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Sara Gago (poster presentation)

Development and validation of a quantitative real-time PCR assay for the early diagnosis of coccidioidomycosis

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Objectives
Coccidioidomycosis is endemic in the southwestern United States and Latin America. Although primary disease is self-limited, coccidioidomycosis also presents with disseminated disease, especially in immunocompromised patients. Early diagnosis of coccidioidomycosis is difficult, as most clinical signs are non-specific, and cultures often negative. Moreover, serology may be also negative, especially early after infection or not indicative of acute infection. Thus, the development of molecular methods for early diagnosis is needed. The aim of this study was the development of a Real-Time PCR assay for the early diagnosis of coccidioidomycosis, and validation of the assay in a murine model of disseminated infection.

Methods
A Real-Time PCR (RT-PCR) was developed for the detection of Coccidioides immitis and C. posadasii DNA. Specific primers and a molecular beacon probe were designed to amplify 267 bp from the ITS2 region of the rDNA. An internal control, based on a jellyfish-derived sequence, was also included in each assay. Standard curves were constructed with PCR results from 5 repetitions of 10-fold dilutions of genomic DNA ranging from 1 ng to 1 fg/μl from C. immitis (CNM-CM 7056) and C. posadasii (CNM-CM 2912), belonging to the mould collection of the Spanish National Center for Microbiology, and C. posadasii strain Silveira. The specificity of the assay was assessed using DNA from 10 unrelated clinical Coccidioides spp. strains and DNA from 17 other fungal species, and also mouse and human DNA. A murine model of disseminated coccidioidomycosis was used for RT-PCR validation. Female 5-week-old CD-1 mice were infected iv with 250 arthroconidia of C. posadasii strain Silveira. Fungal burdens were determined by CFU and RT-PCR in spleen, lung, and liver on days 5, 10 and 14 days postinfection. Uninfected mice served as controls. CFU and CE (conidia equivalent) values were correlated.

Results
The RT-PCR assay showed high reproducibility (r > 0.99) and specificity (100%); the lower detection limit was 1 fg of genomic DNA per microliter of sample. Fungal burdens after 5, 10, and 14 days of infection were higher by RT-PCR than by CFU enumeration for all tissues analyzed; RT-PCR was negative for blood on those days. No PCR inhibition was detected. Spearman correlations of RT-PCR and CFUs results showed a significant correlation for lungs (p < 0.05) but not for liver or spleen (p > 0.05). Comparative linear regression analysis of RT-PCR and CFUs showed similar slopes (p > 0.05) for temporal burden of infection in all organs, indicating RT-PCR, while more sensitive,
is equivalent to CFU for disease progression.

Conclusions
We have demonstrated the utility of a fast, sensitive, and specific RT-PCR for the detection of coccidioidomycosis in clinical samples from mice. RT-PCR appeared to better reflect the progression of infection. Further studies are warranted.

Raquel Sabino (poster presentation)

Surveillance of environmental fungi, with focus on Aspergillus, in a Portuguese Central Hospital
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Objectives
Because immunocompromised patients are more prone to acquire nosocomial infections caused by fungi isolated from the environment, e.g. Aspergillus, this study aimed to screen the hospital environment for the presence of fungi and to understand their epidemiology in the different hospital wards analyzed.

Methods
During one-year period, four seasonal samplings, i.e., air and hard surface, were performed. A total of 101 air samples and 99 surface samples were collected from the Hematology, Oncology, and Intensive Care Unit (ICU) wards of a Portuguese Central Hospital. Aspergillus isolates were plated for growth as single colonies on malt extract agar with chloramphenicol to check the colony purity and observe colonial morphology. The universal fungal primers ITS1 and ITS4 were used to amplify DNA from all Aspergillus isolates, amplimers were sequenced, and isolates identified to the species-complex level.

Results
Aspergillus was the most frequently recovered fungal genus (20.9%), followed by Cladosporium (18.7%), and Penicillium (17.2%). Thirty-five Aspergillus isolates were collected from the wards with hematological patients (bone marrow transplant and hemato-oncology wards), whereas 15 isolates were recovered from ICU. Among Aspergillus isolates from the hospital environment, those belonging to the species-complexes of versicolores (n = 26; 32.5%), nigri (n = 12; 15.0%), flavi (n = 11; 13.7%), and circumdati (n = 6; 7.5%) dominated. Hemato-Oncology was the ward with higher fungal counts, whereas the bone marrow transplant ward, which is protected by HEPA-filtration of the supply air, showed the lowest numbers in all sampling periods. A significant association (p = 0.001) was found between the season and the Aspergillus complexes...
isolated, with spring and summer having a larger number of different species-complexes detected in the hospital's air and on the surfaces. Nevertheless, air counts showed that the autumn was the season with the highest proportion of Aspergillus (one third of the total number of fungi detected). This could be due in part to the presence of construction work near these wards.

Conclusion
The knowledge of the epidemiology of environmental fungi in each hospital may allow the establishment of preventive or corrective measures to decrease nosocomial fungal infections.

Steffi Rocchi (poster presentation)

Evaluation of fungal risk for immunocompromised patients alternatively hospitalized in haematology ICU and at home
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Objectives
Despite chemoprophylaxis and all standard preventive measures, Invasive Fungal Infection (IFI) incidence remains high in hematology patients. In France, quarterly controls are recommended in haematology intensive care units (ICU). However, little is known about the indoor environment of haematology patients when they return home between two hospitalization periods, when they can be considered as chronically immunosuppressed. The aim of our study was to assess the fungal exposure of these patients at home and to compare it with that of the hospital, especially when IFI was diagnosed. We sought to demonstrate the interest of also measuring fungal exposure at home to assess the overall fungal risk in such patients.

Methods
Fifty-three patients from a haematology ICU were included in the study. Fungal exposure was assessed by quantifying opportunistic molds at hospital during hospitalisation and in patients' homes. Environmental surveillance was performed weekly in the haematology ICU with five air samples using the MAS100TMImpactor in the corridor and four air samples in patients' homes after hospitalization. Cultures were carried out on DG18 media. Clinical, biological and radiological data were collected according to consensual criteria of EORTC/MSG for IFI classification. In addition to usual risk factors such as severe and prolonged neutropenia (<500 polynuclear neutrophils/mm3 of blood and >15 days), mild neutropenia (0.5G/L-1G/L), corticoid therapy and all situations which could result in mold exposure in the 3 months prior to IFI diagnosis were also analyzed.

Results
IFI was diagnosed for 14/53 patients. In hospital, 80% of
weekly controls were negative for opportunistic species, 17.4% detected low level of opportunistic species and 2.4% showed uncommon concentrations of A.fumigatus spores in corridor air (9, 14 and 25 CFU/m3). Some patients who developed IFI were hospitalized during these peaks. The air in most homes (34/53) had a low level of total mold (less than 170 CFU/m3) but opportunistic molds (A.fumigatus, A. flavus, Mucor spp., Lichtheimia spp., Rhizomucor spp. and Rhizopus spp.) were found in 69.8% of homes with A. fumigatus identification in 58.4% of cases (31/53). Twelve homes out of 53 had a ratio of opportunistic mold to total mold as high as 10% and 4 homes had more than 20% of opportunistic mold.

Our study established that 6 IFI patients/14 could have been exposed to opportunistic molds at home and in hospital, 3/14 only in hospital and 5/14 only at home. These last 5 patients were living in homes among the 25% having the highest rate of opportunistic molds. The percentage of opportunistic molds at home was a significant predictor variable for the development of IFI in this study.

Conclusion
This study emphasizes the fact that preventive measures should be aimed not only at the hospital but also the home environment. Monitoring fungal contamination in homes of immunosuppressed patients, focusing on opportunistic molds, could contribute to detecting IFI risk and would be a relevant continuation in patient surveillance.

Deborah Lockhart (poster presentation)

**Aspergillus fumigatus GNA1: fragment screening gets groovy**

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Background & objective
Diseases due to Aspergillus fumigatus present a spectrum of clinical, diagnostic and therapeutic challenges. A new generation of antifungal agents is required to address the toxicity and emerging reports of resistance in existing therapies. The cell wall of A. fumigatus (Af) represents a drug target. This dynamic interlaced polysaccharide structure is essential for survival of the fungus. Chitin is an integral component of the cell wall consisting of linear b (1–4) linked N-acetyl-D-glucosamine (GlcNAc). The Hexosamine biosynthetic pathway provides the sole source of the sugar nucleotide precursor UDP-GlcNAc. A new potential antifungal target is glucosamine-6-phosphate Nacetyltransferase (AIGNA1). This enzyme N-acetylates glucosamine-6-phosphate using acetyl-CoA to N-acetyl-glucosamine-6-phosphate.
as an intermediary step in UDP-GlcNAc biosynthesis. Fragment-based lead drug discovery provides a complementary and contrasting approach to traditional high-throughput methods through the elaboration of weakly binding small molecules. Here we assess the "druggability" of AfGNA1 using a fragment screen.

Methods and results
Purified AfGNA1 was chemically biotinylated and a fragment screen based on bio-layer interferometry (Octet Red, Forte Bio) performed to assess the binding of fragments to the target protein. Screening the Dundee Drug Discovery Unit fragment library gave a preliminary hit rate of 5.7% (37/652 with a response rate > 0.02 nm). A subset of seven fragments demonstrated stoichiometric binding with equilibrium dissociation constants in the micromolar range. X-ray crystallography was used to (a) confirm the hits and (b) determine the binding mode. Structural analysis of AfGNA1 in complex with fragment (A) illustrated the fragment binds in a groove behind the sugar substrate. This combined with initial kinetic data suggests fragment (A) may elicit a conformational change in the active site resulting in partial inhibition of AfGNA1 activity.

Conclusion
Fragment screening explores a diverse range of chemical space in assessing the binding capabilities of a target. This work suggests AfGNA1 may be a "druggable" antifungal target with fragment screening identifying a series of ligand efficient molecules binding in a groove adjacent to the active site. Iterative cycles of medicinal chemistry and structural biology are required to optimise this chemical ‘anchor’ by extending into the active site to generate highly potent drug-like lead molecules.

Sebastian Heimann (poster presentation)

Different doses of micafungin for prophylaxis of invasive fungal diseases: A web-based non-interventional trial in four large university hospitals in Germany
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Objectives
Treatment indications of new antifungals in clinical practice often deviate from the strict criteria used in controlled clinical trials. Under routine clinical conditions, beneficial and adverse effect not previously described in clinical trials may be observed. The aim of this study was to describe customary prescription and treatment strategies of micafungin (MIC).

Methods
A registry was set up on www.ClinicalSurveys.net and physicians from German tertiary care centers were invited to provide retrospective information on cases they had treated with MIC. Documentation comprised demographic information, underlying disease, efficacy, safety, and tolerability of MIC. Types of invasive fungal disease
(IFD) were defined by the EORTC/MSG criteria.

Results
A total of 125 episodes of patients hospitalized between 10/2009–01/2012 were documented, of which seven episodes had to be excluded due to incomplete documentation. The most common underlying disease of patients receiving MIC was hematological malignancy (116, 98.3%), followed by stem cell transplantation (104, 88.1%). Risk factors for contracting IFD were antibiotic treatment > 3 days (115, 97.5%), central venous catheterization (112, 94.9%), parenteral nutrition (50, 42.4%), and other (66, 55.9%). Micafungin was administered as prophylaxis (PPX) in 106 (89.9%) and for treatment of possible, probable or proven IFD in 12 (10.1%) patients, respectively. In the group of antifungal PPX, mean duration of MIC treatment was 22.8 days (95% CI: 20.4 - 25.3); 53 of the patients (50%) received a dosage of 50 mg/day, while the other 53 (50%) received 100 mg/day. For the different doses, prophylactic outcome was rated as success in 42 (79.2%) vs. 52 patients (98.1%; p = 0.002). Fifty-five patients (51.9%) were treated with posaconazole before initiation of MIC, 30 (28.3%) also received amphotericin b inhalation during PPX with MIC. Four patients (3.8%) developed a proven IFD while being treated with 50 mg/day MIC, compared to no patients treated with 100 mg/day. At the end of MIC PPX, 24 (22.6%) patients were switched to fluconazole and 61 (66.1%) patients to posaconazole. Six patients (5.7%) died due to unknown or other reasons than an IFD.

Conclusion
Our study demonstrates clinical effectiveness of MIC PPX in patients at high risk of contracting IFD. In most cases, MIC was part of a multi-modal antifungal PPX strategy. Investigators reported better outcomes in patients receiving therapeutic doses of MIC for PPX.

Katharina Becker (oral presentation)

Allergic bronchopulmonary Aspergillosis patients’ serum can modulate Aspergillus-induced immune responses by increasing IL-6 and decreasing IL-5

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Objectives
Allergic Bronchopulmonary Aspergillosis (ABPA) is a T helper (Th) 2 mediated hypersensitivity reaction in the lung against the species of the mould genus Aspergillus, most common of which is A. fumigatus. It occurs most often in patients with asthma and cystic fibrosis and has a high prevalence in India. We screened sera from
84 Indian patients with ABPA for cytokine and chemokine profiles and performed functional stimulation assays to decipher the immune-modulatory capacity of ABPA sera.

Methods
27 different cytokines were measured in the sera of 84 ABPA patients and 12 healthy controls (HC) from the same geographical area by a magnetic bead immunoassay. 17 ABPA patients were treated with itraconazole whereas 67 did not receive antifungal treatment. Peripheral blood mononuclear cells (PBMCs) of five healthy volunteers were stimulated with Aspergillus conidia in the presence of 40 individual ABPA sera and 12 HC sera. IL-6, TNFa, IL-5, IL-13 and IFNc ELISA were performed on supernatants.

Results
Cytokines derived from innate immune cells (IL-6, IL-1ra, IL-12), Th2 cells (IL-4), Th1 cells (IFNc), hematopoietic cells (GMCSF, G-CSF) and the angiogenetic growth factor, VEGF were significantly elevated in antifungal-naive ABPA sera compared to HC sera. In contrast, TNFa or Eotaxin were not significantly different. Interestingly, the concentrations of the elevated cytokines in ABPA sera were at a normal level in all 17 patients treated with itraconazole. Functional stimulation assays revealed a significantly higher Aspergillus-induced IL-6 production in the presence of ABPA sera in all five experiments with PBMCs from healthy volunteers, while Aspergillus-induced TNFa was not modulated. IFNc was not modulated in these functional assays, however IL-5 was significantly lower in 4 out of 5 PBMC experiments.

Conclusion
We describe here in a large cohort of ABPA patients that sera from patients with ABPA show an elevated proinflammatory cytokine profile rather than the classical Th2 cytokine profile. Increased serum concentrations could be restored to the level of healthy controls through antifungal treatment with itraconazole, suggesting a correlation of the fungal load with immune stimulation. Moreover, the immunomodulatory capacity of sera from patients with ABPA is characterised by increasing Aspergillus-induced IL-6 responses, but not TNF, and decreasing IL-5 production, which are unexpected findings that need further investigation.