulphate appears to be dispensable *in vivo*. A double *cysBA; mecaA* mutant, which represents a cysteine auxotroph, displays a significant reduction in virulence, suggesting that cysteine availability is limited in the murine lung. Surprisingly, the methionine synthase-encoding gene *metH* is essential, proven via heterokaryon rescue and conditional promoter replacement by a doxycycline-dependent TetOn module. The latter approach furthermore permitted verification of this gene’s *in vivo* requirement.

Conclusions:
In this study we demonstrate the importance of sulphur assimilation and particularly of sulphur-containing amino acids for *A. fumigatus* virulence, thereby broadening our understanding of this fungus’ pathobiology and providing novel targets for antifungal therapy.
Differential Regulation of the Host Response to *Aspergillus fumigatus* by Nod Receptors

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Purpose:
Invasive aspergillosis remains one of the most severe complications in immunocompromised patients. Screening for polymorphisms, that can make these patients potentially more susceptible to develop aspergillosis, can prove to be beneficial in preventing invasive infections. Several studies demonstrate that the intracellular pattern recognition receptor nucleotide-binding oligomerization domain (NOD)2 plays an important role in the host defense against *Aspergillus*. The purpose of our study was to investigate how polymorphisms in NOD2 influence the host response to *A. fumigatus*. Additionally, we investigated whether the other NOD receptor, NOD1 and the RICK kinase (shared by both receptors) are also involved in the host response to *Aspergillus*.

Methods:
Human peripheral blood mononuclear cells (PBMCs) with polymorphisms in NOD2 (rs2066847 and rs2066842), NOD1 (ND1+32656 T/GG) and RICK (rs42490) were stimulated with *Aspergillus* to investigate how these variations modulated the innate cytokine response. To compare the effect of polymorphisms with complete deficiency of these receptors, splenocytes of wildtype, NOD1-/-, NOD2-/- and RICK-/- mice were stimulated with *Aspergillus*. Immunofluorescence staining for NOD1 was performed with anti-NOD1 antibody on monocytes that were exposed to FITC-labeled conidia.

Results:
We found that PBMCs with polymorphisms in NOD2 and cells of NOD2-/- patients responded with both decreased innate and IL-17, IL-22 and IFNγ responses to *Aspergillus* stimulation. This decreased production of T-cell cytokines correlated with a decreased proliferation of IL-17+, IL-22+, and IFNγ+ T-cells upon stimulation with *Aspergillus*. By immunofluorescence staining we were able to demonstrate that NOD1 localizes to phagocytosed conidia in monocytes, suggesting a role for NOD1 in *Aspergillus* recognition. In contrast to NOD2, PBMCs with a polymorphism in NOD1 responded with increased innate cytokine responses compared to wildtype PBMCs. Complementary, we found that NOD1-/- murine splenocytes also responded with increased innate and T-cell responses to *Aspergillus* stimulation. RICK kinase is responsible for the downstream signaling of both the NOD receptors. We found that a polymorphism in RICK results in an increased cytokine response to *Aspergillus*. Furthermore, RICK-/- murine splenocytes demonstrated a similar increased cytokine response to *Aspergillus* similar to NOD1-/- splenocytes.

Conclusions:
Our data demonstrates that polymorphisms in the NOD receptors are differentially regulating cytokine signaling in response to *Aspergillus*. On the one hand NOD2 is required for induction of responses while on the other hand NOD1 localizes to phagocytosed conidia and together with RICK reduces *Aspergillus*-induced cytokine responses. To discern whether these polymorphisms are either advantageous or detrimental our future experiments are aimed to investigate the role of these polymorphisms in susceptibility to invasive aspergillosis.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
Purpose:
Aspergillus fumigatus (Af), the most common agent of invasive aspergillosis (IA), is an environmental fungus that forms airborne conidia. Humans inhale Af conidia daily and asymptomatic clearance depends on the functional and numeric integrity of the respiratory innate immune system, with a predominate role for neutrophils. In neutropenic patients, conidial germination into tissue-invasive hyphae (filaments) is the central step in IA pathogenesis.

Although the Toll-like receptor adaptor protein MyD88 and the C-type lectin receptor signal transducer CARD9 are implicated in conidial recognition and in mounting inflammatory response following conidial challenge, their role in controlling neutrophil function during respiratory fungal infection remains poorly understood. We have examined the coordination of MyD88 and CARD9 in building a protective immune response against inhaled conidia in the lung.

Method:
We infected mice by intratracheal instillation of Af conidia. We assessed lung inflammation by histology, and measured the levels of cytokines and chemokines released by in situ hybridization and enzyme-linked immunosorbent assay. To investigate neutrophils function, we infected mice with the fluorescent Aspergillus reporter (FLARE) strain that simultaneously report the phagocytic and conidiacidal activity of leukocytes at a single encounter resolution. Conidial uptake and viability in neutrophils of the FLARE infected mice were measured by flow cytometry.

Results:
Investigating neutrophil function post-infection, we found that MyD88 deficiency led to enhanced murine susceptibility to respiratory fungal infection, delayed neutrophil recruitment and reduced chemokine secretion in the lung of infected mice at 10 h post-infection. Our results further indicate that MyD88 signalling in radioresistant cells is critical for generating this early neutrophil response. However, using a fluorescent reporter of fungal viability, we found that MyD88 is dispensable for neutrophil conidial uptake and killing in the lung.

In contrast, CARD9 signaling controls neutrophil recruitment to the lung at 36 h and 72 h post-infection. Consistent with biphasic control of neutrophil recruitment by MyD88 and CARD9, we found that the MyD88 and CARD9 double knockout mice have severely impaired neutrophil recruitment at both phases of infection, and develop and succumb to IA rapidly following experimental infection.

Conclusion:
In sum, our data demonstrate that MyD88 and CARD9 act in a sequential manner to control neutrophil recruitment to infected lungs, demonstrating coordinate regulation of neutrophil influx by TLR and CLR signaling pathways that operate predominately in non-hematopoietic and hematopoietic cells, respectively.
Purpose: The D receptor and D metabolizing enzymes are expressed by immune cells. Interest in the effect of D on aspergillosis stems from the demonstrated D effects, in humans and mice, on T & B cells, including Tregs, cytokine production, macrophages, antigen-presenting cells, antimicrobial peptides; Toll-like, Dectin-1 and mannose receptors; & microbial killing, all relevant to the importance of Th1/Th2 and effector phagocytes in aspergillosis. Favorable D effects have been shown in tuberculosis. D deficiency increases Th2 responses to \textit{A. fumigatus} (Af), and D suppresses Th2 responses to Af in cystic fibrosis-ABPA. Another issue is whether D could modulate the pro-inflammatory effects of amphotericin B (AmB) therapy in aspergillosis.

Method: Groups of 10 five-week-old female CD1 mice were infected intravenously (IV) with (3-8) x 10^6 Af condida. In 6 experiments, doses of 0.08, 2 or 4 mcg/kg calcitriol (active form of D) were given intraperitoneally (IP), and/or AmB deoxycholate 0.4, 0.8, 1.2, 1.8, 3.3, or 4.5 mg/kg IP or 0.8 or 1.2 mg/kg IV. Calcitriol doses were selected to range from doses used in humans, up to those below doses shown to decalcify murine bones. Doses of calcitriol, AmB (or control diluents) were given 5 times each, on alternate days (QOD), to minimize drug-drug interactions (in single experiments calcitriol was also studied daily x 10 or QOD x 4, or AmB daily x 10 or QOD x 3). D treatment began on the day of challenge, and survival was followed for 10 days. In 1 experiment, posaconazole 5mg/kg PO daily was also studied +/- calcitriol.

Results: In no experiments did any dose of calcitriol alone significantly worsen or enhance survival or affect residual CFU in survivors. Calcitriol also did not affect antifungal efficacy. In a representative experiment, 0.8 or 1.2 mg/kg AmB IV +/- 2 mcg/kg calcitriol increased survival p≤0.01, but the AmB regimens with calcitriol were not different (p=0.2-0.8) than without calcitriol, and calcitriol alone was identical to controls.

Conclusion: In this model of disseminated invasive aspergillosis, calcitriol did not affect outcome nor influence antifungal efficacy. Studies of other aspergillosis models would be of interest.
ISOLATION AND IDENTIFICATION OF AIRBORNE FUNGI AND THEIR RELATION TO ALLERGIC DISEASE IN SULAIMANI CITY, IRAQ

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Purpose:
Airborne fungi and allergic disease.

Methods:
Isolation of fungi & determination of total IgE.

Results:
The airborne fungi were isolated by settle plate method in different areas of Sulaimani city during two seasons; (Autumn October 2008) and (Spring April 2009). These results were obtained from current study: a total of 24.09 × 10² CFU belonging to 20 genera with a group of yeasts and 24 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%.

Conclusion:
Most of the isolated fungi are associated with allergy disease.
MALDI-TOF-MASS-SPECTROMETRY OF PROTEIN EXTRACTS FOR ASCERTAINMENT OF PHYLOGENETIC RELATIONSHIPS BETWEEN CLINICAL ISOLATES OF *ASPERGILLUS FUMIGATUS* AND *A. NIGER*

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**Purpose:**

*Aspergillus fumigatus* and *A. niger* are common causative agents of aspergillosis in humans. The possibility of nosocomial infection and antifungal drug resistance demand the creation of universal methods for typing and “parentage” *Aspergillus* clinical strains. There was a lot of successful attempts to study *Aspergillus* molecular epidemiology using genetic methods such as microsatellite typing, rep-PCR, RAPD-PCR, etc. Novel perspectives in this field appeared since the beginning of MALDI-TOF-based identification of filamentous fungi. This paper analyzes the possibility of using different processing methods of protein mass spectra obtained by MALDI-TOF-MS for the *Aspergilli* identification and molecular phylogeny.

**Methods:**

21 strains of *A. fumigatus* and 19 strains of *A. niger* isolated from clinical specimens during 2010 – 2013 years are preserved in Russian Collection of Pathogenic Fungi were studied. Isolates were previously identified by morphology and sequencing of ITS1, ITS2 and β-tubulin regions (Genetic Analyzer 3500, Applied Biosystems). Strains were sub-cultivated on Sabouraud dextrose broth without agitation at 37°C overnight. Protein extraction from colonies was performed according to Bruker standard protocol with formic acid and acetonitrile. MALDI-TOF-mass spectrometry was made with using Autoflex speed TOF/TOF (Bruker Daltonics). Collected spectra were identified with using “Fungi Library” database, then converted into MSP and compared by MSP-dendrogram, hierarchical (5th degree) principal component analysis (PCA) and matrix of composite correlation index (CCI).

**Results:**

Rate of identification varied in wide range: for *A. fumigatus* strains – 1,833 – 2,234, for *A. niger* strains 1,726 – 2,071. Spectra of several strains were identified with low rate, but MSP-dendrograms show these spectra were closely related with reference-spectra of *A. fumigatus* and *A. niger* from database respectively. PCA-clustering allowed to create the most detailed classifications, which coincided in generally with the results of PCA-dendrograms (fig. 1), except some (sub)groups containing single isolates. CCI-matrices showed these single strains (mass-spectra) were the most deviated from studied isolates. In summary, both strains of *A. fumigatus* and strains of *A. niger* were subdivided into 5 groups and subgroups. MSP-dendograms, in contrast, were unsuitable for separation of strains (spectra) groups.
Fig 1. PCA-dendrograms and intraspecific classification of studied strains.

**Conclusion:**
The ascertainment of phylogenetic relationships between *Aspergillus* spp. strains by MALDI-mass-spectra analyzing tools (e.g. MSP-dendrogram) is helpful not only for epidemiological studies, but although for primary species identification. CCI-matrix is suitable for demonstration a few atypical isolates. Hierarchical PCA-clustering and dendrogram are probable methods of choice for construction of subspecies classification of *Aspergillus* strains.
ASPERGILLUS FUMIGATUS (AF) BIOFILM AND THE EFFECT OF
PSEUDOMONAS AERUGINOSA (PA) PHENOTYPES

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Purpose:
Pa and Af frequently infect the airways of patients with cystic fibrosis (CF), and form biofilms there. Our purpose was to examine the impact of Pa isolates from CF or non-CF patients, various colonial phenotypes, and spent culture filtrates of these Pa isolates on the development of established Af biofilm.

Methods:
26 clinical Pa isolates (16 non-CF isolates (nonCF); 5 CF mucoid variant (CFMuc), 5 CF non-mucoid (CFNMuc)), and one Af strain (10AF) were studied. To form Af biofilm (AfBF), an inoculum of 3 x 10⁵ conidia in fresh RPMI+10% fetal bovine serum was grown on flat, polystyrene disks in 12-well tissue culture plates for 16 hours before challenge with 3 x 10⁷ live Pa cells, spent culture filtrates (SCF) from Pa grown 16 hrs in planktonic (PK) or biofilm (BF) conditions, or fresh medium (controls). After 24 hrs further incubation, AfBF metabolic activity was evaluated using an XTT assay. Statistical differences were evaluated using one-way analysis of variance (ANOVA) with a Tukey post-hoc test; statistical significance was considered p ≤ 0.05.

Results:
Pa CFNMuc isolates inhibited AfBF reduction of XTT (p<0.01), whereas nonCF and CFMuc Pa isolates had no significant effect. CFNMuc Pa isolates inhibited AfBF to a greater extent than either nonCF or CFMuc isolates (p<0.001 both comparisons). SCF grown under either PK or BF conditions from CFNMuc isolates were inhibitory to the AfBF (p<0.001 for both conditions), whereas SCF grown under either condition from nonCF or CFMuc isolates had no significant effect. SCF grown under PK or BF conditions from CFNMuc isolates was more inhibitory than that from nonCF or CFMuc isolates grown under the same respective conditions (p<0.001 for all 4 comparisons). Additionally, SCF grown as BF from CFNMuc isolates appeared more inhibitory than SCF grown under PK conditions from CFNMuc isolates; there did not appear a difference in inhibition between SCF grown under PK or BF conditions for nonCF or CFMuc isolates. Serial dilutions of fresh complete media with distilled water indicated the observed inhibition with SCF was not merely due to the prior exhaustion of SCF, but to soluble, Af-inhibiting component(s) produced by CFNMuc Pa, particularly under BF conditions.

Conclusion:
Live cells or spent media from planktonic or biofilm cultures of CFNMuc Pa significantly inhibited the metabolic activity of preformed Af biofilm. This suggests that CFNMuc isolates inhibit the further growth of already established Af biofilm, and do so to a greater extent than nonCF or CFMuc isolates. An hypothesis is that the transition from non-mucoid to mucoid Pa during colonization of CF airways plays a role in enabling development of Af biofilm, thus augmenting Af establishment.
CHARACTERIZATION OF *ASPERGILLUS* SPECIES ISOLATED FROM CEREALS IN AFLATOXIGENIC HOT ZONES IN KENYA

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**Purpose:**
Fungi of the genus *Aspergillus* is an important agricultural pathogen due to its ability to produce mycotoxins and to cause diseases in plants, humans and animals. Kenya has been experiencing repeated aflatoxin episodes with disastrous results. During the aflatoxicoses episodes in Kenya observed in 2004 and 2005 it was documented approximately 200 deaths with 500 acute illnesses. This study aimed to characterize the isolated *Aspergillus* species from which cereals in aflatoxicogenic hot zones in Kenya by using phenotypic and genotypic features; and to determine the potential *Aspergillus* mycotoxins.

**Methods:**
100 grain samples were collected from the grain markets in Eastern Province and transported to Nairobi Mycology Laboratory for mycology analysis. Isolation and phenotypic characterization was done using potato dextrose agar and malt extract agar mediums. Genotypic characterizations were based on camodulin encoding genes. Gene sequences were assembled with sequence navigator.

**Results:**
The most frequently isolated *Aspergillus* species from all samples were; *Aspergillus awamori* (47%), *Aspergillus mini sclerotigenes* (21%), *Aspergillus flavus var. columnaris* (10%), *Aspergillus tamari* (5%), *Aspergillus fumigatus* (5%) respectively. The ability of these isolates to produce the mycotoxins; Ochratoxins A and aflatoxins were confirmed by using Envirologix test kit. From the results we can conclude that 47% of the cereals were contaminated with *Aspergillus awamori* species which is responsible for the production of Ochratoxin A toxins.

**Conclusion:**
There is need for further investigating on other *Aspergillus* species than the traditional green *Aspergillus*. To set up public mycotoxilogical food safety standards and program to safeguard the public from mycotoxin exposure. Such information is essential for intervention strategies aimed at reducing mycotoxin in Kenya.
PSEUDOMONAS AERUGINOSA (PA) INHIBITION OF ASPERGILLUS FUMIGATUS (AF) IS DEPENDENT ON THE SOURCE, PHENOTYPE AND GROWTH CONDITIONS OF THE BACTERIUM

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Purpose:
Co-colonization by Pa and Af in airways of cystic fibrosis (CF) patients is associated with worse clinical outcomes. Our objective was to study in vitro the action of Pa on Af metabolism and the ability of Af to establish a biofilm (AfBF).

Methods:
One Af strain (10AF), and 26 clinical Pa isolates (16 non-CF isolates (nonCF), 5 CF mucoid variant (CFMuc), 5 CF non-mucoid (CFNMuc)) were studied. Biofilms were developed on polystyrene disks in RPMI +10% fetal bovine serum in 12-well plates. Starting with 3x10^5 conidia, AfBF occurred in 16 h. 3x10^7 live Pa cells or spent culture filtrate (SCF) from Pa grown under planktonic (PK) or biofilm (BF) conditions, or fresh medium (controls), were added to conidia initially, and after 16 h fresh medium was substituted for 24 h further incubation. Af metabolic activity was quantified by the XTT assay. Statistical differences were evaluated using one-way analysis of variance (ANOVA) followed by a Tukey post-hoc test; statistical significance was considered p ≤0.05.

Results:
All Pa isolates inhibited the metabolism of Af and AfBF development (p<0.001). CFMuc and CFNMuc isolates were more inhibitory than nonCF (p<0.001), and CFNMuc isolates were more inhibitory than CFMuc isolates (p<0.01). SCF from Pa cultures alone, from all 3 Pa types, grown under either PK or BF conditions, were inhibitory to the Af (p<0.001, all 6 comparisons). SCF from PK nonCF was less inhibitory (p<0.001) than that from either CFNMuc or CFMuc isolates; the 2 CF types were not significantly different. SCF from nonCF Pa grown as BF was not different than that from CFMuc isolates, but SCF from CFNMuc isolates was significantly more inhibitory than those from nonCF or CFMuc (p<0.001). Furthermore, SCF from all 3 types of Pa grown as BF appeared more inhibitory than SCF from Pa grown in PK conditions. Serial dilution experiments of intact media with distilled water indicated the inhibition noted with SCF was not due merely to the effect of prior exhaustion of SCF, but due to inhibitory substances for Af produced by Pa.

Conclusions:
Pa isolates were inhibitory to Af, though isolates from CF patients are more inhibitory, and non-mucoid CF isolates are most inhibitory. The inhibition, and the differences between Pa isolates, appear the result of soluble inhibitory substances, produced to a greater level when Pa was grown under biofilm conditions. Thus, direct microbial contact was not required. Since the development of mucoid Pa is a late development in CF airways, an hypothesis is that this development is less inhibitory to Af, allowing the formation of Af biofilm in the airways, explaining why Af establishment in airways usually occurs later in CF disease following conversion of environmental non-mucoid to non-mucoid Pa phenotypes in situ, to mucoid types.
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IDENTIFICATION OF GENES REGULATING CELL WALL PERMEABILITY AND ANTIFUNGAL RESPONSES IN *ASPERGILLUS FUMIGATUS*

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Purpose:
Identification of novel antifungal drug targets is critical due to the increased incidences of systemic fungal infections in the immunocompromised populations. Studies in *Candida albicans* have attempted to identify novel transcription factors that may be associated with regulating antifungal drug tolerance. With a library of mutants composed of systematic deletions in ca 350 transcription factors, we decided to investigate novel global regulators of cell wall permeability and antifungal responses in *Aspergillus fumigatus*.

Methods:
We began our studies by screening for mutants that exhibit increased resistance or sensitivity to cell wall binding agents congo red (CR) and calcofluor white (CFW). These agents disrupt cell wall assembly by binding to nascent chitin chains, thereby interrupting cross-linking with beta-glucan polymers leading to a weakened cell wall.

Results:
Presently, we have identified five mutants which show resistance to both CR (300 µg/ml) and CFW (150 µg/ml) and one specifically to CR. Amongst these, two were mutants in C2H2 zinc finger transcription factors, one RNA polymerase II elongin subunit A and three C6 transcription factors. One C2H2 mutant is the previously characterized CrzA-encoding transcription factor, demonstrated to be involved in morphology and pathogenesis of *A. fumigatus*. Conversely, 22 mutants exhibited increased sensitivity to CR (50 µg/ml) and six mutants to both CR and CFW (40 µg/ml). The majority belonged to the C6 family (ten) while the rest spanned C2H2, bZIP, and GATA amongst others.

Conclusions:
Future work will involve RNA-sequence analyses on these mutants, to glean information on the genes involved in/ associated with modification of cell wall permeability.
DEACETYLATION OF ASPERGILLUS FUMIGATUS GALACTOSAMINO-GALACTAN IS REQUIRED FOR ADHESION AND VIRULENCE

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Purpose:
To determine if deacetylation of A. fumigatus galactosaminogalactan is a required for its pathogenic function.

Methods:
Galactosaminogalactan production and adherence to polystyrene were assayed using standard protocols. Primary amine content was quantified using trinitrobenzene sulfonic acid (TNBSA). For Δuge3 and Δagd3 mutants cross-culture, conidia of one mutant were inoculated into the culture supernatant of the other mutant, and adherence to polystyrene was assayed. To determine virulence in an invertebrate model, 10⁶ conidia of fungal strains were inoculated into wandering Galleria mellonella larvae, and survival was monitored over the course of 10 days. For in silico analysis and structural modeling, amino acid sequence was obtained from NCBI and various bioinformatics and modeling tools were used.

Results:
Although the Δage3 mutant produced wild-type amounts of galactosaminogalactan, it was not deacetylated. Non-deacetylated galactosaminogalactan was unable to adhere to the surface of hyphae, and could not support biofilm formation. In silico analysis of Agd3 predicted a signal peptide suggestive of extracellular secretion of this enzyme. Consistent with this observation, co-culture of the Δagd3 mutant with culture supernatants from the galactosaminogalactan deficient Δuge3 mutant restored biofilm production. The converse of this experiment, where Δuge3 mutant biofilm production is restored by co-culturing with Δagd3 mutant culture supernatants was also true, suggesting that Agd3 mediated deacetylation occurs in the extracellular space. The Δagd3 mutant had attenuated virulence in Galleria mellonella. Structural modeling revealed that the putative polysaccharide deacetylase domain of Agd3 is structurally similar to bacterial polysaccharide deacetylases involved in the production of bacterial exopolysaccharide.

Conclusion:
These data support the hypothesis that deacetylation of galactosaminogalactan is required for its pathogenic function, likely because deacetylation generates a cationic polysaccharide that can adhere to negatively charged surfaces such as the fungal cell wall, host cell membranes, and plastic.
ISOLATION AND MOLECULAR IDENTIFICATION OF *Aspergillus* spp FROM ICU, INDOOR AND OUTDOOR ENVIRONMENTS OF HOSPITALS, NORTHERN IRAN

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**Purpose:**  
*Aspergillus* spp is able to germinate and grow over a wide range of temperatures and pH conditions, increasing its ability to adapt to adverse environments. While exposure to *Aspergillus* spores is universal, many causal arguments for hospital acquisition of IA can be put forward.

**Methods:**  
From 500 plates of SDA was placed the ground in inside and outside of the ICU of Mazandaran hospitals, then, we identified the approximates according to macroscopic and microscopic morphology. DNA sequencing of the ITS1 and ITS4 regions and also differentiation of species were conducted by the method High Resolution Melting Real-Time PCR. Species identification was based on sequence similarity using BLAST searches. Sequences were compared to those derived from type strains deposited in the Gen Bank data base to identify isolates to the species level.

**Results:**  
In total, 350 *Aspergillus* strains were obtained from ICU, indoor and outdoor environments of the hospitals in Mazandaran province. 15% of the colonies were *Aspergillus fumigatus*, 35% *Aspergillus niger* and 50% were *Aspergillus flavus* colonies. In this study, with molecular method there was 100% homology between the *Aspergillus* isolates of indoor and outdoor the ICU, also we recorded some isolates as a new strains in Gene Bank.

**Conclusion:**  
The melting real-time PCR is a rapid, can serve as an accurate and suitable method with a specificity of 100% for the identification of *Aspergillus*.

**Keywords:**  
Real-Time PCR, *Aspergillus*, ICU, HRM, ITS
Molecular Characterization of <i>Aspergillus fumigatus</i> CrzA Targets by Chromatin Immunoprecipitation - DNA Sequence (Chip-seq)

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Purpose:
<i>Aspergillus fumigatus</i> is an important primary and opportunistic pathogen as well as a major allergen. The most important genetic components for the infection establishment are transcription factors that can activate different programs for virulence regulation and pathogenicity. Calcineurin is a central signaling molecule in most eukaryotic cells and many of these activities are dependent on the transcription factor CrzA that becomes transcriptionally active after dephosphorylation by calcineurin. In <i>A. fumigatus</i>, several signaling pathways are dependent on CrzA and must be activated during the adaptive process into the mammalian host. The identification of <i>A. fumigatus</i> genes regulated by CrzA may provide greater insight into this important signal transduction pathway.

Methods:
Chromatin Immunoprecipitation technique followed by DNA sequencing (ChIP-seq) for CrzA. ChIP-seq is a powerful tool to evaluate the location of DNA-binding proteins in vivo and this assay provides a wide base for the identification of genes which have their mRNA accumulation modulated by CrzA.

Results:
Bioinformatics analysis of the fragments sequenced identified putative promoter regions that control 103 adjacent genes. Some of these genes are involved in the cAMP-dependent signaling during conidial germination (for instance schA), in the activation of MAPKK activity involved in osmosensory signaling pathway (for instance ssk1 and ssk2), in calcium ion homeostasis, and also related to endoplasmic reticulum stress. The analyses of these promoter regions by MEME and MDScan identified two common motifs as calcineurin-dependent response elements (CDRE) possibly involved in the regulation by CrzA: 5' - AGGCTG - 3' or 5' - CAGCCT - 3'. We validated these data by performing RT-PCR for 10 selected genes and Electrophoretic Mobility Shift assays (EMSA) for two of them.

Conclusion:
Our results showed that mRNA accumulation of 9 of these genes is significantly decreased in the delta crzA mutant while these two promoters regions contain as CDREs 5'-CACAGCCC-3' and 5'-CAACCT-3'. Moreover, we constructed null mutants for the genes schA (Afu1g06400), ssk1 (Afu3g12530), ssk2 (Afu1g10940) and ckb2 (Afu1g09950), and studied their phenotypes. The delta schA mutants strain showed attenuated virulence in a murine model of invasive pulmonary aspergillosis.

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HYPOXIA PROMOTES DANGER-MEDIATED INFLAMMATION VIA RAGE IN CYSTIC FIBROSIS


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Purpose:
Hypoxia regulates the inflammatory/anti-inflammatory balance via the receptor for advanced glycation end products (RAGE), a versatile sensor of damage-associated molecular patterns. The multi-ligand nature of RAGE places this receptor in the midst of chronic inflammatory disease. Our objective is to characterize the impact of the hypoxia/RAGE pathway on pathogenic airway inflammation preventing effective pathogen clearance in Cystic Fibrosis (CF) and elucidate the potential role of this danger signal in pathogenesis and therapy of lung inflammation.

Methods:
We employed in vivo and in vitro models to study the impact of hypoxia on RAGE expression and activity in human and murine CF, the nature of the RAGE ligand and the impact of RAGE on lung inflammation and antimicrobial resistance in fungal and bacterial pneumonia.

Results:
Sustained expression of RAGE and its ligand S100B was observed in murine lung and human epithelial cells and exerted a proximal role in promoting inflammation in murine and human CF, as revealed by functional studies and analysis of the genetic variability of AGER in patients with CF. Both hypoxia and infections contributed to the sustained activation of the S100B/RAGE pathway, being RAGE up-regulated by hypoxia and S100B by infection via Toll-like receptors. Inhibiting the RAGE pathway in vivo with soluble (s)RAGE reduced pathogen load and inflammation in experimental CF while sRAGE production was defective in CF patients.

Conclusions:
A causal link between hyper-activation of RAGE and inflammation in CF has been observed, such that targeting pathogenic inflammation alleviated inflammation in CF and measurement of sRAGE levels could be a useful biomarker for RAGE-dependent inflammation in CF patients.
IL-1 BLOCKADE AS A POTENTIAL THERAPEUTIC TARGET IN ASPERGILLOSIS

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Purpose:
Aspergillosis is a life-threatening disease that occurs in immunodepressed patients when infected with *Aspergillus fumigatus*. The progressive decline of pulmonary function is due to a vicious cycle of airways infection and inflammation. Intracellular danger sensing protein complexes called inflammasomes have been identified and functionally characterized in inflammatory and non-inflammatory cells, with their relevance to airway diseases. We hypothesized that the heightened inflammatory response due to inflammasome activation could be responsible for life-threatening bacterial and fungal infections in Chronic granulomatous disease (CGD) or Cystic Fibrosis (CF) and that blocking IL-1 receptor could improve inflammation in both Chronic granulomatous disease (CGD) or Cystic Fibrosis (CF).

Methods:
CGD or CF mice were infected with *A. fumigatus* and treated with anakinra (a recombinant form of the naturally occurring IL-1 receptor antagonist) daily. Mice were monitored for survival, local fungal growth, inflammatory cell recruitment and lung histopathology as well as parameters of inflammasome activation.

Results:
We found that the heightened inflammatory response to *A. fumigatus* was associated with an increased activation of the inflammasome and production of IL-1β in experimental CGD and CF. Anakinra successfully controlled inflammasome-dependent IL-1β production in response to infectious or danger signals in CGD and CF mice, an activity to which the restoration of autophagy greatly contributed.

Conclusion:
Abnormal activation of inflammasome-dependent mechanisms are demonstrable in CGD and CF, such that blocking IL-1 could be a potential therapeutic option to dampen pathogenic inflammation in CGD or CF.
ASPERGILLUS FUMIGATUS CALA BINDS TO β1 INTEGRINS AND MEDIATES HOST CELL INVASION AND VIRULENCE

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Purpose:
During the initiation of invasive disease, Aspergillus fumigatus adheres to, invades, and damages pulmonary epithelial cells. However, the fungal factors and host cell receptors that mediate these host cell interactions are currently poorly understood. The A. fumigatus CalA protein is predicted to be an adhesin by bioinformatics analysis. Also, purified recombinant AfCalA binds to laminin. We aim to identify the role of CalA in mediating adherence and host cell interactions of A. fumigatus.

Methods:
The cellular localization of CalA was determined by expression of a mCherry-tagged allele of calA. A ΔcalA deletion and ΔcalA+calA complemented strains of A. fumigatus were constructed. The capacity of these strains to adhere to, invade, and damage A549 pulmonary epithelial cells and human umbilical vein endothelial cells (HUVECs) was analyzed. A549 cell membrane proteins that bound to A. fumigatus were isolated and identified by NanoLC-MS/MS. Antibody blocking and siRNA knock down were used to determine the role of β1 integrin in A549 cell invasion by A. fumigatus. The mouse fibroblast cell lines GD25 (β1 integrin knock out) and β1AGD25 (expressing functional β1A integrin) were used to investigate the role of CalA and β1 integrin in mediating host cell invasion. Finally, the virulence of the various strains was evaluated in the corticosteroid-treated mouse model of invasive pulmonary aspergillosis.

Results:
CalA-mCherry localized mainly to the cell surface of swollen conidia and the proximal portion of germlings. Scanning electron microscopy revealed that the ΔcalA mutant formed abnormally curved hyphal tips when grown on A549 cells. Both conidia and germlings of the ΔcalA mutant had wild-type (WT) adherence to A549 cells and laminin. However, the ΔcalA mutant had 51% lower A549 cell invasion (p<0.005) and caused 67% less A549 cell damage (p<0.001) than the WT or ΔcalA+calA complemented strains. Furthermore, the ΔcalA mutant displayed 70% less HUVEC invasion (p<0.001) compared to the control strains. Although expression of AfCalA alone in Saccharomyces cerevisiae had no effect on adherence to or invasion of A549 and HUVECs, co-expression of AfCalA and the Candida albicans adhesin Alsl increased adherence to HUVECs by 55% and enhanced invasion by 114%, as compare to in S. cerevisiae expressing CaAlsl alone. Wild-type A. fumigatus hyphae was found to bind to β1 integrin in A549 membrane proteins extracts, while the ΔcalA mutant bound poorly to this protein. Both siRNA knockdown and antibody inhibition of β1 integrin inhibited A549 cell invasion by WT A. fumigatus by 40% (p<0.001). Also, both WT A. fumigatus and CalA-expressing S. cerevisiae had minimal invasion of GD25 cells, which lack β1 integrins, but high invasion of β1AGD25 cells in which β1 integrin expression was restored. Finally, mice infected with the ΔcalA mutant had significantly longer survival (p<0.05) than mice infected with the control strains.

Conclusions:
CalA is the first A. fumigatus invasin to be identified. It interacts with β1 integrins on host cells to mediate host cell invasion and is necessary for maximal virulence during invasive pulmonary aspergillosis in mice.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
TRANSCRIPTIONAL REGULATION OF THE HYPOXIA RESPONSE IN
*ASPERGILLUS FUMIGATUS* BY A FAMILY OF STEROL REGULATORY
ELEMENT BINDING PROTEINS

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Purpose:
Sterol Regulatory Element Binding Proteins (SREBPs) are basic helix-loop-helix (bHLH) transcription factors conserved from fungi to human. In mammals, three SREBPs, SREBP-1α, SREBP-1c and SREBP-2 have been characterized and they are involved in cholesterol biosynthesis and lipid metabolism. Studies in fungi have shown that SREBPs are critical for growth in low oxygen conditions (hypoxia) as well as regulation of sterol biosynthesis genes. Adaptation to hypoxia is particularly important for pathogenic fungi to develop diseases given that sites of fungal infection in host are hypoxic. Our lab has reported the importance of the *A. fumigatus* SREBP, SrbA in growth in hypoxia, drug susceptibility, and virulence. Recently our lab identified additional SREBPs and we aim to understand how these 3 SREBP transcription factors coordinate gene regulation in response to hypoxia and develop a novel therapeutic approach to inhibit activation of this virulence associated genetic network *in vivo*.

Methods:
RNAseq and Chromatin Immunoprecipitation (ChIP)-sequencing were performed with wild type and a srbA null mutant in normoxia or hypoxia. Based on the genome-wide analyses we identified additional SREBPs, designated SrbB and SrbC. We generated a series of deletion mutants with all possible SREBP gene combinations. To understand genetic relationships among the three SREBPs, we performed quantitative real-time PCR and ChIP-quantitative PCR. To test virulence, we utilized our steroid murine model of invasive pulmonary aspergillosis.

Results:
srbB is transcriptionally regulated by SrbA through SrbA direct binding on the srbB promoter. In hypoxia, a srbB null mutant shows reduced growth and biomass production compared to wild type, whereas a srbC null mutant shows enhanced hypoxia growth compared to wild-type. Notably, both ΔsrbB and ΔsrbC mutants show attenuated virulence compared to WT in the murine aspergillosis model. Double or triple knock-out mutants show that deletion of srbA is dominant to that of srbB or srbC in terms of hypoxia growth. Interestingly, restored srbB expression partially complements hypoxia growth in ΔsrbA. In early exposure to hypoxia (30 min) SrbB is a negative regulator of srbA and srbC transcription by direct promoter binding, and SrbB and SrbC transcriptionally regulate each other in a negative feedback loop. Moreover, mRNA levels of two major ergosterol biosynthesis genes, *erg11A* and *erg25A* is almost completely lost in the ΔsrbAΔsrbBΔsrbC triple mutant in normoxia and hypoxia compared to other mutants and wild type.

Conclusion:
Our data suggest that SrbA, SrbB, and SrbC are involved in hypoxia adaptation, virulence, and antifungal drug responses in *A. fumigatus*. Therefore, understanding transcriptional regulation of the hypoxia response by SREBPs will provide new critical information to augment existing antifungal therapies and/or develop new therapeutic approaches.
MOLECULAR DETECTION OF *ASPERGILUS FUMIGATUS* AND SECTION *FUMIGATI* BY MULTIPLEX REAL TIME PCR USING ITS AND β-TUBULINE SEQUENCES

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**Purpose:**
The aim of this work was to develop a multiplex real time PCR to quickly and easily distinguish species of section *Fumigati* from other fungi and specifically identify *A. fumigatus* in one step, avoiding PCR inhibitions.

**Methods:**
We designed primers and specific Taqman probes using as a target internal transcribed spacer regions and β-tubulin gene, to detect members of section *Fumigati* and *A. fumigatus* species, respectively. We also designed an internal control, with specific primers and a Taqman probe, to detect inhibition of the reactions. Finally, several PCR facilitators was studied and included in PCR mixtures to avoid false negatives due to PCR inhibitions. The sensitivity and efficiency of the multiplex real time PCR assay was tested with DNA extracted from hyphae and conidia from *A. fumigatus* AF-293 and from a collection of 67 different strains. Moreover, we checked the performance of the method with 38 human bronchoalveolar lavage samples.

**Results:**
The multiplex real time PCR was optimized using genomic DNA extracted from *A. fumigatus* AF-293 showing sensitivity and mean efficiency of 20 fg and 82.16%, respectively, in the detection of section *Fumigati*; and 50 fg and 101.23% in the specific detection of *A. fumigatus*. Furthermore, the method developed also allows the quantification from 20 ng to 200 fg of *A. fumigatus* genomic DNA in samples and, in particular, detection of germinated conidia (down to 1 germinated conidium vs. 10³ non-germinated conidia). In addition, the method was successfully tested on a collection of microorganisms and on several bronchoalveolar lavage samples. On the other hand, we also included an internal control and another specific Taqman probe for its detection to analyze the PCR inhibition. The inclusion of some PCR facilitators (polyethylene glycol and BSA) together with the dilution of samples (1:2) made possible to avoid false negatives due to PCR inhibitions in all bronchoalveolar lavages assayed.

**Conclusion:**
The multiplex real time PCR assay developed in this study has demonstrated to be an excellent method to detect DNA of *A. fumigatus* in bronchoalveolar lavage samples, even though the *Fumigati* probes designed do not provide the best results. In addition, we confirmed the detection of hyphae and germinated conidia (even a single one), but not non-germinated conidia (below of 10³ conidia/ml). Finally, we were able to avoid the PCR inhibition when the reaction was applied to human samples.

**Note:** This abstract has been selected for oral presentation.
SIGNALLING PROCESS AND ACTIVATION OF SLTA, A TRANSCRIPTION FACTOR INVOLVED IN CATION/ALKALINITY STRESS RESPONSE

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Purpose:
To sense and adapt to abiotic stress conditions some fungi, such as Aspergillus nidulans, have developed regulatory mechanisms to control gene expression. Cation and alkaline pH stress responses are mediated by diverse pathways in A. nidulans. We focus on the signalling process and the activation of SltA transcription factor involved in both responses.

Methods:
Isolation of spontaneous extragenic suppressor mutations of the lethal phenotype caused by certain null vps alleles, made possible to identify new slt loci, i.e. sltB. Using reverse genetic strategies we generated and characterized mutant strains carrying null alleles of sltB and/or sltA to understand their biological function. Fluorescent and epitope tagged proteins allowed visualization and understanding its molecular phenotype through immunodetection.

Overexpressing sltB, by means of the ethanol-inducible promoter alcA, we determined the transcriptional relationships between SltA and SltB in their respective null allele genetic backgrounds.

Results:
Here we present our latest model concerning the signalling process and the activation of SltA, in addition to its transcriptional regulatory activity. Signalling of SltA requires its proteolytic processing, an extreme mechanism of post-translational modification that shares with PacC. Additionally, we have discovered and characterised SltB, a signalling element of SltA. sltB gene encodes for a protein of 1272 amino acids, also specific to filamentous fungi, with two putative functional domains. The SltB-pseudokinase domain is needed to process the native SltA 78 kDa form into a 32 kDa form. The second domain is similar to a trypsin-like protease, and our data suggest that SltB undergoes an auto-proteolytic process through this protease activity. Finally, we have determined that SltB is expressed in a SltA dependent manner.

Conclusion:
A model of regulation of SltA through SltB activity is presented for this novel cation/alkaline pH regulatory pathway in filamentous fungi.
ROLE OF *ASPHERILLUS NIDULANS* SLTA TRANSCRIPTION FACTOR AND PROTEIN KINASE HALA IN A MURINE MODEL OF INVASIVE ASPERGILLOSIS

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**Purpose:**
Invasive aspergillosis caused by *Aspergillus nidulans* is infrequent in humans but pathogenicity originated by this mold is characterised by its aggressive behaviour in the host. Stress responses are essential for fungal pathogens to adapt to the environment as well as to evade host anti-microbial defence mechanisms. In *A. nidulans* two important proteins required for cation homeostasis are the protein kinase HalA and the transcription factor SltA. This study assessed the possible role of these proteins individually or in combination in virulence of *A. nidulans* in a murine model of invasive pulmonary aspergillosis.

**Methods:**
Six-week-old CD1 male mice were immunosuppressed intraperitoneally with cyclophosphamide during pre- and post-infection and subcutaneously with hydrocortisone acetate prior to infection. Prototrophic *A. nidulans* strains were generated carrying a single or double combination of null alleles of *sltA* and *halA*. Conidia suspensions (40 µL) from the *A. nidulans* wild type, *DsltA*, *DhalA* and *DhalA DsltA* strains were inoculated intranasally from suspension of 2x10⁶ conidia/mL. Mice were weighted daily and were sacrificed when a 20% reduction in body weight was detected or after 14 days from day of infection.

**Results:**
The protein kinase HalA and the transcription factor SltA are not involved in fungal tolerance to calcium stress but instead double mutant strains lacking both HalA and SltA need a rich source of exogenous calcium for growth. Our data shows that a null *sltA* mutant displayed attenuated virulence while deletion of *halA* did not affect to infection. The attenuated virulence was reversed in *DsltA sltA*'-complemented strain. Interestingly mice infected with double null *sltA;halA* mutant showed decreased survival rate in comparison to those infected with a *DsltA* mutant.

**Conclusion:**
Therefore, elevated calcium requirement shown by the double *sltA halA* null strain is essential for fungal growth but not for disease development. Furthermore, our results indicate that SltA is an important virulence factor which pathogenicity is dependent on the presence of the protein kinase HalA.
THE ROLE OF PROTEIN KINASES IN ASPERGILLUS FUMIGATUS HYPOXIA ADAPTATION AND VIRULENCE

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Purpose:
Fungi encounter a broad range of conditions in their natural environment as well as in a host during infection. The ability to adapt to a variety of conditions has contributed to the ubiquitous nature of Aspergillus fumigatus in the environment and to the success of this fungus as a pathogen. One condition that Aspergillus faces in the environment and within the host is rapidly changing oxygen tension. Recently, it has been shown that fungal lesions in murine lungs are characterized in part by hypoxic regions (with oxygen tension ≤1%). Intriguingly, the ability of Aspergillus fumigatus to cause disease in a murine model of invasive pulmonary aspergillosis depends on the presence of the master regulator of the hypoxia response, SrbA. Here we aim to identify protein kinases that are crucial for this adaptation to hypoxia with hopes of identifying fungal-specific kinases that are required for virulence of Aspergillus fumigatus.

Methods:
We have used two methods to identify candidate protein kinases that are essential for Aspergillus fumigatus hypoxia adaptation. First, an Aspergillus nidulans kinase deletion collection has been screened under ambient air (~20% oxygen) and hypoxic (1% oxygen) conditions to find kinases that are required for growth in hypoxia. Additionally, protein kinases that are transcriptionally induced in response to hypoxia, based on an RNA-sequencing data set of wild type A. fumigatus shifted from ambient air to hypoxic conditions, will be chosen as candidates. Null mutants of candidate kinases identified in these 2 screens will be generated in Aspergillus fumigatus, and growth in hypoxia and virulence will be assessed.

Results:
Screening of the A. nidulans kinase knockout revealed 10 kinases that have a 20% or greater defect in hypoxic growth compared to wild type. The kinases identified include: Stk47, PtkA, NikA, SepH, Ssn3, Plk1, CmkA, MpkA, Snf1 and Stk19. Orthologs of these kinases in Aspergillus fumigatus are mostly uncharacterized, and generation and characterization of the null mutants is ongoing in our laboratory. From the RNA-sequencing data set, 23 kinases were identified as being induced in response to hypoxia. These include many uncharacterized serine/threonine kinases and histidine kinases, including one putative histidine kinase, AFUB_101210, which is an ortholog of MpkA in A. nidulans, which was identified in our initial screen.

Conclusions:
Our preliminary results show that promising candidates for kinases required for adaptation to hypoxic conditions are present in the A. fumigatus genome. In our ongoing work, we hope to characterize these kinases mutants in Aspergillus fumigatus to better understand their role in this organism and their relationship to the master hypoxia transcriptional regulator, SrbA. Full characterization of the protein kinases involved in hypoxia signaling in A. fumigatus is expected to enhance our understanding of hypoxia’s impact on IPA outcomes.
Purpose/Methods:
Invasive pulmonary aspergillosis (IPA) remains a relevant clinical issue in patients with graft-versus-host disease, largely due to immunosuppressive treatments with high dosages of corticosteroids. The mortality related to IPA infection can be up to 90% in heavily treated patients, thus underlining the need for well tolerable prophylactic interventions aimed at reducing infection frequency. posaconazole is the most recently approved triazole, with potent anti-
Aspergillus activity, currently used in the prophilaxis of IPA. Limitations to the use of posaconazole are related to drug-drug interaction and considerable side effects. PTX3 is an innate immunity multimeric glycoprotein characterized by an opsonic activity against Aspergillus and proven to prevent IPA in mouse and rat models. In order to investigate the potential benefit of combining posaconazole antifungal activity with the immune modulatory function of PTX3, the efficacy of the simultaneous administration of these two molecules in prophylaxis was evaluated in rats immunosuppressed with Cortisone acetate and intratracheally administered with A. fumigatus conidia to elicit pulmonary infection.

Results:
Overall survival (37% posaconazole, 35% PTX3 and 90% combination), mean survival time and lung fungal burden indicate synergic activity of PTX3 and posaconazole. Similarly, evaluation of myeloperoxidase (MPO) as a marker of neutrophils activity in the lungs of infected rats clearly indicates a synergic function of PTX3 and posaconazole in strengthening immune response against the fungus.

Conclusion:
The results highlight the potential clinical benefit of combining oosaconazole and PTX3, particularly in those clinical cases where reduction of triazole dosage would be desirable to minimize side effects.
PLATELET-RICH PLASMA MODULATES THE IMMUNE RESPONSE OF DENDRITIC CELLS AND MACROPHAGES IN ASPERGILLUS FUMIGATUS INFECTIONS

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Purpose:
Invasive aspergillosis (IA), caused by an infection with the ubiquitous mold A. fumigatus, is associated with local thrombosis, bleeding and distal hemorrhagic infarctions indicating that local hemostasis is altered at the side of infection. Important components of hemostasis are thrombocytes, also called platelets that lead to clot formation upon activation. On the other hand platelets are also involved in pathogen recognition and elimination. It is also shown that they modulate the immune response of the adaptive immune system. However, the role of thrombocytes and their influence on innate immune cells during A. fumigatus infections are not well understood.

Methods:
Isolated CD14-positive monocytes of healthy donors were differentiated into monocyte-derived-dendritic cells (moDCs) and monocyte-derived macrophages in RPMI 1640 supplemented with 10% FBS, IL-4 and GM-CSF or M-CSF, respectively. The platelet-rich plasma was generated by centrifugation of fresh blood in acid-citrate-dextrose solution and platelet concentration measured by Sysmex® analysis. moDCs and macrophages (primed with IL-4 for 18h) were co-cultivated either with A. fumigatus germ tubes (MOI 1), PRP (1:30) or a combination of both stimuli for 6 h, respectively. A gene expression profiling was performed with Affymetrix HG-U219 arrays (p<0.05, fold change >2, n=3). Data were analyzed with ingenuity® software. Phagocytotic capacity of FITC-labeled conidia, reduction of the growth of germlings and maturation markers of moDCs were additionally quantified.

Results:
In the genome wide gene expression analysis, macrophages and moDCs were compared in their response to A. fumigatus germlings. Interestingly, the combined stimulation of PRP and A. fumigatus had a stronger effect on gene expression in moDCs and Macrophages than one of the stimuli alone. Only 10% of regulated genes were identical in PRP stimulated moDCs compared to macrophages. Furthermore, stimulation by A. fumigatus with and without PRP resulted in an overlap of regulated genes of only 22% or 30% in both cell types, respectively. Therefore, we concluded that different canonical pathways were switched on in moDCs and macrophages. Fold changes of inflammatory genes induced by A. fumigatus like CXCL1/3 or IL-1a/b were altered in the presence of PRP. Changes were cell-type specific and differed in moDCs and macrophages. PRP also enhanced maturation of moDCs (CD83 and CD86 but not CD40, 80 and HLA-DR) and phagocytosis capacity of macrophages. The growth of germ tubes was significantly reduced in when dendritic cells were co-incubated with PRP.

Conclusion:
Even though generated from the same precursor, moDCs and macrophages differ in their response to A. fumigatus germlings. While in moDCs, PRP seem to induce a more pronounced inflammatory response, PRP might induce a higher recruiting of other immune cell populations by macrophages. PRP also enhanced the phagocytosis of conidia by macrophages. Furthermore, PRP reduced fungal growth when co-cultivated with macrophages. Therefore, we hypothesize that platelets not only have a direct antimicrobial effect but also a regulatory influence on cells of the innate immune system.
HYPOXIA AND HYPOXIA-INDUCIBLE FACTOR 1α MODULATE THE 
RESPONSE OF HUMAN DENDRITIC CELLS AGAINST ASPERGILLUS 
FUMIGATUS

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Purpose:
Immunocompromised patients are highly susceptible for the development of fungal infections including invasive aspergillosis (IA). In mice and men, hypoxic microenvironments are present at sites of A. fumigatus invasive growth in the lung. Hypoxia and signalling via hypoxia-inducible factor (HIF) 1α are known to influence functions of immune cells, including dendritic cells (DCs). DCs are of particular importance in initiating both innate and adaptive immunity against A. fumigatus conidia and hyphae during IA. Here, we evaluate the role of hypoxia and HIF-1α signalling in the immune response of human DCs against A. fumigatus in vitro.

Methods:
Monocytes (CD14+/CD1a-) were isolated from PBMCs of healthy donors and differentiated into DCs (CD14-/CD1a+) by five days incubation with IL-4 and GM-CSF. To study HIF-1α function, DCs were electroporated with siRNA directed against HIF-1α mRNA 24 h prior to the experiments to knock down HIF-1α. Immature DCs were stimulated with A. fumigatus germ tubes at normoxia or hypoxia (1 % O2), followed by characterization of DC metabolism, maturation and gene expression profile using genome-wide microarrays.

Results:
DCs consumed more glucose and released more lactate in the cell culture media when stimulated with A. fumigatus at either normoxia or hypoxia, or when cultivated at hypoxia without stimulus, and this switch in energy metabolism was at least in part dependent on HIF-1α. Up-regulation of relevant maturation and co-stimulatory molecules (CD40, CD80, CD83, CD86 and HLA-DR) as well as of the homing-receptor CCR7 was reduced on DCs stimulated with A. fumigatus at hypoxia compared to normoxia. This effect seemed to be independent of HIF-1α, as both, HIF-1α silenced DCs and control DCs showed similar results. Gene expression profiles of DCs stimulated with A. fumigatus for 6 h at normoxia or hypoxia revealed a minor influence of hypoxia alone on DC gene expression compared to stimulation with A. fumigatus at either normoxia or hypoxia. However, knock-down of HIF-1α significantly affected gene expression in A. fumigatus stimulated DCs, leading to differential expression of app. 250 genes at normoxia and 1000 genes at hypoxia. A first pathway analysis suggests reduced immune functions of HIF-1α silenced DCs, indicating that this transcription factor might be important in the immune response against A. fumigatus.

Conclusions:
Our results suggest that hypoxia and HIF-1α signaling have substantial impacts on DC function, which might also influence the course and outcome of IA in immunocompromised patients. DC maturation was impaired after stimulation with A. fumigatus at hypoxia compared to normoxia. In future studies, we will evaluate the influence of hypoxia and HIF-1α signalling on the ability of DCs to initiate a T-cell response against A. fumigatus. We will further analyze the gene expression profile of HIF-1α silenced DCs at normoxia and hypoxia to understand the underlying pathways and the role of this transcription factor in the response of human DCs against A. fumigatus.
THE β-1,3-GLUCANASE ENG FAMILIES OF *ASPERGILLUS FUMIGATUS* ARE IMPORTANT FOR CONIDIAL CELL WALL MORPHOGENESIS

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**Purpose:**
Beta-1,3-glucan is produced by a variety of different organisms ranging from prokaryotes to higher plants. In fungi it constitutes a major cell wall component being responsible for rigidity of the cell wall structure. Softening of the cell wall is absolutely required during morphogenetic events like germination and branching and would be under the dependence of cell wall specific glycosylhydrolases such as endo-beta-1,3-glucanases and chitinases. We investigated a survey of all potential endo-β-1,3-glucanases found in the *A. fumigatus* genome.

**Methods:**
Different fungal and bacterial glucanases were used as protein queries for BLAST searches to identify possible homologs in the genome of the *A. fumigatus* strain Af293.

**Results:**
We have identified eight genes coding for potential endo-beta-1,3-glucanases that belongs to 2 families. ENGL1 belongs to the family GH81 while ENG2 to ENG8 are GH16 hydrolytic enzymes. While ENGL1 and ENG2 have already been cloned and characterised, here we report the construction of the quadruple deletion strain Afeng2-5 depleted of four GH16_MLG1_glucanases. All four genes were expressed in the wild-type strain during germination and also at later time-points. Additionally all four corresponding proteins are localised close to the cell wall. Eng2 is bound to the plasma membrane by a GPI anchor, Eng4 and Eng5 by a transmembrane helix and Eng3 is secreted, allowing it direct contact with cell wall glucans.

**Conclusion:**
The quadruple mutant showed fragile conidia. As there is still glucanases activities in the quadruple mutant, the quintuple Afengl1/eng2-5 is currently constructed.
A NOVEL TRANSCRIPTIONAL REGULATOR ATRR OF ASPERGILLUS FUMIGATUS IS REQUIRED FOR AZOLE RESISTANCE, HYPOXIA GROWTH, AND EXPRESSION OF ABC TRANSPORTER GENE CDRIB

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Purpose:
Although azoles are major agents for treatment of aspergillosis, resistance to the drugs in a main causative species Aspergillus fumigatus is prevailing in this decade. In most cases, amino acid substitutions are found in an azole-target protein Cyp51A of resistant strains. Although much attention has been paid to the Cyp51A mutation regarding azole resistance, molecular mechanism underlying regulation of Cyp51A as well as transporters that contribute to efflux and sequestration of azole is obscure. In a study of related species Aspergillus oryzae, we have found that a putative transcription factor AtrR was involved in azole resistance and the expression of transporter genes. We here characterized the corresponding AtrR of A. fumigatus with regard to azole resistance, growth under hypoxic condition, and regulation of ABC transporter gene expression.

Methods:
We constructed an atrR gene deletion strain and the complemented strain (Co-atri). To characterize atrR mutant, growth in the presence of azole drugs, expression of cyp51A and the other genes of ergosterol biosynthesis pathway (erg3, erg24A, and erg25A), and growth under hypoxic condition (approximately 1% O2) were investigated. Furthermore, expression of the ABC transporter Cdr1B/AbcC that was recently reported to be associated with azole resistance was also examined.

Results:
Deletion of atrR resulted in an increased sensitivity to azole drugs such as miconazole, itraconazole, and ketokonazole, but not to micafungin and amphotericin B in A. fumigatus. Quantitative real-time PCR analysis showed that cyp51A expression in the atrR mutant was significantly low. In addition, the gene expression levels of erg3, erg24A, and erg25A in the mutant were lower than those in WT (Af293). The atrR mutant grew comparable to WT under normoxic condition. However, the mutant was unable to grow under hypoxic condition, whereas WT strain could grow normally. The cdr1B expression was induced in response to azole addition in WT. In atrR mutant, however, the induction was not observed and the basal level of the cdr1B expression was decreased.

Conclusion:
Results of the present study indicate that AtrR of A. fumigatus plays a crucial role in azole resistance by regulating cyp51A expression as well as cdr1B that were involved in azole resistance. AtrR was also shown to be required for hypoxic growth, suggesting an important role in pathogenicity.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
PREVALENCE OF CRYPTIC SPECIES OF ASPERGILLUS ISOLATED FROM CLINICAL SAMPLES IN BRAZIL

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**Purpose:**
Aspergillus spp. are ranked as the second or third most common cause of opportunistic invasive fungal infection in tertiary care hospitals. In clinical practice, these infections are difficult to diagnose and delayed treatment may increase mortality. Only few medical centers are able to perform accurate identification of Aspergillus at species level in Latin America.

The aim of this study was to analyze the distribution of Aspergillus species among clinical samples isolated from 133 patients admitted in 12 medical centers in Brazil.

**Methods:**
The identification of Aspergillus species was performed based on macromorphology, micromorphology and thermotolerance of the colonies, as well as sequencing analyses of ITS region, calmodulin and β-tubulin genes using the Basic Local Alignment Search Tool (BLAST). Phylogenetic analyses using Bayesian inference and maximum parsimony methodologies were performed to characterize isolates with inconsistent identification by sequencing of the mentioned DNA targets.

**Results:**
The table below illustrates the results of species identification and the species distribution of the isolates included in this study. Despite all effort, 5 isolates from Nigri and 3 isolates from Flavi section could not be identified at species level.

<table>
<thead>
<tr>
<th>Section (n. of isolates)</th>
<th>Species Identification (n. of isolates)</th>
<th>Source (n. of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clavati (1)</td>
<td>A. clavatus (1)</td>
<td>Respiratory tract biopsy (1)</td>
</tr>
<tr>
<td>Circumdati (1)</td>
<td>A. ochraceus (1)</td>
<td>Respiratory secretion (1)</td>
</tr>
<tr>
<td>Flavipedes (1)</td>
<td>A. flavipes (1)</td>
<td>Tissue biopsy (1)</td>
</tr>
<tr>
<td>Ústí (1)</td>
<td>A. calidoustus (1)</td>
<td>Tissue biopsy (1)</td>
</tr>
<tr>
<td>Terrei (2)</td>
<td>A. terreus (2)</td>
<td>Tissue biopsy (1), Ear secretion (1)</td>
</tr>
<tr>
<td>Nidulantes (5)</td>
<td>A. tennesensis (3)</td>
<td>Respiratory tract secretion (2), Skin biopsy (1)</td>
</tr>
<tr>
<td></td>
<td>A. tabacinus (1)</td>
<td>Respiratory secretion (1)</td>
</tr>
<tr>
<td></td>
<td>A. creber (1)</td>
<td>Respiratory secretion (1)</td>
</tr>
<tr>
<td>Nigri (13)</td>
<td>A. awamori (3)</td>
<td>Respiratory secretion (2), Skin biopsy (1)</td>
</tr>
<tr>
<td></td>
<td>A. foetidus (1)</td>
<td>Respiratory secretion (1)</td>
</tr>
<tr>
<td></td>
<td>A. niger (4)</td>
<td>Respiratory secretion (3), Tissue biopsy (1)</td>
</tr>
<tr>
<td></td>
<td>Aspergillus sp. 2 (4)</td>
<td>Respiratory secretion (3), Ear secretion (1)</td>
</tr>
<tr>
<td></td>
<td>Aspergillus sp. 3 (1)</td>
<td>Aspirate (1)</td>
</tr>
<tr>
<td>Flavi (37)</td>
<td>A. flavus / A. oryzae (29)</td>
<td>Skin biopsy (1), Respiratory tract biopsy (4), Respiratory secretion (16), Tissue biopsy (8)</td>
</tr>
<tr>
<td></td>
<td>A. arachidicola (1)</td>
<td>Respiratory tract biopsy (1)</td>
</tr>
<tr>
<td></td>
<td>A. nomius (1)</td>
<td>Respiratory secretion (1)</td>
</tr>
<tr>
<td></td>
<td>Aspergillus sp. 1 (3)</td>
<td>Respiratory secretion (2), Respiratory tract biopsy (1)</td>
</tr>
<tr>
<td></td>
<td>A. tamarii (2)</td>
<td>Tissue biopsy (1), Respiratory tract biopsy (1)</td>
</tr>
<tr>
<td></td>
<td>A. transmontanensis (1)</td>
<td>Respiratory tract biopsy (1)</td>
</tr>
<tr>
<td>Fumigati (72)</td>
<td>N. pseudofischeri (1)</td>
<td>Tissue biopsy (1)</td>
</tr>
<tr>
<td></td>
<td>A. fumigatus (71)</td>
<td>Skin biopsy (3), Respiratory tract biopsy (10), Respiratory secretion (46), Tissue biopsy (12)</td>
</tr>
</tbody>
</table>
Conclusion:
This was the first Brazilian study to demonstrate a high prevalence of cryptic species among clinical
*Aspergillus* strains including rare human pathogens as *A. arachidicola, A. tabacinus, A. tennesseensis, A. creber* and *A. transmontanensis*. These results reinforce the importance of accurate identification of *Aspergillus* at species level, since the cryptic species may present variable susceptibility profiles against the antifungal drugs.
CHARACTERIZATION THE RTFA GENE IN ASPERGILLUS FUMIGATUS

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Purpose
Invasive aspergillosis by Aspergillus fumigatus is a leading cause of infection-related mortality in immunocompromised patients. This population group includes individuals infected with HIV, cancer patients undergoing chemotherapy, those individuals with genetic immune deficiencies and those with hematological malignancies. The number of patients who fall into these categories is steadily increasing. In order to discover potential genetic targets to control A. fumigatus infections, we have characterized rtFA, a gene encoding a conserved putative RNA polymerase II elongation factor. Recently, work from our laboratory has shown that the rtFA ortholog in the model fungus Aspergillus nidulans influences growth, conidiation, and secondary metabolism. In the present study we are investigating the regulatory output of rtFA in the opportunistic pathogen A. fumigatus.

Methods
With this purpose, we generated an A. fumigatus rtFA deletion mutant, a complementation strain where the rtFA wild-type allele was incorporated in the rtFA deletion mutant, and an rtFA over-expression strain. These strains were compared with the isogenic wild-type control strain, CEA10. Predicted expression profile of rtFA in these strains was confirmed by qRT-PCR. Colony growth was determined by colony diameter in point-inoculated Czapek-Dox cultures. The strains were allowed to grow for five days. Quantification of conidial production was performed with an hemacytometer under a light microscope. Protease activity was measured using an Azo-Casein assay. Oxidative stress response was evaluated adding increased concentrations of menadione to the culture medium. All the experiments were carried out with three replicates.

Results
Deletion of A. fumigatus rtFA resulted in a strain with a notable reduction (30%) in colony diameter compared to the wild-type strain. Complementation of the rtFA deletion strain with the rtFA wild-type allele was able to restore wild-type phenotype. Deletion of rtFA also resulted in colonies with delayed but slightly increased conidiation levels compared to the controls. Interestingly, the rtFA deletion strain was also deficient in protease activity. In addition, the rtFA deletion strain presented higher sensitivity to oxidative stress, being unable to grow in the presence of 20 µM menadione, condition that allowed normal growth in the wild type strain. The over-expression strain presented wild-type phenotype.

Conclusion
The notable decrease in colony diameter of the rtFA deletion strain compared to the control strains indicates that rtFA is necessary for normal growth in A. fumigatus. Furthermore, the delay and increase in conidiation detected in the absence of rtFA suggests that this gene is a regulator of conidiation. In addition, the rtFA mutant lacks protease activity, and presents growth inhibition in an oxidative stress environment. For all these reasons, rtFA is a promising genetic target with high potential against aspergillosis. Current studies in our laboratory are focused on further characterizing the effect of rtFA on A. fumigatus secondary metabolism and pathogenicity.
UNUSUAL FORMS OF CHRONIC PULMONARY ASPERGILLOSIS IN PATIENTS WITH SOLID TUMORS

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Background:
Aspergillus spp. can cause acute invasive disease in severely immunocompromised patients. Nonetheless, there are few reported cases of solid tumors complicated with chronic pulmonary aspergillosis (CPA).

Methods:
A retrospective observational cohort study performed in patients with primary lung cancer or secondary lung metastasis complicated with CPA in three referral hospitals.

Results:
From 2008 to 2011, 14 episodes of CPA were diagnosed, including 11 (78.6%) probable and 3 proven (21.4%). Nine patients (64.3%) had primary lung cancer. Thirteen patients (92.9%) had more than one local or systemic predisposing factor for CPA. No patient had previous fungal colonization. Aspergillus spp. was isolated in 6 specimens of bronchoalveolar lavage, 6 sputum, 2 biopsies, and 1 percutaneous lung puncture. At the time Aspergillus spp. was isolated, the most common radiologic findings on chest computed tomography (CT) were cavitory masses, and development or expansion of cavitation in existing masses or nodules (10/14, 71.4%). On CT follow-up, most patients (8/12, 66.7%) had new cavity formation or expansion of one or more existing cavities. All patients were treated with azoles and two underwent surgery. Ten (71.4%) patients died after Aspergillus spp. was detected (median time 73 days, IQR 33-243): 2 (20%) deaths were CPA-attributable and 6 (60%) were related.

Conclusions:
Primary lung cancer and secondary lung metastasis seem to be triggering factors for Aspergillus spp. implantation, and predispose to CPA. Once localized in the damaged lung, the mold can grow and cause or expand cavities. In lung cancer patients, Aspergillus spp. detection is associated with a very poor prognosis.
Purpose:
This study intended to characterize fungal contamination in a totally indoor composting plant located in Portugal. Besides conventional methods, molecular biology was also applied to overcome eventual limitations.

Methods:
Air and surfaces samples from one composting plant were collected through impaction and swab methods, respectively. The analyzed places inside this plant were: maturation park, waste screw, maintenance workshop, room process control, pre-treatment and centrifugues. After laboratory processing and incubation of the collected samples, quantitative and qualitative results were obtained, with identification of the isolated fungal species. For molecular analysis, air samples of 250L were also collected from the same sampling sites using the impinger method. Molecular detection of *Aspergillus fumigatus*-complex was achieved by Real Time PCR (RT-PCR).

Results:
Nine different species of filamentous fungi were identified in air samples with a total of 982 isolates. *Aspergillus* genus showed the highest prevalence (90.6%) of isolates. The complexes *Nigri* (32.6%), *Fumigati* (26.5%) and *Flavi* (16.3%) were the most prevalent fungi in air sampling. Four different species were isolated in surfaces samples with a total of 1810000 isolates. *Aspergillus* genus also showed the highest prevalence (60.8%). *Mucor* sp. (39.2%), and the *Aspergillus* complexes *Nigri* (30.9%) and *Fumigati* (28.7%) were the most frequently found. Regarding the results obtained from molecular methods, *A. fumigatus* complex was detected in one more sampling site (maintenance workshop) than using conventional methods, and was also detected in the air samples from two sampling sites that were not isolated with conventional methods (maturation park and room process control).

Conclusion:
The complementarity of conventional and molecular methodologies was corroborated in this study, since it was possible to achieve a more realistic scenario regarding *A. fumigatus* complex exposure assessment by the use of both methods. The results obtained highlight the need to apply conventional and molecular methods to assess occupational fungal exposure in highly contaminated settings.
Efficacy and Toxicity in Clinical Practice of Antifungal Combination Therapy for Proven or Probable Invasive Mold Diseases in Hematopoietic Stem Cell Transplantation

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3Division of Hematology, UCSC, Roma, Italy

Introduction:
This multicentre observational study reported efficacy and toxicity of antifungal combination (Combo) therapy as treatment of proven or probable invasive mold diseases (IMDs) in hematopoietic stem cell transplantation (HSCT) recipients.

Materials and Methods:
Between June 2006 and June 2012, 30 cases of IMDs (16 proven and 14 probable) treated with Combo were reported from 7 HSCT Centers in Italy. Median age of HSCT patients was 29 yrs (range 2-61) and 26% had less than 18 yrs. Acute leukemia was their most common underlying hematologic disease (17/30; 57%); 10/30 (33%) of cases were in complete remission and 20/30 (67%) had refractory or relapsed hematologic disease. The main site of infection was lung with or without other sites. The causative moulds were: Aspergillus sp in 22 cases (74%), Zygomycetes in 4 cases (13%), Fusarium sp in 3 cases (10%) and Paecilomyces sp in 1/30 (3%).

Results:
The most used combo therapy were caspofungin+voriconazole (13/30 pts-43%), caspofungin + liposomal amphotericin B (L-AmB) (7/30 pts-22%), and L-AmB+voriconazole (8/30 pts-26%). The median duration of combo therapy was 30 days (range 7-154). The overall response rate (ORR) was 67% (20/30 responders) without significant differences between the combo regimens. The granulocyte (PMN) recovery did not significantly influenced the response to combo therapy in HSCT recipients. Only 29% of patients experienced mild and reversible adverse events (hypokalemia, ALT/AST increase, creatinine increase). The mortality IFD related was 24%.

Conclusion:
This observational study indicates that combo therapy was well tolerated and effective in HSCT with proven or probable IMDs. The most used combo regimens in clinical practice were caspofungin+voriconazole (with a response rate of 80%) and caspofungin+ L-AmB (response rate 70%). The ORR was 67%.
POSACONAZOLE PLASMA CONCENTRATIONS: CORRELATION OF EARLY TROUGH LEVELS (DAY 4) WITH STEADY-STATE CONCENTRATIONS

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Purpose:
Low posaconazole plasma concentrations (PPCs) may be associated with breakthrough invasive mould infections (IMI) among patients with hematological malignancies. Generally it is considered that steady-state plasma concentrations of posaconazole are achieved after 6 to 10 days. The purpose of this study was to correlate PPCs measured early (day 4 of prophylaxis) with those obtained during steady state.

Methods:
The study was conducted at the Division of Hematology, Medical University of Graz, Austria. Hematologic patients receiving posaconazole with initial trough PPCs obtained on day 4 of prophylaxis and then subsequent PPCs obtained between day 7 and 8 (defined as “early steady state”) as well as between days 10 and 14 (defined as “late steady state”) were included. The two steady state time points were always compared to concentrations of day 4. PPCs above the target of 0.5 mg/L were defined as satisfactory and those below the target as low PPCs.

Results:
A total of 37 patients with PPCs obtained at all 3 time points were included. 8 patients (22%) had PPCs below 0.2 on day 4. PPCs remained unchanged in all patients at early steady state, while at late steady state 1/8 had a satisfactory PPC and 3/8 PPCs between 0.2 and 0.4. 11 patients (30%) had PPCs between 0.2 and 0.5 at day 4. At early steady state 2 of those 11 patients had satisfactory levels and one a PPC <0.20, while PPCs remained unchanged in 8/11 patients. At late steady state PPCs had decreased in 2 to <0.20 and increased in 3 to >0.50, while they remained unchanged in 6/11 patients. The majority of included patients (n=18; 49%) had satisfactory PPCs on day 4. PPCs decreased below 0.5 in 4/18 patients at early and 3/18 patients at late steady state (each compared to day 4). Concentrations remained satisfactory in 14/18 patients at early steady state and 15/18 patients at late steady state, although accumulating to twice or more of the initial day 4 PPC in 4 patients at early and 6 at late steady state.

Conclusion:
A positive correlation was found between PPCs obtained on day 4 and those on days 7/8 and 10-14. In patients with insufficient PPCs on day 4 only 4/19 had satisfactory PPCs at late steady-state. In patients with PPCs >0.5 on day 4 concentrations remained satisfactory in nearly 80% at both steady-state time points. Bigger studies are needed to evaluate potential benefits of early PPC measurements and early intervention (e.g. modification of intake procedure and dosage).
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Purpose:
Timely diagnosis is a key factor in successful treatment of invasive fungal infections (IFI) at the ICU. Beta-D-glucan (BDG) is a component of the fungal cell wall of most fungi including *Aspergillus*, *Candida*, *Fusarium* and *Pneumocystis*. BDG serum testing has been shown to be a useful marker for early detection and therapy monitoring of IFIs in the ICU. The exact role of BDG in clinical routine/IFI management remains, however, unclear. The purpose of this study was to evaluate a preemptive approach with BDG as a marker for guidance of AF in the ICU.

Methods:
ICU patients with clinical suspicion of invasive fungal infections admitted between April 2013 and September 2013 at the Medical University of Graz, Austria were included in the analysis. All patients were seen by the Infectious Disease (ID) service and clinical decision was made together with ICU physicians whether or not to initiate systemic preemptive antifungal therapy (AF) depending on clinics and candida scores. BDG testing was performed always prior to initiation of antifungal treatment and in addition to routine diagnostic measures. Beta-D-glucan testing was performed automatically on the coagulation automat providing results within 24 hours. On the following day when BDG test results were available preemptive antifungal therapy was either discontinued (in case of negative BDG results, i.e. <60 pg/mL) or initiated/continued (in case of positive results, i.e. >120 pg/mL). BDG testing was repeated in case of results between 60 and 120.

Results:
In total 66 pts were included in the analysis. According to clinical decisions by ID and ICU physicians preemptive AF were started in 40 pts, while in 26 pts no AF therapy was initiated. One day later when BDG test results were available BDG results led to discontinuation of preemptive AF in 13 patients, initiation of AF in 7 patients, while in 46 patients the clinical decision was confirmed by BDG results (27 pts with AF, 19 patients without AF). While the majority of probable and proven IFI cases were predicted by the test, BDG resulted negative in two cases diagnosed later as probable invasive aspergillosis (diagnosed by BAL GM 4 and 9 days later) and one case of probable invasive candidiasis (diagnosed 4 days later). In all 3 patients AF would have also not been initiated according to clinical decision.

Conclusion:
BDG seems to be a promising tool to guide antifungal therapy in ICU patients. Prospective studies evaluating our IFI management approach are needed to confirm our results.
GALACTOMANNAN ANTIGEN ASSAY FROM BRONCHOALVEOLAR LAVAGE FLUID IN DIAGNOSIS OF INVASIVE PULMONARY ASPERGILLOSIS IN INTENSIVE CARE UNITS PATIENTS

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Purpose:
Invasive pulmonary aspergillosis (IPA) is an important infection in critically ill patients including patients of intensive care units (ICU). Different diagnostic tools are available and since its mortality is high, it is vital to start the antifungal therapy as soon as possible. Knowing the epidemiology of this disease in each ICU and area will help to better and more rapid management of such patients. The aim of this study is to determine the frequency of IPA based on the level of galactomannan in bronchoalveolar lavage fluid in ICU of Al-Zahra hospital, Isfahan, Iran.

Methods:
This was a cross sectional study, which was conducted in Al-Zahra hospital, Isfahan, Iran, between 2010 to 2011. The study population was all the patients admitted to ICU and were suspected to have invasive Aspergillus spp pneumonia. The level of galactomannan in bronchoalveolar lavage was measured and demographic data were gathered by the questionnaire.

Results:
The frequency of IPA in this study was calculated as 2.43% while galactomannan level in bronchoalveolar lavage fluid of this patient (2.50) was significantly higher than others (0.03±0.02).

Conclusion:
Larger studies are required to determine the exact frequency of IPA and the best antifungal therapy for it.
AFLP GENOTYPING, IN VITRO ANTIFUNGAL SUSCEPTIBILITY AND EVALUATION OF ANTIFUNGAL COMBINATIONS OF AMPHOTERICIN B, VORICONAZOLE AND ANIDULAFUNGIN AGAINST 126 CLINICAL ASPERGILLUS TERREUS SPECIES COMPLEX ISOLATES FROM A TERTIARY CARE CHEST HOSPITAL, DELHI, INDIA

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Purpose:
Aspergillus terreus is the predominant etiological agent of invasive aspergillosis (IA) in certain medical centers around the world including those in Houston, Texas, and Innsbruck, Austria, suggesting ecological specificity for this pathogen. Aspergillus terreus is of serious concern because of resistance to amphotericin B (AMB) and isolates from different parts of the world reveal different patterns of AMB susceptibility. Moreover, A. terreus is a complex, comprising many newly recognized species such as A. alabamensis, A. terreus var. floccosus, A. terreus var. africanus, A. terreus var. aureus, A. hortai and A. terreus, therefore, molecular techniques are mandatory for its definitive identification. In the present investigation 126 A. terreus complex isolates were (a) analyzed employing amplified fragment length polymorphism (AFLP) and calmodulin gene sequencing (b) studied for in vitro activity against AMB, azoles and echinocandins. Additionally, we evaluated in vitro combination regimens using voriconazole, anidulafungin and AMB in 20 isolates for synergistic activity.

Methods:
One hundred twenty six A. terreus species complex isolates originating from 118 patients collected during 2009-2013 from V. P. Chest Institute, Delhi were investigated. Data included demographics, information on underlying disease, identification of the clinical disease entity or condition due to A. terreus such as IA, aspergilloma, chronic necrotizing pneumonia, allergicbronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA) or colonization. The isolates were sequenced using calmodulin gene and subjected to AFLP analysis. In vitro antifungal susceptibility profile was determined using CLSI M38-A2 broth microdilution method. Microdilution broth checkerboard technique based on the CLSI was used to evaluate antifungal combinations against 20 clinical isolates of A. terreus.

Results:
Of 126 A. terreus isolates, 114 were identified as A. terreus, 7 as A. cryptoterreus and two as A. hortai and 3 were not identifiable. Sixty-two (54.3%) of 114 patients were colonized with A. terreus. Of 52 (45.6%), patients with infection, 21 had ABPA, 12 had IA, 11 aspergilloma, and 8 CPA. Among the 12 patients with IA, 7 (58.4%) had disseminated disease and the deaths of 8 patients (66.6%) were attributed to A. terreus infection. AFLP showed that Indian A. terreus isolates clustered separately from CBS reference strains. All of the isolates were resistant to AMB (MIC40 4µg/ml). All azoles, posaconazole (MIC50 0.125µg/ml), itraconazole (MIC50 0.25µg/ml), voriconazole (MIC50 0.5µg/ml) and isavuconazole (0.5µg/ml) had good in vitro activity. Also, the 3 echinocandins revealed low MICs. Combinations study of voriconazole with anidulafungin showed additive effect in 12 (60%) isolates with FIC index (0.52-0.62) and synergy in 6 (30%) while 2 isolates had indifference. The interaction between voriconazole and AMB was additive (FIC index, 0.52-0.64) for all 20 isolates. Similarly, anidulafungin with AMB also showed additive FIC index values barring two isolates which revealed antagonistic effect.

Conclusion:
Invasive and allergic diseases caused by A. terreus are common at our institution. Azoles and echinocandins demonstrated good in vitro activity. Indian A. terreus isolates represented a single clade by AFLP, representing a possible geographical endemicity. Combination of voriconazole with anidulafungin and AMB warrants further investigations.
GALLERIA MELLONELLA AS ALTERNATIVE MODEL TO STUDY THE IN VIVO EFFICACY OF AMPHOTERICIN B AGAINST ASPERGILLUS TERREUS INFECTIONS AND ITS INFLUENCE ON LARVAL IMMUNE RESPONSE

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Background:
Infections with Aspergillus terreus are of major concern, due to its high likelihood of dissemination and its intrinsic resistance to amphotericin B (amB). The reason for this resistance is not known yet. Recently, three clinical isolates, with distinct morphological variations, have been found to be amB susceptible in vitro.

Methods:
Efficacy of amB treatment and its influence on the larval immune system were investigated in the invertebrate model G. mellonella. Proteomic analysis of larval haemolymph, haemocyte counts and post-treatment infection studies were performed. Additionally, putative difference in virulence potential of the respective isolates was analyzed by correlating survival rates with physiological attributes and in vitro killing ability of larval haemocytes.

Results:
Treatment with amB only showed success in the groups infected with amB-susceptible strains, which reflected our in vitro data. Furthermore, amB administration resulted in an increased number of circulating haemocytes. Proteomic studies showed different protein expression of proteins which have immune function. Pre-treatment of larvae with amB also increased their resistance to Staphylococcus aureus infection, indicating a general ability of amB to prime the insect’s immune system. Larval survival rates differed in the early time points of infection for the various isolates tested. The amB-resistant isolate T90, showed lowest mortality rates in the early time points of infection. This is in correlation with a slower germination rate of T90, and the highest fungal damage caused by haemocytes in vitro.

Conclusion:
This work demonstrated that G. mellonella is a useful model to determine the in vivo efficacy of amB against A. terreus isolates, and study the virulence potential of different isolates. Our results showed that antifungal treatment in vivo correlates with in vitro susceptibility data. Furthermore, one has to be aware of a non specific influence of antifungal drugs on the larval immune response.
CONTROL OF AZOLE RESISTANCE ASPERGILLUS FUMIGATUS BY USING OF RNAI BASED ON CYP51A GENE

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Purpose:
Drug resistant in environmental and clinical isolates of Aspergillus has revealed in recent decades has been detected especially in A. fumigatus. Two important factors which lead to route of action and azole resistance mechanism is either long term exposure to azoles in patients with aspergillosis or confront toazole fungicides in agriculture fields. Anyhow the evolution of drug resistance is more likely to proceed by the mutations in amino acids or less by MDR genes overexpressed. Majority cases of azole resistance A. fumigatus are related with mutations in the azole drug target enzyme encoded by the cyp51A gene. Therefore, here we evaluated RNA silencing technology for decreasing of resitancy to azole treatment in patients with aspergillosis.

Methods:
siRNA sequences was designed to target the mRNA sequence of the cyp51A. The 21-nucleotide siRNA was designed on the basis of the cDNA sequence of the Cyp51A gene of A. fumigatus. The sense and antisense sequences were 5’-UGGCAAGCACAAGGACGUUAA-3’ and 5’FAM-UUAACGUCCUUGUGCUUGCCA-3’ respectively. Also a scramble sequence which is unrelated and random siRNA of the mentioned target with the sense and antisense oligonucleotides 5’-GAUGGCAUACCUAGGAGAACA-3’ and 5’-CUACCGUAUGGAUCCUCUUGU-3’ was obtained from a distinct site and synthesized as a negative control. The antisense strand of siRNA was labeled with the FAM fluorescent dye to monitor the entrance of siRNA in mycelia. To investigate cyp51A gene silencing in siRNA treated germinated conidia (15, 20, 25 and 50nM), azole resistant Aspergillus was cultured on Czapek-dox broth, then quantitative changes in expression of the cyp51A gene were analyzed by measuring the cognate cyp51A mRNA level by use of a RT-PCR assay. Statistical analysis was performed by using of R software version 3.0.1.

Results:
With fluorescence microscopy, it was approximately appraised which all mycelia with used concentrations had been transfected by siRNA successfully. Compared with the positive control, expression of the cyp51A gene was significantly reduced by siRNA at concentrations of 50 nM (P=0.05). In addition, siRNA at mentioned concentration, revealed to decrease MIC effectively.

Conclusion:
The correlations between changes in cyp51A gene expression and MIC suggests RNA silencing technology could be used as a efficient means for control of azole resistancy in patients with aspergillosis.

Keywords:
Azole resistance, Aspergillus fumigatus, RNAi, cyp51A gene
STUDY THE ANTIFUNGAL ACTIVITY OF NEW IMIDAZOLE, BENZIMIDAZOLES, TRIAZOLES, BENZOTRIAZOLES AND TETRAZOLES DERIVATIVES AGAINST ASPERGILLUS FLAVUS AND ASPERGILLUS FUMIGATUS

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Purpose:
Aspergillus species are associated with diseases ranging from simple onychomycosis to life-threatening infections in immunocompromised patients. During the past two decades, resistance to established antifungal drugs has increased dramatically and has made it crucial to identify novel antimicrobial compounds. In this study, the antifungal activity of novel imidazole, benzimidazoles, triazoles, benzotriazoles and tetrazoles derivatives against Aspergillus spp were examined and the possible association of their structure and effects were determined.

Methods:
Five new derivatives of triazoles, three of benzotriazoles, five of imidazoles and three of benzimidazoles were designed as inhibitors of cytochrome P450 14a-demethylase. Moreover, nine derivatives of benzimidazoles and 13 of tetrazoles were also been synthesized in the presence of nano-TiCl4.SiO2 as a reusable and efficient catalyst. The chemical structures of the new compounds were confirmed by elemental and spectral (1H-NMR and Mass) analyses. The antimicrobial activities of the synthetic compounds have been tested against the A. flavus (ATCC 64025) and A. fumigatus (ATCC 14110) by broth microdilution method as recommended by CLSI.

Results:
Inhibition studies showed that some of the tested compounds, in particular 1-(diphenylmethyl)-1H-imidazole and 1-[(4-chlorophenyl)(phenyl)methyl]-1H-imidazole, exhibited strong antifungal activities against the tested fungi followed in activity by 2-(2,3-dihydroxyphenyl)-1H-5-nitrobenzimidazole and 2-phenyl-1H-benzimidazole. None of the tetrazoles exhibited the antimicrobial activities at the examined concentrations.

Conclusion:
These results suggest that the derivatives should be investigated further for possible use in antimicrobial products.
Purpose:
The objective of this study was to investigate the airborne fungi in sheep house environment and potential evaluation of possible risk of respiratory diseases.

Methods:
The study was conducted in one building for sheep. In total were taken 15 air samples, weekly, in the middle of each week in the same period (between 13:00 and 14:00 h). In each trial were collected four air samples. One technique was applied for the airborne fungi trapping: the sedimentation method. In this method were used Petri dishes with the radius of 90 mm, that were filled with 15 ml of (Sabouraud dextrose agar) The plates were exposed in the same spaces at each trials: in the front, in the left and right side of the middle and on the end of sheep house). The exposure time for the sedimentation plates was 10 min. Then the plates were incubated at 26±2°C for fungi. The fungal colonies developed were counted after 3, 5, and 7 days.

For the identification of isolates, the purified fungal cultures were transferred onto malt extract, Czapek. Their cultural and morphological characteristics were studied employing light microscopy. Identification to the genus and/or species level was done by rate of growth, texture and the pigmentation of cultures (Laura, Charles and Arnold, 1998).

Results:
Ten species ascribe to eight fungal genera were isolated and identified from the sheep house investigated.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Number of isolates</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>23</td>
<td>30.7</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>14</td>
<td>18.7</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Penicillium spp</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Rhizopus spp</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Fusarium spp</td>
<td>4</td>
<td>5.3</td>
</tr>
<tr>
<td>Curvularia spp</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Rhinocladiella spp</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>Alternaria spp</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Exserohilum spp</td>
<td>1</td>
<td>1.3</td>
</tr>
</tbody>
</table>
RANDOM AMPLIFIED POLYMORPHIC DNA AS A NOVEL METHOD FOR MAKING A LINK BETWEEN THE *ASPERGILLUS* CLINICAL AND HOSPITAL ENVIRONMENTAL ISOLATES

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**Purpose:**
Aspergillosis is known to be an airborne infection and the nosocomial infections are associated with constructions and increased dust loads in hospital indoors. Our main object was to find the environmental sources of *Aspergillus* species causing hospital acquired infections.

**Methods:**
The clinical and environmental samplings were performed during 18 months from spring 2010 to summer 2011 in an educational hospital. A morphological diagnosis was used including media culture, for the first identification of all isolated *Aspergillus* species. For the random amplified polymorphic DNA (RAPD) assay, extraction of DNA was performed using manual phenol-chloroform method followed by PCR with six random primers. The results of RAPD were compared between the clinical *Aspergillus* isolates and hospital indoor isolates.

**Results:**
Application of primer in RAPD was resulted different patterns for the pairs 16, 36 and 237, but same pattern for the pair of 31 including; case and environmental *Aspergillus* isolates. P2 resulted identical patterns for *Aspergillus* groups 16, 31 and 237. Comparison of clinical and environmental revealed the pairs 32 and 45 are only *Aspergillus* strains with identical RAPD patterns. The *Aspergillus* isolated from the case no(32) BAL sample was completely similar to that of relevant air conditioner sample in RAPD analysis. Also, banding patterns of *A. flavus* isolated from case no(45) sinus discharge was similar to those of wall swabs.

**Conclusion:**
The hospital sources for the *Aspergillus* clinical isolates included air condition and walls and RAPD-PCR analysis can play a trivial role in finding the hospital sources of *Aspergillus* clinical isolates.
**Aspergillus terreus** as an emerging cause for pulmonary fungus ball: A case report

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**Introduction:**
Based on epidemiological studies, *Aspergillus terreus* has been demonstrated as an emerging fungal agent of pulmonary fungus ball. Here we describe a case of pulmonary fungus ball due to *Aspergillus terreus* in a 30-year-old Iranian female.

**Case:**
The patient had a history of bilateral pulmonary echinococcal (hydatid) cyst surgery. However, clinical signs/symptoms were weight loss, dyspnea and chronic persistent cough but not fever and haemoptysis. No evidences were seen for fasciolosis, HIV and tuberculosis infections.

Dichotomously branched hyphae were observed in direct microscopy of bronchoalveolar lavage (BAL). Several inoculation of BAL samples on sabouraud’s medium was indicative for *Aspergillus Section Terrei*. Sequencing of RNA polymerase II (RBP2) and Beta tubulin (BT2) genes confirmed the identity of isolate as *A. terreus*. Oral therapy with itraconazole provided complete resolution of aspergilloma in the patient and no evidence of infection was detected in the mycological and pathological inspection of BAL samples after 3 months follow-up.

**Conclusion:**
In conclusion, it is recommended to apply molecular biology techniques for rapid, reliable and accurate identification of fungal pathogens as well as for selecting effective antifungal therapy.

**Keywords:**
*Aspergillus terreus*, Aspergilloma, RBP2
**IN VIVO MANIPULATION OF LUNG OXYGEN CONTENT TO REDUCE FUNGAL GROWTH DURING MURINE INVASIVE PULMONARY ASPERGILLOSIS**

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**Purpose:**

Aspergillus fumigatus is a filamentous fungus with the potential to cause invasive disease in immune compromised hosts, known as invasive aspergillosis. Recent studies have shown that adaptation to hypoxia present at the infection site microenvironment is one of the major traits of this fungus that allows it to cause disease. A. fumigatus has also been shown to block angiogenesis, which may contribute to the infection site hypoxia and contribute to poor disease outcomes. It remains to be fully defined why treatment with current anti-fungal drugs (polyenes, azoles and echinocandins), that are so effective *in vitro*, still results in high mortality rates. Perhaps importantly, transcripts for genes encoding the targets of these drug classes show significant changes in response to hypoxia. Given the negative impact of hypoxia on IPA outcomes, we hypothesized that hyperbaric oxygen treatments could relieve tissue hypoxia, increase angiogenesis, alter fungal metabolism to enhance susceptibility to antifungal drugs, and thus overall reduce fungal growth and tissue invasion to improve IPA outcomes.

**Methods:**

To test our hypothesis we conducted *in vitro* and *in vivo* experiments to examine the impact of hyperbaric oxygen (100% O₂ at 2.5 ATA) on fungal physiology. Experiments were conducted with HBO therapy alone or in combination with existing antifungal drugs. *In vitro* fungal metabolic activity in response to HBO treatments was measured with the XTT assay. Analysis of fungal gene expression of select target genes was conducted with qRT-PCR. For *in vivo*, studies a leukopenic mouse model of invasive pulmonary aspergillosis was utilized. After establishment of infection for 12 hours, two hyperbaric treatments of 2 hours each were given at 24-hour interval (36 hours and 60 hours post inoculation, effectively). Lungs were harvested and fungal burden was measured with qRT-PCR analyses of fungal 18S rDNA.

**Results:**

We observed that *in vitro* hyperbaric oxygen treatments significantly reduced fungal metabolic activity by 90% as measured by the XTT assay compared to normoxic and hypoxic conditions. Importantly, leukopenic mice given two hyperbaric treatments had a significant reduction in fungal burden as measured by quantitative real time PCR. No oxygen toxicity was observed in control mice after three hyperbaric treatments (2 hours each) as measured by their survival for 10 days after the last treatment.

**Conclusion:**

Preliminary data suggest hyperbaric oxygen treatments significantly inhibit fungal metabolic activity *in vitro* and is capable of reducing the fungal burden *in vivo*. Ongoing studies are assessing whether this reduction in fungal burden improves murine IPA survival rates. Moreover, ongoing studies are examining the mechanism of hyperbaric oxygen inhibition of fungal growth to identify new novel antifungal drug targets. Finally, experiments are underway to test the efficacy of hyperbaric oxygen in combination with existing antifungal drugs.

**NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.**
ANTIFUNGAL SUSCEPTIBILITY AND THE FREQUENCY OF ASPERGILLUS FUMIGATUS-RELATED SPECIES OF CLINICAL STRAINS COLLECTED AS “A. FUMIGATUS” FROM VARIOUS AREA IN JAPAN

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\textbf{Purpose:}
Azole resistance among clinical isolates of \textit{Aspergillus fumigatus} is becoming a serious problem in some countries. In Japan, only a few surveillance reports regarding the antifungal susceptibility of clinical isolates of \textit{A. fumigatus} are available, and the nationwide situation remains unknown. The aim of this study was to determine the present status of azole resistance in \textit{A. fumigatus} in Japan and the frequency of \textit{A. fumigatus}-related species.

\textbf{Methods:}
We employed 178 clinical isolates collected as \textit{A. fumigatus} from 1987 to 2008 at our institution. To find \textit{A. fumigatus}-related species, all isolates were re-examined from morphology and highest growth temperature, and those with atypical phenotypes were further analyzed genetically. The antifungal susceptibility of \textit{A. fumigatus} sensu stricto isolates was tested based on the CLSI M38-A2 broth microdilution method.

\textbf{Results:}
In our collection, 171 isolates were confirmed to be \textit{A. fumigatus} sensu stricto and the remaining 7 isolates (4.0%) were found to be related species of \textit{A. fumigatus}, i.e. \textit{A. lentulus} (2 isolates) and \textit{A. udagawae} (5 isolates). Only 1 (0.6%) and 2 isolates (1.2%) showed elevated MIC to voriconazole and itraconazole, respectively.

\textbf{Conclusion:}
Our study disclosed that the frequency of azole resistance in \textit{A. fumigatus} still remains low in this collection.
THE SUBCLINICAL ENVIRONMENTAL INVESTIGATIONS HAVE PROVEN TO PROVIDE DIAGNOSTIC LEADS TO THE CLINICIANS. MYCOSES INVOLVING ASPERGILLUS AND OTHER GENERA. CASE STUDIES, EXPOSURE ASSESSMENT AND FINDINGS

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Background:
In clinical practice it is very important to find out if the particular symptoms are related to a specific allergen or pathogen, and if exposure in a home or working environment has a causative effect.

Purpose:
The methods of identification of the environmental factors (Aspergilli and other fungi) that are clinically relevant by causing allergic, toxic, and potentially invasive effect in non-immunosuppressed patients and moreover in the immunocompromised individuals may be of interest to the physician has been an elusive challenge for hygienists.

Methods:
23 highly integrated and differentiated (allergy, immunology and toxicology) investigations were conducted where fungal causes were suspected. Patient medical history, current diagnosis, applied differentials with targeted and focused exposure sampling leading to relevant data collection with subsequent identification and reporting were performed in our centre.

Results:
Clinically based investigations of human habitations where suspected fungal were identified caused have provided relevant information to clinicians showing fungi as diagnostic leads in cases where such agents were suspected to be present. This confirms validity of the methods for sample collection, identification and reporting have had outcomes for patient health and recovery.

Conclusion:
In cases of suspected fungal related illnesses subClinical environmental investigations when prescribed by the dotor have been shown effective in showing clinical relevancy of suspected fungal agents may provide additional diagnostic leads to the physicians.
SUITABILITY OF BIS(METHYLTIO)GLIOTOXIN FOR INVASIVE PULMONARY ASPERGILLOSIS DIAGNOSIS

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Purpose:
Invasive pulmonary aspergillosis (IPA) is a severe disease caused in 90% of the cases by the mold Aspergillus fumigatus. It affects not only to immunocompromised patients but also to critically ill patients or patients with chronic obstructive pulmonary disease. Its mortality is high, thus being fundamental its early diagnosis and treatment.

Mold produces severe secondary metabolites which contribute to exacerbate the pathologic effects observed in the infected host. These metabolites can be measured for IPA diagnosis. One of these metabolites is bis(methylthio)gliotoxin (bmGT). It remains stable in body fluids and can be measured for IPA diagnosis.

The objective of this study is to study bmGT suitability for IPA diagnosis. We compared it with galactomanan(GMN) results.

Methods:
GMN was detected with a commercial assay (Platelia Aspergillus Ag-BioRad). GT and bmGT were detected with HPTLC. Clinical data from 91 patients was collected. 4 bronchoalveolar lavages (BAL) and 333 serums were analyzed for both GMN and bmGT. A positive result for GMN was considered those higher than 0.7ng/ml. Any result of bmGT was considered positive.

Results:
Both techniques were positive for 3 patients (3.2%). Both were negative for 53 patients (58.2%). GMN was positive and bmGT was negative in 16 patients (17.6%). GMN was negative and bmGT was positive in 14 patients (15.5%). GMN was weak (<0.7ng/ml) and bmGT was positive in 5 (5.5%) cases.

From patients with weak GMN and positive bmGT, one had sinusopathy and improved after voriconazol treatment. One patient had CMV infection according with the ground-glass CT scan image, he also received voriconazol. Impotantly, a patient with nodules and opacities was treated with voriconazol. A. fumigatus was isolated in his bronchoalveolar lavage. Another patient has C. parapsilosis bacteremia.

Among the patients with GMN-/bmGT+, one was haematological and had ground-glass image in CT scan. A renal recipient had an opportunistic infection suggesting image and was treated with caspofungin. Two patients showed nodules in CT scan, one of them improved with antibiotics and antivirals and the other had an infection due to Mycobacterium abscessus.

Among the group with GMN+/bmGT-, two patients were haematological and had ground-glass image in CT scan but they also had CMV infection, one of this patients received caspofungin. An oncological patient had ground-glass finding and required voriconazol. Three patients had pneumonia and were in treatment with piperaciline-tazobactam, one received voriconazol. Rest of
patients didn't have clinical signs or radiological findings of respiratory infection. However, four of them received antifungal treatment.

**Conclusion:**
In 61.4% of the cases two techniques agree. In five cases of weak positives for GMN, bmGT was positive supporting the IPA diagnosis. Curiously, these five patients received voriconazol. In four of them, treatment with voriconazol produced a decrease in bmGT levels in following sample. Thus, we can conclude that bmGT helps in the diagnosis and evolution monitoring of the patients with suspected IPA.

More studies are necessary for clarifying the cases in which two assays are in disagree, probably it is necessary to fix a cutoff for bmGT with the aim of avoiding these false positives results.
COINFECTION OF INVASIVE PULMONARY ASPERGILLOSIS AND PNEUMOCYSTIS JIROVECII IN SEVERELY IMMUNOCOMPROMISED PATIENTS: A DIAGNOSTIC AND CLINICAL CHALLENGE

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Purpose:
Invasive infections caused by Aspergillus spp. as well as Pneumocystis jirovecii pneumonia (PcP) are serious diseases each in severely immunocompromised patients. We report on six patients (pts) with pulmonary coinfections of Aspergillus species and Pneumocystis jiroveci and a history of severe immunosuppression caused by the underlying disease or due to administration of immunosuppressants and subsequent low CD4 lymphocyte counts.

Methods:
We retrospectively screened electronic records and hospital charts of patients with proven/probable invasive pulmonary aspergillosis (IPA) contained within our clinical data bank. In a timeframe of 11 years we identified 95 patients with proven/probable IA according to 2008 EORTC/MSG Criteria for whom PcP diagnostics (immunoflourescence testing or immunostaining) had been performed additionally due to suspicious lung infiltrate patterns.

Results:
In 95 severely immunocompromised patients with proven/probable IPA and atypical lung infiltrates we found six patients (6.3 %) to harbour Pneumocystis jirovecii as an additional pathogen. Pulmonary infiltrates observed in chest computed tomography (CT) showed infiltration patterns corresponding to both infections. Three of these patients had an underlying malignant disease (Non-Hodgkin Lymphoma (n=1), glioblastoma (n=2)), whereas two patients had been HIV-positive with low CD4-cell count and one patient suffered from lupus erythematoses. Patients with malignant disorders succumbed to the infection whereas patients without malignancy survived the combined infection.

Conclusion:
In immunocompromised patients with IPA, the prevalence of PcP coinfection represents a rare complication, however especially in patients with malignant disorders mortality is high. A heightened clinical awareness and stringent and accurate diagnostic work-up encompassing molecular diagnostic tools and biomarkers in BAL is recommended to avoid delayed diagnosis and establish the microbiologic etiology in immunocompromised patients with atypical infiltrates in chest CT.
MONITORING ASPERGILLUS BIOMARKERS: A TWO CENTRES APPROACH

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Purpose:
Invasive aspergillosis (IA) remains a major complication in patients with haematological malignancies (HM) and after allogeneic haematopoietic cell transplantation (HCT). In these patients, IA is the most common cause of mortality due to infection. Early detection of Aspergillus infections would have the potential to facilitate a more effective management of invasive disease. The optimal blood fraction for detecting Aspergillus DNA has still to be determined and different fractions will provide different DNA sources. Free circulating fungal DNA (DNAemia) is likely to be available in plasma and serum, but the differences in obtaining these cell free fractions (presence of blood clot) may alter the availability of free DNA.

Therefore we evaluated the sensitivity of different blood fractions (plasma and serum) for the detection of free circulating Aspergillus DNA in patients with HM.

Methods:
The centres in Cardiff and Wuerzburg collected consecutive samples from HM patients undergoing chemotherapy or HCT. Blood samples were collected twice weekly from all study patients classified according to the European Organization for Research and Treatment of Cancer consensus criteria. In detecting Aspergillus DNA in plasma (centre 1) or serum (centre 2) methods conforming with European Aspergillus PCR Initiative recommendations were used. Galactomannan and PCR assays were performed prospectively. Patients with less than 3 samples were excluded from this case control study.

Results:
In total 65 patients were included over a one-year study period. There were 12 probable and 10 possible IA cases as well as 43 unclassified patients who served as controls.

Overall, 696 blood samples were collected. The PCR positivity for centre 1 testing 388 plasma samples was 10.8% and for centre 2 testing 308 serum samples was 3.3%. Centre 1 detected 9 of 10 probable cases (90%), one possible IA case (100%) and 13 of 27 unclassified patients, three of which were also GM ELISA positive. Ten of the 13 unclassified patients showed only one single positive PCR result. If a threshold of two PCR positive results was used 8 of 10 probable cases (80%) remained positive and specificity increased to 89%. Centre 2 detected 2 of 2 probable cases (100%), 2 of 9 possible cases (22%) and 1 of 16 unclassified patients.

PCR positivity rate in centre 1 was 15.3%, 15.5% and 7.8% and for centre 2 8.9%, 3.5% and 0.9% for probable, possible and unclassified patients, respectively. Negative Predictive Values (NPV) were high for centre 1 and 2 (0.93 and 1.00; possible IA cases were excluded for the analysis).

Conclusion:
PCR positivity rates appear greater in plasma compared to serum. However, both blood fractions, plasma and serum, allowed the detection of probable IA cases with high sensitivity. Plasma showed a higher positivity rate in unclassified patients. Specificity can be increased, if plasma is used as an add-on biomarker or if at least 2 positive PCR results are considered to start treatment. Further research testing the concomitant serum samples at centre 1 and plasma samples at centre 2 is being performed to permit direct performance comparison.
Objective:
Invasive aspergillosis (IA) and candidiasis (IC) are leading causes of morbidity and mortality in haematological patients. Publications about combination of IC and IA in haematological patients are limited.

Methods:
The prospective study was conducted during the period 2012-2013 y.y. Diagnosis of IA and IC was made according to EORTC/MSG criteria (2008).

Results:
We observed 3 hematological patients with IA and IC. The mean age of our patients was 42 years (range 36-58), male and female ratio 1:2. Underlying conditions were: acute myeloid leukemia – 2, non-Hodgkin’s lymphoma - 1.

Test «Platelia Aspergillus EIA» (Bio-Rad) was positive in all of patients in blood and BAL. A. flavus in BAL - 1 patient.

Diagnosis of IC was confirmed by culture of blood. The agents of IC were C.krusei, C. parapsilosis and unidentified Candida.

The main sites of IA and IC were lungs (100%), sinuses (67%), skin and soft tissues – (33%). More then one organs were affected in all of patients.

Antifungal therapy was performed all of patients: voriconazole - 100%, fluconazole – 67%, amphotericin B deoxycholate – 67%%, caspofungin – 67%%, amphotericin B lipid complex – 33%, micafungin – 33%. Combination therapy voriconazole + amphotericin B, voriconazole+ caspofungin, voriconazole+ micafungin was used for 67% patients.

Duration of antifungal therapy was 11 - 167 days (median - 120). Necrosectomy of nasal soft tissues was performed in 1 patients. Overall survival at 12 weeks was 2/3 of patients.

Conclusion:
Combination therapy can be successful in the treatment of combination of invasive aspergillosis and candidiasis in haematological patients.
ANTIFUNGAL ACTIVITIES OF HERBAL PLANTS ESSENTIAL OILS AGAINST ASPERGILLUS SPECIES

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3Horticultural Sciences, Faculty of Agriculture, Shiraz University, Shiraz, Iran
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Purpose:
Aspergillus species are causative agent of fungal infections named aspergillosis. Herbal plants and their aromatic products have potential antimicrobial activities and used in treatment of many diseases in traditional medicine. Their aromatic products commonly used as preservative in food industries, cosmetics and pharmaceutical products. The aim of this study was to evaluated antifungal activities of five herbal plants essential oils against Aspergillus species.

Methods:
Essential oils of five herbal plants including: Nepeta cataria, Myrtus communis, Mentha piperita, Ocimum sanctum and Carum copticum were prepared and their compounds were analyzed by Gas chromatography mass spectrometry. Antifungal activities of these oils were evaluated against standard strain of Aspergillus fumigatus and Aspergillus flavus by microdilution method using CLSI protocol.

Results:
All of the compounds reveal antifungal activities in the range of 0.125 to 4 microliter per milliliter. The best antifungal activity exhibited for Ocimum sanctum on Aspergillus species in compared with the other essential oils.

Conclusion:
In the face of increasing antibiotic resistance, natural products and phytochemicals are being examined as potential sources of novel antimicrobial agents. Presenting the new antimicrobial agents from natural resources are one of the main priorities of the pharmaceutical and food processing industries.
IN VITRO ANTIFUNGAL SUSCEPTIBILITY TESTING OF CLINICAL ISOLATES OF ASPERGILLUS FLAVUS AGAINST TREE TRIAZOLES

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³Department of Medical Parasitology and Mycology, Kurdistan University of Medical Sciences, Sanandaj, Iran

Purpose:
Aspergillus flavus is the second leading cause of invasive and non-invasive aspergillosis. The occurrence of A. flavus is worldwide especially in tropical and subtropical regions. The vital importance of A. flavus has been grown in the last years. Presently appearance of resistance to antifungal agents among Aspergillus species is dramatically increasing. Therefore, in the present study, we evaluated the in vitro activity of three antifungal agents against A. flavus isolated from different sources.

Methods:
In total, 50 strains of A. flavus which have been isolated from various specimens (nail, bronchoalveolar lavage, paranasal sinus) from suspected patients of aspergillosis patients. All strains were identified based on conventional methods and subsequently confirmed by DNA sequencing of the β-tubulin gene. The minimal inhibitory concentrations (MIC) of itraconazole, voriconazole, and posaconazole were determined using the broth microdilution method in accordance with the guidelines of Clinical and Laboratory Standards Institute (CLSI) document M38-A2.

Results:
The resulting MIC90 s for all A. flavus strains was in increasing order, as follows: posaconazole (0.25 μg/ml); voriconazole (0.5 μg/ml) and itraconazole (0.75 μg/ml). The results showed that the activity of posaconazole was significantly higher than other tested azole agents.

Discussion:
The present study based on in vitro activity showed that posaconazole followed by voriconazole might have a potent activity with a best choice of alternative to previous antifungal drugs, for aspergillosis and confirmed that the resistance of Aspergillus species to other agents, and are contradictory to cure rates achieved by patients who have used it to treat aspergillosis. However, clinical effectiveness of new generations remains to be determined.
EVALUATION OF THE \textit{IN VITRO} POTENCY OF ITRACONAZOLE, VORICONAZOLE, AND POSaconazole AND RESISTANCE IN \textit{ASPENULLUS} ISOLATES FROM THE UNITED STATES

NP Wiederhold, AW Fothergill, DI McCarthy, C Sanders, DA Sutton

1Pathology, UTHSCSA, San Antonio, USA

\textbf{Purpose:}
The triazoles itraconazole, voriconazole, and posaconazole are important agents for the treatment of infections caused by \textit{Aspergillus} species. However, increased resistance to these agents in \textit{Aspergillus fumigatus} isolates has been reported in some areas of the world, including many parts of Europe. Our objective was to evaluate the \textit{in vitro} potency of itraconazole, voriconazole, and posaconazole against common \textit{Aspergillus} species, and determine the rates of resistance to these agents in isolates collected from institutions in the United States.

\textbf{Methods:}
The antifungal susceptibility database in the Fungus Testing Laboratory at the UT Health Science Center San Antonio was queried for itraconazole, voriconazole, and posaconazole MIC data against \textit{A. fumigatus}, \textit{A. terreus}, \textit{A. flavus}, and \textit{A. niger} isolates from 2001 through 2013. This database is populated with antifungal MIC data against fungal isolates sent to our mycology reference laboratory from institutions across the U.S. Susceptibility testing was performed according to CLSI guidelines. The MIC50, MIC90, and geometric mean (GM) MIC values were determined. Differences in GM MIC values were assessed for significance by ANOVA with Tukey’s post-test for multiple comparisons. Isolates were also classified as resistant based on proposed clinical breakpoints (voriconazole & itraconazole > 4 \(\mu\text{g/ml}\), posaconazole \(\geq 1 \mu\text{g/ml}\); Verweij et al. \textit{Drug Resist Update} 2009). Differences in the number of isolates classified as resistant were assessed for significance using Fisher’s exact test.

\textbf{Results:}
Posaconazole had the most potent activity of each of the triazoles tested. The GM MIC values of this agent (range 0.131 – 0.313 \(\mu\text{g/ml}\); \(p < 0.0001\)) were significantly lower than those of itraconazole (0.232 – 0.979 \(\mu\text{g/ml}\)) and voriconazole (0.471 – 0.801 \(\mu\text{g/ml}\)) against each of the four species evaluated. Similarly, itraconazole was significantly more potent than voriconazole against \textit{A. fumigatus}, \textit{A. terreus}, and \textit{A. flavus}. In contrast, voriconazole was more potent than itraconazole against \textit{A. niger} isolates. Azole resistance was highest in \textit{A. fumigatus} against each agent (range 2.58\% - 4.06\%) and for itraconazole and posaconazole against \textit{A. niger} isolates (6.87\% and 5.46\%, respectively). In \textit{A. fumigatus} there was a trend towards increased voriconazole resistance in isolates tested from 2007 to 2013 compared to the earlier period of 2001 to 2006 (4.23\% vs. 2.42\%; \(p = 0.0721\)). In 425 \textit{A. fumigatus} isolates for which MIC values were available for each agent, resistance was observed in 7.06\% with 2.35\% of isolates demonstrating resistance to all three triazoles.

\textbf{Conclusions:}
Each of the triazoles demonstrated \textit{in vitro} activity against \textit{Aspergillus}, with posaconazole being the most potent agent against each species. Resistance was observed for each agent against \textit{A. fumigatus} and for itraconazole and posaconazole in \textit{A. niger}. In addition, there was a trend towards increased voriconazole resistance in \textit{A. fumigatus} over time. Further surveillance studies are needed to monitor for triazole resistance in \textit{Aspergillus} species in the U.S.
<table>
<thead>
<tr>
<th>Aspergillus spp.</th>
<th>Parameter</th>
<th>Itraconazole</th>
<th>Voriconazole</th>
<th>Posaconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. fumigatus</strong></td>
<td>No. Isolates Tested</td>
<td>1162</td>
<td>1819</td>
<td>886</td>
</tr>
<tr>
<td>MIC Range</td>
<td>&lt;0.03 – &gt;16 μg/ml</td>
<td>0.06 - &gt;16 μg/ml</td>
<td>&lt;0.03 – &gt;16 μg/ml</td>
<td></td>
</tr>
<tr>
<td>MIC50</td>
<td>0.5 μg/ml</td>
<td>0.5 μg/ml</td>
<td>0.25 μg/ml</td>
<td></td>
</tr>
<tr>
<td>MIC90</td>
<td>1 μg/ml</td>
<td>1 μg/ml</td>
<td>0.5 μg/ml</td>
<td></td>
</tr>
<tr>
<td>GM MIC</td>
<td>0.537 μg/ml</td>
<td>0.586 μg/ml</td>
<td>0.191 μg/ml</td>
<td></td>
</tr>
<tr>
<td>% Resistant</td>
<td>2.58%</td>
<td>3.74%</td>
<td>4.06%</td>
<td></td>
</tr>
<tr>
<td><strong>A. terrus</strong></td>
<td>No. Isolates Tested</td>
<td>157</td>
<td>336</td>
<td>193</td>
</tr>
<tr>
<td>MIC Range</td>
<td>≤0.03 – 1 μg/ml</td>
<td>0.125 – 8 μg/ml</td>
<td>≤0.015 – 1 μg/ml</td>
<td></td>
</tr>
<tr>
<td>MIC50</td>
<td>0.25 μg/ml</td>
<td>0.5 μg/ml</td>
<td>0.125 μg/ml</td>
<td></td>
</tr>
<tr>
<td>MIC90</td>
<td>0.5 μg/ml</td>
<td>1 μg/ml</td>
<td>0.25 μg/ml</td>
<td></td>
</tr>
<tr>
<td>GM MIC</td>
<td>0.232 μg/ml</td>
<td>0.471 μg/ml</td>
<td>0.131 μg/ml</td>
<td></td>
</tr>
<tr>
<td>% Resistant</td>
<td>0%</td>
<td>0.89%</td>
<td>0.52%</td>
<td></td>
</tr>
<tr>
<td><strong>A. flavus</strong></td>
<td>No. Isolates Tested</td>
<td>181</td>
<td>338</td>
<td>177</td>
</tr>
<tr>
<td>MIC Range</td>
<td>&lt;0.03 – 2 μg/ml</td>
<td>0.125 - &gt;16 μg/ml</td>
<td>≤0.03 – 2 μg/ml</td>
<td></td>
</tr>
<tr>
<td>MIC50</td>
<td>0.5 μg/ml</td>
<td>0.5 μg/ml</td>
<td>0.25 μg/ml</td>
<td></td>
</tr>
<tr>
<td>MIC90</td>
<td>1 μg/ml</td>
<td>1 μg/ml</td>
<td>0.5 μg/ml</td>
<td></td>
</tr>
<tr>
<td>GM MIC</td>
<td>0.315 μg/ml</td>
<td>0.606 μg/ml</td>
<td>0.207 μg/ml</td>
<td></td>
</tr>
<tr>
<td>% Resistant</td>
<td>0%</td>
<td>1.48%</td>
<td>2.82%</td>
<td></td>
</tr>
<tr>
<td><strong>A. niger</strong></td>
<td>No. Isolates Tested</td>
<td>233</td>
<td>341</td>
<td>183</td>
</tr>
<tr>
<td>MIC Range</td>
<td>&lt;0.03 – 8 μg/ml</td>
<td>0.06 – 4 μg/ml</td>
<td>≤0.03 – 4 μg/ml</td>
<td></td>
</tr>
<tr>
<td>MIC50</td>
<td>1 μg/ml</td>
<td>1 μg/ml</td>
<td>0.25 μg/ml</td>
<td></td>
</tr>
<tr>
<td>MIC90</td>
<td>2 μg/ml</td>
<td>2 μg/ml</td>
<td>0.5 μg/ml</td>
<td></td>
</tr>
<tr>
<td>GM MIC</td>
<td>0.979 μg/ml</td>
<td>0.801 μg/ml</td>
<td>0.313 μg/ml</td>
<td></td>
</tr>
<tr>
<td>% Resistant</td>
<td>6.87%</td>
<td>0.88%</td>
<td>5.46%</td>
<td></td>
</tr>
</tbody>
</table>
THE USE OF THE SERUM GALACTOMANNAN ASSAY IN HIGH-RISK HEMATOLOGY PATIENTS ON PRIMARY PROPHYLAXIS WITH POSACONAZOLE: TOWARDS AN EFFICIENT DIAGNOSTIC STRATEGY

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3Microbiology, Hospital Universitario de Bellvitge, Barcelona, Spain

Purpose and Methods:  
We have recently reported that primary antifungal prophylaxis with posaconazole is effective to prevent invasive mould infections in high-risk hematology patients, with only 5 cases of probable/proven breakthrough invasive aspergillosis (IA; 1.9%) in a series of 262 consecutive high-risk episodes (161 AML/MDS, 79 allogeneic transplantation, 22 graft-versus-host disease) from 121 patients in our center between June 2007 and June 2011 [Sánchez-Ortega; P950 EBMT 2013]. The management algorithm for this series included a comprehensive diagnostic approach for symptomatic patients as well as preemptive serum galactomannan tests (GM; Platelia, Bio-Rad) performed twice weekly from the start of the risk episodes. Based on the sensitivity (70%) and specificity (90%) of serum GM tests in hematology patients (Pfeiffer, CID 2006) and a 1.9% real-life incidence of IA, the negative predictive value of the test remains very high (>99%) but the expected positive predictive value would drop sharply to 12%, potentially limiting the diagnostic performance of the assay when used as a screening technique in this setting. Here, we present an analysis of our current biweekly serum GM test screening strategy in high-risk hematology patients on posaconazole primary prophylaxis and describe possible improvements of its use in response to the low pre-test incidence of IA.

Results:  
A total of 2972 GM tests were performed in 262 high-risk episodes (median 11 per episode, 3-30). In the vast majority of episodes (188, 71.7%), all GM tests were negative. In all 5 episodes of IA, positive GM tests in the serum (Optical Index [OI] >0.7 x1 or OI >0.5 x 2 consecutive samples) and in the BAL (OI >1 x1) contributed to the diagnosis (Table). However, in 30 additional episodes (11.4%) all positive GM tests were false positive results (FP, 1-6 per episode; OI: 0.59-2.85) as defined as those positive tests performed on patients who remained on posaconazole prophylaxis without receiving other antifungal therapy, survived the risk episode and never developed other criteria of IA. In 87% of these episodes (n=26) all FP GM tests were performed as a screening test in asymptomatic cases. In only 4 episodes (13%) such FP tests were performed as part of the diagnostic workup in persistently febrile symptomatic patients (p<0.001) (Table).

Conclusion:  
Our experience in high-risk hematology patients receiving effective antifungal prophylaxis with posaconazole suggest that the serum GM assay provides no clinical or diagnostic benefit when performed as a surveillance test in asymptomatic patients. In this screening setting tests were always negative or FP. However, serum GM tests remain very useful as part of the management strategy for symptomatic patients, both for their contribution to the diagnosis of the small percentage of breakthrough IA, as well as for its high negative predictive value. Overall, these data suggest that the diagnostic accuracy of GM tests can be improved when used in high-risk hematology patients on posaconazole primary prophylaxis who yield a low pre-test incidence of IA.
Table. Distribution of high-risk episodes according to GM tests results

<table>
<thead>
<tr>
<th>Distribution of episodes according to GM</th>
<th>Num. episodes</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM ALL NEGATIVE</td>
<td>188</td>
<td>17.7%</td>
</tr>
<tr>
<td>GM NON-EVALUABLE (i.v. antifungal therapy)*</td>
<td>39</td>
<td>15%</td>
</tr>
<tr>
<td>Toxicity</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Oral intolerance</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Candida spp infection</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Persistent fever and negative GMN tests</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Persistent fever and positive GMN tests</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>GM POSITIVE IN INVASIVE ASPERGILLOSIS</td>
<td>5</td>
<td>1.9%</td>
</tr>
<tr>
<td>Serum GM positive tests (n=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number positive GM tests: median 4 (1-13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OI: median 0.76 (0.7-1.74)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAL GM positive tests (n=3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number positive GM tests: median 1 (1-2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OI: median 7.23 (3.49-7.35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM FALSE POSITIVE</td>
<td>30</td>
<td>11.4%</td>
</tr>
<tr>
<td>Asymptomatic patients without clinical suspicion of IA</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Symptomatic patients with clinical suspicion of IA</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

* Episodes in which i.v. antifungal therapy was started.
DIRECT DETECTION OF AN ASPERGILLUS FUMIGATUS-SPECIFIC BREATH VOLATILE ORGANIC METABOLITE PROFILE FOR THE DIAGNOSIS OF INVASIVE ASPERGILLOSIS

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2Dana-Farber Cancer Institute, Boston, MA, USA
3Harvard Medical School, Boston, MA, USA
4Draper Laboratory, Cambridge, MA, USA

Purpose:
Aspergillus fumigatus emits a complex bouquet of volatile organic compounds (VOC) in vitro, distinctive from the VOC profile of other pathogenic mold species. A VOC profile of the terpene and sesquiterpene compounds camphene, α- and β-pinene, limonene, α-bergamotene, and farnesene is particularly characteristic of A. fumigatus in vitro and distinguishes A. fumigatus from other pathogenic molds, including other Aspergillus species. We assessed whether we could discriminate patients with invasive aspergillosis (IA) from patients without IA by directly detecting these volatile metabolites of fungal origin in their breath.

Methods:
We collected tidal breath from patients with suspected invasive fungal disease (IFD) and concurrent ambient air control samples onto thermal desorption traps designed to retain VOCs of diverse size, boiling points, and polarity from November 2011 through September 2013. We used gas chromatography-mass spectrometry to assess for Aspergillus fumigatus-specific VOCs in these breath samples, minus ambient air.

Results:
Of 64 patients, 25 (39%) were female, 54 (84%) were patients with hematologic malignancy, 24 (38%) were allogeneic stem-cell transplantation recipients, 8 (13%) were solid organ transplant recipients, 27 (42%) had prolonged neutropenia, 55 (86%) were receiving T-cell immunosuppressants and 12 (19%) corticosteroids. These characteristics were comparable in 34 patients with EORTC/MSG proven (5) or probable (29) IA and 30 patients with nodular pneumonia caused by other IFD or other infectious processes. While some of the volatile metabolites produced in vitro by A. fumigatus were equally present in patients with or without IA (camphene, α- and β-pinene, limonene), a combination of the sesquiterpenes farnesene and β-vatirenene and the farnesene derivative geranylacetone correctly discriminated patients with IA from patients with other IFD or other infectious processes. While some of the volatile metabolites produced in vitro by A. fumigatus were equally present in patients with or without IA (camphene, α- and β-pinene, limonene), a combination of the sesquiterpenes farnesene and β-vatirenene and the farnesene derivative geranylacetone correctly discriminated patients with IA from patients with other IFD or other infectious or inflammatory pneumonias in 60/64 (94%) patients, with 94% sensitivity and 93% specificity (Figure). Aspergillus niger, which has a distinct VOC profile from A. fumigatus in vitro, was ultimately identified as the causal etiology of pneumonia in one of the IA patients who had no farnesene, β-vatirenene, or geranylacetone in their breath sample. One patient with breath farnesene and geranylacetone was initially classified as not having IA by EORTC/MSG criteria, but on autopsy was found to have invasive pulmonary aspergillosis.

Conclusion:
In breath sampled from patients with suspected IFD, an Aspergillus fumigatus-specific VOC profile of farnesene, β-vatirenene, and geranylacetone accurately and noninvasively discriminates clinically comparable patients with IA from patients with other causes of pneumonia.
**NOTE:** THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
RAPID DISCRIMINATION OF *ASPERGILLUS FUMIGATUS* CYP51A RESISTANCE MUTATIONS BY PYROSEQUENCING

L Novak Frazer1*, R Masania2, DW Denning1,2, MD Richardson1,2

1Manchester Academic Health Science Centre, University of Manchester, Manchester, UK
2Mycology Reference Centre, National Aspergillosis Centre, University Hospital of South Manchester, Manchester, UK

**Purpose:**
A significant proportion of patients who attend the National Aspergillosis Centre suffer from azole resistance in *Aspergillus fumigatus* attributable to mutations in the target site (cyp51A). Our challenge is to develop an assay to elucidate whether the resistance observed clinically in patients, who are unresponsive to long-term azole therapy and have been confirmed to be positive for *Aspergillus* infection or colonisation by qPCR, is due to mutations in the cyp51A target.

**Methods:**
Primers were designed using Qiagen Assay Design software. DNA extracted from wild-type *A. fumigatus* and isolates confirmed by Sanger sequencing to be carrying cyp51A mutations were processed with Qiagen QIAamp DNA Mini Kits. Amplification of cyp51A for pyrosequencing was carried out on an Applied Biosystems Veriti PCR. Pyrosequencing was carried out on a Qiagen PyroMark Q24 platform. All pyrosequencing results were confirmed by Sanger sequencing.

**Results:**
We have developed a pyrosequencing assay which can detect all the mutations involved in azole resistance that have been described to date (TR/L98, G54, M220, TR46/Y121/T289). The processing time, from PCR to pyrosequencing result is 6h. We have processed WT and mutant isolates to confirm the validity and reliability of our assay.

**Conclusion:**
We have shown that cyp51A mutations can be monitored quickly with this novel pyrosequencing assay. We will also process *Aspergillus* PCR-positive patient sputum and BAL samples for the presence of these mutations, with the intention of providing this assay as a routine service for future patients undergoing azole therapy. The aim is to have the results available to clinicians within 24h of a positive *Aspergillus* PCR. The assay is amenable to processing several patient samples simultaneously and may be useful in quickly determining the genetic cause of azole therapy failure. The presence of WT and mutant *A. fumigatus* cyp51A sequences can be detected simultaneously and the ratio quantified as well. The advantage of using pyrosequencing is that this technique can be adapted easily to detect new cyp51A gene mutations in *A. fumigatus* as they arise. In addition, mutations in other genes involved in resistance to triazoles and other antifungal drugs can be monitored in *A. fumigatus* and in other fungal species. It is envisaged that the results of this assay will have a positive impact on patient treatment and antifungal stewardship.
DETECTION OF ASPERGILLUS ANTIBODIES BY A NEW INDIRECT HAEMAGGLUTINATION ASSAY

MD Richardson1,2,*, ID Page1,2, RMK Rautemaa-Richardson1,2, DW Denning1,2

1National Aspergillosis Centre, University Hospital of South Manchester, Manchester, UK
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Purpose:
Measuring Aspergillus antibodies is an important part of the diagnostic pathway for allergic bronchopulmonary aspergillosis (ABPA) and chronic pulmonary aspergillosis (CPA). It may represent a major public health issue on a global scale as 20-35% of patients develop Aspergillus antibodies following tuberculosis treatment and 63% of these develop pulmonary aspergillosis within 3 years. The worldwide 5 year period prevalence of (CPA) secondary to tuberculosis in, for example, the Congo and Nigeria has been estimated at between 0.8 and 1.37 million cases, with 43 cases per 100,000 population. Detection of specific antibodies provides key diagnostic evidence in chronic aspergillosis. There are numerous EIA formats for quantifying antibodies but these are not suitable for use in developing countries with limited laboratory resources. Haemagglutination tests involve coating erythrocytes with antigens. Erythrocytes clump together when antibodies cross-react with antigens on more than one cell and become visible to the human eye. The method also detects all Aspergillus antibody types. Its simplicity and speed (~2 hours) and commercial production make it highly suited for epidemiological and prevalence studies in low and middle resource countries. The goal was to compare the efficacy of an indirect haemagglutination assay designed to detect Aspergillus agglutinating antibodies with an agar double diffusion system used for the detection of Aspergillus precipitins.

Methods:
Serum samples from patients with a diagnosis of chronic pulmonary aspergillosis were tested by ELI.H.A. Aspergillus indirect haemagglutination (ELITech MICROBIO, Signes, France), and by an Aspergillus immunodiffusion system (Microgen Bioproducts Ltd, Camberley, UK). For the indirect haemagglutination assay sera were serially diluted to 1:2560. For the immunodiffusion assay patient sera were diluted to 1:16. The antigens used in these assays were a combination of cytoplasmic (somatic) and culture filtration extracts.

Results:
In the indirect haemagglutination assay sera with a titre <1:320 were considered to be a non-significant reaction. Sera with a titre equal to 1:320 were considered to be an indeterminant reaction. Titres ≥1:640 indicated a significant reaction in favour of aspergillosis. A positive precipitin reaction in the immunodiffusion test signified the presence of Aspergillus precipitating antibodies. The performance of positive control sera in both assays was excellent, and reproducible. All sera positive in one test were positive in the other. The concordance between titres determined by either the indirect haemagglutination assay or the precipitin test were varied. High precipitin titres (1:8) were recorded as >1:2560 in the agglutination assay. Precipitin titres of 1:4 were all in the positive range of the agglutination assay. Sera recorded as weak precipitin positive (titre of 1:2) were all positive in the agglutination test but with a range of agglutinin titres (1:320 – >1:2560). The immunodiffusion test takes 5 days to perform. The total performance time of the indirect haemagglutination test is 3 hours.

Conclusion:
The EliTech indirect haemagglutination assay for the detection of Aspergillus antibody in patients with chronic manifestations of pulmonary demonstrated has many advantages compared with precipitin tests: it was rapid, very user friendly and easy to read. This is an ideal near point-of-care test for field studies and for community clinics. Furthermore, this test can be used for screening patients and support our efforts to understand the global epidemiology of chronic pulmonary aspergillosis.
**ASPERGILLUS AS A CAUSE OF FUNGAL EMPYEMA THORACIS: EXPERIENCE OF A CANCER CENTER**

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**Purpose:**
Empyema thoracis due to *Aspergillus* is rare and information about this condition is scarce. Moreover, the interpretation of a positive pleural fluid for this mold is challenging. To that end, we retrospectively evaluated patients with a pleural culture for *Aspergillus* spp. in order to describe the experience of a cancer center.

**Methods:**
We reviewed the records of all patients with at least one pleural fluid culture growing *Aspergillus* spp (01/2005 - 03/2013) at MD Anderson Cancer Center, Houston, TX. All clinical and demographical information was obtained in order to characterize patients with empyema thoracis due to *Aspergillus*. Subjects who did not receive antifungal therapy or were subsequently found to have a negative pleural culture within 7 days were considered contamination.

**Results:**
We identified 8 such patients; median age was 55 yrs. (19 – 74 yrs.) and 4 of them were female. Among them, 6/8 patients were considered to have a true empyema and received systemic antifungal therapy accordingly. All but one of these patients had a hematological neoplasia. On the contrary, the 2 patients in whom the culture was considered a contaminant had an underlying solid organ tumor. Pleural fluid characteristics did not vary between patients with true infection and contamination. Four out of 6 patients with true infection had concomitant involvement of other sites (3 cases of pneumonia and 1 sinusitis). With regard to the species, *A. fumigatus* was the most frequently recovered (4 occasions), followed by 1 *A. terreus*, 1 *A. niger* and 2 *Aspergillus* sp. Only 2 patients with true *Aspergillus* empyema survived, both of them had no evidence of invasive disease in other sites.

**Conclusion:**
Empyema due to *Aspergillus* is rare and is typically seen in highly immunosuppressed patients with hematologic cancer. This condition carries a high mortality and it is usually seen in the setting of concomitant invasive fungal infection.
MYCOLOGICAL PROFILE OF FUNGAL SINUSITIS: A RETROSPECTIVE STUDY OVER A 7-YEAR PERIOD IN A TERTIARY CARE HOSPITAL IN RIYADH, SAUDI ARABIA

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Purpose:
Fungal rhinosinusitis (FRS) includes a wide spectrum of fungal diseases ranging from colonization and mildly symptomatic to invasive and fatal disease. The fungi causing FRS have a great diversity and regional variation in the incidence has been reported.

Our aim was to look at the mycological profile and clinical aspects of fungal sinusitis in a tertiary hospital in Riyadh, Saudi Arabia.

Methods:
This is a retrospective study conducted on samples submitted to the clinical mycology laboratory from 2005 to 2011. Samples including sinus secretions, and tissue specimens were processed and examined by direct microscopy and microbiology culture.

Results:
562 suspected patients were included in the study with a total number of 1362 samples. Samples per patient ranged from 2-9 samples. Male to female ration was 1.4: 1. A total of 138 (24.6%) were positive for fungal hyphae by direct microscopy examination and 129 (23%) were positive by culture. Out of 138 patients with positive culture, 71.3% were allergic fungal sinusitis cases. Nasal tissues were positive for fungal element in 26 patients (18.8%). Aspergillus species were the most common fungi isolated (77.5%) mainly A. flavus (79%). Bipolaris species were ranked second with 22.4%.

Conclusion:
In this study, the fungi associated of fungal sinusitis were different than that of western countries. Allergic fungal sinusitis was the most common type. sp was the most common causative agent in both allergic and invasive forms of the disease.
Purpose:
Pulmonary aspergillosis has a number of different manifestations. Classically pulmonary aspergillosis in immunocompetent patients presents as a saprophytic infection in a pre-existing cavity. However, pulmonary *Aspergillus* disease can present as a nodule(s), without cavitation, which may be mistaken for malignancy. The purpose of this study is to review the presentation, radiology and histological features of nodules caused by *Aspergillus* spp.

Methods:
Sixty-eight patients who had histological features of aspergillosis on lung biopsy, from 2003-2013 were identified. These patients radiology was then reviewed. Patients with cavitating lung lesions, aspergillomas, or those without parenchymal lung abnormalities were excluded. Patients with a diagnosis of invasive aspergillosis were also excluded. Demographic data and laboratory data was recorded on each patient. in addition to their clinical presentation.

Results:
Seven patients with pulmonary nodules and histology features diagnostic of aspergillosis were identified. The mean age of the patients was 58 years (range 46-67). Five (71%) were men. The mean Charleston co-morbidity score was 3.7 (2-6). Five patients (71%) were former smokers. None of the patients was in receipt of immunosuppressive drugs, the mean lymphocyte count pre-operatively was 1.5 x 10⁹/L (range 1.1-1.9, laboratory normal range 1.5-4), two patients (29%) were mannose binding lectin deficient. Three patients (43%) did not have an elevated *Aspergillus* IgG. Three patients had a single nodule identified on computerised tomography (CT), two had two nodules and two had three nodules present. The mean size of the nodules was 2cm (range 1.3-3.2cm), none had cavitation radiographically. Five patients had lesions in the upper lobes on CT (four in the right upper lobe), while one patient had nodules in the right lower lobe and one in the left lower lobe. Only one patient had significant lymphadenopathy on CT. On presentation, all the patients complained of cough, five complained of dyspnoea, two complained of weight loss, and one complained of haemoptysis. On histological examination, five patients underwent lung biopsy. All five had evidence of fibrosis, granulation tissue and fungal hyphae were visualised. The remaining two patients had bronchoscopy and broncho-alveolar lavage; inflammatory cells and branching hyphae were identified.

Conclusion:
Pulmonary nodules are a less common manifestation of aspergillosis in immunocompetent patients. Their natural history is not yet defined, although in this series all of the patients presented with cough. These nodules may be difficult to distinguish from other lung pathology on CT findings alone.
COMBINATION OF *Aspergillus* MITOCHONDRIAL AND RIBOSOMAL QPCR FOR EARLY DIAGNOSIS OF INVASIVE ASPERGILLOSIS IN HEMATOLOGY PATIENTS

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**Purpose:**  
A combination of two quantitative *Aspergillus* PCR assays, targeting a mitochondrial and a ribosomal target (mtQPCR), have proved efficacy for diagnosis of invasive aspergillosis in hematology patients with risk factors and a positive galactomannan antigen (GM) (1). However, in that study, *Aspergillus* circulating DNA detection using mtQPCR was done only when GM was positive, and we have never assessed mtQPCR for screening at risk patients. The aim of the present study was to assess the performance of mtQPCR for screening at risk patients in a Hematology Intensive Care Unit.

**Methods:**  
The study was performed in the Hematology Intensive Care Unit from Besançon University Hospital from March 2012 to September 2013. GM detection (Platelia *Aspergillus* (Bio-Rad, France)) and mtQPCR were performed on the same serum sample, twice a week, in all patients with risk factors for invasive aspergillosis. Risk factors, clinical, radiological and biological data were prospectively recorded using the information sheet from the French network for surveillance of IA.

**Results:**  
Twenty five patients were diagnosed with probable or proven invasive aspergillosis according to 2008 EORTC/MSG criteria. 13/25 patients had a positive mtQPCR. Both mitochondrial and ribosomal QPCR assays were positive in 5/13 patients. Mitochondrial QPCR assay was the only positive in 1/13 patient, and ribosomal QPCR assay was the only positive in 7/13 patients. Out of the 13 patients with positive mtQPCR, 9/13 had a positive GM at the same time (GM index > 0.5), and 4/13 had a negative GM at the time of the positive mtQPCR. The first positive GM was obtained from 10 to 30 days after the first positive PCR for 3 out of these 4 patients. For the 4th patient, a positive *A. fumigatus* culture from a sputum was obtained 15 days after the first positive PCR.

**Conclusion:**  
Screening at risk patients using both mtQPCR and GM on the same serum sample is easily feasible in routine clinical setting. Our results confirm the usefulness of combination of biomarkers to improve early diagnosis of invasive aspergillosis.

(1) Millon et al, J Clin Microbiol 2011, 49:1058-1063
GALACTOMANNAN TESTING OF SERUM AND BRONCHOALVEOLAR
LAVAGE FLUID FOR DIAGNOSIS OF INVASIVE ASPERGILLOSIS IN
DIFFERENT HEMATOONCOLOGICAL PATIENTS IN SAINT-PETERSBURG,
RUSSIA

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Purpose:
To analyze the utility of galactomannan (GM) test for the diagnosis of invasive aspergillosis (IA) in
different groups patients with hematological malignancies in St. Petersburg, Russia.

Methods:
The study included 651 samples: 390 serum and 261 bronchoalveolar lavage fluid (BAL) from 360
hematological patients (313 specimens of neutropenic patients and 338 specimens of bone marrow
transplant recipients) with IA in Saint-Petersburg between 1998 and 2013 yy. Diagnosis of IA was
based on EORTC/MSG 2008 criteria. Detection of GM was performed with “Platelia Aspergillus
EIA” (Bio-Rad Laboratories). As controls, 216 samples were collected from the hematological
patients without IA. The test was considered as positive with cut-off of ≥ 0,5 (serum) and ≥ 1,0
(BAL). We calculated the sensitivity, specificity, positive predictive value and negative predictive
value for the GM assay. Two-tailed p values of < 0,05 were considered statistically significant.

Results:
The patients were: adult - 84%, children - 16%. The mean age of patients was 37 years (range 1-77),
male - 54%, female - 46%. Sensitivity of GM test in adult neutropenic patients and in bone marrow
transplant recipients was identical (72% vs.73%). Positive results of GM test in BAL specimens of
neutropenic children with IA was revealed more often, than at bone marrow transplant recipients
group (91% vs. 63%, P=0,002). In neutropenic patients with IA a higher level GM index (2, 0 – 7, 5)
was found in the BAL samples more frequently in children, than in adult (60% vs. 23%, P=0,012)
and was similar in different groups bone marrow transplant recipients. The prognostic value of
positive and negative result of the GM test in serum samples of neutropenic patients and bone
marrow transplant recipients were 0,77 (95%CI 0,71-0,87) and 0,86 (95%CI 0,83-0,88), in BAL
samples - 0,85 (95%CI 0,79-0,94) and 0,84 (95%CI 0,78-0,96), respectively.

Conclusion:
This study indicates that sensitivity, prognostic value of positive and negative result of GM test in
neutropenic patients and in bone marrow transplant recipients depends on the type of biological
specimen and varies for adult and children.
THE TEMPORAL SEQUENCE OF THE TRANSITION FROM ASTHMA THROUGH ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS TO CHRONIC PULMONARY ASPERGILLOSIS

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Introduction:
Allergic bronchopulmonary aspergillosis (ABPA) is an uncommon complication of asthma, occurring in 0.7-4.1% of cases in secondary care. There are an estimated 4,800,000 cases of ABPA worldwide. Development of chronic pulmonary aspergillosis (CPA) in patients with ABPA is a well-recognised but poorly understood phenomena. Whilst oral itraconazole is frequently used in the management of patients with ABPA, voriconazole and posaconazole is often used in those who develop CPA. The temporal sequence of the transition from asthma through ABPA to CPA is not well described.

Method:
Patients with problematic CPA in the UK are cared for at one national centre, the National Aspergillosis Centre (NAC), based at the University Hospital of South Manchester. These patients are screened for a dual diagnosis of ABPA and CPA. In cases referred to the NAC prior to 1st of June 2012 in whom a dual diagnosis was suspected, the full medical records were reviewed and discussed by a multi-disciplinary team to make judgements on the likely onset of asthma, ABPA and CPA. ABPA was defined as the highest serum total IgE of greater than 1000 IU/L, or raised serum anti-Aspergillus IgE (or positive Aspergillus skin prick test), a history of asthma, with compatible symptoms of ABPA. CPA was defined as positive Aspergillus precipitins, or highest serum anti-Aspergillus IgG of >40 mg/L with radiological findings consistent with CPA (Nodule disease, lobar shrinkage and fibrosis, pleural capping, and cavity formation with or without fungal balls).

Results:
Of all patients referred to the NAC prior to 1st of June 2012 (392 patients), 42 were recorded as having a co-existing diagnosis of asthma, ABPA and CPA. On review of the medical records, 20 were considered to have a clear progression from asthma, through ABPA to CPA. Seven patients did not have CPA, five patients did not to have ABPA but a Th-2 response to chronic pulmonary aspergillosis, with a high total IgE. Six patients were considered to have developed CPA due to causes other than ABPA. The remaining four patients had too much missing data in the medical records to allow timing and diagnostic judgements to be made. Of the 20 patients included, twelve were female and 8 were male. Figure 1 shows the ages at which the 20 patients developed asthma, ABPA and CPA, including long-term asthma remissions. Patients one to 15 developed chronic cavitary pulmonary aspergillosis, patients 16 and 17 developed Aspergillus nodule disease, and patients 18, 19 and 20 all developed the radiological appearance of CPA but remained anti-Aspergillus IgG negative. The patient documented on line 2 is case 44 in the case histories from www.aspergillus.org.uk.

Discussion:
The overlapping criteria for the diagnosis of CPA and ABPA make the diagnosis of CPA in patients with ABPA challenging. There is considerable range in the length of time the patients in this series had asthma and ABPA prior to the onset of CPA. A clearer definition of CPA in ABPA is needed to guide diagnosis and criteria for escalation in antifungal therapy.
Figure 1: The age at which patients developed asthma (and long-term asthma remissions), allergic bronchopulmonary aspergillosis and chronic pulmonary aspergillosis.

**NOTE:** THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
The purpose of this work is to study the distribution of different minimal inhibitory concentrations (MICs) to amphotericin B (AmB) and itraconazole (ITZ) among clinical strains of *Aspergillus spp.*, which were isolated in Saint-Petersburg, Russia.

Methods:
In this study were used clinical strains of *Aspergillus spp.* from Russian collection of pathogenic fungi (numbers of strains tested to ITZ / AmB are in brackets): *A. fumigatus* (20/11), *A. flavus* (12/10), *A. oryzae* (1/1), *A. niger* (18/13), *A. terreus* (3/3), *A. glaucus* (2/2), *A. nidulans* (1/1), *A. clavatus* (1/0), *A. sydowii* (1/1), *A. candidus* (1/1) and *A. calidoustus* (1/0). Strains were previously isolated mainly from respiratory substrates (BAL and sputum), but also from external ear, corneal scraping, autopsy materials, cerebrospinal fluid and some other sites. Susceptibility testing was performing according protocol EUCAST E.DEF 9.1.

Results:
The results are performed in Table 1. There are MICs for rare clinical isolates obtained in our study (ranges or values in μg/ml for ITZ/AmB are in brackets; NT – not tested): *A. oryzae* (0,125/4), *A. glaucus* (0,25 - >16/0,0625), *A. clavatus* (4/NT), *A. candidus* (<0,0313/4), *A. calidoustus* (>16/NT).

Table 1: The comparison of MICs to studied antimicotics

<table>
<thead>
<tr>
<th>Species</th>
<th>Antifungals</th>
<th>This study</th>
<th>EUCAST data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIC range or value, μg/ml</td>
<td>Resistance, %</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>ITZ</td>
<td>0,0625 — 16</td>
<td>4,8 %</td>
</tr>
<tr>
<td></td>
<td>AmB</td>
<td>0,25 — &gt;16</td>
<td>14,3 %</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>ITZ</td>
<td>0,0313 — &gt;16</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>AmB</td>
<td>0,5 — 8</td>
<td>5,6 %</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>ITZ</td>
<td>0,0625 — &gt;16</td>
<td>9,1 %</td>
</tr>
<tr>
<td></td>
<td>AmB</td>
<td>1 — &gt;16</td>
<td>—</td>
</tr>
<tr>
<td><em>A. terreus</em></td>
<td>ITZ</td>
<td>0,125 — 1</td>
<td>0 %</td>
</tr>
<tr>
<td></td>
<td>AmB</td>
<td>&gt;16</td>
<td>—</td>
</tr>
<tr>
<td><em>A. nidulans</em></td>
<td>ITZ</td>
<td>0,25</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>AmB</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td><em>A. sydowii</em></td>
<td>ITZ</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>AmB</td>
<td>2</td>
<td>—</td>
</tr>
</tbody>
</table>
Conclusion:
As can be seen from the table, obtained MICs ranges are in generally close to ranges performed by EUCAST, except ranges for *A. flavus* to ITZ as well as to AmB because of resistant strains with more high values of MICs. Proportions of resistant strains of *A. fumigatus* to AmB, strains of *A. niger* to AmB and strains of *A. flavus* to ITZ are higher with comparison to EUCAST data. However, to determine the statistical significance of these features the study on extended selection of isolates is needed.
**Aspergillus Species Is a Frequent Finding in the Sputum of Iranian CF-Patients**

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**Introduction:**
Mutations in CFTR result in a defective mucociliary clearance with production of viscous and sticky bronchial mucus that facilitates colonization of the airways by various microorganisms which contribute to the progressive deterioration of lung function. Therefore, considerable attention has been paid to prevention and control of microbial growth, which has resulted in improvement of patient management and in a marked increase in the life expectancy of the patient. The objective of this study was to investigate the frequency of Aspergillus species from respiratory specimens of Iranian CF patient, including the evaluation of the in vitro susceptibility of the species of antifungal drugs to improve the antifungal therapy in patients.

**Material and Methods:**
Forty-two patients diagnosed to have CF, based on positive sweat chloride tests, a characteristic clinical manifestation, or on mutations in CFTR protein (29 males and 13 females), enrolled in the Respiratory Research Centre. Aspergillus species identification was performed using conventional criteria, with confirmation by sequencing of partial beta-tubulin.

**Results:**
The most frequently isolated filamentous fungi were *A. terreus* (37.5%), followed by *A. fumigatus* and *A. oryzae* (25%). A higher proportion of patients with persistent Aspergillus colonization had ABPA than those with no organisms. Finally, male were numerically (but not significantly) more often persistently colonized with Aspergillus. *A. terreus* was the predominant species which colonized in the majority of CF cases that probably due to resistant to antifungal drug like amphotericin B.

**Conclusion:**
However, in the present study the frequency of Aspergillus species are low, the possibility of fungal colonization and consideration of their clinical significance in these patients is highly recommended. Future studies and more factors which may be responsible for the increased isolation of fungi in CF need to be investigated.

**Keywords:**
Cystic fibrosis, CFTR gene, fungal colonization, Aspergillus Spp, in vitro antifungal susceptibility
PHARMACOKINETICS AND EFFICACY OF ISAVUCONAZOLE FOR TREATMENT OF EXPERIMENTAL INVASIVE PULMONARY ASPERGILLOSIS

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Purpose:
New antifungal agents are needed to improve outcome in immunocompromised patients with invasive pulmonary aspergillosis (IPA). The third-generation triazole isavuconazole (ISA) in vitro demonstrated superior hyphal growth inhibition and lower MICs against *A. fumigatus* in comparison to that of voriconazole (VRC). We therefore studied the pharmacokinetics, efficacy, and safety of ISA in treatment of experimental IPA in persistently neutropenic rabbits.

Methods:
Treatment groups included rabbits receiving orally administered BAL8557 prodrug equivalent to active compound (ISA or BAL4815) of 20 (ISA20), 40 (ISA40), and 60 (ISA60) mg/kg/day. Control groups consisted of untreated controls (UC) and VRC-treated animals at 15 (VRC) mg/kg Q12 PO. ISA treated rabbits received loading dose of BAL8557 prodrug equivalent to isavuconazole 90 mg/kg PO 24 h after endotracheal inoculation of *A. fumigatus*. Isavuconazole treatment was administered once daily thereafter for up to 12 days.

Results:
There was a significant reduction of residual fungal burden (CFU/g) in ISA40 and ISA60 treated rabbits vs that of VRC or UC (p<0.001). As measures of organism-mediated pulmonary injury, lung weights, and pulmonary infarct score were significantly lower in ISA40 and ISA60 treated rabbits in comparison to that of VRC and UC (p<0.001). Rabbits treated with ISA40, ISA60 significantly prolonged survival in comparison to that of UC (p<0.001). In addition, rabbits treated with ISA40 and ISA60 demonstrated significantly prolonged survival in comparison to that of VRC treated (p<0.05). ISA20 and VRC treated rabbits showed prolonged survival vs untreated controls (p<0.05). ISA40 and ISA60 treated animals demonstrated a significant decline of galactomannan antigenemia (GMI) during therapy following day 4 in comparison to progressive GMI of VRC treated rabbits, and untreated controls (p<0.01). There was significantly lower GMI in bronchoalveolar lavage (BAL) from rabbits treated with ISA40 and ISA60 in comparison to that of VRC or untreated controls rabbits (p<0.001). There also was significant decrease of plasma (1→3)-β-D-glucan concentrations ISA40 and ISA60 treated rabbits in comparison to that of VRC or untreated controls rabbits (p<0.05). These outcome variables correlated directly with ISA exposure measured by AUC$_{0-24}$.

Conclusion:
In summary, rabbits treated with ISA40 and ISA60 demonstrated significant dose-dependent reduction of CFU/g, decreased pulmonary injury, prolonged survival, lower GMI in serum and BAL, lower plasma (1→3)-β-D-glucan concentrations in comparison to that of VRC and untreated controls with a direct pharmacodynamic relation between exposure and outcome.
CO- INFECTION OF PROBABLE INVASIVE PULMONARY ASPERGILLOSIS AND CUTANEOUS *FUSARIUM* INFECTION IN A PATIENT WITH PYODERMA GANGRENOSUM: A CASE REPORT

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**Purpose:**
Invasive fungal infections (IFIs) have an increased frequency in immunocompromised patients and are major causes of morbidity and even mortality.

**Case Presentation:**
We report an unusual case of probable invasive pulmonary aspergillosis (IPA) caused by *Aspergillus flavus* and fusarial skin infection caused by *Fusarium proliferatum* in patient with chronic pyoderma gangrenosum (PG) which was previously controlled with oral prednisolone, cyclosporine. The diagnosis of probable IPA and cutaneous fusariosis caused were made on the basis of direct microscopic examination, culture of endobronchial washing and bronchoalveolar lavage (BAL) and tissue samples which was confirmed by PCR sequencing. Lung biopsies was not performed because of his critical condition. The patient underwent systemic antifungal therapy with voriconazole. The treatment failed and the patient expired 12 days following hospitalization.

**Conclusion:**
This report highlights the rarity of coexistence of IPA and a chronic fusarial skin infection and thereby reinforcing the physicians attention towards the possibility of invasive fungal infection in the immunosuppressed patients.
ANTIFUNGAL DRUG INTERACTIONS DATABASE AND APPS FOR IPHONE AND ANDROID

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Introduction:
Drug:drug interactions (DDIs) account for 3-5% of serious adverse reactions, which themselves are common causes of hospital admission and sometimes death. In 433 patients >60 years old taking at least 2 agents, the incidence of DDI-related ADRs was 6.5%. Systemic azole antifungal medications have a high potential to cause DDIs with many drugs. Some interactions reduce the efficacy of azoles (ie rifamycins), others the efficacy of the interacting drug (ie low dose ritonavir), or lead to excess concentrations of the interacting drug (ie ciclosporin, warfarin, digoxin, benzodiazepines etc). Some interactions occur with amphotericin B and echinocandins. Both medical professionals and patients (& carers) need support in this area because of the range and complexity of possible interactions. We have created a quick reference for guidance.

Methods:
Information on interactions with itraconazole, voriconazole, posaconazole, fluconazole, amphotericin B, micafungin and caspofugun has been collated from a number of sources: manufacturers’ Summary of Product Characteristics, Stockley’s Drug Interactions, consideration of the effects of each drug on CYP P450 isoenzymes & p-glycoprotein, clinical considerations and primary literature. For each interacting pair of drugs the interaction is classified as of ‘major’ (red), ‘moderate’ (amber) or ‘minor’ (green) significance. ‘Major’ interactions are those that could cause significant harm, even if rare & which require avoidance, with ‘minor’ interactions generally increasing the risk of one or more adverse effects. Where no interaction is expected there is no entry. For each interacting pair of drugs, patients are informed of the action their doctor should be taking & effects to look out for. Doctors are provided with the mechanism of the interaction, evidence of an existing interaction and action to take.

Results:
We have constructed a searchable database of interactions between systemic antifungal drugs and every other prescribed drug currently available to provide a reference for both doctors and patients. The information is provided free of charge at The Aspergillus Website (www.antifungalinteractions.org) and for convenience and maximum accessibility is also available as an APP for smartphones both for iPhones and Android devices (‘Antifungal Interactions’). There are 739 drugs listed and 8 antifungals. 398 interactions are rated as minor, 1375 moderate and 443 severe, a total of 2216 recorded interactions. The user simply chooses the drug they wish to investigate from a list and with one button click they will be taken to a ‘traffic light’ system of warnings - green for minor, orange for moderate and red for severe interaction. If there is no interaction noted or known this is also indicated. The online database is updated regularly with additional publications and constant review. The app database is updated every 6 months. It will shortly be available in a new form that will be more useful to doctors and other medical professionals, written out in a far more detailed format for professional use.

Conclusion:
A DDI resource is available for antifungal drug interactions, to reduce adverse drug reactions and loss of antifungal efficacy.
Purpose:
While previously rarely considered to be the cause of infections, Aspergillus species are now known to be the major cause of disease and mortality in immunocompromised patients. Most clinical strains of fungi are able to demonstrate resistance to antimycotic medications. Given the significance of invasive aspergillosis in healthcare, this study aims to assess the effects of lyticase on fungi of Aspergillus fumigatus species in combination with antifungal medication.

Methods:
Lyticase is an exoenzyme produced by soil bacteria that is able to cleave the mannoprotein complex of fungal cell walls. Previously we had shown that lyticase successfully causes cell wall lysis of Candida and enhances the effects of various antifungal agents by several times [Sachivkina et al., 2013]. In this investigation, the object of study is the pathogenic strain of fungi Aspergillus fumigatus, isolated from the lung of a dead animal (dog) in the veterinary clinic, as well as widely known antymycotic drug nystatin 25,000 units. The enzyme lyticase was obtained from culture broth of Cellulomonas cellulans AS-870 strain, which is stored in the National Collection of Microorganisms of the State Research Institute of Genetics and Selection of Industrial Microorganisms. The already-ready enzyme Lyticase 20,000 units («Sigma», Germany) could have also been used in the experiment, but the product yielded by our technology exceeded the specific activity of commercial drug.

Aspergillus sp. grow well on standard nutrient media, which is why the liquid medium Saburo was used. Titration of the antymycotic drug Nystatin was carried out on a microplate in liquid medium at different concentrations, decreasing by two times from well to well. Equal concentrations of Aspergillus was seeded in row A, which initially contained the concentration of 2500 IU of nystatin. Lyticase was added to row B in a concentration ratio of 1:1, containing the same nystatin titer. Analogous with this set-up, row C contained 250 units of nystatin, and row D contained 250 units of nystatin with the addition of 250 IU of the enzyme lyticase in a 1:1 ratio. Aspergillus was seeded in all rows equally. After 5 days, the results were obtained. A seeding was carried out onto a dense Saburo medium from the microplate.

Results:
On the fifth day the following results were obtained from the microplate: row A had no growth in the 4th well (at a concentration of 156,25 IU of nystatin), growth retardation was noted in well A5 (78,125 units). Row B (with the addition of lyticase) showed a full halt in growth in well 6 (39,06 IU of nystatin), growth retardation was observed from B7 (19,53 IU) to B11 (1,22 IU). In row C with the initial concentration of 250 units, a full stop was observed in well C1 (125 IU), stunted growth was noted in wells from C2 (62,5 IU) to C3 (31,3 IU). A halt of growth was observed in row D starting from well D3 (31,3 IU), growth retardation was seen in wells D4 (15,6 IU) to D10 (0,24 IU). Moreover, the prevalence of mycelial growth over the growth of the sporangium in rows with lyticase was noted. After seeding on the solid medium, an offset of fungal growth was noted. Complete cessation of growth occurred in wells A3 (312.5 IU), B5 (78,125 IU), D2 (62,5 IU). Based on these results, we found that the enzyme lyticase increases the effect of the antymycotic medication by four times.

Conclusion:
The results obtained in this study could shape future treatment plans of patients suffering from fungal infections, by potentially utilizing combined therapy with lyticase as to significantly reduce the risk of side effects, which are a substantial drawback of almost all modern antifungal medications. Such combined therapy would considerably improve the treatment of patients at risk of an Aspergillus infection.
COMPARISON OF *ASPERGILLUS* SPECIES-COMPLEXES DETECTED IN DIFFERENT ENVIRONMENTAL SETTINGS

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**Purpose:**

Samples from different environmental sources were screened for the presence of *Aspergillus*, and the distribution of the different species-complexes was determined in order to understand differences among that distribution in the several environmental sources and which of these species complexes are present in specific environmental settings.

**Methods:**

Four distinct environments (beaches, poultries, swineries and hospital) were studied and analyzed for which *Aspergillus* complexes were present in each setting. After plate incubation and colony isolation, morphological identification was done using macro- and microscopic characteristics. The universal fungal primers ITS1 and ITS4 were used to amplify DNA from all *Aspergillus* isolates, which was sequenced for identification to species-complex level. SPSS v15.0 for Windows was used to perform the statistical analysis.

**Results:**

Thirty-nine isolates of *Aspergillus* were recovered from both the sand beach and poultries, 31 isolates from swineries, and 80 isolates from hospital environments, for a total 189 isolates.

Eleven species complexes were found total. Isolates belonging to the *Aspergillus Versicolores* species-complex were the most frequently found (23.8%), followed by *Flavi* (18.0%), *Fumigati* (15.3%) and *Nigri* (13.2%) complexes.

A significant association was found between the different environmental sources and the distribution of the several species-complexes (p<0.001); the hospital environment had a greater variability of species-complexes than other environmental locations (10 in hospital environment, against nine in swine, eight in poultries and seven in sand beach). Isolates belonging to *Nidulantes* complex were detected only in the hospital environment, whereas the other complexes were identified in more than one setting.

**Conclusion:**

Because different *Aspergillus* complexes have different susceptibilities to antifungal drugs, and different abilities in producing mycotoxins, knowledge of the species-complex epidemiology for each setting may allow preventive or corrective measures to be taken toward decreasing professional workers or patient exposure to those agents.
NONCANONICAL AUTOPHAGY IS A TARGET OF FUNGAL CELL WALL MELANIN

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Purpose:
Activation of noncanonical autophagy via the engagement of signaling receptors regulates phagosome maturation and participates in many physiologic responses, including the killing of bacterial and fungal pathogens. In Aspergillus fumigatus, the predominant airborne fungal pathogen in humans, dormant spores are immunologically inert because cell wall immunostimulatory molecules are covered by a layer of hydrophobic rodlet protein RodA and melanin. Upon germination of A. fumigatus spores, surface exposure of β-glucan triggers autophagy protein recruitment to the phagosome and facilitates fungal killing. Whether masking of immunostimulatory molecules is the sole mechanism of the fungus to evade noncanonical autophagy is currently unknown.

Methods:
We investigated the mechanism of autophagy protein recruitment in the phagosome of A. fumigatus mutants lacking RodA and/or cell wall melanin by immunostaining and western blot analysis. The contribution of autophagy in killing of A. fumigatus was evaluated in Atg5 defective macrophages.

Results:
Surprisingly, we found that genetically enforced β-glucan surface exposure in dormant spores of A. fumigatus upon the removal of RodA layer (ΔrodA mutant) failed to activate noncanonical autophagy. In contrast, lack of cell wall melanin in dormant spores of ΔrodA/alb1 and ΔpksP mutants was necessary and sufficient to trigger robust LC3+ phagosome formation via β-glucan-dependent activation of NADPH oxidase. Cell wall melanin in the absence of RodA or upon exogenous melanin complementation in ΔrodA/alb1 spores did not interfere with β-glucan-mediated signaling regulating cytokine release, but selectively blocked noncanonical autophagy by inhibiting NADPH oxidase. Importantly, melanin-defective A. fumigatus mutants displayed increased susceptibility to killing that was dependent on noncanonical autophagy as it was restored in Atg5 defective macrophages.

Conclusion:
Collectively, we identified noncanonical autophagy as a molecular target of fungal cell wall melanin.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
IDENTIFICATION OF THE DNA SEQUENCE TO WHICH BINDS THE ZAFA TRANSCRIPTIONAL ACTIVATOR OF ZINC HOMEOSTASIS IN *ASPERGILLUS FUMIGATUS*

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Purpose:
*Aspergillus fumigatus* virulence depends on many different fungal capacities, including its capability to uptake zinc from the extreme zinc-limiting environment provided by the lungs of susceptible individuals. In this fungal pathogen the ZafA transcription factor regulates zinc uptake and zinc homeostasis under zinc starvation and is essential for virulence. For this reason, we investigated how ZafA recognizes the promoter regions of genes whose transcription regulates in order to discover new functions regulated by ZafA that could be essential for fungal virulence.

Methods:
We have hypothesized that ZafA might bind to a DNA consensus sequence located in the promoter regions of the genes most strongly induced by ZafA such as those involved in zinc uptake (*zrfA*, *zrfB* and *zrfC*). Thus, we expressed and purified the C-terminus of the ZafA protein that contains the putative DNA binding domain and used DNA fragments from the *zrfA*, *zrfB* and *zrfC* promoters to perform EMSA analyses and fluorescent DNase I footprinting assays. In addition, we performed a statistical analysis of the distribution of the zinc responsive motif at a genome-wide level and confirmed by northern blot the expression of some selected genes.

Results:
EMSA assays revealed that ZafA does bind to the promoters of the *zrfA*, *zrfB* and *zrfC* genes. By using a fluorescent DNase I footprinting assay we identified the protected sequences presumably recognized by ZafA in the promoter regions of these genes. A comparison of all protected regions revealed a consensus sequence to which ZafA binds or zinc responsive sequence (ZR). A search for this motif at a genome-wide level revealed that it was present in the promoter regions of 67 coding sequences. Nevertheless, degenerated versions of the ZR motif in up to two nucleotides indicated that it was also present in the promoters of 1,440 coding sequences. The statistical analysis of the distribution of all these ZR motifs at a genome-wide level revealed that the functional ZRs were most likely located within 1.2 kb upstream of the predicted AUGs start codons, as shown by northern blot analyses of some selected genes.

Conclusions:
ZafA either up-regulates or down-regulates gene expression through binding to one or more zinc responsive motifs located at the promoter regions of the genes that regulate. This study has allowed us to discover new functions regulated by ZafA that could be essential for fungal virulence.
113 EFFECT OF PH AND ZINC AVAILABILITY ON PACC PROCESSING IN ASPERGILLUS FUMIGATUS

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Purpose:
The zinc adquisition has been proven to be an essential trait for Aspergillus fumigatus virulence. This function relies on the alkaline zinc transporter ZrfC, which is required for optimal fungal growth under alkaline zinc-limiting conditions. The expression of zrfC is strongly induced by ZafA under alkaline zinc-limiting conditions because PacC, which regulates pH homeostasis in Aspergillus, modulates somehow the ZafA transcriptional activation activity. In addition, our previous work suggested that the repression of zrfC under acidic zinc-limiting conditions was PacC-dependent, thus suggesting a novel function for PacC that, until now, has been proposed to be inactive in acidic media.

The metabolic regulation by PacC has been widely described in A. nidulans, where is produced in an inactive form (called p72) and then proteolized by a two-step mechanism to generate the active form of the protein (called p27). Thus, as a first approach to investigate the PacC-mediated repression of “alkaline” genes such as zrfC, we tested whether the activation of the Af-PacC protein was as that most widely accepted for An-PacC.

Methods:
To afford these experiments we constructed strains of A. fumigatus that express a Myc-tagged PacC wild-type protein (Af-PacCwt) and Myc-tagged PacC mutant versions that mimic the growth of the wild type at both acid (Af-PacCacid) and alkaline pH (Af-PacCalkaline). The growth capacity of these mutant strains was tested under acidic and alkaline conditions and their defect on gene expression was analyzed by northern blot using the zrfB (an “acidic” gene) and zrfC (an “alkaline” gene) genes as probes. Afterwards, we analyzed by western-blot the processing pattern of the Myc-tagged PacC proteins from all strains cultured under acidic and alkaline zinc-limiting conditions.

Results:
We observed that An-PacC was processed as expected according to previous studies, although some processing was readily observed the in acidic defined media. In contrast, Af-PacCwt was processed in all conditions tested regardless of pH and zinc availability, although processing occurred at a larger extent in alkaline media. Both the Af-PacCacid and Af-PacCalkaline mutants processed partially under all conditions tested.

Conclusion:
These results suggest that the Af-pal pH-signalling pathway might not influence Af-PacC processing as directly as the An-pal pathway triggers An-PacC processing. Further investigations will be required to understand the Af-pal route and its influence in Af-PacC function.
THE ROLE OF CASPASE 1 INFLAMMASOME AND IL-18 DURING COLONIZATION INFECTION

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Purpose:
Patients with chronic lung disease such as cystic fibrosis, are commonly colonized by Aspergillus fumigatus. While a minority of these patients will develop allergic bronchopulmonary aspergillosis (ABPA), even in the absence of ABPA Aspergillus colonization is associated with increased numbers of pulmonary exacerbations and declining lung function. The inflammatory response to colonization with A. fumigatus hyphae in the absence of ABPA is poorly understood. We hypothesized that the inflammasome may play an important role in governing the host response to airway colonization with A. fumigatus.

Methods:
Mice were injected intratracheally with agar beads embedded with A. fumigatus conidia. Fungal galactomannan content in the lung was quantified as a measure of disease progression. Host immune response was measured by leukocyte recruitment and cytokine profile by EIA.

Results:
Our initial studies found that pulmonary colonization was associated with increased pulmonary levels of IL-1β in wild type B6 mice. Caspase 1 is the canonical protease responsible for pro-IL-1β, we therefore investigated the outcome of an infection in caspase 1 deficient mice. Caspase 1 deficient mice were found to be hyper-susceptible to fungal colonization and displayed an increased in fungal burden during the first week of infection. Interestingly, this increased fungal burden was associated with normal levels of IL-1β but decreased levels of IL-18, and increased levels of neutrophil recruitment.

Conclusion:
These results suggest that the caspase-1 inflammasome mediates protection against fungal infection in an IL-1β-independent fashion and that IL-1β processing during hyphal colonization is mediated by a non-canonical caspase 1 independent pathway. Further we hypothesize that IL-18 processing by caspase 1 plays a role in controlling the infection.
EPITHELIAL INVASION OF ASPERGILLUS FUMIGATUS IS GENETICALLY REGULATED AND REQUIRES THE TRANSCRIPTION FACTOR PACC

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Purpose:
A hallmark of invasive lung diseases caused by Aspergillus fumigatus is the penetrative growth of fungal hyphae which destroy pulmonary tissue. Although the respiratory epithelium represents the first point of contact with the host, the molecular basis of the host-pathogen interaction at this interface remains, for aspergilloses, poorly characterised. We established that the transcription factor PacC is required for pulmonary invasion as ΔpacC mutants are non-invasive, and attenuated for virulence, in a leukopenic model of infection. The purpose of this study was to interrogate the molecular basis of this critical deficit.

Methods:
To assess the effect of A. fumigatus spores on epithelial cells (ECs), wild type and ΔpacC strains were co-incubated with A549 monolayers and the number of detaching cells was enumerated at 16 hours of co-incubation. The assay was repeated using fungal culture filtrates, cell wall extracts, and heat killed hyphae to determine the role, respectively, of secreted proteins, cell wall components and physical perturbation in monolayer decay. To identify gene functions under PacC regulatory control during murine infection we performed transcriptional profiling of the A. fumigatus ATCC46645 clinical isolate and an isogenic ΔpacC mutant using germlings extracted from bronchoalveolar lavages (BALs) of leukopenic mice, after 4, 8, 12 and 16 hours of infection. To determine the effect of spore internalisation upon epithelial integrity a modified nystatin assay was used to measure the number of wild type and ΔpacC spores internalised during the first 4 hours of host-pathogen contact. This assay was also performed in the presence of Cytochalasin D and a Dectin-1 blocking antibody to study the contribution of actin and Dectin-1, respectively, to the internalisation process. Susceptibility of the isolates to caspofungin was measured in vitro according to the EUCAST specifications, and during murine infection by A. fumigatus viable counts and histological analysis of lung sections.

Results:
Using our in vitro infection assays, we found that A. fumigatus-mediated decay of A549 monolayers occurs via two temporally and mechanistically distinct processes, involving an initial contact-dependent mechanism, followed by damage caused by soluble effectors. Clues to the molecular basis of these perturbations were provided by transcriptional profiling of the ΔpacC mutant during murine infection which revealed aberrant expression of secreted proteases and remodelling of the fungal cell wall during infectious growth. Cell wall components and internalisation of spores by ECs contribute to epithelial decay during the early phase of the host-pathogen interaction. PacC mutants are deficient in both of these processes and nystatin protection assays revealed that ΔpacC spores are internalised less efficiently by ECs relative to the respective parental isolates. Importantly, pH non-sensing mutants were demonstrated to be more susceptible to caspofungin treatment, both in vitro and during murine infection.
Conclusions:
Epithelial invasion is a genetically regulated trait, and is regulated by the PacC transcription factor, which mediates distinct phases of host perturbation during pulmonary aspergillosis. In light of the hypersensitivity of infecting ΔpacC mutants to cell wall-active antifungal agents, our study identifies PacC signalling as a premier target for antifungal therapy.
MODIFICATION OF EXOPOLYSACCHARIDE COMPOSITION CAN INCREASE THE VIRULENCE OF NON-PATHOGENIC ASPERGILLUS SPECIES

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Purpose:
Differences in the composition or quantity of Aspergillus nidulans galactosaminogalactan may contribute to the reduced virulence of this organism

Methods:
Gene transcript levels were measured by real-time RT-PCR. Galactosaminogalactan production and composition, adherence to polystyrene, cell wall morphology, β-glucan masking property, and ability to induce apoptosis were assayed. Strains of A. nidulans that heterologously expressed A. fumigatus uge3 (An-Uge3) or overexpressed A. nidulans ugeB (An-UgeB) were constructed. The virulence of A. fumigatus Af293 and the various A. nidulans strains was assessed in mice that were immunosuppressed with either cortisone acetate alone or cortisone acetate plus cyclophosphamide. The mice were inoculated intranasally with 10⁶ conidia per strain. Virulence was assessed by survival and histopathologic analysis of the infected lungs.

Results:
Wild-type A. fumigatus and A. nidulans produced similar levels of galactosaminogalactan, however the GalNAC to galactose ratio was markedly reduced in A. nidulans. A. nidulans expression of ugeB was considerably lower than A. fumigatus expression of uge3. Both An-Uge3 and An-UgeB strains had increased production of galactosaminogalactan, which had a higher GalNAC content. The control strain of A. nidulans had low adherence to polystyrene and poor induction of leukocyte apoptosis, as compared to wild-type A. fumigatus. However, the An-Uge3 and An-UgeB strains adhered to polystyrene and induced leukocyte apoptosis similarly to A. fumigatus. Both the An-Uge3 and An-UgeB strains had significantly increased virulence in corticosteroid treated, non-neutropenic mice, and the virulence of these strains was similar to A. fumigatus. Histologic examination of the infected lungs showed an increase in lesion size and invasion in mice infected with An-Uge3. In contrast, the virulence of the An-Uge3 and An-UgeB strains in leukopenic mice was similar to wild-type A. nidulans, suggesting that the increased virulence of these strains is mediated through effects of galactosaminogalactan on leukocytes.

Conclusion:
Our results demonstrate that one mechanism for the low virulence of A. nidulans is that the galactosaminogalactan produced by this organism is significantly different from that produced by the more pathogenic A. fumigatus. Furthermore, these data provide additional support for the key role of galactosaminogalactan in adherence, host cell interactions, and virulence of A. fumigatus.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
FIBCD1 BINDS TO ASPERGILLUS FUMIGATUS CHITIN AND MODULATES THE INFLAMMATORY RESPONSE INDUCED BY CELL WALL POLYSACCHARIDES, PROTEASES AND INTACT ORGANISM

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Aim:
We have recently identified and characterized fibrinogen C domain-containing protein 1 (FIBCD1) as a homotetrameric type II transmembrane protein expressed by epithelial cells. In the present study, we investigate the role of FIBCD1 in the immune reaction against Aspergillus fumigatus (A. fumigatus), a pathogenic fungus involved in the development of asthma and allergy in western society. We try to understand the collective and individual role of fungal cell wall polysaccharides i.e. chitin and β-glucan and propose FIBCD1 as a novel Pattern Recognition Receptors (PRRs) which can recognize fungal cell wall chitin and modulate the innate immune response.

Methods:
We utilized fluorescence microscopy, pull down binding assays along with in vitro stimulation studies to test our hypothesis which were further confirmed using in vivo studies.

Results:
We show that FIBCD1 binds to the A. fumigatus cell wall, which is primarily composed of chitin, β-glucan, and galactomannan, in a calcium- and acetate-independent manner. We demonstrate that FIBCD1 recognises chitin-rich zones in the fungal cell wall in different cellular stages of A. fumigatus and that FIBCD1 is expressed in ciliated airway epithelial cells from patients with aspergillosis. We show that conidia, AIF, chitin, and β-glucan induce IL-8 secretion from A549 lung epithelial cells in a time- and dose-dependent manner. Furthermore, our results indicate that FIBCD1-transfected A549 cells display reduced IL-8 secretion compared to sham-transfected A549 cells when stimulated with any of these cell wall preparation and that this reduction is significantly increased when the polysaccharide is a FIBCD1 ligand. Furthermore, to evaluate the role of A. fumigatus chitin, which binds to FIBCD1, we studied the role of alkali insoluble polysaccharides in vivo. We demonstrated that Aspergillus fumigatus alkali-insoluble cell wall fragments (AIF) induced enhanced immune responses when compared with individual cell wall polysaccharides. Intranasal administration of AIF induced eosinophil and neutrophil recruitment, chitinase activity, TNF-α and TSLP production in mice lungs. Selective destruction of chitin or β-glucan from AIF significantly reduced eosinophil and neutrophil recruitment as well as chitinase activity and cytokine expression by macrophages, indicating the synergistic effect of the cell wall polysaccharides when presented together as a composite PAMP.

Conclusion:
Collectively our in vivo and in vitro data indicate that chitin and β-glucan play an important roles in activating innate immunity when presented as composite cell wall PAMPs and FIBCD1 is present in human airway epithelial cells, binds A. fumigatus cell wall PAMPs i.e chitin and influences the inflammatory response in the lung.
Background:
Zinc is required for many cellular processes that are essential for fungal growth and survival. S100A8 and S100A9 are proteins abundant in neutrophils which compete for free Zn2+, thereby limiting fungal growth. We examined the role of neutrophil S100A8/A9 in killing wild-type *Aspergillus fumigatus* and *A. fumigatus* strains with mutations in the transcription factor ZafA, zinc importers ZrfA/ZrfB and ZrfC and secreted protein Aspf2, all of which comprise the *A. fumigatus* zinc uptake system in zinc limiting environments.

Purpose:
The purpose of this study is to examine the role of neutrophil S100A8/A9 in control of *A. fumigatus* growth *in vivo* and *in vitro* and to determine whether *A. fumigatus* zinc uptake allows for fungal survival in the presence of S100A8/A9.

Methods:
Wild-type or mutant strains of *A. fumigatus* were incubated with neutrophils from S100A9-/- and control C57BL/6 mice in low (RPMI 1640) or high Zn2+ (RPMI + ZnSO4) media, and fungal growth was quantified by total chitin. The role of S100A8/A9 was also examined in a well-established murine model of corneal disease.

Results:
C57BL/6 neutrophils inhibited the growth of wild-type and all mutant strains of *A. fumigatus* in low Zn2+, which was ablated in by adding exogenous Zn2+. In contrast to C57BL/6 neutrophils, S100A9-/- neutrophils were unable to control the growth of wild-type, ΔZrfA/B and ΔAspf2 strains in low Zn2+, but did limit the growth of ΔZafA and ΔZrfC strains. Growth of wild-type *A. fumigatus* in the corneal stroma was significantly higher in S100A9-/- mice compared to C57BL/6 mice, although there was no difference in neutrophil infiltration.

Conclusions:
Neutrophil S100A8/A9 regulates growth of wild-type *A. fumigatus in vivo and in vitro* by competing for available Zn2+. The susceptibility of ΔZrfC and ΔZafA mutants to killing by S100A9-/- neutrophils suggests a role for additional mechanisms of zinc control by neutrophils and indicates that ZafA and ZrfC are essential for *A. fumigatus* survival in the presence of neutrophils.
Purpose:
Functional characterization of fungal genes using synthetic siRNAs has become a powerful reverse genetics tool and emerged as a novel small RNA-based therapeutics for human diseases. However, prediction and validation of a single siRNA duplex specific to a target gene is often ineffective in terms of silencing efficacy, off-target effects and is cost-intensive. With the explosion of fungal genomic information, there is an increasing need to analyze gene function in a rapid manner. In the present study, we have developed a fast, specific and efficient gene silencing approach for elucidating gene function in model filamentous fungus *Aspergillus nidulans* using RNase III-diced-siRNAs (d-siRNAs). This method was applied for identification and *in vitro* validation of novel RNAi targets to combat *A. fumigatus* infections.

Methods:
Stable expression of reporter *GFP* gene eases the examination of new RNAi induction route. So, we optimized *Agrobacterium* mediated genetic transformation in *A. nidulans*. The d-siRNAs for all the target genes were produced by digestion of *in vitro* transcribed double stranded RNAs using RNase III enzyme.

Results:
We demonstrated that the reporter *GFP* gene stably introduced into *A. nidulans* can be effectively silenced by treatment of *GFP*-d-siRNAs and effective knockdown of (AnrasA and AnrasB) the endogenous genes. We elucidated the function of an uncharacterized Ras homolog, *rasB* gene in *A. nidulans*, which was found to be involved in hyphal growth and development. We found that the silencing potency of d-siRNA was higher as compared to synthetic siRNA duplex, targeting AnrasA. Silencing was shown to be sequence-specific, since expression profiles of other closely related Ras family genes in d-siRNA treated AnrasA and AnrasB silenced lines exhibited no change in gene expression. Further, we applied this efficient silencing approach for exploring gene function and identifying novel target genes for development of siRNA-based therapeutics to combat emerging infections of human pathogenic fungus *A. fumigatus*. We have targeted genes involved in cell wall biosynthesis, nutrient uptake in invasive growth, polyamine biosynthesis, cellular signaling, metabolic regulation and asexual development and we examined the silencing effect on growth and life cycle of *A. fumigatus in vitro* and we selected these candidate genes for *in vivo* studies.

Conclusion:
We have developed and applied a fast, specific and efficient gene silencing approach for elucidating gene function in *Aspergillus Spp*. We also identified and *in vitro* validated several RNAi targets for development of siRNA-based therapy against *A. fumigatus* infections.
POLO-LIKE KINASE REGULATES COLONY GROWTH, CONIDIATION AND CELL POLARITY IN ASPERGILLUS FUMIGATUS

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Purpose:
Protein kinase is a key enzyme that functionally changes the target protein by phosphorylation. It is involved in the regulation of many cellular pathways including signal transduction. In mammalian cells, polo-like kinase (Plk) plays essential roles in the regulation of both mitosis and cytokinesis, and is an attractive target for the development of antitumor agents. To explore the possibility of using Plk as a target for antifungal therapies, we investigated the involvement of a Plk homolog, AfPLK1, in the regulation of hyphal growth in Aspergillus fumigatus.

Methods:
The A. fumigatus ΔakuA strain AfS35 was transformed with a deletion cassette comprised of 1-kb regions upstream and downstream of AfPLK1 flanking a hygromycin resistance cassette, generating the ΔAfplk1 disruptant strain. To generate a complemented strain, the ΔAfplk1 strain was transformed with a DNA cassette containing AfPLK1 and a pyrithiamine resistance gene.

Results:
The successful construction of a deletion strain demonstrated that AfPLK1 was not essential for the growth of A. fumigatus. The ΔAfplk1 disruptant strain exhibited a reduced colony growth rate of approximately 60% compared with the wild-type strain on PDA or AMM agar plates at 37°C. Conidiation by the ΔAfplk1 disruptant strain dramatically decreased because of the development of immature conidiophores. In liquid AMM media, branching of subapical hyphae was observed in 50% of hyphal cells of the disruptant grown at 37°C for 11 h, whereas the wild-type and complemented strains did not show any branching of hyphae under these conditions.

Conclusion:
AfPlk1 plays an important role in the regulation of hyphal elongation, conidiation, and cell polarity. The possibility of its use as a potential target for the development of novel antifungal agents against aspergillosis will be examined in future animal studies.
NEW CUSTOMIZED EXPRESSION MICROARRAY FOR *ASPERGILLUS FUMIGATUS*: EFFECT OF TEMPERATURE ON THE TRANSCRIPTOME DURING THE EARLIEST STEPS OF GERMINATION

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Purpose:
The aim of this study was to validate a new customized expression microarray designed by our group that covering the entire genome of *A. fumigatus* to improve our understanding of the pathogenesis of this mold. In this study, we wanted to analyze the effect of temperature on the transcriptomes of this fungus at the first steps of germination in order to identify functional changes and expressed genes related to virulence.

Methods:
The microarray was designed using the information available from the NCBI database and a commercial microarray design system (e-Array of Agilent Technologies, Santa Clara, CA, USA). This microarray includes 9,630 genes of *A. fumigatus*, 62 quality control genes and positive and negative controls included automatically by the design system. In order to validate this microarray, we studied the transcriptome of germinated conidia at 37 and 24°C after 6.5 and 18 h of incubation, respectively. Three independent extractions of total RNA obtained from each condition were hybridized with the microarray, and the expressions results were statistically compared. The expression results were validated by the selection of twenty overexpressed genes and their retrotranscription qPCR analysis. Finally, differentially expressed genes were classified according to their biological function.

Results:
The microarray data revealed 1,253 genes that were differentially expressed at 24 or 37°C. Retrotranscription qPCR results confirmed the differential expression of the selected genes at different temperatures with a Pearson’s correlation value of 0.89. According to our results, *A. fumigatus* modifies the expression of genes related to metabolism, cell rescue, defense and virulence, transport and energy in order to adapt to new conditions. Furthermore, some genes related to virulence were also overexpressed in the earliest steps of conidial germination but only following growth at a high temperature. Some of these overexpressed genes encoded proteins mainly related to a wide range of allergens (Asp F1, Asp F2 and MnSOD), while others are genes known to be involved in gliotoxin biosynthesis (GliP and GliZ) or nitrogen (NiiA and NiaD) or iron (HapX, SreA, SidD and SidC) metabolism.

Conclusion:
This study provides strong evidence to suggest that there is a connection between host temperature and the adaptation and virulence of *A. fumigatus*. It seems that simply with a heat shock of 37°C for a few hours, which could be correspond to the initial contact with human host, the fungus begins a quick process of adaptation, consisting of an increase in the germination rate and change in gene expression. These changes could help to colonize the lung and, ultimately, to infect the host, if the immune system is weakened. Moreover, the microarray used provides the opportunity to carry out more expression studies comparing the transcriptome of this pathogen growing under different laboratory conditions, and even during murine or human cell infections, and such experiments would provide a great deal of data that could improve our understanding of *A. fumigatus* pathogenesis.
THE INTERPLAY BETWEEN THE MICROBIOTA AND MYCOBIOTA IN THE HUMAN LOWER RESPIRATORY AIRWAYS

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Purpose:
Investigation of the lung microbiome is a relatively new field. Although the lungs were classically believed to be sterile, recently published investigations have identified microbial communities in the lungs of healthy humans. In pathogenic conditions, multiple investigations have documented the complex microbial ecology of bacterial communities in the lung. The roles of bacterial communities in the control of significant pathogens and in the generation of mucosal immunity and tolerance in the lung are of increasing scientific interest. Less is known about the mycobiota and its interplay with lung microbiota.

Methods:
To this purpose we have analyzed bronchoalveolar lavage samples (BAL) from different clinical cohorts. Bacterial metagenomic analysis was matched with microbial (bacterial and fungal) culture techniques in addition to laboratory and clinical data of fungal colonization and/or infection.

Results:
In patients with yeast infection, members of the Proteobacteria phylum increase from 6% to 46% and those of Actinobacteria phylum from 4% to 20%. In contrast, Firmicutes decrease from 88% to 34%. In the case of mould infection, Proteobacteria reached 82%, 79% of which were Pseudomonas sp, the Firmicutes decreased to 18%, with abundance of Staphylococcus sp over Streptococcus sp, and Actinobacteria disappeared. In condition of mixed fungal infection (yeasts plus moulds) bacterial communities are comparable to that of patients with yeast infection.

Conclusion:
These data suggest distinct relationships between yeasts and moulds and bacterial communities in the lung.

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LYSINE ACETYLATION AS A NOVEL APPROACH TO COMPROMISE HSP90 FUNCTION IN VIRULENCE AND ANTIFUNGAL RESISTANCE IN ASPERGILLUS FUMIGATUS

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Purpose:
The heat shock protein 90 (Hsp90) is an essential chaperone in eukaryotes. In fungi, Hsp90 has a crucial role in virulence and antifungal resistance mechanisms. Post-translational modifications such as phosphorylation and acetylation are known to play important roles in Hsp90 function and interactions with client proteins. Acetylation-mimetic mutations compromise Hsp90 function in Saccharomyces cerevisiae, without impacting antifungal resistance. Acetylation of Hsp90 can be induced by lysine deacetylase inhibitors, but these drugs were not found to be active against Candida albicans. The objective of this study was to determine the as yet unknown sites of phosphorylation and acetylation in Aspergillus fumigatus and to assess their role in Hsp90 function.

Methods:
Our Hsp90-EGFP strain (genetic fusion of Hsp90 to the enhanced green fluorescent protein) was used for isolation of the Hsp90 macromolecular complex by GFP-Trap affinity purification. Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis was performed after phosphopeptide (TiO2) enrichment and treatment with a lysine deacetylase inhibitor for the detection of phosphorylated and acetylated sites of Hsp90, respectively. Phosphorylated and acetylated residues identified by LC-MS/MS were mutated to non-phosphorylable residues (alanine) or to acetylation/deacetylation-mimetic residues (alanine/arginine).

Results:
Three phosphorylated residues (S49, S288, T681) of Hsp90 were detected by LC-MS/MS. However, mutation of those residues to alanines did not result in any phenotypic consequence on hyphal growth under standard and various stress conditions. Acetylation of Hsp90 was recovered only after treatment with deacetylase inhibitors, suggesting that Hsp90 is deacetylated under normal conditions. Acetylation-mimetic mutations of both K27 and K271 resulted in altered Hsp90 function and decreased virulence in a murine model of invasive aspergillosis (45% survival vs 20% in the wild-type, p=0.02). Deletion or acetylation-mimetic mutation of K27 only was sufficient to increase susceptibility to both caspofungin and voriconazole, while response to heat stress was conserved. This effect was partially reversed by a deacetylation-mimetic mutation, suggesting that K27 is crucial and should be deacetylated for proper Hsp90 function in antifungal resistance pathways. To further support the potential deleterious effect of acetylation on Hsp90 function and A. fumigatus growth, we tested the in vitro effect of the lysine deacetylase inhibitor trichostatin A (TSA) against A. fumigatus. In contrast to previous reports in C. albicans, TSA was active against the wild-type (MIC50=1 ug/ml) and reduced resistance to caspofungin in an echinocandin-resistant strain.

Conclusion:
Lysine acetylation cripples Hsp90 function and impacts fungal growth and virulence. Most importantly, the K27 residue of Hsp90 has a key role in antifungal resistance pathway which is unique to A. fumigatus. Lysine deacetylase inhibitors such as trichostatin A are active against A. fumigatus and may represent a novel strategy to combat invasive aspergillosis.
DIGITAL DROPLET PCR: A PROMISING METHOD FOR RARE EVENTS DETECTION FOR INVASIVE ASPERGILLOSIS DIAGNOSIS

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Purpose:
Molecular diagnosis of invasive aspergillosis (IA) has still no contenting sensitivity for clinical routine diagnosis despite vigorous efforts of standardisation. Real-time PCR is a fast method for identifying fungal load in immunocompromised patients. However, the detection of small amounts of fungal DNA in clinical samples makes the diagnosis difficult. These small amounts of fungal DNA may be an explanation for the non-adequate diagnostic sensitivity and high amounts of human genomic DNA and may lead to unspecific amplifications. Digital droplet PCR (ddPCR) could be helpful to improve sensitivity and specificity of this molecular technique for diagnosis of IA. The purpose of this study was to test clinical samples by use of ddPCR in comparison to real-time PCR results.

Methods:
For detection limit of the real-time PCR fungal DNA was extracted from whole blood samples from healthy volunteers spiked with A. fumigatus conidial concentrations ranging from $10^2$ to $10^6$ conidia/ml. Human whole blood samples without fungi served as a negative control. DNA of serum samples from patients with probable IA (n=5) and serum samples from patients without IA (n=5) were extracted and tested with ddPCR and real-time PCR. The ddPCR technique separates the sample into a large number of droplets (20,000 droplets/20 µl of reaction volume) and a single amplification is carried out in each droplet individually.

Results:
The absolute detection limit was 1 conidia/µl (Cq 37) for real-time PCR and 2 events for ddPCR. The cut off level based on negative control results were Cq > 40 for real-time PCR and < 2 events for ddPCR. For real time PCR none of the positive serum samples was detected as positive sample (Cq > 40 for all samples) and one sample out of five negative IA patient samples was detected positive (Cq 39) due to the detection limit. With ddPCR three out of the five positive serum samples were detected as positive with 5.6, 2.9 and 4.4 events. All other samples including negative patient serum samples were detected as negative (0 to 1.5 events).

Conclusion:
None of the clinical samples from patients with probable IA gave positive results by use of real time PCR. One out of the five negative serum samples was detected positive. As fungal burden in the bloodstream of patients is low, a high degree of analytical sensitivity and specificity is needed to avoid false negative and false positive results.

With ddPCR three out of five serum samples of patients with probable IA were tested positive and all negative samples were tested negative. One of the potential advantages of ddPCR versus real-time PCR is the capability to obtain absolute quantification without external references and robustness to variations in PCR efficiency leading to more precise results.

Due to the fact that serum samples from patients with probable IA were taken early in the course of IA, the ddPCR showed excellent results at an early stage of disease. Using this new PCR technique is an innovative approach and represents a good tool for detection of IA by enhancing diagnostic capabilities.
MORTALITY DUE TO INVASIVE ASPERGILLOSIS IN GRAFT-VERSUS-HOST DISEASE AFTER EXPERIMENTAL ALLOGENEIC BONE MARROW TRANSPLANTATION CAN BE REDUCED BY TRANSFER OF DONOR CD4⁺CD25⁺ REGULATORY T CELLS

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

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

Results:
After infection with Aspergillus fumigatus all animals with GVHD died within 10 d, whereas 60% of the animals without GVHD survived for more than 35 d after infection. Survival of recipients protected from GVHD after co-transplantation of donor CD4⁺CD25⁺ Treg cells was significantly better than that of unprotected recipients with GVHD. Interestingly, initial clearance of the fungus from the lung after i.t. infection, or from spleen, liver, lung, kidney and brain after i.v. infection, was rapid and comparable in mice with and without GvHD. Later on, fungi (re)appeared in the kidneys and fungal load increased significantly in moribund animals. Lymphocytes isolated from spleen and liver of infected animals with GVHD and restimulated in vitro with germinating conidia secreted significantly more pro-inflammatory TNF, IFN-γ and IL-6 than those from control mice. Co-transplanted donor Treg cells did not interfere with early pathogen clearance, but normalized the dysregulated cytokine secretion.

Conclusion:
Our data show that co-transplantation of donor CD4⁺CD25⁺ Treg cells protects mice not only from lethal GvHD but also from infection-related co-mortality. Currently, we are investigating whether the destruction of the kidneys by late outgrowth of residual fungal conidia is the main cause of death or whether an uncontrolled anti-fungal immune response is primarily reponsible for the high morbidity and mortality of opportunistic infections in GVHD.
CHARACTERIZATION OF HUMAN NATURAL KILLER CELL RECEPTORS RECOGNIZING ASPERGILLUS FUMIGATUS

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Purpose:
Natural killer (NK) cell recruitment by chemokines is a fundamental host defense mechanism during invasive aspergillosis in neutropenic mice. Mice double depleted in neutrophils and NK cells showed a twice fold mortality compared to neutropenic mice with normal NK cells. However, the exact mechanisms of the interaction between human NK cells and A. fumigatus are still unknown. Previously, our group was able to show that NK cells exhibit a cytotoxic effect against A. fumigatus germtubes by releasing IFNγ which induces fungal damage. Here, we further characterize the interaction of human NK cells and A. fumigatus germtubes to identify potential pathogen recognition receptors (PRRs) on the NK cell surface.

Methods:
NK cells were isolated from PBMCs of healthy donors by negative selection (MACS) and treated with Pro-Leukine overnight. NK cells were then either stimulated with A. fumigatus germtubes (MOI 0.5), or with IL15 and Pro-Leukine (positive control) or left untreated for 3h, 6h, 9h and 12h respectively, followed by analyses of NK cell activating receptors. Different pretreatments (Untreated, Pro-Leukine or IL2 pretreated) of NK cells and stimulation with different NK cell targets (K562, LPS, inactivated Candida (C.) albicans and inactivated A. fumigatus germtubes) were used to confirm receptor deregulation.

Results:
After stimulation of NK cells with A. fumigatus, the expression of the activation markers CD69 and CD137 significantly increased. Interestingly, a time dependent downregulation of the NK cell characterization marker CD56 was observed on the NK cell surface. However, this downregulation was not due to induced apoptosis in these cells. Furthermore, in highly activated NK cells the CD56 positive cells were reduced to a lesser extent than in untreated and Pro-Leukine pretreated cells. Treatment of NK cells with different targets such as K562, LPS, inactivated C. albicans and inactivated A. fumigatus revealed no reduction of CD56 on the surface of NK cells. In addition, the activation receptors NKp30, NKp44, NKG2D showed only a slight reduction in Pro-Leukine pretreated NK cells stimulated with A. fumigatus compared to the negative control.

Conclusion:
Treatment of NK cells with A. fumigatus induces NK cell activation and leads to a stringent reduction of CD56 on the cell surface. This downregulation was not induced by apoptosis of these cells and it was shown that the decrease of CD56 positive cells was only induced by living A. fumigatus germtubes while it was not present on NK cells stimulated with K562, LPS, inactivated C. albicans and inactivated A. fumigatus germtubes. These results suggest a role of CD56 in the recognition of A. fumigatus. Future experiments with blocking antibodies of CD56 and the NK cell activation receptors should provide an indication whether NK cell activation is also induced by CD56 or not. Fluorescence microscopy analyses will be performed to monitor localization of A. fumigatus and binding to CD56. These experiments will help to understand the interaction of human NK cells and A. fumigatus and identify the potential PRRs.
Background:
Chronic pulmonary aspergillosis (CPA) is a slowly progressive destructive disease, usually of the upper lobes, which is characterised by chronic inflammation and a failure to halt the intra-cavitary growth of *A. fumigatus* (usually). TH1 responses have been found to be crucial for effective defence against *Aspergillus* spp. TH17 immunity has been recently as well associated to the defence of fungal infections.

Methods:
We performed active cytokine-profiling by inducing whole blood cultures collected from 150 CPA patients in parallel with healthy controls. Bloods were taken at the NAC in Manchester and then sent by courier to the Cambridge Cytokine Laboratory for experimental activation on the same day and subsequent analysis.

Production of various cytokines including IFN-g, IL17a, TNF-a, IL-6, IL-12 and IL-10 after *in vitro* whole blood stimulation of by a variety of stimuli including PHA, LPS, Beta-Glucan, Zymosan, BCG, IFN gamma, IL-12, and IL-18. Cytokines were measured by ELISA or multiplexed particle based flow cytometry.

Results:
We have extended our analysis of a previously presented small cohort to more than 150 CPA patients applying an array of stimulations targeting T-, Nk-, and Monocyte subsets.

Results show a highly significant impairment of IL17 production after polyclonal T-cell stimulation with PHA (means: 394pg/ml vs 87 pg/ml; p <0.0001) across the cohort.

Impaired IFN gamma was evident after all stimulations but was most pronounced after induction with LPS (means 131 pg/ml vs 37 pg/ml; p < 0.0001), after correction on lymphocyte numbers.

Distinct response pattern could be identified showing (1) overall low IFN gamma production (2) Selectively low production after T-cell stimulation (3) Normal T-cell induced IFN gamma but impaired (innate) induced NK-cell derived IFN gamma production.

We noted as well increased production of the inflammatory cytokines TNF and IL-6. Patients had also significantly increased numbers of monocytes (means 0.37 vs 0.55; p<0.0001), which are a major source of these inflammatory cytokines. Stratifying on monocyte counts normalised the production after most stimuli *in vitro*.

Conclusion:
Results suggest significant impairment of IFN gamma and IL17 mediated immunity with a major involvement of TH17 and NK-cell subsets.
STRUCTURAL CHARACTERIZATION OF THE NEW IMMUNOSUPPRESSIVE GALACTOSAMINOGALACTAN PRODUCED BY *ASPERGILLUS FUMIGATUS*

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**Purpose:**
The galactosaminogalactan (GAG) is a new antigenic polysaccharide secreted by the human opportunistic fungal pathogen *Aspergillus fumigatus* during infection particularly during invasive aspergillosis. GAG is also an adhesin involved in the biofilm formation on abiotic support. Structure and function have been investigated.

**Methods:**
Immunological studies of GAG were carried on humans cells and murine model. Structural characterization was performed by carbohydrate analysis using specific chemical degradations, mass spectrometry and NMR.

**Results:**
In murine model, GAG promotes fungal development in immunocompetent mice due to its immunosuppressive activity that is associated to a decrease of neutrophil infiltrates in lung. Particularly, GAG induced neutrophil apoptosis. Carbohydrate analysis showed that this polysaccharide is a linear heterogeneous galactosaminogalactan composed of α1-4 linked galactose and α1-4 linked N-acetylgalactosamine residues where both monosaccharides are randomly distributed and with variable percentage of galactose per chain. The apparent Mr was estimated by gel filtration chromatography, this polysaccharide was eluted as a polydisperse homogenous polymer between 10 and 1000 kDa with an average of 100 kDa. A specific glycosyltransferase has been identified. The deletion mutant does not produce GAG anymore and its phenotype is currently analysed.

**Conclusion:**
GAG is the first fungal described immunosuppressive polysaccharide that favours the fungal infection.
HYPOXIA INDUCIBLE FACTOR 1 ALPHA IS REQUIRED FOR PULMONARY INNATE IMMUNE RESPONSE TO ASPERGILLUS FUMIGATUS

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Purpose:
While much effort is being placed in identifying new antifungal drug targets and compounds, an alternative and increasingly important area of investigation is finding therapies that work at increasing the host response and defense to fungal infections in immune compromised hosts. Recent studies have implicated a role for hypoxia inducible factor 1 (HIF1α) in the regulation of inflammation and host defense responses to microbial pathogens. Thus, the goal of my studies is to define the role of HIF1α in pulmonary immune responses to A. fumigatus.

Methods:
We utilized the glucocorticoid model of immunosuppression by triamcinolone in CD-1 mice to determine the involvement of HIF1α in the susceptibility to fungal infection by A. fumigatus. The HIF1α agonist L-mimosine was used in an ex vivo macrophage model as a treatment for glucocorticoid suppression, as measured by HIF1α protein and XTT-fungal damage. The in vivo involvement of HIF1α for clearance and defense against A. fumigatus was determined using a myeloid-specific lysozyme-M cre-recombinase knockout mouse of HIF1α (HIF-KO) compared to littermate controls. We used flow cytometry, fungal burden, neutrophil migration and luminex analysis to investigate HIF1α's role in pulmonary control of two A. fumigatus strains with different inflammatory profiles: wild type CEA10 (CBS144.89) and null ΔorlA mutant.

Results:
We observed that A. fumigatus could stabilize HIF1α protein in macrophages, independent of oxygen availability, and in lungs of infected mice. This protein stabilization of HIF1α was prevented by treatment of macrophages and mice with glucocorticoids, corresponding to a decrease in the antifungal activity of the macrophages. Therapeutic treatment of glucocorticoid-suppressed macrophages with a HIF1α agonist partially restored their antifungal activity.

Utilizing the HIF-KO mice, we observed that HIF1α is required for survival, fungal clearance, neutrophil recruitment, and inflammatory responses early during infection with the inflammatory strain CEA10. The early neutrophil recruitment defect at 8 and 12hr post infection in the HIF-KO mice was demonstrated to be due to a defective cytokine response, as ex vivo neutrophils were able to respond and migrate in the presence of infected littermate BALFs but not infected HIF-KO BALF. Cytokine analysis of the BALF from infected littermate and HIF-KO mice found decreased levels of KC. Addition of KC to levels found in littermate BALF into the HIF-KO BALF significantly restored the migration defect. Additionally, the hyper-inflammatory strain ΔorlA was able to restore the neutrophil defect in the HIF-KO mice, further supporting a signaling defect.

Conclusion:
Our data suggest that myeloid HIF1α is required for initiating the correct inflammatory signal and responses in order to control and clear A. fumigatus infection. The data strongly suggest that the timing of this response is critical for determining the outcome of the infection. Ongoing studies will determine whether the in vivo restoration of the signaling defect in HIF-KO mice will improve recruitment and survival. Additionally, this work supports the strategy of utilizing HIF1α as a therapeutic target in specific immunosuppressed populations with fungal infections. Future studies will seek to further define the mechanisms by which HIF1α helps mediate antifungal immune responses in the lung.
**ASPERGILLUS FUMIGATUS – DECIPHERING THE ROLE OF THE CHITIN SYNTHASE FAMILIES I AND II BY MULTIPLE GENE TARGETING DELETION**

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**Purpose:**
Chitin is an essential component of the fungal cell wall (CW), contributing for the mechanical strength and shape of the fungal cell. In *Aspergillus fumigatus* (the most ubiquitous opportunistic human-fungal pathogen), there are eight chitin synthase (*CHS*) genes belonging to the two families. Our aim was to understand the function of each *CHS* genes using single and multiple gene deletion strategy.

**Methods:**
Single and multiple deletions of the *CHS* were performed using β-reg/six system (Hartmann et al., 2010). Deletion mutants’ phenotypic (radial growth, conidiation, mycelial morphology) analyses were performed. Mutants were analyzed for their conidial/mycelial CW chitin content, CHS-activity, susceptibility to antifungal drugs/CW perturbing agents and pathogenicity.

**Results:**
β-Reg/six system allowed us to obtain single up to quadruple *chs*-deletion mutants belonging to the two families (family I – Δ*chsACBG* and Δ*csmA*B*chsFG* belonging to family II). Of the family I *CHS*, there was reduced conidiation, altered mycelial morphotype and reduced CHS activity in the Δ*chsG* and Δ*chsACBG* mutants; but all the mutants were as virulent as the wild-type strain. Among family II *CHS*, phenotypic defects were mainly associated with *CSMA* deletion mutant. Despite of the significant morphological growth phenotypes of the quadruple mutants, the chitin contents of the entire single and multiple deletion mutants were equal/non-significantly reduced compared to the parental strain.

**Conclusions:**
For the first time we did multiple *CHS* deletion in the filamentous fungus *A. fumigatus*, which has highlighted the complex overlapping role of the different *CHS* genes inside and in-between the two chitin synthase families.

**NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.**
UNDERSTANDING THE ROLE OF SEPTINS IN *ASPERGILLUS FUMIGATUS* GROWTH AND DEVELOPMENT

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**Purpose:**
*A. fumigatus* hyphal growth is required for invasive disease, and both the cell wall and septa provide critical structural support for proper hyphal extension. Septins are conserved GTPases involved in several cellular processes, such as cell wall integrity, cytokinesis, and septation. The *A. fumigatus* genome contains five genes encoding for septins: AspA, AspB, AspC, AspD, and AspE. AspE is specific to filamentous fungi. Although the localization of septins has been studied in *A. fumigatus*, how each of these septins contributes to hyphal growth and virulence has never been examined. Therefore, an understanding of their precise functions in hyphal morphogenesis will not only lead to fundamental knowledge but also to potential identification of novel antifungal targets.

**Methods:**
We generated six septin deletion strains (*ΔaspE*, *ΔaspD*, *ΔaspB*, *ΔaspEΔaspD*, *ΔaspEΔaspB* and *ΔaspEΔaspDΔaspB*) in the *A. fumigatus akuKU80* background to examine the role of these septins in *A. fumigatus* growth and pathogenesis. Radial growth was assessed on solid media, conidiation quantified, and inter-septal distances measured by staining with 0.1% aniline blue. To determine the role of septins in cell wall integrity, each septin deletion strain was treated with cell wall inhibitors caspofungin, a β-glucan synthase specific inhibitor, and nikkomycin Z, a chitin synthesis inhibitor.

**Results:**
Single, double, or triple deletion of the septin genes did not result in any significant radial growth defect under normal growth conditions. However, the *ΔaspD*, *ΔaspB*, *ΔaspEΔaspD*, *ΔaspEΔaspB* and *ΔaspEΔaspDΔaspB* strains have a significant reduction in conidiation compared to wild-type strain. In contrast to the inter-septal distances of the apical and subapical compartments of the wild-type strain (76.30 µm, 23.15 µm), the *ΔaspE* (170.61µm [P<0.001], 44.13µm [P<0.001]), *ΔaspB* (177.96µm [P<0.001], 60.09µm [P<0.001]), *ΔaspD* (96.55µm [P=0.0025], 31.60µm [P<0.001]), *ΔaspEΔaspD* (162.62µm [P<0.001], 46.06µm [P<0.001]), and *ΔaspEΔaspDΔaspB* (255.63µm [P<0.001], 92.44µm [P<0.001]) strains exhibited an increase in inter-septal distances of both compartments. Interestingly, the apical compartment of the *ΔaspEΔaspB* double deletion strain was also significantly larger than the apical compartment of *ΔaspE* and *ΔaspB* strains [P=0.002]. The *ΔaspB*, *ΔaspEΔaspB* and *ΔaspEΔaspDΔaspB* strains were also more susceptible to nikkomycin Z and caspofungin. Additionally, the caspofungin paradoxical effect (increase in growth following exposure to higher concentrations of caspofungin) was slightly abrogated following aspB deletion.

**Conclusion:**
We demonstrated that septin genes are dispensable for basal growth of the fungus, but important for maintenance of inter-septal distances through regular septation, conidiation, and for cell wall integrity. Specifically, deletion of aspB resulted in increased susceptibility to nikkomycin Z and caspofungin. This is the first exploration of septins in a human 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.
**Introduction:**

*A. fumigatus (Af)* colonizes the airways of 25-50% of cystic fibrosis (CF) patients, with some further progressing to allergic bronchopulmonary aspergillosis (ABPA). Even if multiple genetic/environmental factors likely determine susceptibility of CF patients (~10%) to develop ABPA, it is still not well understood why most CF patients colonized by *Af* do not develop ABPA while some do. Polymorphonuclear neutrophils (PMN), essential actors in the innate response to *Af* hyphae, can recognize *Af* via pattern recognition receptors such as dectin-1 and its cognate fungal ligand b-glucan. Dectin-1 has been shown to regulate exocytosis and other PMN functions. We hypothesized that the expression of dectin-1 at the surface of sputum PMN in CF-ABPA patients would be higher compared to CF patients without ABPA, whether chronically colonized with *Af* or not.

**Method:**

We present results from a longitudinal cohort study here comparing blood and sputum PMN dectin-1 levels. Cell surface dectin-1 was measured by flow cytometry on freshly isolated PMN without any *in vitro* activation in 10 CF-ABPA patients and two control groups: CF patients colonized with *Af* but without ABPA (CF-AC; n=10), and CF patients without *Af* colonization or ABPA (CF; n=14). The overall goals of this longitudinal study are to evaluate the interactions between *Af* and innate immune cells in CF blood and airways as accessed via induced sputum.

**Results:**

In all three CF groups, surface dectin-1 levels were increased on sputum PMN as compared to their blood counterparts (CF-ABPA: p=0.0035; CF-AC; p<0.001 and, CF, p<0.001). Furthermore, in sputum, surface PMN dectin-1 levels showed a trend toward increase in CF-ABPA patients compared to CF-AC (+128%, p=0.29) and CF patient groups (+112%, p=0.39). In our cohort CF-ABPA patients experienced more pulmonary exacerbations, were more frequently co-infected with *P. aeruginosa*, and had more diabetes than controls. In contrast, co-infection with *S. aureus* or *S. maltophilia* was similar in CF-ABPA and controls.

**Conclusion:**

CF-ABPA patients are distinguished from CF patients without ABPA (whether chronically colonized by *Af* or not) by ongoing innate immune activation as manifested by upregulated blood basophil surface CD203c (Gernez et al, *JCF* 2012; 11:502-10; Moss et al, *JCF* 2013; 12:S46). Additionally we show here that sputum but not blood PMN show an upregulation of dectin-1 as another feature of this innate response, which may be differentially increased in ABPA. Further patients will have to be enrolled to clearly evaluate this last finding.
133 EFFECT OF β-GLUCAN SUPPLEMENTATION ON THE GROWTH AND CELL WALL STRUCTURE OF ASPERGILLUS SPP.

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Purpose:
β-Glucan is one of the main component of fungal cell wall polysaccharides. It was detected in the culture supernatants of Aspergillus and Candida. β-Glucan has been reported to show various biological activities such as production of the mediators of the inflammation in vivo and in vitro. However, only few studies have examined the effect of β-glucan on the fungal cell itself. In this study, we investigated the effect of β-glucan supplementation on the growth and cell wall structure of Aspergillus spp.

Methods:
Aspergillus fumigatus was cultured in synthetic media supplemented with β-glucan (curdlan and laminarin). The morphological changes in Aspergillus induced by curdlan and laminarin were observed under a the microscope and compared. The sodium hypochlorite (NaClO) oxidized cells of the fungus body in media supplemented with or without β-glucan were prepared and the structure of these cells analyzed by carbon-nuclear magnetic resonance (C-NMR).

Results:
In β-glucan added medium, the promotion of the growth assessed by increase in the turbidity of the medium was determind and compared with that of the control medium on day 1. Hyphal growth was promoted in liquid and solid-cultures with addition of β-1,3-glucan. Glucose and dextran did not induce growth. In the control medium, α-1,3-glucan was the main component, but in the β-glucan added medium, the peak ratio of β-1,3-glucan increased. We compared the reactive oxygen species (ROS) production by macrophages that were stimulated in the A. fumigatus dried cells with β-glucan addition. A. fumigatus dried cells in β-glucan added medium induced higher ROS production than A. fumigatus dried cells in the control medium.

Conclusion:
The addition of β-glucan in the culture medium influenced the growth of the fungi and induced qualitative changes in the cell wall structure. Because β-glucan is detected in the mycology-culture supernatant, the relativity between these changes and the pathogenicity of Aspergillus spp has an interest.
How Does the Phylogenetic Species Concept Correlate with Biological Characteristics of the Pathogenic Species in Aspergillus Section Fumigati?

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Purpose:
Aspergillus section Fumigati contains twelve clinically relevant species. Among those species A. fumigatus is the most frequent agent of invasive aspergillosis followed by A. lentulus and A. viridinutans. Prior phenotypic and phylogenetic analysis concluded that they are separate species, with A. lentulus more closely related to A. fumigatus than it is to A. viridinutans. Since these three species are heterothallic, we aimed to find how phylogenetic distance correlates with biological relatedness judged by their ability to interbreed.

Methods:
Strains tested were clinical and environmental isolates of A. lentulus, A. viridinutans and, A. fumigatus and four indeterminate clinical isolates (Aspergillus unsp.) from the section Fumigati. Phylogenetic analyses were carried out with the sequences of ITS, benA, RPB2, Tsr1 and Mcm7. Thermotolerance was tested on malt extract agar at 25, 30, 37 and 42°C. Antifungal susceptibility was assayed according to the CLSI M38-A2. Virulence was tested on larvae of Galleria mellonella, mice immunosuppressed with hydrocortisone and mice with chronic granulomatous disease (CGD). Mice were inoculated via post-pharyngeal method. Mating crosses were arranged pairwise with strains of opposite mating type on oatmeal agar and incubated at 30°C.

Results:
Phylogenetic analysis confirmed A. fumigatus, A. lentulus and A. viridinutans are phylogenetically distinct and also showed that the Aspergillus unsp. isolates form an independent clade, apart from the other three Aspergilli. All isolates were able to grow at temperatures ranging from 25 to 42°C except A. viridinutans, whose maximum growth temperature was 37°C. Highest resistance to amphotericin B, itraconazole and voriconazole was found among the Aspergillus unsp. isolates followed by A. lentulus, A. fumigatus and A. viridinutans in decreasing order. Virulence assays in larvae of G. mellonella and CGD mice showed that A. lentulus and A. viridinutans were less virulent than A. fumigatus. Among the four Aspergillus unsp. strains, two were more virulent than A. fumigatus in G. mellonella. In CGD mice, however, all four were avirulent. Assays in non-neutropenic balbC mice immunosuppressed with hydrocortisone revealed that all species, except for A. viridinutans, were equally virulent. Mating was not observed for A. fumigatus × A. viridinutans, A. fumigatus × A. lentulus or A. lentulus × A. viridinutans. The complete sexual cycle was only observed in a cross between one of the Aspergillus unsp. strains (strain labeled as ASP1) with A. fumigatus and in ASP1 × A. viridinutans. SEM morphology of the ascospores produced by ASP1 × A. fumigatus was clearly distinct from the ascospore morphology produced by mating between A. fumigatus isolates.

Conclusion:
Inter-species mating between A. fumigatus and ASP1 (which is phylogenetically more distant from A. fumigatus than A. lentulus) suggests that ASP1 is biologically close enough to A. fumigatus to produce hybrid progeny when mating type incompatibility can be overcome. Our findings underscore the importance of weighing both genetics and biology in understanding each taxon. It will be interesting to know whether the inter-species F1 hybrids created in the ASP1 × A. fumigatus cross are fertile. The analyses with these hybrids are in progress.
INDUCTION OF MUC5AC EXPRESSION TO ASPERGILLUS FUMIGATUS IN AIRWAY EPITHELIAL CELLS AND SUPPRESSIVE EFFECTS BROUGHT ABOUT MACROLIDE ANTIBACTERIAL AGENTS

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Purpose:
Pathology of chronic pulmonary aspergillosis has many parts which are not elucidated. It is thought that local immunity of lungs is important to long-term colonization of Aspergillus spp. MUC5AC is main mucin protein constituting airway mucus. It has been already reported that MUC5AC production from the respiratory tract is stimulated by infection of Chlamydomphila pneumoniae, Haemophilus influenzae, Legionella pneumophila and Mycoplasma pneumonia. Mucus secretion plays a role in host protection of mucosal surfaces against pathogens. However, excessive mucus secretion affects mucociliary transport system and contributes chronic respiratory infectious disease. Therefore we examined that induction of MUC5AC expression to Aspergillus fumigatus in airway epithelial cells and suppressive effects brought about macrolide antibacterial agents.

Methods:
Airway epithelial cells (NCI-H292, ATCC® Number: In CRL-1848TM) were cultured in RPMI 1640 medium (GIBCO RPMI 1640). NCI-H292 cells were stimulated for 24 hours in the presence of the A. fumigatus (5233, ATCC® Number: Culture of 13073TM) culturing supernatant. We regulated concentration of culture supernatant adjustment with β-D glucan, from 100pg/ml to 1000pg/ml. The concentrations of MUC5AC protein in culture supernatant were measured by ELISA. We used lipopolysaccharide as a positive control to inhibition. As a control, cells were treated with culture RPMI medium alone. Furthermore we added macrolide antibiotics (azithromycin or clarithromycin) to NCI-H292 cells just before exposure to A. fumigatus culture supernatant, to determine the effect on MUC5AC expression. Concentration of azithromycin and clarithromycin are used at 50μg/ml.

Results:
The level of secreted MUC5AC increased with culture supernatant of A. fumigatus addition in a dose-dependent manner. MUC5AC protein levels were measured by ELISA and given in terms of % above control. It resulted more than 200% above control at highest concentration stimulator. Macrolide antibiotics reduced the level of secreted MUC5AC protein at 50μg/ml. This effect was seen from 20μg/ml and dose-dependently. There was no difference in the inhibition MUC5AC production between azithromycin and clarithromycin.

Conclusion:
Our experiments have demonstrated an effect of macrolides on mucin overproduction induced by A. fumigatus culture supernatant stimulation of airway epithelial cells. Our findings suggest that suppressive of excessive mucus secretion may be able to reduce colonization of A. fumigatus. It is thought that the effect of the macrolide on pulmonary aspergillosis will have to examine it more in future.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
α-GLUCANASE INHIBITS CONIDIAL AGGLUTINATION OF ASPERGILLUS FUMIGATUS AND PROMOTE INFLAMMATORY CYTOKINE PRODUCTION FROM THP-1

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Purpose:
α-glucan is one of the main component of cell wall of Aspergillus fumigatus swollen conidia, hyphae and extracellular matrix and it covers β-glucan. We examined the influence of α-glucan-lytic enzyme α-glucanase (mutanase) from Bacillus circulans for growth of A. fumigatus and production of inflammatory cytokines from THP-1 stimulated with A. fumigatus.

Methods:
First, we evaluated expression of cell wall α-glucan and β-glucan sequentially using immunofluorescent stain with α-glucanase. Secondly, A. fumigatus were cultured in shaking incubator with α-glucanase for 3 and 24 hours and assessed agglutination of conidia. Thirdly, we assessed conidial adhesion to human lung epithelial cell H292 with α-glucanase. Finally, human monocytic cell THP-1 were stimulated with LPS and germinating conidia of A. fumigatus preincubated for 12 hours with α-glucanase. We assessed production of inflammatory cytokines.

Results:
Fluorescent intensity of cell wall α-glucan and β-glucan were observed after swollen conidia, although they were not expressed on resting conidia. The intensity of α-glucan reduced with α-glucanase. Conidial agglutination in shaking culture and conidial adhesion to H292 cell layer were inhibited by α-glucanase. THP-1 produced inflammatory cytokine interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-α) stimulated with germinating conidia. Additionally, α-glucanase induced production of IL-8 and TNF-α from THP-1 stimulated by Aspergillus conidia, although it had no influence on the production by LPS.

Conclusions:
On Aspergillus cell wall, β-glucan, which is recognized from host immune cells by Dectin-1, is covered with α-glucan in growing phase. Previous reports suggest that α-glucan is not essential for growth of Aspergillus. Our results suggest that α-glucanase inhibits initial agglutination and adhesion of conidia and promotes production of inflammatory cytokines lysing α-glucan covering β-glucan in growing phase.
137 ACTIVATION AND EVASION OF INNATE IMMUNITY BY ASPERGILLUS FUMIGATUS

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Purpose:
Phagocyte recruitment is essential for effective clearance of Aspergillus fumigatus. Once pathogens invade the human body, they are opsonised by serum components such as activated complement proteins and antibodies. Complement can be activated via antibody-antigen complexes, via the recognition of sugars and via spontaneous activation. Activation of the complement system results in deposition of complement component C3b on the outer cell wall of the pathogen and the generation of small complement molecules in fluid phase, mediating chemotaxis and inflammation. In response to inflammatory cytokines, endothelial cells of the blood vessel express P-selectin. P-selectin glycoprotein ligand 1 (PSGL-1), a cell surface receptor expressed on leukocytes, interacts with P-selectin leading to leukocyte rolling on endothelial cells. After firm adhesion, leukocytes transmigrate through the endothelial layer into the tissue and migrate via a gradient of chemotactants to the site of infection. Leukocytes recognize the pathogen by sensing deposited C3b molecules and antibodies on the outer cell wall. This opsonisation process is indispensable for efficient phagocytosis and subsequent killing and clearance of the invading pathogen.

Methods:
In this study we investigated the processes of extravasation of phagocytes and the opsonisation, phagocytosis and clearance of A. fumigatus. We demonstrate that complement is essential for phagocytosis by neutrophils of swollen conidia and germ tubes. Although antibodies bind both morphotypes, antibodies only are insufficient for opsonisation and phagocytosis. Interestingly, anti-Aspergillus antibodies are required to activate the complement system. This C3b mediated opsonisation results in efficient phagocytosis and is essential in the killing of A. fumigatus by neutrophils.

Results:
Previous studies showed that A. fumigatus possesses strategies to evade the innate immune system. No factors have previously been identified that interfere with phagocyte extravasation. Therefore, we focussed on the potential of A. fumigatus to modulate cell rolling, the primary process upon phagocyte recruitment. Using immunoprecipitation with immobilized PSGL-1 we identified a previously uncharacterized secreted protein that binds to the phagocyte surface. Antibody competition experiments reveal that the secreted protein binds to the ligand binding part of PSGL-1. Moreover, post-translational modifications of PSGL-1, which are crucial for P-selectin binding, are also essential for the binding of the secreted protein.

Conclusion:
In conclusion, we show that antibody mediated activation of complement is essential for efficient phagocytosis and killing of A. fumigatus. We identified a previously uncharacterized protein secreted by A. fumigatus that binds PSGL-1 expressed on the phagocyte surface and potentially functions as a virulence factor that can hamper recruitment of phagocytes to A. fumigatus infected tissue.
**ASPERGILLUS FUMIGATUS AND ASPERGILLUS TERREUS VIRULENCE IN THE GALLERIA MELLONELLA INSECT MODEL**

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**Introduction:**
Aspergillus spp. have emerged as important pathogens in Cystic Fibrosis (CF) patients, most commonly causing Allergic Bronchopulmonary Aspergillosis (ABPA). However many CF patients may be colonised without displaying any signs or symptoms of ABPA. *A. fumigatus* and *A. terreus* are among the most commonly isolated fungal pathogens from CF patients. In this study the virulence of a collection of *A. fumigatus* and *A. terreus* strains isolated from CF patients were compared using the *Galleria mellonella* insect model.

**Methods:**
*A. fumigatus* (n=14) and *A. terreus* (n=7) isolates from CF patients were collected. All isolates from the study were confirmed as *A. fumigatus* or *A. terreus* by sequencing of the ITS region. *G. mellonella* were inoculated with a range of concentrations of *Aspergillus* conidia and larval survival was monitored over 7 days. Virulence was compared for all isolates and the lethal dose 50 (LD50) was calculated for all isolates.

**Results and Conclusions:**
The *A. fumigatus* isolates showed variable levels of virulence in the Galleria model. Likewise the *A. terreus* isolates showed different levels of virulence. However no statistical difference was observed between *A. fumigatus* isolates. One *A. terreus* isolate (AT5429) was significantly more virulent than the others (p<0.001). *A. fumigatus* isolates caused a significantly higher mortality than *A. terreus* with the exception of one *A. terreus* isolate (AT5429) (p<0.01). The *A. terreus* isolate AT5429 caused a similar level of mortality to that of the *A. fumigatus* isolates suggesting that some *A. terreus* strains may be as virulent as *A. fumigatus*. LD50 calculations of these isolates revealed no significant difference between *A. fumigatus* isolates. However LD50 calculations for *A. terreus* isolates showed a significant difference between all isolates. There was a significant difference between LD50 calculations for *A. fumigatus* and *A. terreus* isolates with the exception of one *A. terreus* isolate (AT5429). Administration of Amphotericin B after exposure to *A. fumigatus* or *A. terreus* significantly reduced mortality in the *G. mellonella* insect model. These results demonstrate that different strains of *A. fumigatus* and *A. terreus* have varying effects on *G. mellonella*. Monitoring the specific strains of *A. fumigatus* and *A. terreus* colonising the airways of CF patients may be of clinical benefit.
THE ZRFC ALKALINE ZINC TRANSPORTER IS REQUIRED FOR ASPERGILLUS FUMIGATUS VIRULENCE AND ITS GROWTH IN THE PRESENCE OF THE ZN/MN-CHELATING PROTEIN CALPROTECTIN

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Purpose:
Zinc is an essential micronutrient, required for the proper function of many proteins in which it plays a catalytic, cocatalytic, and/or structural role. Therefore, to grow within the lungs of a susceptible host Aspergillus fumigatus must be able to obtain zinc from the tissue. The bioavailability of this element in the living tissues is, however, very low since most of it is tightly bound to proteins. Moreover, its availability can be further decreased upon infection by the neutrophil-released protein calprotectin. We previously demonstrated that the correct regulation of zinc homeostasis by the transcriptional factor ZafA is essential for A. fumigatus virulence. In this work we investigated the role of the main zinc permeases – ZrfA, ZrfB and ZrfC - in the pathobiology of A. fumigatus.

Methods:
We investigated the virulence of various single, double and triple A. fumigatus mutant strains in two different mouse models of invasive aspergillosis: neutropenic (cyclophosphamide-cortisone treated) and non-neutropenic (cortisone treated) mice. In addition, we analyzed histological sections of infected lungs and performed immunohistochemistry localization of calprotectin. Furthermore, we tested the growth capacity of several strains in the presence of recombinant human calprotectin (rhCP). 

Results:
The sole presence of ZrfC is sufficient for full virulence, proving that this is the major transporter devoted to scavenge and uptake zinc from living tissues. zrfA and zrfB genes are not required in the presence of ZrfC, but can partially compensate its absence as a result of the upregulation of their transcription. The ZrfC scavenging capacity is largely dependent on its N-terminus, which is absent in the ZrfA and ZrfB proteins. Furthermore, we show that zrfC enables A. fumigatus to overcome the Zn/Mn-chelating capacity of calprotectin, which may explain the higher relevance of ZrfC for virulence in the non-neutropenic mice. Therefore, we propose that the presence or absence of calprotectin might be one of the major reasons of the different susceptibility to infection depending on the immunosuppression regime administered.

Conclusions:
Our results prove that zrfC confers A. fumigatus the capacity to grow in the alkaline zinc-limiting environment provided by the lung tissue of immunosuppressed individuals. This finding extends our knowledge about the pathobiology of A. fumigatus and might anticipate the development of new antifungal therapies to treat IPA based on inhibiting fungal zinc uptake.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
NEW INSIGHTS INTO THE PATHOGENESIS OF INVASIVE PULMONARY ASPERGILLOSIS: REAL TIME IMAGING OF HYPOXIA AND INFLAMMATION

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Purpose:
Aspergillus fumigatus, is responsible for relevant diseases especially in immunocompromised individuals. Among these, life-threatening invasive pulmonary aspergillosis (IPA) results in mortality rates ranging from 30–90%. While investigating the role of immune effector cells in host defense against A. fumigatus using a bioluminescent A. fumigatus reporter strain, we recently showed that one day post-infection lungs from cortisone acetate-treated mice displayed severe tissue necrosis and hypoxia (Brock et al 2008, Ibrahim-Granet O et al 2010, Galiger et al 2013). Development of hypoxia was confirmed by Grahl et al in 2011 using the hypoxia marker pimonidazole hydrochloride on lungs sections from euthanized mice with IPA. In this study we focused on the in vivo real-time monitoring of hypoxia and inflammation within the lungs of mice from three immunologically distinct murine models of invasive pulmonary aspergillosis, i.e. cortisone acetate versus cyclophosphamide treatment versus CXCR2 knock-out mice (Ibrahim-Granet et al BMC 2010).

Methods:
To monitor disease progression, hypoxia and inflammation we used bioluminescence imaging and fluorescence molecular tomography systems (Perkin Elmer) on mice infected with the luminescent A. fumigatus strain. Here, (i) fungal growth was quantified by bioluminescence, (ii) hypoxia was quantified by the cell surface expression of carbonic anhydrase (CAIX) and (iii) inflammation was determined through the pan cathepsin proteases known to be produced by inflammatory cells. In addition, inflammation was investigated from lung tissues homogenates by studying the inflammatory cytokine patterns by ELISA.

Results:
Three days following infection hypoxia was detected under all infectious conditions regardless the immune status of mice. Even immunocompetent mice developed hypoxia levels five times higher than mock-infected (control) animals although no invasive aspergillosis developed. However, hypoxia was most pronounced in susceptible CXCR2 KO mice (28 times higher than control) followed by corticosteroid and cyclophosphamide treated animals. In the CXCR2 KO mice, the high level of hypoxia is associated with an overwhelming inflammatory cytokines levels in the lungs homogenate. Regarding the cathepsin level, in immunocompetent animals, cathepsin was hardly detectable within the lungs of the mice. However, in corticosteroid animals the presence of cathepsin was more pronounced (11 times compared to control). Interestingly, in CXCR2 KO, showing the highest level of hypoxia, the presence of cathepsin was only moderate (3 times compared to control). This indicates that the pan cathepsin proteases activity is not necessarily linked to the severity of the infection and the role of these proteases in the clearance of the infection needs further investigations.

Conclusion:
This is the first study that correlates hypoxia with fungal growth in real-time from in vivo models of murine IPA. Importantly our data brings new insights on the contribution of hypoxia to lung inflammation during fungal infection. Impact of hypoxia on the innate immune system during invasive pulmonary aspergillosis has to be investigated.
MID1/CCH1/YVC1 CALCIUM CHANNELS ARE INVOLVED IN ASPERGILLUS FUMIGATUS VIRULENCE

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Purpose:
Calcium-mediated signaling mechanisms are widely employed in eukaryotes and are implicated in the regulation of diverse biological processes including gene expression, exocytosis, cytoskeletal rearrangement, cell morphology and in fungal pathogenesis. Although the high affinity Ca²⁺ channel, Cch1, and its subunit Mid1 have been investigated and evaluated in yeast and some filamentous fungi, little is known about the function of their homologs in Aspergillus fumigatus.

Methods:
Here we have characterized the yeast homologues Cch1 (Afu1g11110), Mid1 (Afu5g05840) and Yvc1 (Afu3g13490) in A. fumigates by using deletion analysis. We also investigated these mutants in a murine model of immunosuppressed aspergillosis.

Results:
Both null mutants midA and cchA showed a reduced diameter of growth when compared to the wild type. However, the ΔcchA mutant rescued its phenotype when grown in the presence of 200 mM of calcium. In contrast, the ΔmidA mutant was sensitive to increasing concentrations of CaCl₂. The ΔcchA mutant is more sensitive to MnCl₂ while Δmid mutant showed increased sensitivity to paraquat, LiCl and MnCl₂ and the double mutant ΔmidA ΔcchA behaved essentially like the ΔmidA. The ΔyvcA mutant exhibited phenotype identical to the wild type. qPCR analyses showed that cchA and midA genes had increased mRNA accumulation in the wild type strain when exposed to calcium, Congo Red and Calcofluor White, while the yvcA gene has increased mRNA accumulation only when exposed to calcium and Calcofluor White.

Conclusion:
The three single null mutants showed attenuated virulence and reduced fungal burden in a murine model of invasive pulmonary aspergillosis. These results strongly indicate that calcium channels are important for A. fumigatus virulence.

Supported by FAPESP and CNPq, Brazil
**ASPERGILLUS LIPOXYGENASE: A NOVEL INSTIGATOR OF ASTHMA?**

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**Purpose:**
Fungi have long been associated with asthmatic/allergy diseases, yet the exact mechanism(s) by which fungi induce asthma is unknown. Human 5-lipoxygenase derived oxylipins, including leukotrienes, induce inflammation, mucus secretion, vasodilation, immune cell recruitment, and bronchial constriction. Increasing evidence suggests that specific oxylipins play critical roles in exacerbating asthmatic phenotypes, with significant differences in oxylipin profiles between asthmatics and non-asthmatics. We investigated the ability of an *Aspergillus fumigatus* lipoxygenase, LoxB, to synthesize oxylipins similar to those detected in humans and whether those oxylipin products could exacerbate acute allergic responses commonly associated with asthma.

**Methods:**
We identified a 5-lipoxygenase homolog, LoxB, within *Aspergillus fumigatus*. loxB deletion and overexpression strains were grown in RPMI media and culture supernatant assessed for oxylipin production using mass spectrometry (n=3). Fungal extract/oxylipin cocktails were prepared by mixing 1:1 oxylipin-containing culture supernatant and soluble antigenic extract solution prepared from the fungal tissue of each respective loxB strain. Cocktail solution was administered to mice (n=9) three times a week over a three week period after which differences in airway hyperresponsiveness, immune cell recruitment, and IgE production were recorded.

**Results:**
Overexpression of LoxB leads to increased production of several oxylipins known to cause airway hyperresponsiveness including hydroxyeicosatetraenoic acids (HETEs) from the human 5-lipoxygenase pathway and hydroxyoctadecanoic acids (HODEs). Murine asthma studies demonstrated that mice treated with fungal cocktail derived from the overexpression loxB strain exhibit elevated airway hyperresponsiveness, increased macrophage and eosinophil recruitment, and higher IgE levels than mice administered wild-type or loxB deletion-derived fungal cocktail.

**Conclusions:**
This study demonstrates the ability of fungal lipoxygenases to exacerbate acute allergic responses. We propose that *A. fumigatus* LoxB is involved in asthmatic diseases, particularly ABPA (Allergic bronchopulmonary aspergillosis). Future work includes characterizing the oxylipins responsible for the acute allergic responses and assessing the impact of other *A. fumigatus* oxygenases and resultant oxylipins on asthmatic and allergenic responses.

**NOTE:** THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.
COMPARATIVE GENOME ANALYSIS OF ASPERGILLUS TANNERI, A. FUMIGATUS, A. PARASITICUS, AND A. FLAVUS PREDICTS MECHANISMS OF DIFFERENTIAL HOST VIRULENCE

L Losada1*, J Sugui2, N Zafar1, V Joardar1, S Pakala1, J Yu3, K Ehrlich1, D Bhatnagar3, JK Chung2, WC Nierman1

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Purpose:
The genus Aspergillus is responsible for several respiratory conditions among them invasive aspergillosis (IA). In the United States, IA is usually caused by A. fumigatus, while A. flavus represents a very small fraction of IA cases. Recently, we described a novel, highly virulent, aggressively invasive, and drug resistant species, Aspergillus tanneri. We sought to understand the differences in virulence in these three species by performing comparative genomics analysis.

Methods:
To this end, we sequenced the genomes of A. tanneri, an additional strain of A. flavus, AF70, and a strain of A. parasiticus, a species not known to cause IA using the paired-end Illumina GAIIx or HiSeq platforms coupled with 5 KB paired-end libraries for improved scaffolding. Assemblies were carried out using Celera Assembler.

Results:
The genomes of A. tanneri and AF70 were assembled into 1715 and 1139 scaffolds, totaling 36.5Mb and 37.2Mb. A. tanneri contained 11,112 predicted protein coding genes. The ‘core’ genome of these aspergilli consisted of 5,834 genes, which means that greater than 50% of genes in A. fumigatus are shared with a divergent group of aspergilli. An additional 1,462 genes were shared between 7 out of 8 genomes, in which most cases, Atan did not have an ortholog. Genomic analysis showed that Atan was no more similar to A. flavus than to A. fumigatus indicating that it is a distinct pathogenic lineage within the aspergilli. Comparative genomics showed that over 5,600 genes were unique to A. flavus strains compared to other aspergilli. Nearly 500 of these genes were unique to either A. flavus 3357 or AF70 alone. In contrast, A. tanneri had over 2,400 unique genes, which corresponds to roughly 20% of its genome. Among the unique genes in each species were several clusters that encode for non-ribosomal peptide synthases and polyketide synthases, suggesting that novel secondary metabolites may play a role in virulence. When analyzed using SMURF to identify secondary metabolite clusters, Atan was predicted to have almost 3 times as many clusters (73 total) as A. flavus or A. fumigatus. A more in-depth comparison of potential virulence traits including proteases, allergens, and metabolic versatility, especially in comparison with the non-human pathogen A. parasiticus will be presented.

Conclusions:
We have sequenced important members of the Aspergillus genus and conducted comparative genomic analysis. The results show a remarkable number of core genes among aspergilli – over 50% of their genomes – even across very distant relatives. The results from this study can be used to explore genetic differences that might explain phenotypic differences including drug resistance, host range, and virulence.

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OLM Medical is a company focused on sales, marketing and business development within the in-vitro diagnostic market. In 2011, OLM Medical entered into a strategic partnership with ISCA Diagnostics, a University of Exeter spin-out company established by Dr Chris Thornton, to offer a rapid diagnostic test built around ISCA’s patented monoclonal antibody specific to the human pathogenic fungi Aspergillus.

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