11th Trends in Medical Mycology

Abstract book

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S02.4

Invasive Aspergillosis and Aspergillus Colonization in Lung Transplant Recipients (LTRs) in a Large Contemporary Cohort

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Objectives: Invasive aspergillosis (IA) is a significant concern for lung transplant recipients (LTRs). Understanding current factors associated with the development of IA is crucial for effective prevention and management strategies. This retrospective cohort study aimed to assess the contemporary risk factors for the development of IA in LTRs overall and separately for those with pre (preAC)- and post-transplant Aspergillus colonization (postAC).

Methods & Materials: The study included all LTRs who underwent lung transplantation at Toronto General Hospital between January 2010 and December 2019, targeted approach is used for antifungal prophylaxis: pre-emptive treatment is only given in the presence of specific risk factors. The participants were followed for one year after transplantation, and diagnosis of IA was established as per ISHLT criteria. Multivariable and univariable logistic regression models were used to evaluate the impact of various factors on the development of IA overall and for LTRs with pre- and postAC separately.

Results: Among the 1358 LTRs, 451 (33%) received preemptive antifungal prophylaxis. 81 LTRs (5.96%) were diagnosed with IA in the first-year post-LT. At IA diagnosis 55/81 (68%) respiratory fungal cultures were positive: Most prevalent were *Aspergillus fumigatus (65%, 36/55), followed by A. niger (9%, 5/55) , A. flavus (5%, 3/55), A. terreus (4%, 2/55), A. versicolor (2/55), and A. species (13%, 7/55).* The median time from transplantation to IA was 100 days (IQR 58-186). In the multivariable analysis (Table A), infection with a respiratory virus (OR 1.94, p=0.028), statin use (OR 0.51, p=0.018), preAC (OR 3.78, p=0.002), postAC (OR 10.10 p=0.000), and pre-emptive fungal treatment post-transplantation (OR 0.14, p<0.001), were identified as factors associated with development of IA in contrast to ATG use (OR 1.23 p=0.60).

A total of 408 participants (30%) had any fungal colonization. 99 patients (7%) had documented preAC, at a median of 74 days (IQR 183-16.5) before transplant. 71/99 (71%) received pre-emptive antifungal treatment. 11/99 (11%) were diagnosed with IA post-transplant. In the univariate analysis

(Table B), the following factors were associated with IA occurrence: CMV viremia (OR 3.95, p=0.040) and postAC (OR 9.64, p=0.005) but not pre-emptive antifungal treatment (OR 0.65, p=0.53). 346 patients (25%) had documented postAC, that occurred at a median of 80 days after transplant (IQR 1-184). 193/346 (56%) received pre-emptive antifungal treatment. In this group 53/346 (15%) were diagnosed with IA within one year post-transplant. In the multivariate analysis pre-transplant aspergillus colonization (OR 3.94, p=0.008) and pre-emptive antifungal treatment (OR 0.15, p<0.001) were associated with IA occurrence. At one year post-transplant 25% of LTRs with IA (20/81) were deceased compared with 12% in LTRs without IA (149/1277, p=0.001).

Conclusion: In this contemporary cohort involving >1300 LTRs IA incidence was <6%. Pre- and postAC were the most important risk factor for the development of IA. Factors associated with IA differed in the presence of pre- or post-transplant Aspergillus Colonization. Highlighting the potential benefits of tailored targeted interventions to mitigate the risk of IA and associated outcomes in lung transplant recipients.

TABLE A. Factors associated with occurrence of Invasive Aspergillosis in all Lung Transplant Recipients

	Univariable Analysis		Multivariable Analysis	
Variable	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	**p-value**
Female Sex	0.65 (0.41-1.05)	0.079	0.67 (0.40-1.12)	0.125
Age	0.99 (0.97-1.00)	0.465	1.01 (0.98-1.03)	0.679
Single Lung	1.40 (0.81-2.41)	0.222	1.33 (0.70-2.56)	0.380
Indication for Lung Transplant				
Other	Reference			
COPD/Emphysema	0.74 (0.33-1.68)	0.481	0.64 (0.25-1.67)	0.363
Interstitial Lung Disease	1.04 (0.52-2.08)	0.914	0.97 (0.43-2.22)	0.951
Pulmonary Hypertension	0.51 (0.16-1.63)	0.256	0.37 (0.10-1.34)	0.131
Cystic Fibrosis	1.57 (0.69-3.57)	0.282	1.48 (0.57-3.85)	0.422
ATG use	1.06 (0.52-2.16)	0.878	1.23 (0.57-2.68)	0.600
CMV-Viremia	1.57 (1.00-2.46)	**0.049**	1.37 (0.83-2.26)	0.211
Infection with Respiratory Virus	1.91 (1.13-3.21)	**0.015**	**1.94 (1.08-3.50)**	**0.028**
Rejection	1.44 (0.84-2.49)	0.186	1.11 (0.61-2.03)	0.736
Statin Use	0.36 (0.23-0.55)	**<0.001**	**0.51 (0.30-0.89)**	**0.018**
Pre-Transplant Aspergillus Colonization	1.59 (0.87-2.93)	0.128	**3.78 (1.65-8.63)**	**0.002**
Aspergillus Colonization post-transplant	6.35 (2.10-4.89)	**<0.001**	**10.10 (6.00-17.02)**	**0.000**
Pre-emptive antifungal Treatment	0.44 (0.25-0.77)	**0.005**	**0.14 (0.07-0.29)**	**0.000**

TABLE B. Factors associated with occurrence of Invasive Aspergillosis in 99 LT recipients with **Pre-Transplant Aspergillus Colonization**

	Univariable Analysis		Multivariable Analysis NA	
Variable	Odds ratio (95% CI)	p-value		
Female Sex	0.69 (0.19-2.44)	0.570		
Age	0.97(0.94-1.01)	0.203		
Single Lung	Omitted			
Indication for Lung Transplant				
Other	Reference			
COPD/Emphysema	0.52 (0.04-6.29)	0.612		
Interstitial Lung Disease	1.05 (0.13-8.24)	0.961		
Pulmonary Hypertension	1.66 (0.13-21.73)	0.697		
Cystic Fibrosis	2.08 (0.36-11.91)	0.409		
ATG use	2.1 (0.21-20.68)	0.525		
CMV-Viremia	**3.95 (1.07-14.64)**	**0.040**		
Infection with Respiratory Virus	1.0 (0.19-5.08)	1.000		
Rejection	1.56 (0.37-6.53)	0.538		
Statin Use	0.22 (0.05-1.08)	0.063		
Aspergillus Colonization post-transplant	**9.64 (1.93-47.59)**	**0.005**		
Pre-emptive antifungal Treatment	0.65 (0.17-2.44)	0.530		

TABLE C. Factors associated with occurrence of Invasive Aspergillosis in 346 LT recipients with **Post-Transplant Aspergillus Colonization**

	Univariable Analysis		Multivariable Analysis	
Variable	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	**p-value**
Female Sex	0.64 (0.35-1.16)	0.465	0.75 (0.38-1.47)	0.401
Age	0.99 (0.98-1.01)	0.955	1.00 (0.97-1.03)	0.885
Single Lung	1.33 (0.64-2.78)	0.473	1.24 (0.53-2.93)	0.614
Indication for Lung Transplant				
Other	Reference			
COPD/Emphysema	1.12 (0.38-3.26)	0.827	1.00 (0.29-3.52)	0.989
Interstitial Lung Disease	1.47(0.55-3.91)	0.434	1.19 (0.38-3.69)	0.762
Pulmonary Hypertension	1.02 (0.26-3.95)	0.973	0.64 (0.14-2.86)	0.566
Cystic Fibrosis	1.57 (0.54-4.61)	0.406	1.94 (0.54-6.91)	0.302
ATG use	0.40 (0.12-1.35)	0.142	0.64 (0.17-2.32)	0.132
CMV-Viremia	1.78 (0.99-3.21)	0.054	1.62(0.84-3.12)	0.151
Infection with Respiratory Virus	1.44 (0.69-3.01)	0.328	1.32 (0.57-3.00)	0.512
Rejection	0.70 (0.32-1.57)	0.395	0.54 (0.23-1.29)	0.190
Statin Use	0.66 (0.36-1.19)	0.173	0.57 (0.28-1.18)	0.132
Pre-Transplant Aspergillus Colonization	1.94 (0.86-4.37)	0.113	**3.94 (1.42-10.90)**	**0.008**
Pre-emptive antifungal Treatment	**0.23 (0.12-0.44)**	**<0.001**	**0.15 (0.066-0.33)**	**<0.001**

CF Cystic Fibrosis, CI Confidence Interval, COPD Chronic Obstructive Pulmonary Disease

S03.4

Differential transcriptomic response dynamics of primary human macrophages to Scedosporium apiospermum infection compared to Aspergillus fumigatus

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Objectives

Scedosporium is the second most prevalent airway colonization fungal agent after *Aspergillus* in cystic fibrosis. Long-term bacterial and fungal airway colonization in CF patients contribute to maintain a chronic inflammation, through the release of various damage- associated molecular patterns, which may be involved in lung injury. We hypothesized that disparities in terms of cell wall structure and conidia morphology between both fungi, may affect interactions with host innate cells, that may ultimately affect fungal survival within the host.

The objectives of this study were i) to perform the first comparative transcriptomic analysis of human macrophages challenged with *S. apiospermum* (Sap) or *A. fumigatus* (Afu) conidia, at two distinct time points, *i.e.* 4 h and 12h, ii) to measure the inflammatory cytokine response and iii) to assess the survival of Sap and Afu conidia after macrophage challenge.

Methods

All experiments were performed with conidia harvested from 9 day-cultures of the wild-type 14462 *Scedosporium apiospermum* strain (Sap) and the wild-type Af 293 *Aspergillus fumigatus* strain. Macrophages derived from human peripheral blood mononuclear cells (PBMCs). RNA sequencing was performed from macrophage lysates obtained after 4h and 12h infection at 37°C with Sap or Afu conidia (MOI 10:1), and from uninfected macrophages. Bioinformatics analysis was conducted to identify differentially expressed genes (DEGs), comparing Sap-infected *vs* uninfected cells, Afu-infected *vs* uninfected cells and Sap-infected *vs* Afu-infected cells, at 4h and 12h ($|\log_2 \text{fold-change}|>1$). Cytokines were measured in culture supernatants after 12h infection at 37°C with Sap or Afu, using a 13plex-bead-based immunoassay LEGENDplex® multi-analyte flow assay (BioLegend). After co-incubation at 37°C for 6h, the killing macrophage of Af and Sap conidia by macrophages was determined by cytometry assay after propidium iodide staining.

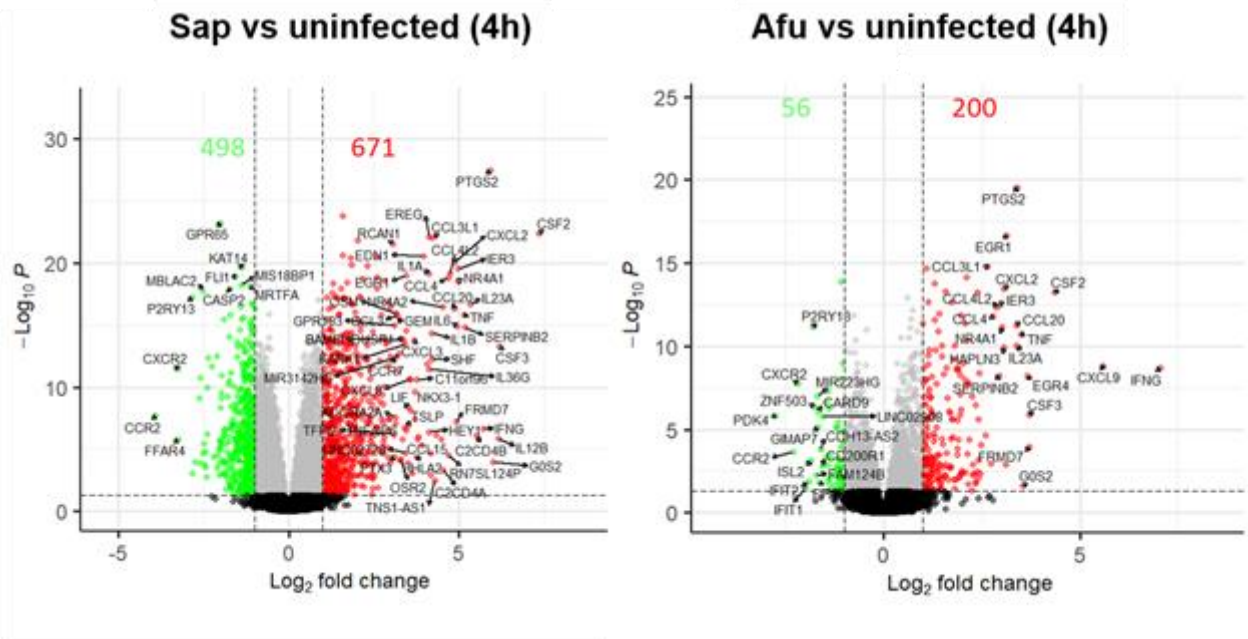
Results

Sap triggered the activation of several pro-inflammatory pathways, which were similar to the pathways activated by Afu. At 4h post-infection, a total of 15 pathways involved in inflammatory processes were differentially expressed in Sap-infected macrophages (FDR of 4.10^{-2} to 7.10^{-13}), of which 12 were concomitantly enriched in Afu-infected macrophages (FDR of 4.10^{-2} to 0), independently of the sense of variation. The four more strongly dysregulated pathways were signaling pathways associated with TNF, IL-17, NF-kappa B, and actors of cytokine-cytokine receptor interactions, commonly described during antifungal response. Interestingly, Sap triggered a stronger and earlier activation of pro-inflammatory pathways, as revealed by the three-fold higher number of over-expressed DEGs at 4h by Sap, compared to Afu (671 *vs* 200). Sap infection also triggered a higher release of IL-6, IL-1β, TNF-α, IL-23, IL-10 ($p<0.001$) and IFN-α2 ($p<0.05$) in culture supernatants, which supported transcriptomic data. If the rate of

infected macrophages was similar at 4h between both fungi, the killing of Sap conidia was higher than Afu conidia after 6h co-incubation (46.6% *vs* 34.5%, p<0.05), reflecting a lower ability of Sap to survive macrophage antifungal mechanisms.

Conclusion

S. apiospermum tailored a stronger and quicker inflammatory macrophage response, which could mediate a facilitated clearance, compared to *A. fumigatus*. An excessive inflammatory response could be deleterious for the host in the long term, especially in the specific context of cystic fibrosis.



S04.1b

Molecular epidemiology of invasive infection due to Trichosporon spp.: increasing of trichosporonosis and emerging of new species in France

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Objectives:
Trichosporon species are basidiomycetous yeast-like considered as emerging pathogen. Fungemia due to *Trichosporon* have increased in recent years in many countries (Taiwan, France, Italy, Spain, USA, Turkey, Brazil). *Trichosporon* fungemia is frequently associated with haematological malignancies, neutropenia, intensive care units (ICU), skin lesions and frequently reported in the pediatric population. Some horizontal transmission were also documented maybe due to the fact that some species are found on inanimate surfaces. Many species among the *Trichosporon* genus are involved in human infections but *T. asahii* remains the leading species in invasive infection. Recently, molecular analyses reclassified *Trichosporon* species in different genera. Epidemiological data based on multicentric survey associated with molecular identification remain relatively rare.

Materials & Methods:
Here we report clinical data of 68 episodes of *Trichosporon* invasive infections based on a national active surveillance program (RESSIF). Thirty three French hospital reported all their cases of invasive infection between January 2012 and December 2022. All isolates were identified by using IGS1 and whole genome sequecencing. The episodes involving *Trichosporon* together with other genus such as *Candida, Aspergillus, Pneumocystis* were considered as mixed infections and were excluded from this analysis (n=13).

Results:
The 68 episodes were due to 10 different species. Of these, 3 species respectively closely related to *T. inkin*, *T. faecale* or *T. coremiiforme* have not been previously described. *Trichosporon asahii* corresponds to the major species involved (n=36) and 5 different IGS haplotypes were identified ; haplotype 1 was the most frequent (16/36) as in most countries. Surprisingly, the second most frequent species was closely related to *T. inkin* (*Trichosporon cf. inkin,* n=19) and has yet to be described. Of note, two of the 10 spceies were in the genus *Cutaneotrichosporon* and one in *Apiotrichum.* Overall, there was a trend towards an increase in the number of cases of invasive *Trichosporon* infection over time in France.
Most isolates were involved in fungemia (n=51) ; 62% of patients were male, the mean age was 45 years and 19% of the patients were less than 15 year-old. The main associated factors were ICU (42%), haematological malignancies (32%) and recent surgery (27%). Pre-exposition to antifungal agents was also frequent (43%) as the presence of foreign devices (77%). Global mortality rate was high (49%). When comparing the characteristics of patients infected with the two major species (*T. asahii* and *T. cf. inkin*) *T. asahii* was significantly associated in fungemia

(p=0.049), while *T. cf. inkin* was more frequently associated with recent surgery (p=0.021) and organ transplant (p=0.011).

Conclusions:
Our data confirm the trend of increasing cases of invasive infections due to *Trichosporon* species and unfortunatly a high mortality rate. The use of molecular data allowed us to 10 distinct species of which 3 do not seem to be described yet. Moreover, among these new species, *T. cf. inkin* ranked second in frequency and was associated with organ transplant and recent surgery. *Trichosporon asahii* remained the leading species with 5 different IGS haplotypes involved in invasive trichosporonosis in France.

S04.2b

Polymorphisms in genes encoding (1,3)-β-D-glucan release components in Pneumocystis jirovecii

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Objectives: Serum 1,3-β-D-glucan (BG) testing is increasingly used to guide the management of suspected *Pneumocystis* pneumonia (PCP). It may be useful as a non-invasive adjuvant tool for PCP diagnosis in patients infected with the human immunodeficiency virus (HIV). Contrastingly, sensitivity is lower among non-HIV patients who develop PCP, especially those suffering from hematological malignancy or solid cancer. Our aim was to assess whether molecular mechanisms could be involved in this reduced sensitivity. For this purpose, we assessed the polymorphism of two genes implicated in BG release from the fungal cell wall: *bgl2* and *ace2*, encoding a β-1,3 endoglucanase and its transcription factor, respectively.

Materials & Methods: This retrospective, monocentric, non-interventional study (July 2014 – December 2021) aimed at sequencing *ace2* and *bgl2* genes of *Pneumocystis jirovecii* isolates obtained from PCP patients who underwent a diagnostic bronchoalveolar lavage (BAL), and for whom a BG result was available. The patients were divided into two groups according to the BG level (positive vs. negative). Sanger sequencing of the two target genes was performed on *P. jirovecii* DNA from BAL samples. Single nucleotide polymorphisms (SNPs) were then identified by sequence alignment with reference genomes, and their frequency has been compared between the two groups of patients. Finally, the genomic sequences were translated into amino acid sequences to assess the impact of SNPs on the corresponding protein.

Results: A total of 22 BAL samples from 21 patients (8 HIV and 13 non-VIH) were analyzed. Among these samples, eight were associated with a negative level of BG, of which seven were collected from non-HIV patients. Both target genes were amplified in 20/22 (91%) samples. Sequence analysis revealed significant polymorphism, with eight SNPs identified for each locus, resulting in four missense mutations. However, these mutations corresponded to poorly conserved residues among phylogenetically related fungi, and were identified regardless of the BG level. Therefore, they do not seem to be responsible for the lower sensitivity of BG assay. Conversely, an absence of amplification of at least one of the two genes was observed for two non-HIV patients, both presenting a negative level of BG. This suggests the existence of deleterious genomic changes that might have hampered the target amplification by PCR and also the release of BG.

Conclusions: This study provides the first evidence of potential involvement of the genetic polymorphism of *P. jirovecii* in false-negative BG results. Moreover, the apparent lack of imputability of some missense mutations suggests that host factors may also affect BG detection, but this issue deserves further investigations.

S04.3b

Candida albicans presence induces metabolic reprogramming and molecular alterations of MB16F10 melanoma cells leading to a more aggressive phenotype

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Objectives:
Candida albicans is an opportunistic fungus that is part of the human microbiota, populating body sites such as oral cavity and gut. There, *C. albicans* presence has been related with tumor promotion and progression. However, in the skin, where *C. albicans* is also commonly found, its association with the aggressive cancer melanoma has not yet been studied. Therefore, the aim of this work was to study the effect of *C. albicans* presence on melanoma cells both *in vitro* and *in vivo*.

Materials & Methods:
For this purpose, melanoma cells were exposed to *C. albicans* and its effect on cell migration, adhesion to endothelial cells and proliferation was measured *in vitro*. In addition, to deepen into molecular mechanisms activated, a transcriptomic analysis was performed using RNA-seq. These results were then confirmed by RT-qPCR for gene expression analysis and Extracellular Acidification Rate (ECAR) assays. Moreover, to study further the routes, specific inhibitors of the pathways in which overexpressed genes appeared in the transcriptomic study were used, analyzing their effect on VEGF production by ELISA and endothelial cell migration promotion.
Furthermore, *in vivo* assays were performed in C57BL/6 mice. For that, melanoma cells were stimulated with *C. albicans* and intrasplenically inoculated into mice. After two weeks, livers were extracted and the number of metastatic foci was counted and affected areas measured.

Results:
First, migration and adhesion assays demonstrated that *C. albicans* is able to enhance both processes in melanoma cells, but no effect was observed on cell proliferation. Moreover, the transcriptomic analysis revealed an increase in the expression of various genes related to the Warburg effect (*Hk2*, *Eno2*) and cancer progression (*Vegfa*, *Jun*, *Cfos*, ...) after the contact with the fungus. The induction of the expression of some of these genes (*Hk2*, *Eno2*, *Cfos*), the Warburg effect and the production of VEGF after *C. albicans* contact with melanoma cells were confirmed by RT-qPCR, ECAR assays and ELISA analysis, respectively. Moreover, the inhibition of the MAPK/ERK pathway showed a reduction of VEGF release and a decrease in endothelial cell migration promotion, indicating the importance of this pathway in the signal transduction induced by *C. albicans* in melanoma cells. Altogether, this indicate that the contact with the fungus causes the development of a more aggressive tumor phenotype.
Finally, *in vivo* assays showed a significantly higher metastatic area, both micro- and macroscopically, in mice inoculated with melanoma cells stimulated with the fungus in comparison to the control.

Conclusions:

Overall, these results indicate that *C. albicans* promotes metabolic and molecular changes in melanoma tumor cells that could lead to increased pro-tumor and metastatic capacity.

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S04.4a

Characterisation of Candida species isolated from the skin of admitted neonates at a regional South African hospital

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Objectives: Microorganisms that colonise the gastrointestinal tract, mucous membranes, skin, monitoring devices, and indwelling lines of admitted neonates are implicated in healthcare-associated invasive infections. Approximately 7%-28% of neonates with a low birth weight (< 1500 g) and *Candida* colonisation develop invasive candidiasis. We aimed to determine the prevalence of *Candida* species colonising the skin of neonates admitted to an 83-bed neonatal unit of a regional hospital in South Africa and to characterise these cultured fungal pathogens.
Materials and methods: Admitted neonates were enrolled into the study between 25 October 2021 and 30 April 2022. At enrolment and every 48-96 hours throughout the duration of the hospital stay, skin samples were collected from around the umbilicus, axilla and the groin area using flocked swabs. These were immediately placed in Amies transport medium and transported to the mycology reference laboratory. *Candida* species were isolated in culture using selective media. Antifungal susceptibility testing was performed for *Candida auris* and *C. parapsilosis* isolates using commercial methods (Sensititre YeastOne, Thermo Fisher Scientific and Etest, bioMerieux); CLSI and tentative CDC breakpoints were applied.
Results: There were 1297 newborn admissions to the neonatal unit during the study period, 102 (7.9%) of whom were enrolled into this study. The median weight of all 102 neonates was 2660 g (interquartile range [IQR], 1840-3300 g), and 41% (42/102) had a gestational age of <37 weeks. The median duration of admission was four days (IQR, 2 – 10 days). A total of 265 skin swabs were collected from 93 of the neonates for the *Candida* colonisation study. *Candida* colonisation was detected in 92 skin swabs from 42/93 (45%) neonates. *C. parapsilosis* was the most common *Candida* species (70%, 64/92) followed by *C. auris* (20%, 18/92), *C. albicans* (10%, 9/92), and *C. krusei* (1%, 1/92). Fourteen of the 42 neonates (33%) were colonised with *Candida* within 24 hours of birth. The odds of colonisation by *Candida* spp was higher among neonates admitted for >72 hours (34/42) compared to neonates admitted for <72 hours (1/13) (OR = 0.02, 95% CI, 0.00-0.18; p<0.001); no such association was found with gestational age or mode of delivery. Six of the 12 neonates who died were co-colonised with ESKAPE bacterial organisms and *Candida.* Most *C. parapsilosis* (70%, 45/64) and *C. auris* (94%, 17/18) isolates were resistant to fluconazole (MIC ≥ 8 µg/mL and ≥32 µg/mL respectively), and 28% (5/18) of *C. auris* isolates were considered resistant to amphotericin B (MIC of ≥2 µg/mL). We identified five neonates whose first isolates of *C. parapsilosis* were susceptible to voriconazole and fluconazole, but whose subsequent *C. parapsilosis* isolates were resistant to both azoles.
Conclusions: Neonates with a hospital admission ≥72 hours were more likely to be colonised with *Candida*. In this neonatal unit with intermittently-reported clusters of candidaemia, *C. parapsilosis*

was the most common colonizing species, followed by *C. auris*. Understanding the prevalence, distribution and susceptibility of *Candida* colonizing isolates in a unit can help guide infection prevention measures, and empiric treatment options for neonates with suspected invasive *Candida* infection.

S04.4b

The role of rhizoferrin in growth and virulence of Rhizopus microsporus

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Objectives:

One essential element for all living organisms is iron. Fungi secrete siderophores to enable chelation and uptake of ferric iron. For clinically relevant mucormycetes it has been shown that a polycarboxylate siderophore, identified as rhizoferrin, is secreted. The aim of this study was to determine the role of rhizoferrin production for growth and virulence of R.microsporus by determining germination, growth, rhizoferrin production and virulence potential of rfs deletion strains in comparison to their wildtype.

Methods and Material:

To elucidate the role of rhizoferrin in virulence, we achieved disruption of *rfs* gene in *Rhizopus microsporus* by applying plasmid free CRISPR/Cas9 system and HR-mediated DNA repair. Homokaryon formation was induced by repetitive plating and confirmed by PCR and Southern Blot analysis. Growth and germination assays were carried out in various media with variable iron concentrations or inpresence of serum. Virulence potential and progression of infection was studied in Galleria mellonella larvae. Rhizoferrin production was determined by CAS assay and is currently confirmed by HPLC analysis.

Results:

Rfs deletion resulted in significantly reduced virulence potential in the *Galleria* mellonella infection model. Further, rfs deletion strains were unable to form hyphae within Galleria larvae and growth reduction/unability under low iron conditions was evident. Currently investigations are carried out to determine if virulence and germination can be restored in the presence of iron or xenosiderophores.

Conclusion:

Rhizoferrin production is inevitable for virulence of R.microsporus.

S04.5b

Innate and adaptive immune response in subjects with CPA secondary to post-pulmonary tuberculosis lung disease

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Objectives: Post-tuberculosis lung disease (PTBLD) is the commonest risk factor for chronic pulmonary aspergillosis (CPA) and 14-25% of the subjects with PTBLD develop CPA. The pathogenesis and the host immune response in chronic pulmonary aspergillosis (CPA) are not well understood. Herein, we investigate the immune response in patients with post-tuberculosis pulmonary sequalae with or without chronic pulmonary aspergillosis.

Materials & Methods: We prospectively compared the innate and adaptive immune responses mounted by patients with PTBLD with or without CPA. We studied the neutrophil oxidative burst (by DHR), classic (serum C3 and C4 levels) and alternative (mannose binding lectin protein levels) complement pathway, serum immunoglobulins (IgG, IgM, and IgA), B and T lymphocyte subset in subjects with PTBLD with or without CPA.

Results: We included 111 subjects (58 CPA and 53 controls) in the current study. The mean±SD age of the study population was 42.6±15.7 years (Table 1). The cases and controls were matched for age, gender distribution and body weight.

Table 1. Demographic, clinical profile and chest imaging finding of the study population

	CPA (n=58)	Control (n=53)	Total (n=111)	P value
Demography				
Age in years (mean±SD)	45.2±15.3	39.8±15.8	42.6±15.7	0.072
Male sex, n (%)	37 (68.3)	31 (58.5)	68 (61.3)	0.697
Weight in kilograms	51 (42-58)	55 (45-60)	52 (43.9-60)	0.257
Clinical profile				
Time since ATT in years	8 (3-20)	3 (2-9)	6 (2-14)	**0.03**
Duration of symptoms	0.5 (0.3-1.5)	0.2 (0-1)	1(0-1)	**0.004**
Cough, n (%)	48 (82.8)	31 (58.5)	79 (71.2)	**0.006**
Fever, n (%)	12 (20.7)	13 (24.5)	25 (22.5)	0.652
Malaise, n (%)	25 (43.1)	19 (35.8)	44 (39.6)	0.446
Dyspnea, n (%)	36 (62.1)	23 (43.4)	59 (53.2)	0.055
Hemoptysis, n (%)	46 (79.3)	16 (30.2)	62 (55.9)	**<0.0001**
A.fumigatus-specific IgG, mgA/L	100 (67-160)	14.7 (10-21)	41 (14.7-111)	**<0.0001**
Imaging findings on CT thorax				
Presence of cavity	58 (100)	37 (69.8)	95 (85.6)	**<0.0001**
Number of cavities	2 (1-3)	1(0-1.8)	1 (1-3)	**<0.0001**
Fungal ball	39 (67.2)	0	39 (35.1)	**<0.0001**
Number of fungal balls	1 (0-1)	-	0 (0-1)	-
Parenchymal fibrosis	42 (72.4)	24 (45.3)	66 (59.5)	**0.006**
Bronchiectasis	23 (39.7)	24 (45.3)	47 (42.3)	0.698
Pleural thickening	41 (70.7)	22 (41.5)	63 (56.8)	**0.006**

ATT: anti-tuberculosis therapy
All values are expressed as median (interquartile range), or number (percentage) unless specified otherwise

Subjects with CPA had impaired neutrophil function (reduced oxidative burst), lower memory T lymphocytes and impaired Th-1 immune response (lower Th-1 lymphocytes) than controls (Table 2). We found no significant difference in the serum complement levels, MBL levels, B-cell subsets, and other T lymphocyte subsets between the two groups.

Table 2. Immunological profile of cases (chronic pulmonary aspergillosis [CPA]) and controls (post-Tb lung disease)

	CPA (n=58)	Controls (n=53)	Total (n=111)	P value
Innate immune pathway				
MBL levels (ng/ml)	199 (62-644)	403 (155-899)	337 (72.4-847)	0.205
MBL deficiency, n (%)	30 (51.7)	25 (47.2)	55 (49.5)	0.705
Serum C3 levels (mg/dl)	183 (156-215)	190 (171-224)	183 (166-218)	0.194
Serum C4 levels (mg/dl)	37 (30-50)	40 (31-50)	38.5 (31.4-50)	0.864
Neutrophil DHR assay				
MFI US	66 (35-115)	50 (38-92)	56 (37-104)	0.677
MFI S	3388 (1247-8708)	6031 (3429-8981)	5490 (1837.5-8843)	**0.047**
Stimulation index	51 (21.1-129.9)	95.2 (42.6-191)	78 (28.2-174.1)	**0.030**
Stimulation index <10	10 (17.2)	4 (7.5)	14 (12.6)	0.211
Adaptive immune pathway				
Serum immunoglobulin G (mg/dl)	19 (16.5-23)	16.1 (13.3-18.1)	17.2 (14.1-21.1)	**<0.0001**
Serum immunoglobulin A (mg/dl)	3 (2.4-4.5)	3.3 (2.5-4.1)	3.2 (2.4-4.1)	0.392
Serum immunoglobulin M (mg/dl)	1.1 (0.9-1.5)	1.2 (0.8-1.8)	1.2 (0.9-1.7)	0.309
B cells subset	N=42	N=38	N=80	
Total B (CD 19+) lymphocytes cells/µL	47 (13-89)	55.9 (14.6-99.6)	48 (13-94)	0.637
Naïve B- cells (Ig D+ CD 27-) cells/µL	20 (2-32)	16.7 (1.4-37)	19 (1.9-32)	0.992
Switched memory B-cells (Ig D- CD 27+) cells/µL	3.8 (1.7-10.6)	7.2 (2.1-18)	4 (1.8-13)	0.157
Unswitched memory B cells (Ig D+ CD 27+) cells/µL	4 (0.4-13.3)	4.1 (0.6-11.6)	4 (0.5-12.7)	0.937
T cell subset	N=50	N=45	N=95	
Total T lymphocytes (CD3+), cells/µL	1031 (739-1499)	1188 (612-1420)	1072 (707-1442)	0.852
Naïve T cells (45 RA+), cells/µL	568 (315-781)	554 (376-835)	560 (343-791)	0.499
Memory T cells (45 RO), cells/µL	462 (293-791)	722 (409-987)	600 (341-872)	**0.021**
Total CD8 T lymphocytes, cells/µL	600 (417-822)	679 (405-1039)	642 (413-962)	0.344
Naïve CD8 T cells (45 RA+), cells/µL	176 (90-294)	174 (120-345)	174 (103-301)	0.384
Memory CD8 T lymphocytes (45 RO), cells/µL	92 (47-215)	142 (77-243)	111 (64-238)	**0.093**
Total CD4 T lymphocytes, cells/µL	937 (721-1134)	1086 (829-1336)	974 (777-1200)	**0.063**
Naïve CD4 T-lymphocytes (45 RA+), cells/µL	269 (165-359)	243 (150-340)	253 (157-348)	0.708
Memory CD4 T-lymphocytes (45 RO), cells/µL	433 (194-566)	440 (313-567)	436 (253-567)	0.311
CD 4 T-lymphocytes subsets	N=40	N=29	N=69	
TH1 (CD4) cells/µL	68 (24-107)	104 (40-198)	74 (27-165)	**0.038**
TH2 (CD4) cells/µL	86 (43-156)	93 (46-175)	86 (44.5-160)	0.627
TH17 (CD4) cells/µL	99 (36-172)	154 (60-233)	105 (44-197)	**0.066**

C3: complement 3; C4: complement 4; MBL: mannose binding lectin; MFI US: median fluorescence intensity of unstimulated neutrophils; MFI SI: median fluorescence intensity of stimulated neutrophils.
All values are expressed as median (IQR), or number (percentage) unless specified otherwise

Conclusions: Subjects with CPA have impaired neutrophil oxidative burst, and a lower Th-1 response than controls.

S04.6b

Comparative performances of non-invasive and invasive respiratory specimens for the molecular diagnosis of Pneumocystis jirovecii Pneumonia : the DANIPOP study.

Cécile Garnaud[1,2], Truong Giang NGuyen[1,2], Salomé Gallet[3], Sophie Lejeune[3], Alexis Bocquet[4], Nicolas Terzi[5], Anne Thiebaut-Bertrand[6], Rebecca Hamidfar[7], Johan Noble[8], Muriel Cornet[1,2], Danièle Maubon[1,2]

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Abstract:

Incidence and morbi-mortality of *Pneumocystis jirovecii* Pneumonia (PCP) are increasing. As for other fungal diseases, the earlier the diagnosis, the better the prognosis. Today, its biological diagnosis relies on *Pneumocystis jirovecii* (Pj) detection in bronchoalveolar lavage (BAL) fluid, as the fungus lives and multiplies at the surface of type I pneumocytes. However, BAL sampling is an invasive procedure which can not always be performed urgently on hypoxemic patients. More superficial respiratory specimens, including sputa, are therefore often sampled for Pj PCR, although their diagnostic performances are not well established to date.

Objectives:

To study the diagnostic value of Pj PCR on non-invasive and/or superficial respiratory samples (oral fluids, sputa and bronchial aspirates (BA)) compared to the current gold-standard (Pj PCR on BAL).

Materials & Methods:

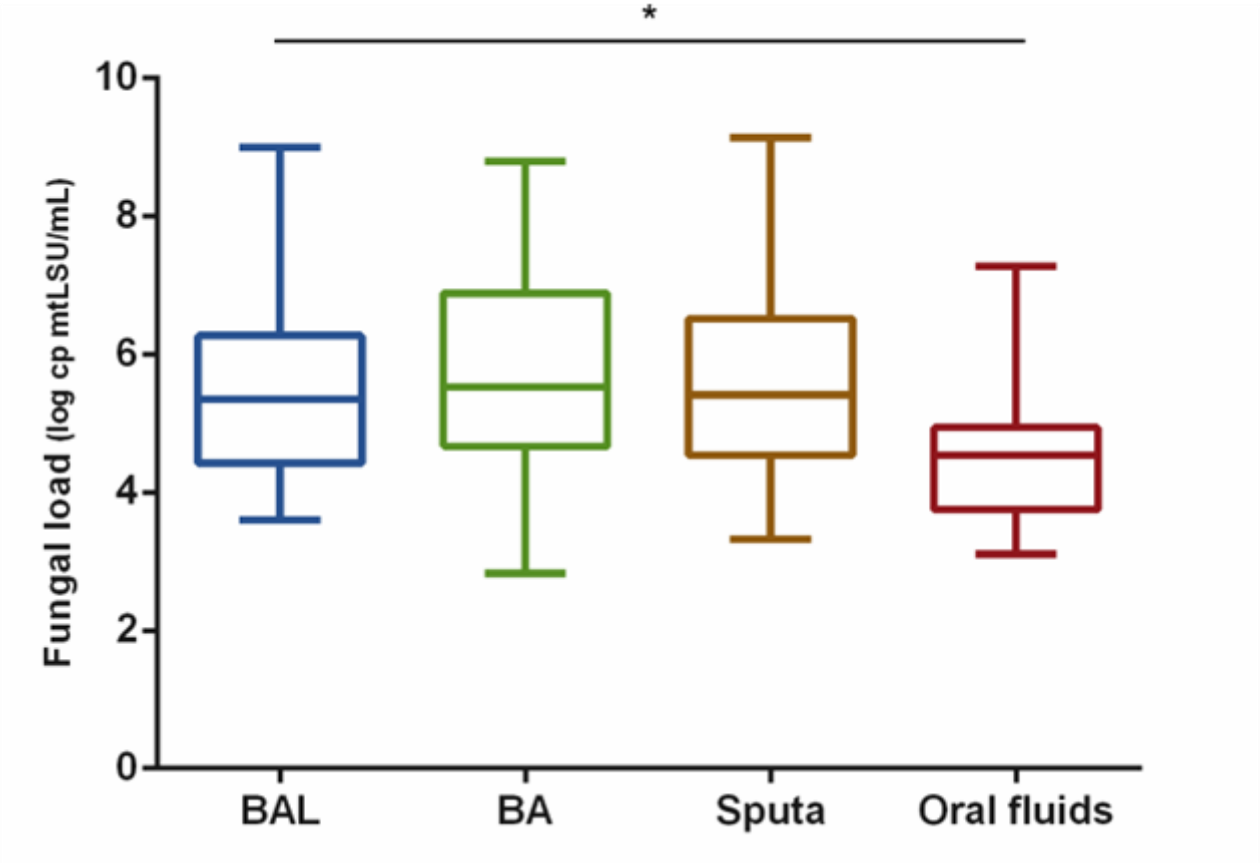
A monocentric case-control prospective study was conducted in the Grenoble Alpes University Hospital (France) from June 2018 to December 2022. Patients with radiological or clinical suspicion of PCP and for which a diagnostic bronchoscopy was planned were included in this study. Oral fluids, sputa and BA were sampled in addition to BAL fluids. Pj fungal load was assessed in all the respiratory samples by an in-house RT-qPCR technique targeting the Pj *mtLSU* gene using the BDMAX (Becton Dickinson) platform. Main clinical, radiological and biological parameters (including serum beta-D-glucans (BDG) levels) were collected for each patient. The study was approved by a French ethics committee and declared to competent authorities.

Results:

Eighty-one patients were included in this study, among which 30 cases and 51 control patients. Main underlying diseases were hemopathies (n=44, 54.3%), solid tumors (n=11, 13.5%) and solid organ transplants (n=11, 13.5%). BA, sputa and oral fluids samples were available in 66.7% (n=20), 66.7% (n=20) and 96.7% (n=29) of the PCP cases, with respective Pj PCR positivity rates of 90.0%, 65.0% and 41.4%. Mean fungal loads in BAL, BA and sputa did not differ significantly between eachothers, but were higher than that in oral fluids (Figure 1). As

expected, serum BDG levels were significantly higher in cases than in controls (502.2 *vs* 98.4 pg/mL, p = 0.004)

Conclusions:
To our knowledge, this is the first study comparing PCP diagnostic performances of four different respiratory specimens sampled at the same time in a single patient. BA and sputa represent an interesting alternative to BAL with comparable fungal loads and no requirement for lavage or invasive procedure, even if a negative PCR on these samples could not definitely exclude PCP.



S04.7b

New insights into the interplay between certain enzymatic ergosterol biosynthesis steps and the antifungal effects of azoles in Aspergillus fumigatus

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Objectives

Azole antifungals are actively used to treat many fungal infections. Depending on the fungal species, azoles have fungistatic or fungicidal activities. We have previously shown that azoles induce the formation of cell wall carbohydrate patches in the pathogenic mold *Aspergillus fumigatus*, which contribute to the fungicidal activity. The mode of action of the azole class of antifungals relies on inhibition of the lanosterol 14α-demethylase (CYP51). In our present study we explored the relevance of several other key enzymes in the ergosterol biosynthesis pathway for growth and viability of *A. fumigatus*.

Methods & Material

We have constructed a set of *A. fumigatus* mutants in which selected enzymes of the ergosterol biosynthesis pathway can be downregulated. The mutants were subsequently analyzed by fluorescence microscopy and with drug susceptibility testing assays.

Results

Many of these enzymes turned out to be essential for viability. The lack of some enzymes appears to be compatible with viability of the mold, but results in a sterile mycelium. Interestingly, inhibition of certain enzymes affects the azole susceptibility of *A. fumigatus* in a manner that is different from what would be expected in yeast.

Conclusion

Our results will help to understand the antifungal activities of agents used to treat infections caused by this pathogen. They also reveal which other enzymes of the ergosterol biosynthesis pathway might represent promising targets for future antifungal treatments.

S05.5

Response of the emergent pathogen Candida auris to oxidative stress

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Objectives:
The high resistance to environmental stresses presented by the emergent pathogenic fungus *Candida auris* is of great concern, mainly because of its ability to persist in hospital centers and create outbreaks. Previous results of our research group confirmed that the growth capability of *C. auris* was higher than other *Candida* species when incubated with many stressor compounds such as Calcofluor White (CFW), Congo Red (CR) and SDS. Among these conditions, the high resistance presented by *C. auris* to oxidative stress generated by H_2O_2 stood out above the rest, which can be of paramount importance due to the implication of this compound in fungal clearance by immune system cells of the host. Therefore, the aim of this study is to analyze the expression of proteins and genes related to oxidative stress response of *C. auris* in presence of H_2O_2 and to generate and characterize a deletion mutant strain of a protein detected in fungal response to oxidative stress by both approaches.

Materials & Methods:
Total protein extract and RNA were extracted after incubation of *C. auris* in absence and presence of 8 mM H_2O_2. The proteomic analysis was carried out by two-dimensional electrophoresis (2-DE) and the identification of the differentially expressed proteins was achieved by mass spectrometry (LC-MS/MS). The expression of 10 genes previously described as relevant on the response of *C. albicans* to oxidative stress was measured at different times of incubation in *C. auris* by RT-qPCR technique. Finally, CRISPR-Cas9 technology was used for the generation of the mutant and complemented strains.

Results:
Firstly, the proteomic analysis showed that, among 350 spots detected on the total extract, four of them were specifically expressed on the oxidative stress condition, and eleven were overexpressed. Secondly, the analysis of gene expression done by RT-qPCR showed the overexpression of *CAT1, TSA1B, CCP1, SOD1* and *SOD6* genes on different incubation times. Finally, a deletion mutant of the protein Tsa1b, detected as specifically expressed on the proteomic analysis and overexpressed on the RNA expression analysis, was generated by CRISPR-Cas9 method. The characterization of the *knock-out* strain showed a greater susceptibility than the wild type (WT) strain towards oxidative stress generated in presence of 8 mM H_2O_2.

Conclusions:
This study revealed changes on the protein and gene expression when growing *C. auris* under conditions of oxidative stress. Among them, the expression of the peroxiredoxin Tsa1b was detected to be altered in response to oxidative stress in both proteomic and RT-qPCR studies. Accordingly, the construction and characterization of the deletion mutant of this protein showed that this *knock-out* strain was more sensitive to H_2O_2-generated stress than the WT strain. The effects of the lack of Tsa1b on the host immune system cells need to be further studied, as well as the usefulness that this could have on a new diagnostic or treatment option.

Funding information:
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S06.3

Decoding the differential immune response in patients with COVID-19 associated Mucormycosis compared to COVID-19 and healthy controls

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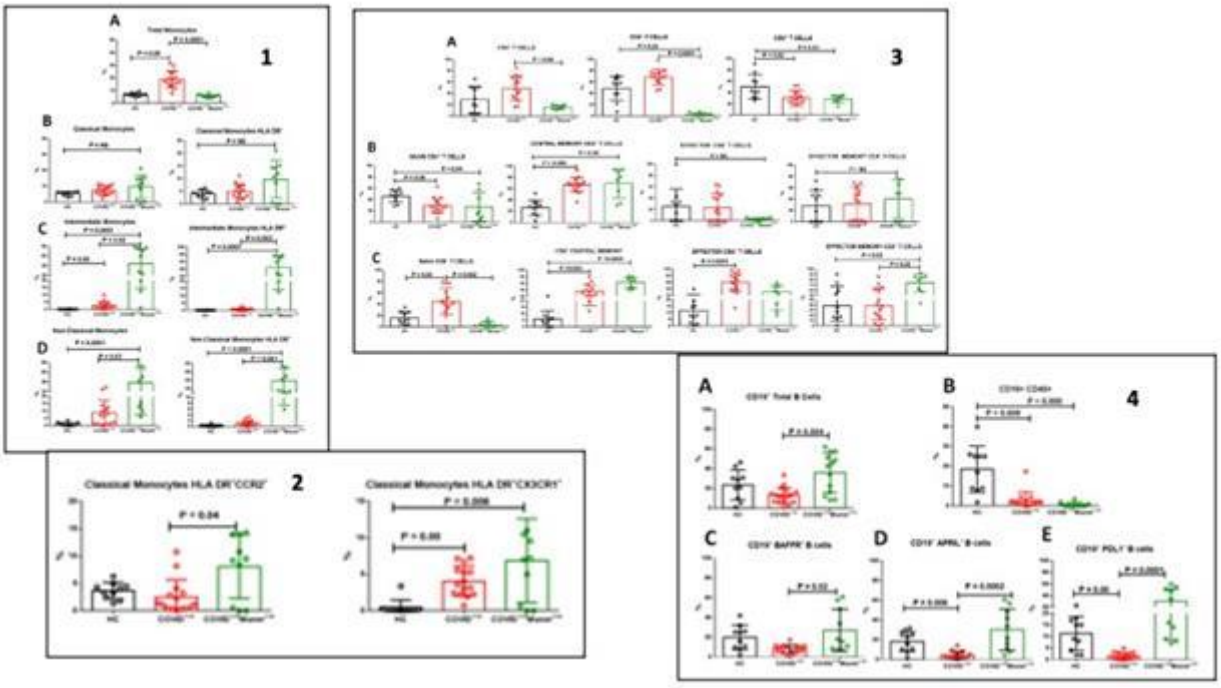
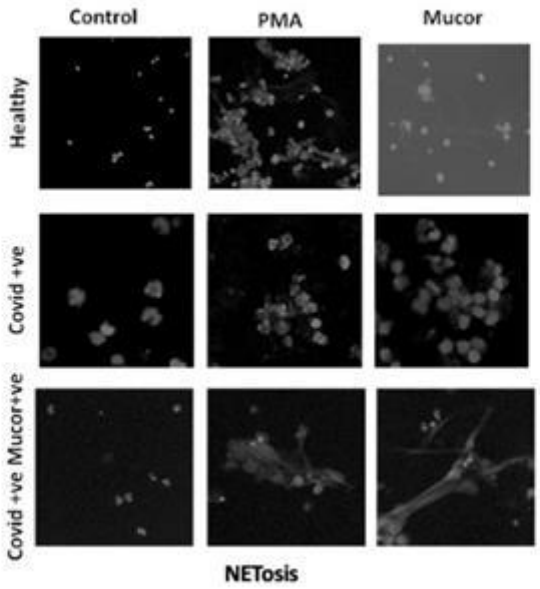
Objectives: Steroid therapy, use of antibiotics, virus induced hyperglycemia, large diabetic/prediabetic population were the postulated causes for massive surge in Mucormycosis in COVID-19 patients. Most studies focused on epidemiology and underlying causes, however difference in immunological response was largely understudied. We aimed to investigate immune responses in COVID-19 associated mucormycosis (CAM) patients compared to COVID-19 without fungal infection and healthy adults.

Materials & Methods: This prospective study was conducted in Institute of Liver and biliary Sciences, India. Neutrophils and monocytes were isolated from plasma samples from patients with CAM (8), COVID-19 (8) and healthy volunteers (8). We assessed tweny-one plasma cytokines using cytokine bead array and analysed immune cells using a detailed multicolour flow cytometry. Oxidative burst activity was determined. Neutrophils extracellular traps (NETosis) assay after stimulation with LPS and IL-8 was analysed using fluorescent microscope.

Results: CAM patients had significantly decreased circulating monocytes compared to Covid 19 patients (Fig1-1A). There was no significant change in the number of classical monocytes ($CD14^{++}16^{-}$) and HLADR positivity on them in the CAM as well as COVID-19 patients compared to healthy controls (Fig1-1B). Increase in the intermediate ($CD14^{++}16^{+}$) and non-classical ($CD14^{+}CD16^{++}$) subsets were observed in CAM (Fig1-1C,D). Dual expression of HLA-DR and CCR2 was increased in CAM, whereas both HLA-DR and CX3CR1 was increased in the COVID-19 and CAM (Fig1-2). ROS producing activity was severly hampered in CAM. Total T cells were significantly low compared to COVID-19 patients. $CD4^{+}$ and $CD8^{+}$ T cell compartment was significantly skewed in CAM (Fig1-3A). In both CAM and COVID-19, $CD4^{+}$ naïve T cells were reduced compared to healthy but the central memory T cells were increased (Fig1-3B). However, in the $CD8^{+}$ T cell compartment, naïve T cells were increased in COVID-19 but not in CAM patients. CD8 central memory T cells were increased in both patient groups compared to healthy, increased effector memory T cells in CAM compared to healthy and Covid 19 (Fig1-3C). Activated B cells were significantly decreased in both patient groups compared to healthy (Fig1-5A,B). B cell-activating factor receptor (BAFFR) and proliferation-inducing ligand (APRIL) expressing B cells were significantly reduced in the COVID-19 but increased in CAM (Fig1-5C,D). Along with this mucor patients also had increased expression of PDL1 compared to COVID-19. Increased NETosis was observed in CAM (Fig2)

Conclusions: Immune response in CAM was significantly different as compared to COVID-19 patients. An increase in the intermediate and non-classical monocyte subsets have a higher activity in response to infection and produce proinflammatory cytokines suggesting increased inflammatory damage. Neutrophils having ability to mix and extrude their DNA and fungicidal molecules creating NET-like structures called NETosis, was significantly higher in CAM, suggesting increased neutrophil lysis. Increased effector memory T cells suggest a disseminated immune response for prolonged time. B cell-activating factor receptor (BAFFR) and APRIL, cytokines regulating B-cell survival, maturation and differentiation and activate cytotoxic T cells to kill infected target cells. Thus there is differential immune response in CAM, cause increased cellular damage and increased mortality in these patients.




Control
PMA
Mucor
Healthy
Covid +ve
Covid +ve Mucor+ve
NETosis

S06.4

Candida albicans induces an inflammatory response and a metabolic reprogramming in liver sinusoidal endothelial cells

Leire Aparicio-Fernandez[1], Dr. Aitziber Antoran[1], Maialen Areitio[1], Oier Rodriguez-Erenaga[1], Leire Martin-Souto[1], Idoia Buldain[1], Aize Pellon[2], David L. Moyes[2], Joana Márquez[1], Beatriz Arteta[1], Aitor Rementeria[1], Andoni Ramirez-Garcia[1]
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Objectives:
Candida albicans is an opportunistic pathogenic fungus that can reach the bloodstream and cause disseminated infections. For its clearance, the main filtering organ in humans is the liver. Once there, *C. albicans* is recognized by liver sinusoidal endothelial cells (LSECs), which constitute the first barrier and the beginning of a host-microbial interaction process. However, the response induced by *C. albicans* in LSECs has been poorly described. Therefore, the aim of this study was to elucidate the molecular response of LSECs against the yeast *C. albicans*.

Materials & Methods:
For this purpose, LSECs were exposed to *C. albicans* for 6 hours and host transcriptomic profile was studied by RNA-seq. The results obtained were then confirmed and further studied by RT-qPCR for gene expression analysis, by Western Blot for protein expression analysis and Extracellular Acidification Rate (ECAR) assays. Furthermore, specific inhibitors of the metabolic pathways in which overexpressed genes were involved were used, studying their effect on IL-6 production by ELISA assays.

Results:
First, the transcriptomic analysis revealed an increase in the expression of various Pattern Recognition Receptors (PRRs; Mincle, Dectin-2, TLR-2...), cytokines and chemokines (IL-6, TNF, CCL-3...) and adhesion molecules (VCAM-1, CADM-1) after the contact with the fungus. All these together would imply the activation of a proinflammatory state and the initiation of an immune response. Indeed, assays performed using Syk, Myd88, Erk and Ap-1 inhibitors indicated that both Toll-like and C-type lectin receptors are relevant to produce IL-6 in response to *C. albicans* recognition and that the pathway activated is mediated by ERK. Accordingly, the RT-qPCR and WB analysis showed the importance of the *Cfos* transcription factor in the signalling pathway of MAPK/ERK pathway induced by the fungus in LSECs.
On the other hand, RNA-seq analysis showed an increased metabolic activity through a reprogramming to aerobic glycolysis (*Eno2*, *Slc1a1*...). This fact was confirmed by ECAR assays and *Eno2*, *Hk2* and *Slc2a1* gene expression analysis by RT-qPCR, and may be related to an increased demand of energy due to the activation of the immune responses.

Conclusions:
Overall, these results indicate that LSECs respond to the presence of *C. albicans* by increasing their metabolic activity and promoting a proinflammatory environment. Specifically, the route activated by *C. albicans* to produce the most overexpressed cytokine IL-6 is dependent on Syk, and Myd88, which activate the MAPK/ERK pathway.

Funding information: This research is funded by Basque Government (IT1657-22). MA, LAP and ORE have received a predoctoral grant from Basque Government (MA and ORE) or UPV/EHU (LAP).

S06.5

The trend of reducing the number of cryptic species – examples in Aspergillus and consequences for diagnostics

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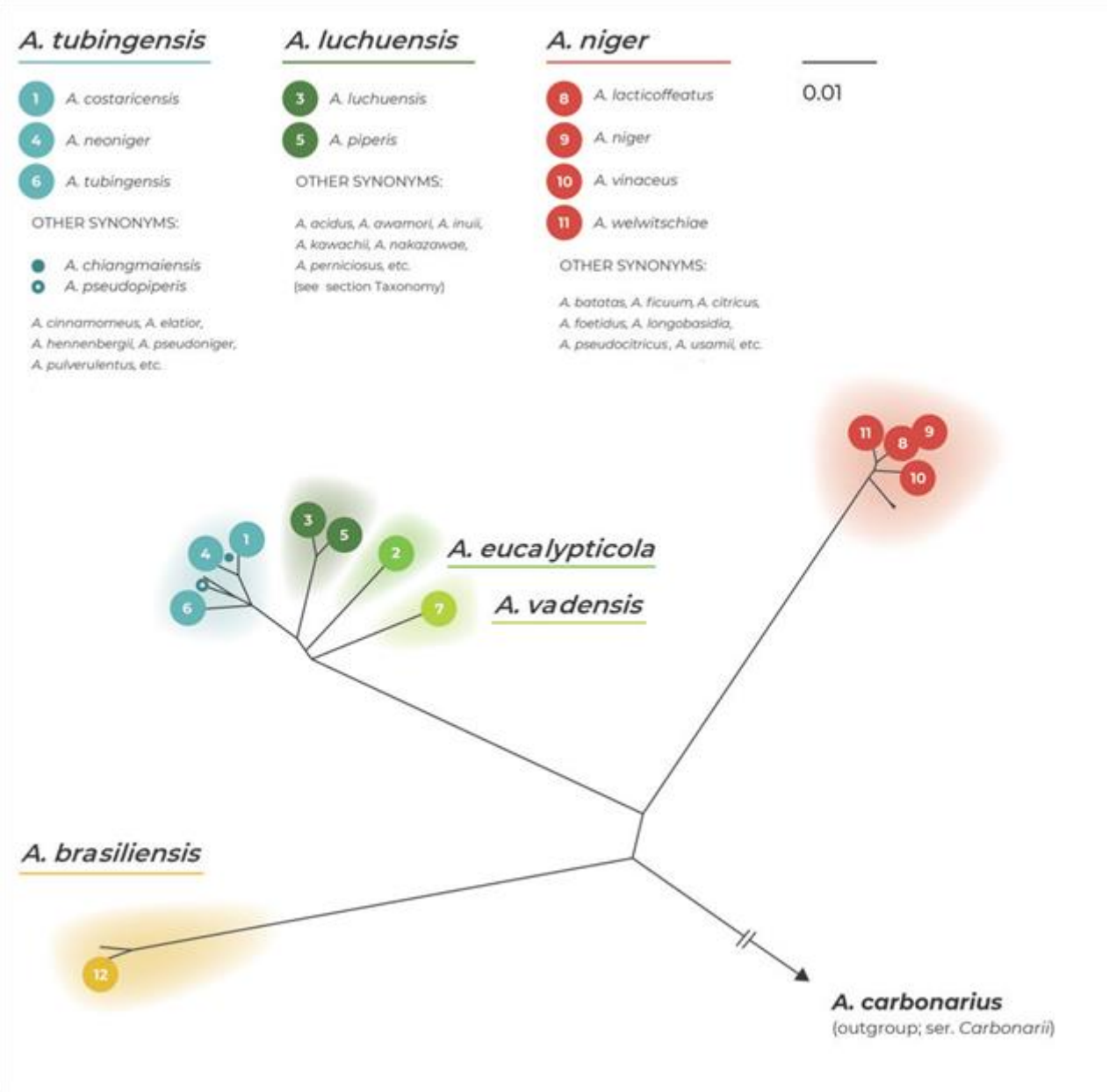
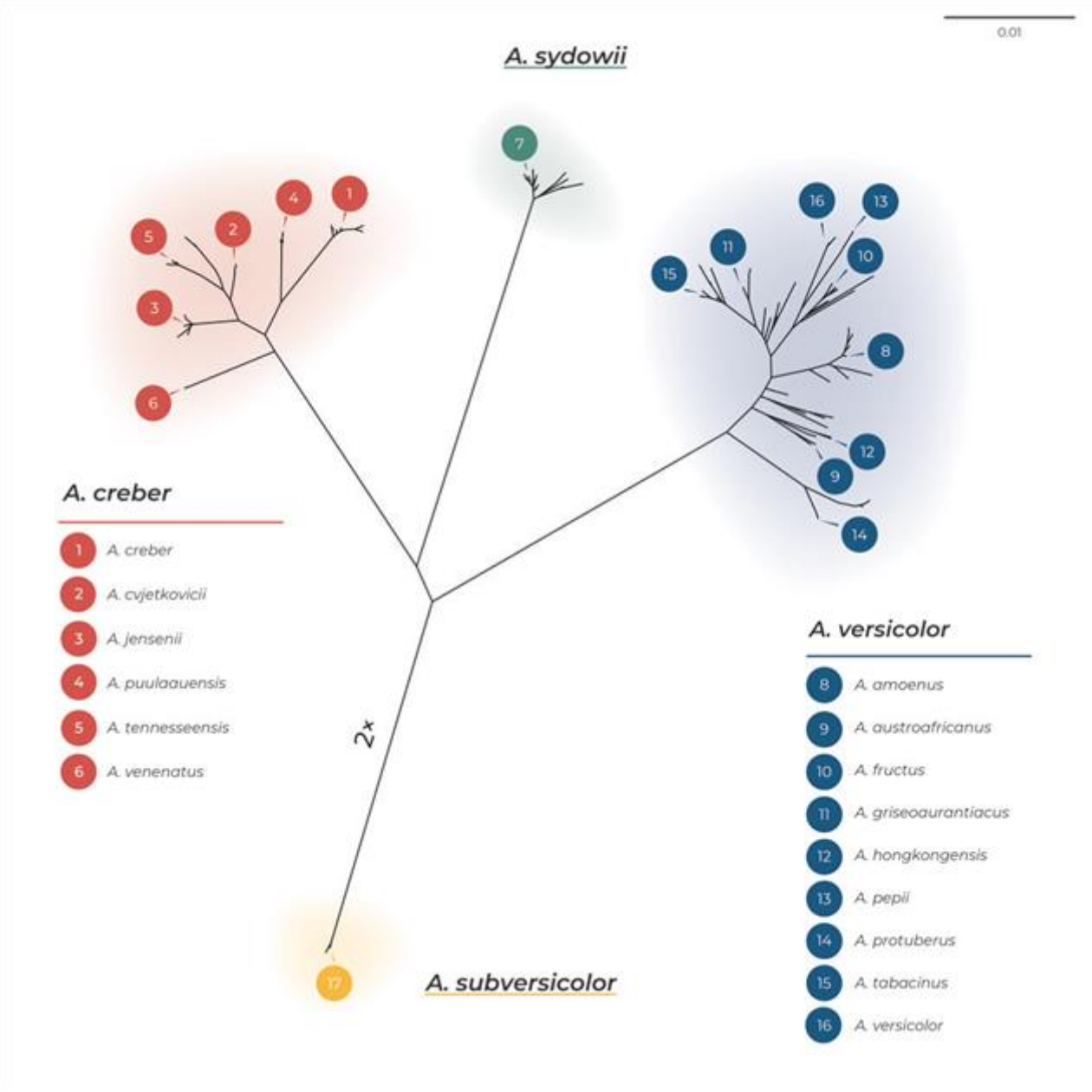
Objectives:
The reliable identification of cryptic species is of great importance because many features show species-specific patterns including virulence and antifungal resistance. Here we demonstrated on the example of several clinically relevant *Aspergillus* species complexes (*A. niger, A. tubingensis* and *A. versicolor*) that the species-level identifications are, however, problematic or impossible for some isolates even when using DNA sequencing or MALDI-TOF mass spectrometry, indicating a possible problem in the definition of species limits.

Materials & Methods:
For the re-evaluation of species boundaries in series *Nigri* and *Versicolores*, we used classical phylogenetic methods, advanced methods based on the multispecies coalescent models, and morphology/physiology. To reveal the true intraspecific variability, we analysed large strain datasets comprising hundreds of samples from different substrates and continents.

Results:
We showed that the phenotypic characters in narrow species frequently expressed a significant intraspecific variability exceeding interspecific variability. The species delimitation methods concordantly supported the recognition of a low number of broad species. The synthesis of the resulting data and the consideration of its practical taxonomic implications have led to a significant reduction in the number of species: from 11 to 4 species in the series *Nigri* (Image 1) and from 17 to 4 species in the series *Versicolores* (Image 2). Thanks to a large enough depth of sampling, we showed that intraspecific genetic variability in commonly used molecular markers is higher than previously expected. On the contrary, insufficient sampling can lead to misinterpretation of boundaries between intraspecific and interspecific variability, and inaccurate taxonomic conclusions.

Conclusions:
The new classification proposed for some *Aspergillus* species complexes with broader species definitions will facilitate species identification that is currently complicated by inconsistent identification results when using sequence data of different genes and the impossibility of finding species-specific mass spectra for some narrow species when using the MALDI-TOF method. We believe that the user community will benefit from the simplified taxonomy and thus easier diagnostics. We expect that the application of a comparable methodology with extensive sampling could lead to a similar reduction in the number of cryptic species in other *Aspergillus* species complexes and fungal genera.


A. tubingensis
1 A. costaricensis
4 A. neoniger
6 A. tubingensis
OTHER SYNONYMS:
A. chiangmaiensis
A. pseudopiperis
A. cinnamomeus, A. elatior, A. hennenbergii, A. pseudoniger, A. pulverulentus, etc.
A. luchuensis
3 A. luchuensis
5 A. piperis
OTHER SYNONYMS:
A. acidus, A. awamori, A. inuii, A. kawachii, A. nakazawae, A. perniciosus, etc. (see section Taxonomy)
A. niger
8 A. lacticoffeatus
9 A. niger
10 A. vinaceus
11 A. welwitschiae
OTHER SYNONYMS:
A. batatas, A. ficuum, A. citricus, A. foetidus, A. longobasidia, A. pseudocitricus, A. usamii, etc.
0.01
A. eucalypticola
A. vadensis
A. brasiliensis
A. carbonarius
(outgroup; ser. Carbonarii)


0.01
A. sydowii
7
4
1
5
2
3
6
16
13
11
10
15
8
12
9
14
A. creber
1 A. creber
2 A. cvjetkovicii
3 A. jensenii
4 A. puulaauensis
5 A. tennesseensis
6 A. venenatus
2x
A. versicolor
8 A. amoenus
9 A. austroafricanus
10 A. fructus
11 A. griseoaurantiacus
12 A. hongkongensis
13 A. pepii
14 A. protuberus
15 A. tabacinus
16 A. versicolor
17
A. subversicolor

S06.6

Intestinal Candida albicans overgrowth in IgA deficiency

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Objectives

Secretory IgA interacts with commensal bacteria, but its impact on human mycobiota ecology has not been widely explored. In particular, it remains unknown whether human IgA-deficiency is associated with gut fungal dysbiosis. Our goal was to study the impact of IgA on gut mycobiota ecology.

Methods

The Fungi-flow method was used to characterize fecal, systemic, and maternal IgA, IgM and IgG responses against 14 representative fungal strains (yeast/spores or hyphae forms) in healthy donors (HD, n= 34, 31 and 20, respectively), and also to compare gut mycobiota opsonization by secretory antibodies in HD (n=28) and patients with selective IgA deficiency (SIgAd n=12). Stool mycobiota composition was determined by ITS gene sequencing in HD (n= 23) and SIgAd (n=17). Circulating CD4+ T cell cytokine secretion profiles were determined by intracellular staining. Impact of secretory IgA, purified from breast milk (n=9), on candida growth and intestinal Caco-2 cell invasion was tested *in vitro*.

Results

Homeostatic IgA binds commensal fungi with a body fluid-selective pattern of recognition. In SIgAd patients, fungal gut ecology is preserved by compensatory IgM binding to commensal fungi. Gut *Candida albicans* overgrowth nevertheless occurs in this condition, but only in clinically symptomatic

patients with decreased Th17/22 T cell responses. Indeed, secretory IgA can reduce *in vitro* budding, adhesion and invasion of intestinal cells by *C. albicans*, and therefore exert control on this pathobiont.

Conclusion

IgA has a selective impact on *C. albicans* ecology to preserve fungal-host mutualism.

S07.4

Shotgun metagenomics for microbiota and mycobiota analysis in a One Health perspective

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[1]Unité Mycologie, GHU Henri Mondor, AP-HP, Créteil, France, [2]Dynamyc Research Group, UPEC, EnvA, USC Anses, Créteil, France, [3]Unité Mycologie-Parasitologie, Hôpital Saint-Louis, AP-HP, Paris, France, [4]Department of Clinical Sciences, FARAH, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium, Liège, Belgium, [5]Ecole Nationale Vétérinaire d'Alfort, Maison-Alfort, France, [6]Unité de Bactériologie, GHU Henri Mondor, AP-HP, Créteil, France, [7]Plateforme GenoBioMics, GHU Henri Mondor, AP-HP, Créteil, France

Objectives: Few studies have used shotgun metagenomics (SMg) to analyse mycobiota and none have evaluated SMg performances for mycobiota and microbiota analyses in different hosts. Here, we compared the results of SMg for the analysis of respiratory and fecal mycobiota, in relation to the other components of the microbiota (bacteria, viruses, parasites) in mammals, including humans, and birds.

Materials & Methods: Human samples came from a biological collection of positive/negative samples for fungi and parasites (Mondor hospital, Créteil, France); rock-pigeons' samples were collected at autopsy in a French Wildlife Centre (CHUV-FS, EnVA, Maisons-Alfort) and healthy dogs were sampled at a Belgium veterinary clinic (Faculty of Veterinary Medicine, Liège). SMg data obtained from these 3 cohorts were re-analysed concomitantly. Overall, 29, 18 and 8 bronchoalveolar lavage (BAL) from human, pigeons, and dogs, and 62 and 18 feces from human and pigeons were studied, respectively. After a mechanical, enzymatic, and chemical pre-treatment, samples were extracted using QiaSymphony®; DNA/RNA libraries were sequenced using NovaSeq (Illumina®) and data were analysed using METAMIC® pipeline. SMg data were compared using Anova and Kruskal-Wallis tests.

Results: We observed significant differences in the median number of DNA/RNA reads/sample according to hosts for BAL and feces. In BAL, the number of DNA reads/sample was significantly lower in pigeons compared to mammals (Table-1, p-value<1.10^{e-5}). All pigeons' BAL (18/18) obtained <10-million-DNA-reads (considered as a quality-threshold for human analysis), while 73% (21/29) and 100% (8/8) of humans' and dogs' BAL passed it. Differences were also observed for RNA-reads according to host, although less marked (Table-1, p-value<0.001). For feces, the median number of reads/samples was also different between dogs and human (p-value=0.01 and 0.005 for DNA or RNA, respectively) but >85% of DNA/RNA libraries passed the 10-million-threshold (88% and 87% for DNA; 95% and 98% for RNA in humans and pigeons, respectively). The percentage of DNA-reads assigned as microorganisms was <1% in BAL regardless of host, whereas a huge contrast was observed in feces (<1% DNA-reads in pigeons; 32% in humans). The bacterial load, measured by the number of DNA-reads/million, was different between hosts (p-value<1.10e-5). In BAL, the bacterial loadwas higher in pigeons than in humans (10-fold) or dogs (2300-fold, Table-1) but in feces, it was higher in humans than in pigeons (26-fold). The fungal load was low in BAL and feces for all hosts [0.2-18.4 DNA-reads/million], with significant differences between hosts in BAL only (p-value=0.002).

Conclusions: SMg performs differently according to the sample (BAL/stools), as previously described, but also according to the host, particularly for BAL. Also, SMg as currently designed may present limitations to deeply study the mycobiota diversity regardless of the host or sample, as suggested by the low number of fungal reads detected. This might be due to the

low fungal loads in animals' microbiota and aggravated by the majority amplification of host nucleic acids in SMg, especially in mammals. Further studies including more samples and hosts are needed to better document SMg performances in a One Health perspective.

Table 1. Performances of Shotgun Metagenomic (SMg) in feces and BAL of pigeons, dogs and humans.

	Total reads, median [min-max]	Ratio microorganisms/total reads (%)	Bacterial reads/million, median [min-max]	Virus reads/million, median [min-max]	Fungi reads/million, median [min-max]	Parasites reads/million, median [min-max]
DNA reads						
BAL						
Pigeons, n=18	680062 [63180-4238968]	0,71	9581,3 [955,1-161695,4]	685,0 [80,0-2609,1]	18,4 [0,0-387,6]	0,0 [0,0-22,6]
Humans, n=29	24554708 [206082-70942128]	0,05	823,1 [55,0-68210,0]	3,7 [0,0-305,7]	6,5 [0,0-947,3]	0,0 [0,0-0,6]
Dogs, n=8	27364322 [19543514-67482964]	0,00	4,7 [1,5-32,3]	0,3 [0,2-0,5]	0,2 [0,1-0,7]	0,2 [0,1-0,5]
Feces						
Pigeons, n=18	17985432 [3738056-56274610]	0,68	12591,2 [41,9-355958,7]	1447,0 [180,6-136301,7]	1,2 [0,1-2297,1]	0,2 [0,0-1,6]
Humans, n=62	39825899 [50520-157998860]	31,87	334414,3 [21144,1-721225,4]	361,7 [17,2-60845,7]	2,4 [0,2-26634,5]	0,5 [0,0-336,5]
RNA reads						
BAL						
Pigeons, n=18	32644228 [11476370-63902022]	2,37	29963,1 [94,1-196033,5]	38,0 [0,3-1529,2]	19,9 [0,4-266,8]	0,6 [0,1-1995,5]
Humans, n=29	19344988 [1082788-41054184]	0,79	8677,4 [409,1-138299,0]	17,9 [1,2-533,0]	27,3 [0,0-860,3]	0,2 [0,0-8,3]
Dogs, n=8	45119268 [25119090-59745908]	0,01	110,7 [28,5-4001,0]	0,5 [0,2-2,8]	0,3 [0,1-1,5]	0,0 [0,0-0,2]
Feces						
Pigeons, n=18	36910852 [7565286-101234018]	3,00	21965,1 [799,1-422954,6]	330,7 [10,3-2097,5]	9,7 [1,1-3673,4]	0,8 [0,0-289,0]
Humans, n=62	28883250 [3022352-48711682]	33,35	349895,8 [911,2-554421,6]	132,6 [1,9-13022,5]	25,8 [0,4-2690,2]	0,6 [0,0-2761,2]

S07.5

First in human data on BSG005, a genetically engineered polyene macrolide evaluated in a double-blinded placebo-controlled Phase 1 trial

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Objectives: BSG005, is a novel genetically engineered polyene macrolide demonstrating enhanced preclinical safety and efficacy profiles in vitro and in vivo compared to the currently marketed similar class of drugs for treatment of invasive fungal infection. We performed a first-in-human, Phase 1, randomized, double-blind, placebo-controlled dose-escalation trial to investigate safety, tolerability, and pharmacokinetics (PK) of BSG005 after single - (SAD) and multiple dosing (MAD) in healthy subjects.

Materials and Methods: We enrolled 38 males and females aged 18 to 55 years, with body mass index 18.0 -32.0 kg/m^2 (inclusive) and body weight of more than 50 kg, and no significant prior medical history or clinically relevant abnormality in clinical laboratory tests in our Phase 1 trial. Each cohort of 6 subjects was randomized 2:1 to receive BSG005 or placebo with sequential dose escalation contingent on safety and PK data review by a safety review committe. In SAD, 24 subjects were enrolled in 4 cohorts receiving a single IV infusion at BSG005 doses of 0.015, 0.035, and 0.1 mg/kg or placebo. In MAD, we enrolled 14 subjects in 2 cohorts to receive multiple IV infusions once daily for 7 days at a dose levels 0.035 mg/kg and 0.05 mg/kg or placebo with 12 subjects completing the MAD part while 2 subjects withdrew consent.

Safety analysis data included enrolled subjects who were administered at least one dose of study treatment with subjects being analyzed according to treatment received.

Results: Demographic and baseline characteristics were similar of subjects receiving BSG005 versus Placebo with mean age of 27.5 years, and mean BMI of 24.51 kg/m^2 (33 Males; 5 Females). Single and multiple IV infusion(s) of BSG005 were safe with no clinically relevant changes in post-baseline analysis of clinical laboratory parameters – including kidney and liver data, vital signs, or clinically relevant abnormalities in electrocardiograms. Infusion-related AEs (IRR) (Grade 1-2) including fever, rigors, chills, headaches were reported for some subjects in SAD cohorts. IRRs were reported to disappear or become miniscule after repeated infusions in MAD cohorts. Injection site reactions (ISR) such as pain, swelling, erythema and phlebitis were present in a dose-dependent manner among all subjects receiving active treatment in the MAD cohorts. PK data showed that systemic exposure increased approximately proportional to dose. The observed systemic clearance was low and the mean half-life after single dose was approximately 7-10 hours. Upon once daily dosing steady state was reached within 4-5 days and minor accumulation was observed after 7 days.

Conclusion: Single and multiple dose intravenous infusions of BSG005 were safe in the healthy subjects. Adverse events were mild to moderate in severity, the majority of AEs being infusion-related in the SAD and phlebitis in the MAD, and no SAEs were seen in the study. Basic PK parameters were established. Our Phase 1 results provide robust evidence of systemic safety enabling the initiation of subsequent studies in patients with invasive fungal infection.

S07.6

Novel antifungals in the pipeline and their activity against Aspergillus section Terrei

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Novel antifungals in the pipeline and their activity against *Aspergillus* section *Terrei*

Objectives:

Novel antifungal agents are needed to combat the growing threat of fungal infections. Invasive fungal infections (IFIs) have remained challenging to treat due to the emergence of drug resistance, practical limitations of conventional antifungals, and the emergence of less common fungal species for which optimal therapies are not well defined. IFIs, namely aspergillosis, cause severe morbidity and mortality, particularly in immunocompromised individuals. Novel therapeutic agents are essential for overcoming drug resistance and providing better treatment options. *A. terreus* is one of the causative agent of aspergillosis, which is naturally less susceptible to amphotericin B (AmB), so it is particularly problematic when it is resistant to other conventional agents, specifically azoles. In this study, the most promising or unique antifungal agents under development are evaluated for their potential efficacy against nearly all currently accepted species in section *Terrei*, including isolates with reduced susceptibility to conventional antifungals.

Materials & Methods:

A total of 100 molecular identified *Aspergillus* section *Terrei* isolates were studied, including AmB-wild type/non-wild type, isavuconazole, posaconazole resistant/susceptible, and voriconazole wild type/non-wild type isolates. Novel antifungals, including manogepix, rezafungin, ibrexafungerp, and olorofim, were assessed for their antifungal activity against *Aspergillus* section *Terrei* isolates, according to the method of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Results:

In general, all tested agents showed potent and consistent activity against tested isolates, exhibiting a geometric mean (GM) and minimum effective concentration (MEC)/minimum inhibitory concentrations (MIC) range respectively as follows: manogepix (0.048 mg/L, 0.032–0.5 mg/L), rezafungin (0.020 mg/L, 0.016–0.5 mg/L), ibrexafungerp (0.071 mg/L, 0.032–2 mg/L), and olorofim (0.008 mg/L, 0.008–0.032 mg/L). In terms of MIC90/MEC90, olorofim had the lowest values (0.008 mg/L), followed by rezafungin (0.032 mg/L), manogepix (0.125 mg/L), and ibrexafungerp (0.25 mg/L).

Conclusions:

In conclusion, the studied set of novel antifungals showed promising and consistent *in vitro* activity against nearly all currently accepted species of *Aspergillus* section *Terrei* regardless of azole and AmB resistance. It appears that the future holds bright prospects for several promising drugs that are developing. Novel antifungal agents could play a pivotal role in treating multi-resistant mold infections, including azole-resistant aspergillosis.

S08.4

Synergistic anti-fungal activity of new, inhaled opelconazole with systemic posaconazole on Mucor circinelloides in an in vitro human alveoli model

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Objectives:
PC945 (opelconazole) Nebulizer Suspension is a new investigational antifungal agent specifically being developed as an inhalation therapy for the treatment of respiratory fungal disease. We previously reported that “inhaled” opelconazole and systemic azoles showed synergic antifungal effects against *A. fumigatus* infection *in vitro* and *in vivo* (Sci Rep, 2019, 9482). This *in vitro* study examined the anti-fungal activity of “inhaled” opelconazole on systemic invasion of *Mucor circinelloides* (*M. circinelloides*) in combination with “systemic” posaconazole using a model of human alveoli.

Materials & Methods:
A model of human alveoli was constructed in transwells consisting of a bilayer of human alveolar epithelial and endothelial cells (Hop-W et al., 2007, JID). Once daily on days 0, 1 and 2, clinically relevant concentrations of either monotherapy opelconazole (1 µg/mL) or voriconazole (1 µg/mL) was added to the apical epithelial compartment (upper chamber) mimicking inhalation treatment; or monotherapy posaconazole (0.1 µg/mL) was added to the basal endothelial compartment (bottom chamber) mimicking systemic treatment. In addition, either opelconazole or voriconazole was added to the upper chamber together with posaconazole added to bottom chamber mimicking inhalation treatment together with systemic treatment. Transwells were infected apically with *M. circinelloides* at a concentration of 1×10^4 conidia/well, one hour after treatment on day 0, and incubated at 35°C. Basal chamber samples, as surrogate markers of fungal invasion into systemic circulation, were collected Day 0 to Day 5 post inoculation, and quantified as CFU using agar culture.

Results:
In the *in vitro* model of human alveoli, combination of “inhaled” opelconazole and “systemic” posaconazole achieved marked inhibition of fungal invasion up to Day 5 post infection (92% inhibition on Day 3, $p<0.05$). This combination provided much greater protection than a combination of “inhaled” voriconazole and “systemic” posaconazole (69% inhibition on Day 3). Monotherapy with opelconazole alone (1 µg/mL; upper chamber) or posaconazole alone (0.1 µg/mL; bottom chamber) showed only modest inhibitory effects on fungal invasion (13% and 30% inhibition on Day 3 post infection, respectively). In contrast, the synergistic effect of opelconazole and posaconazole was not observed in a broth micro-dilution MIC assay.

Conclusions:
In this study, a combination therapy mimicking inhaled opelconazole and systemic posaconazole was shown to inhibit *M. circinelloides* systemic invasion to a greater extent than monotherapy using either compound alone. In addition, this combination was more potent against *M. circinelloides* than a combination of apical voriconazole and basal posaconazole. Inhaled opelconazole therefore has the potential to be used in combination with established systemic antifungal drugs for the treatment of *M. circinelloides* infection in humans and further studies are recommended.

S08.5

Olorofim treatment of mould IFD in patients with limited or no treatment options: Results from a Phase 2b open-label study

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Objectives: Olorofim is the first orotomide antifungal, selectively inhibiting fungal dihydroorotate dehydrogenase (DHODH), a key enzyme in fungal pyrimidine biosynthesis, disrupting many intracellular functions and ultimately leading to cell death. Olorofim is active against a range of mould species: *Aspergillus* (including azole-resistant and cryptic species), pan-resistant moulds (e.g., *Lomentospora prolificans, Scopulariopsis* spp.) and dimorphic fungi (e.g., *Coccidioides* spp.*).* Olorofim does not have activity against yeasts or Mucorales. The drug is given orally and cleared by multiple CYP450 isoenzymes.

Materials & Methods: Patients with limited or no treatment options for proven invasive mycoses or probable pulmonary invasive aspergillosis (IA, as per 2020 EORTC-MSGERC criteria) received olorofim (loading dose of 150mg BID on day 1, followed by 90mg BID) for 84 days +/- 6 days (main phase), with extended therapy beyond day 90 allowed (NCT03583164, Study 32). The primary endpoint was DRC-adjudicated overall response rate by pathogen at day 42 using the EORTC-MSG response criteria. Overall response at day 84 and ACM were key secondary measures.
Results: 203 patients were enrolled with 202 cases meeting the modified ITT (mITT) criteria: *Aspergillus* species (101, including 22 cases with azole-resistant strains), *L. prolificans* (26), *Scedosporium* spp. (22), *Coccidioides* spp. (41), *Scopulariopsis* spp (6) and other fungi (8). Overall Success (complete or partial response) was 28.7% at day 42 and 27.2% at day 84. Excluding patients with coccidioidomycosis (none of whom achieved Overall Success due to inability to prove fungal eradication in the 84-day study period), Overall Success was 36.0% at day 42 and 34.2% at day 84. When stable disease (including coccidioidomycosis) was counted as success, the success rate was 75.2% and 63.4% respectively. For patients with coccidioidomycosis, benefit (symptoms/signs) was obtained in 31 patients (75.6%, CI 59.7,87.6) achieving complete or partial Clinical Response at day 42 and 30 (73.2%, CI 57.1, 85.8) at day 84.
ACM was 11.4% and 15.8% at day 42 and 84 respectively (IA: 17.8% and 25.7%; non-IA infections 5.0% and 5.9% respectively).

Safety: Olorofim was generally well tolerated. In the main phase, the median dosing duration was 84 days (max 99 days). Including extended therapy, median treatment duration was 308 days (min 89 days; max 988 days). Altered hepatic biochemistry at least possibly due to olorofim was seen in 9.9% of study subjects overall (main phase + extended therapy) and was managed by dose reduction/pause. Altered hepatic biochemistry led to permanent discontinuation in 2.5%. Gastrointestinal intolerance to olorofim, generally self-limiting, was noted in 9.9%.

Conclusions: Olorofim is an oral, mechanistically novel agent with activity against a range of mould infections including many species which are difficult to treat with current standard of care. Olorofim has an acceptable risk-benefit profile in a well-defined population of patients with limited or no treatment options with infections due to moulds including species considered resistant to all approved antifungals. The data demonstrate the challenges of using the EORTC-MSG response criteria for infections such as coccidioidomycosis, for which the response criteria have not been validated. A Phase 3 study of olorofim in IA is now enrolling.

Table 1: DRC-adjudicated Overall Success response rate and ACM at Day 42 and Day 84 for the overall study population and per disease subtype (mITT Analysis Set, Overall patient population: N=202).

	DRC-adjudicated overall response rate n (% success)		ACM n (%)	
	Day 42	**Day 84**	**Day 42**	**Day 84**
Overall (N = 202)	58 (28.7)	55 (27.2)	23 (11.4)	32 (15.8)
***Aspergillus* spp. (N = 101)**	35 (34.7)	34 (33.7)	18 (17.8)	26 (25.7)
***Lomentospora prolificans* (N = 26)**	11 (42.3)	11 (42.3)	3 (11.5)	3 (11.5)
***Scedosporium* spp. (N = 22)**	8 (36.4)	5 (22.7)	2 (9.1)	2 (9.1)
***Scopulariopsis* spp.* (N=6)**	5 (83.3%)	5 (83.3%)	0 (0)	0 (0)
Other olorofim-susceptible Fungi (N = 8)	1 (12.5)	2 (25) []	0 (0) []	1 (12.5) []
***Coccidioides* spp.# (N = 41)**	0 (0) [0.0,8.6]	0 (0) [0.0,8.6]	0 (0) [0.0, 8.6]	0 (0) [0.0, 8.6]

*This group includes: 4 patients infected with *Scopulariopsis* spp only and 2 patients with coinfection; 1 *L. prolificans* and 1 *Aspergillus* spp. These 2 patients are included in the tallies for both fungi therefore total number of infections =204
#See text for explanation of outcomes in *Coccidioides* infections

S08.6

SCY-247, a Second-generation IV/Oral Triterpenoid Antifungal: In Vitro Activity Against Broad-spectrum of Fungal Pathogens, and Dose-Dependent Tissue Distribution In Vivo

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Objectives: There are limited treatment options for the treatment of invasive and severe mucocutaneous fungal infections. Combined with the increase in *Candida* resistance to the commercially available antifungals and the increase in *Candida glabrata* infections, globally, there is a need for novel treatment options to prevent and treat these serious and life-threatening fungal diseases. SCY-247 is a novel second-generation IV/oral triterpenoid antifungal with activity against *Candida* spp., *Aspergillus* spp., and dimorphic fungi. In this study, the activity of SCY-247's to inhibit a broad panel of fungi was evaluated *in vitro.* Additionally, pharmacokinetics (PK) evaluation of this antifungal was performed.

Materials & Methods: SCY-247 was tested *in vitro* against a panel of wild-type isolates of *Candida* spp. (n = 50), *Aspergillus* spp. (n = 20), and dimorphic fungi (5 each of the following genera- *Coccidioides*, *Histoplasma*, *Blastomyces*). Additionally, *in vitro* testing of SCY-247 was performed against echinocandin-resistant *Candida* (n = 34) and *Aspergillus* (n = 18) isolates. *In vitro* Time-Kill studies were performed against *Candida auris* and *Aspergillus fumigatus*. Preliminary *In vivo* murine model of *Candida* infections was conducted to evaluate SCY-247 levels in the plasma, brain, lung and kidney with three doses at 10 mg/kg, 20 mg/kg, and 40 mg/kg.

Results: SCY-247 showed potent activity against *Candida* spp. (MIC_{50} of 0.5 μg/ml), *Aspergillus* spp. (MEC_{50} of 0.063 μg/ml), and against the dimorphic fungi, (MEC_{50} = 0.25 μg/ml). Against echinocandin- and azole-resistant isolates SCY-247 had an MIC range for *Candida albicans* of 0.125-4 μg/ml, *Candida glabrata* <0.03-1 μg/ml, and MEC range for *Aspergillus* of 0.03-4 μg/ml. SCY-247 showed fungicidal activity against *Candida auris* (MFC values of 2 μg/ml and 4 μg/ml). Interestingly, SCY-247 showed some concentration dependent fungicidal activity against *Aspergillus fumigatus*. In a mouse model, SCY-247 showed dose-proportional exposures in plasma and various tissues (Table 1 shows levels of SCY-247 in various tissues).

Conclusions: SCY-247 is a second-generation triterpenoid antifungal with potential to be a future IV and oral treatment option for serious fungal infections.

Table 1 SCY-247 *In Vivo* Plasma and Tissue levels at 4 hours (mouse model)

Dose (mg/kg)	Cmax (mg/ml)	AUC0-24 (mg*hr/ml)	Brain (mg/g)	Kidney (mg/g)	Lung (mg/g)	Liver (mg/g)	Spleen (mg/g)
10	2.5	41	0.2	29	25	57	17
20	5.1	98	0.4	59	47	140	42
40	9.2	201	0.9	132	110	684	72

S10.3

A seven-year prospective cohort study on invasive aspergillosis at a tertiary care hospital in Madrid, Spain

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Objectives: The continous epidemiological and microbiological changes make it advisable to review the invasive aspergillosis series every few years. The objective of this study was to provide a comprehensive description of our extensive most recent IA case series, including clinical, epidemiological, and microbiological findings, and the clasification of all the patients according to new definitions.

Materials & Methods: We conducted a prospective cohort study of IA cases diagnosed in accordance with local and international guidelines. Study period from October 2014 to October 2021. A predefined protocol was followed, which included demographic, clinical, radiological, and microbiological data, as well as information on antifungal therapy and outcomes. The cases were reviewed and discussed by a multidisciplinary team known as the COMIC Study Group.

Results: A total of 173 patients were diagnosed with IA during the study period, with 72.8% being male, median age of 63 (IQR 54-72). Table 1 describes main clinical characteristics of IA cases. Among the main underlying conditions, only 28.9% had hematologic malignancies, 29.5% were non-immunosuppressed patients in the intensive care unit (ICU), 11% had solid tumors, 9.2% had liver disease, 7.5% were solid organ transplant (SOT) recipients, 6.4% had chronic obstructive pulmonary disease (COPD), and 4.6% had advanced HIV infection. Neutropenia(<500cells/mm3) was observed in 23.1% of cases. Interestingly, viral infections were clinically associated with IA in 55.5% of cases. The virus involved were: SARS-Cov-2 in 54.2% followed by CMV in 41.7%, influenza 17.2%(68.8% influenza A and 31.2% influenza B), adenovirus 2.1%, respiratory syncytial virus (RSV) 1%, parainfluenza 1% and HSV 1%. High-dose corticosteroid therapy had been administered to 75.1% of patients. In terms of clinical presentation, IA was pulmonary in 93.1%, extrapulmonary in 2.9%, disseminated with central nervous system (CNS) involvement in 2.3%, and disseminated IA without CNS involvement in 1.7%. Classic CT-scan signs were present in 65.5% of patients (76/116).

Regarding the classification of IA according to different definitions, the EORTC/MSG criteria classified 54.3% of patients as probable, 7.5% as possible, 5.2% as proven, and 32.9% as unclassifiable. When considering viral co-infections according to the IAPA-CAPA definition, 3.5% and 23.1% of cases met the criteria for probable and putative IA, respectively. According to Bulpa et al., 20.2% of COPD cases were classified as probable IA, and according to Blot et al., 38.2% of cases in the ICU were classified as putative aspergillosis. The distribution of the classification according to the different definitions is outlined in Table 2.

Voriconazole was used as main antifungal treatment in 48% of cases, liposomal amphotericin B(L-AMB) 14.5%, isavuconazole 15%, posaconazole 1.2%, L-AMB followed by an azole in 17.9%, and combination therapy was deemed necessary 13.3%, primarily involving echinocandins. The overall mortality rate was 57.2%, with IA-related mortality accounting for 34.1% of cases.

Conclusions: To the best of our knowledge, this represents the largest single-center series of IA cases reported in recent years and confirms the significant change in underlying conditions. Our study also highlights the difficulty in classifying IA, with a high proportion of patients not fulfilling the classic EORTC/MSG criteria(32.9%).

Table 1. Main clinical characteristics of IA cases

	N = 173 (%)
Gender - male	72.8
Age – years (IQR)	63 (54-72)
Neutropenia (<500 cel/mm^3)	23.1
Viral co-infection	55.5
Received high dose of corticosteroids (prednisone >20 mg/day)	75.1
Clinical presentation	
Pulmonary	93.1
Extrapulmonary	2.9
Disseminated with CNS involvement	2.3
Disseminated without CNS involvement	1.7
CT-scan classic signs	65.5 (76/116)
Microbiology results	
Serum positive GM	62/163 (38.0)
Serum positive BDG	47/122 (38.5)
BAL positive GM	74/99 (74.7)
Positive PCR	25/53 (47.2)
Etiology of infections*	
Aspergillus fumigatus	108 (62.4)
Aspergillus flavus	18 (10.4)
Aspergillus nidulans	5 (2.9)
Aspergillus lentulus	7 (4.0)
Aspergillus niger	8 (4.6)
Aspergillus terreus	5 (2.9)
Other *Aspergillus* spp	7 (4.0)
No isolation	34 (19.7)
Antifungal treatment	
Voriconazole	48.0
Isavuconazole	15.9
Posaconazole	1.2
L-AMB	14.5
L-AMB followed by an azole	17.9
Combination therapy (+echinocandins)	13.3
Mortality	
Overall	57.2
Related to IA	34.1

*Some episodes are mixed infections

IA: invasive aspergillosis; **IQR:** interquartile range; **CNS:** central nervous system; **L-AMB:** liposomal amphotericin B; **GM:** galactomannan; **BAL:** bronchoalveolar lavage; **BDG**: 1,3 β-D-glucan

Table 2. Classification according different invasive aspergillosis definitions

	Possible N (%)	Probable/Putative N (%)	Proven N (%)	Non-classified N (%)
EORTC/MSG	13 (7.5)	94 (54.3)	9 (5.2)	57 (32.9)
AspICU	-	66 (38.2)	-	107 (61.8)
IAPA	-	6 (3.5)	-	167 (96.5)
CAPA	8 (4.6)	40 (23.1)	-	125 (72.3)
Bulpa		35 (20.2)	2 (1.2)	136 (78.6)

EORTC/MSG: European Organization for the Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group; **AspICU:** *Aspergillus* Intensive Care Unit; **IAPA**: Influenza-associated pulmonary aspergillosis; **CAPA:** COVID-19-associated pulmonary aspergillosis

S10.4

Application of PCR/HRM in detection/identification of both COVID-19 and non-COVID-19 associated mucormycosis from fresh clinical specimens using Mucorales specific primers

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Abstract:

Objectives:
Mucormycosis in both COVID-19 and non-COVID-19 individuals is a fungal emergency featured by angioinvasion, aggressive tendency for progression, high morbidity and mortality, underscoring the importance of its timely diagnosis and management**.** Here, we investigated the utility of high-resolution melt analysis (HRMA) as a non sequence method using *Mucorales*-specific primers targeting a 188 bp region of the 18S region of rDNA for detection/identification of *Mucorales* DNA from fresh specimens in comparison with diagnostic value of direct and histopathological examinations and culture.

Materials & Methods:
Specimens collected from patients with suspicion of invasive fungal infections were prospectively chased up initially for mucormycosis diagnosis through histopathological examination and direct microscopy (15 % KOH) as reference methods. Fungal culture was also performed. Identification of *Mucorales* isolates was performed using PCR with pan-fungal ITS primers. For molecular diagnosis of those specimens showed broad aspetate hyphae suggestive of mucormycosis, HRM analysis using *Mucorales*-specific primers were attempted**.** Identification via PCR/HRM was assessed according to the similarity of melting curve to each of the reference melting curve generated by the mucormycete culture strains identifed (served as standard strains for HRM) in the current study through PCR & sequencing.

Results:
Overall, 36 cases (35 proven and 1 probable cases) of mucormycosis out of 353 investigated specimens were diagnosed. Five out of 36 cases were COVID-19 associated mucormycosis patients Rhino-orbito-cerebral mucormycosis (50%) was the most common presentation. Uncontrolled diabetes (55.5%) and then hematological malignancies (36%) were the most prevailing risk factors for mucormycosis. Fresh specimens of 34 cases were available for culture and molecular detection. Isolation was possible in 25 (73.5 %) specimens**.** Regarding molecular diagnosis, the PCR/HRM enabled detection and identification of *Mucorales* in all of the cases (100 %) with a turnaround time of <5 h (regarding DNA extraction step)

(Figure 1). None were culture positive only. *R. oryzae* (27/34, 79%) was predominantly identified. The sensitivity, specificity, negative and positive predictive value of PCR/HRMA was all 100%**.**

Conclusions:
Through direct molecular diagnosis, the deficiency of conventional diagnosis is overcome, the necessity for culture based molecular identification will be eliminated and turnaround time to establish the diagnosis is reduced. Using PCR/HRM, the detection rate of mucormycosis in a shorter time increased in comparison with culture. PCR/HRM is novel technique showed superiority over the semi-nested PCR, histopathology, culture and ITS sequencing in terms of cost-effectiveness, rapidness and sensitivity for diagnosis and identification to species level when a sequencing facility is not available. As there is no serological test, PCR/HRM can be used as a confirmatory test to rule in or rule out mucormycosis in culture negative cases particularly when atypical aseptate hyphae or septate and non-septate hyphae suggestive of mixed infections are observed in histopathology**.**

Figure footnote:

Figure 1. Melt curve analysis demonstrating the discrimination of mucormycosis positive tissue from negative one and identification of different mucormycete species by *Tm*.

S10.5

A set of novel antigenic markers for the serodetection of Scedosporium/Lomentospora in cystic fibrosis patients

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Objectives:

Scedosporium and *Lomentospora* are emergent fungal pathogens ranking second among filamentous fungi causing chronic colonizations of the airways of patients with Cystic Fibrosis (pwCF). This may lead to chronic inflammation, lung damage and even to life-threatening invasive disease in cases of severe immunosupression. The detection of these fungi is currently performed by non-standardized and low-sensitivity culture-based methods. With this concern, the aim of this study is to gain insights into the immunodiagnosis of *Scedosporium/Lomentospora* fungi in pwCF, by identifying the specific antigens recognized by serum IgG of pwCF that may serve as diagnostic targets for future serological tests.

Materials & Methods:

A serological proteome analysis was performed to identify *Scedosporium boydii* antigens recognized by serum IgG from pwCF with positive cultures for *Scedosporium/Lomentospora* (Scedo+), *Aspergillus* (Asp+) and with negative cultures for both fungi (Scedo-/Asp-). The same procedure was used to detect infection-associated antigens employing sera from mice with *Scedosporium/Lomentospora* disseminated infection. The proteome and immunome of *S. boydii* was resolved by two-dimensional western blotting using pooled sera of each group. The most immunoreactive antigenic proteins were identified by mass spectrometry (LC-MS/MS) and bioinformatic analyses were performed to characterize the function and cellular location of the identified proteins. Finally, the specific antigens were purified by electroelution and tested against individual samples to determine the recognition prevalence among pwCF and to assess the diagnostic utility.

Results:

The immunoproteomics-based study showed that fungal glycoproteins strongly immunoreacted with sera from pwCF, with the peptidorhamnomannans being highly recognized by Scedo+ sera. Moreover, 17 immunoreactive proteins, specifically recognized by Scedo+ pooled sera, were identified by LC-MS/MS. Metabolic proteins with catalytic activity and stress-related proteins represented the main functional groups among the identified antigens.

On the other hand, when sera from mice with disseminated infection were used, the reactivity profile showed to be more specific towards protein antigens. A total of 6 immunoreactive proteins were identified by LC-MS/MS, most of them involved in metabolic processes with binding function.

After in-gel purification, the identified antigens were immunoblotted against individual serum samples from pwCF. Among them, the heat shock protein 70 kDa (HSP70), identified as an infection-associated antigen, was recognized by 90 % of Scedo+ CF sera. In addition, 6-

phosphogluconate dehydrogenase (6PGDH), 3-ketoacyl-CoA thiolase (KAT) and phosphoenolpyruvate carboxykinase (PEPCK) also stood out for their high immunoreactivity prevalence rates (67-81 % of Scedo+ sera).

Conclusions:

HSP70, 6PGDH, KAT and PEPCK proteins emerge as novel promising diagnostic targets which may be very useful for the development of new methods of serological diagnosis of *Scedosporium/Lomentospora* infections in pwCF.

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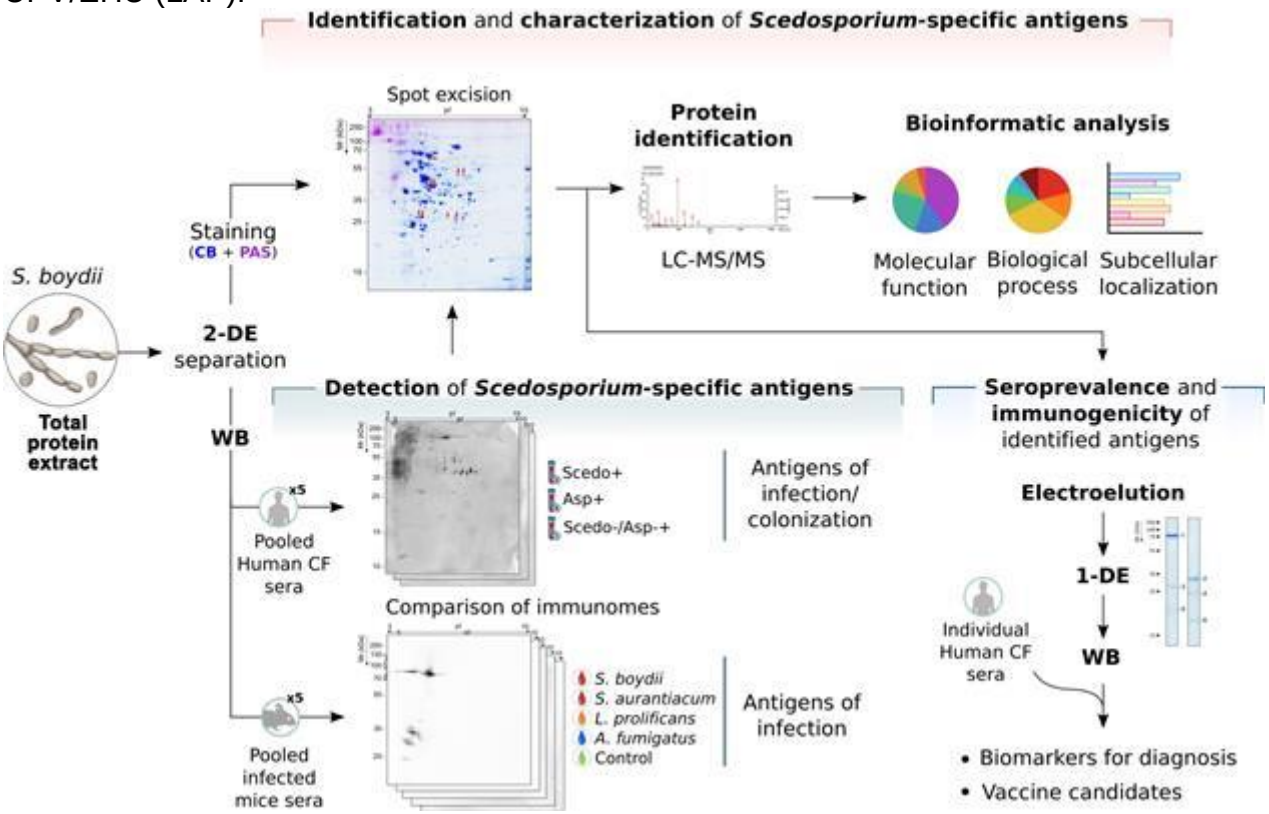

S10.6

Immunotherapy with nebulized pattern recognition receptor agonists improves clinical outcomes and pulmonary immuno-pathology in mice with influenza-associated pulmonary aspergillosis

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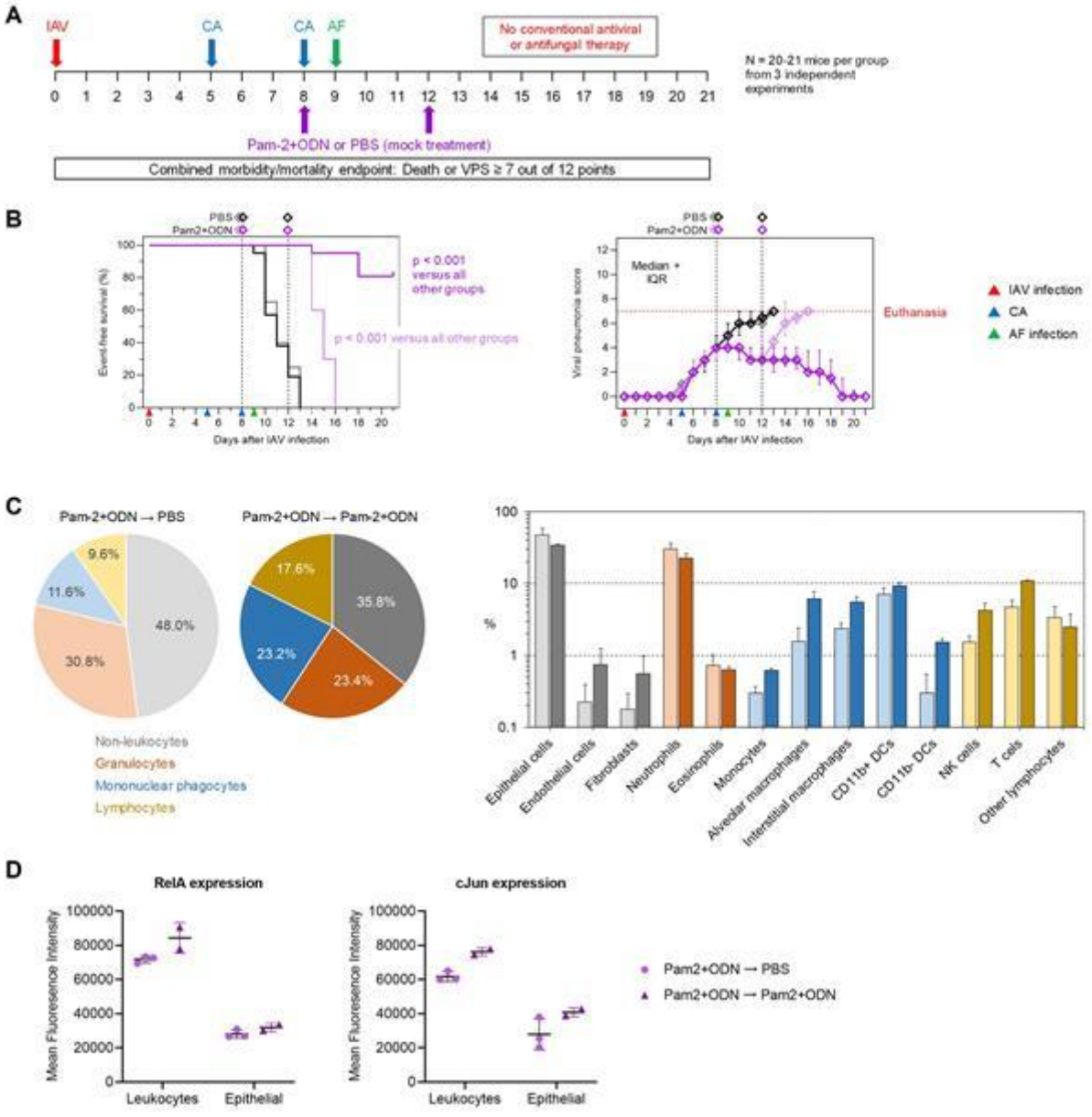
Objectives: Influenza-associated pulmonary aspergillosis (IAPA) is a feared and deadly super-infection in patients with severe influenza infection, especially those with underlying immunosuppression or corticosteroid therapy. Given the poor outcomes of IAPA despite conventional antifungal therapy, there is a critical unmet need to develop adjunct immunomodulatory strategies. Previously, aerosolized Toll-like receptor (TLR) agonists Pam2+ODN have shown promising preclinical and early clinical results in viral pneumonia, but data regarding their benefit in sequential viral/fungal pneumonia is lacking. Therefore, we herein studied the impact of immunotherapy with Pam2+ODN on infection outcomes and pulmonary immunopathology in a corticosteroid-immunosuppressed murine model of IAPA.

Methods & Materials: Eight- to ten-week-old female BALB/c mice were infected with 7.5% of the 90% lethal dose of a mouse-adapted influenza A/H3N2 strain, delivered by aerosolization. Mice then received two intraperitoneal injections of 10 mg cortisone acetate (CA) or mock injections on days 5 and 8 after influenza infection. On day 9, mice were intranasally challenged with 50,000 *Aspergillus fumigatus* (AF) conidia (**Fig. 1A**). Mice received a 30-minute nebulization of either phosphate-buffered saline (mock treatment) or the synergistic TLR 2/6/9 agonists Pam2+ODN (4 µM/1 µM). Treatment was given before (single-dose, day 8) or before and after AF infection (dual-dose, days 8 and 12, **Fig. 1A**). Infection severity was scored daily using the viral pneumonia score (VPS, 0=healthy to 12=moribund). To assess therapeutic efficacy, a combined morbidity and mortality endpoint was used, with an event defined as either death or reaching a VPS ≥7. Composition and activation of immune and epithelial cell populations in lung tissue was analyzed by histopathology (hematoxylin & eosin stain), conventional flow cytometry, and imaging flow cytometry on day 14.

Results: All mock-treated CA-immunosuppressed mice with IAPA reached the combined morbidity/mortality endpoint by day 13. Single-dose Pam2+ODN therapy led to universal event-free survival until day 13 but all mice reached the morbidity/mortality endpoint by day 16 (**Fig 1B**). Dual-dose Pam2+ODN therapy led to 80% event-free survival until day 21 ($p<0.001$ versus all other groups), with all survivors fully recovering (VPS=0, **Fig. 1B**). Consistent with these findings, histopathology revealed that Pam2+ODN-treated animals, especially those receiving dual-dose treatment, had significantly reduced fungal burden and hemorrhagic lesions. Additionally, mice receiving dual-dose Pam2+ODN showed a globally increased leukocyte-to-epithelial cell ratio in lung tissue homogenates, especially enhanced pulmonary recruitment of mononuclear phagocytes, natural killer cells, and T-lymphocytes (**Fig. 1C**), which predominantly displayed a Th1 memory phenotype. Moreover, both leukocytic (CD45+) and epithelial (EdCah+) cell populations showed activation of antimicrobial and pro-survival signaling via RelA and cJun (**Fig. 1D**).

Conclusions: Immunomodulatory treatment with nebulized Pam2+ODN strongly improved morbidity and mortality in an otherwise highly lethal CA-immunosuppressed IAPA model. Moreover, Pam2+ODN enhanced recruitment of mature mononuclear phagocytes to the lung, induced antimicrobial

pathways, and led to more favorable, type 1-prone T-helper cell polarization that is commonly associated with fungal clearance. Ongoing in-depth omics-based analyses of the pulmonary immune environment are expected to yield further insights into the immuno-protective activity of Pam2+ODN in post-viral aspergillosis, possibly facilitating further refinement of this facile, topical immunotherapy.



S12.4

Assessment of aspergillus-specific T cells during invasive pulmonary aspergillosis: Optifil study preliminary results

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ObjectivesInvasive pulmonary aspergillosis (IPA) is a life-threatening mycosis occurring in hematology patients. Optimal duration of antifungal therapy for IPA has not been clearly defined and response to treatment is mainly evaluated based on conventional imaging. We conducted a multicentric pilot study (OPTIFIL) assessing several radiological and biological parameters as markers of therapeutic response and clearance of *aspergillus* infection, including antigen-specific T cell immunity. Indeed, impaired T-cell mediated immunity is thought to play a role in susceptibility to IPA. We report here the analysis of host T cell response to *Aspergillus* during therapeutic management of IPA.
Methods: Thirty-eight patients with hematological malignancies/hematopoietic stem cell transplantation and diagnosed with proven or probable IPA were included in the study. *Aspergillus*-specific-T cells producing IFN gamma or IL-10 were enumerated by ELISPOT assay at inclusion (less than 5 days after IPA diagnosis), week-6 and week-12. An *Aspergillus fumigatus* lysate as well as overlapping peptide pools for *Aspergillus* catalase B, Crf1, Cu-Zn superoxide dismutase (Sod), glucanosyltransferase (Gel1), serine hydroxymethyltransferase (Shmt), putative peroxiredoxin Pmp20 and enolase Asp f22 were used as antigens during overnight cultures of peripheral blood mononuclear cells (PBMC) standardized for T cell counts.
Results; Due to lack of sampling, transportation delays, insufficient numbers of T cells in PBMC samples or death during follow-up, ELISPOT assays could be performed with at least three antigens in 45% (17/38) of patients at inclusion, 50% (15/30) at week-6 and 50% (13/26) at week-12. At inclusion, 10 out of 17 patients (58%) showed detectable IFN-gamma-producing T cells to at least one antigen (70±96 spots/10^6 T cells) and 9 patients (52%) showed detectable IL-10-producing T cells (294±650 spots/10^6 T cells). IL-10 basal production was high. A positive correlation was observed between *Aspergillus*-reactive T cell numbers and lymphocyte counts (p=0,0037; r=0,7). Three patients died before week-6 (n=2) or showed infection progression (n=1) at week-6. None of them had detectable *Aspergillus*-specific T cells at the time of IPA diagnosis. Among the thirteen patients with a complete or partial response at week-6, eight (62%) displayed IFN gamma-specific T cells at inclusion. No relation between antigen-specific T cell numbers (at inclusion/week-6) and week-12 outcome or lymphocyte counts was evidenced. At week-12, 83% of the surviving patients showed a complete or partial resolution of the infection and 75% of them displayed significant numbers of IFN-gamma or IL-10 producing T cells.
Conclusion:These preliminary data suggest that lack of antigen-specific T cells at IPA diagnosis may be predictive of early unfavorable outcome under antifungal therapy.

S12.5

A MULTINATIONAL REPORT ON SARS-COV-2 INFECTION OUTCOMES IN PEOPLE WITH CF AND ASPERGILLUS INFECTION OR ABPA

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Objectives
Aspergillus infection is associated with worse respiratory outcomes in people with CF (pwCF) and is a well-recognised complication of severe SARS-CoV-2 infection. The aim of this observational cross-sectional study was to examine the association of pre-existing *Aspergillus* infection or allergic bronchopulmonary aspergillosis (ABPA) in pwCF and severity of subsequent COVID-19.

Methods
Data on SARS-CoV-2 infections in pwCF from January 2020 to June 2021 were collected by the European Cystic Fibrosis Society Patient Registry. The primary outcome was COVID-19 severity measured by hospitalisation. Logistic regression models were used to assess the association between *Aspergillus* infection/ABPA in the previous year and COVID-19 severity outcomes. The models were adjusted for age, lung transplant and lung function as these have been previously identified as risk factors for worse COVID-19 outcomes in pwCF.

Results
1095 pwCF were diagnosed with SARS-CoV-2 and information on pre-existing *Aspergillus*/ABPA status was available from 807 (73.7%). Of these 19% had had preceding *Aspergillus* infection (n=115) or ABPA (n=27) or both (n=11). PwCF and SARS-CoV-2 in the *Aspergillus*/ABPA group (n=153), in comparison to the non-*Aspergillus*/ABPA group (n=654), were older (median age 25 years versus 21 years), had a higher rate of CF related diabetes (30.2% versus 21.0%), and had increased co-infection with *Pseudomonas aeruginosa*. There were no significant differences in terms of lung function, BMI z-scores, genotype, lung transplantation, or CFTR modulator treatment. PwCF and SARS-CoV-2 in the

Aspergillus/ABPA group were more likely to be hospitalised (p=0.005) and their disease course was more likely to be complicated by sepsis (p=0.008).

Conclusions

We show that pre-existing *Aspergillus* infection and ABPA in pwCF is associated with increased rates of hospitalisation and sepsis in COVID-19.

Table 1: Characteristics of COVID-19 in patients with Cystic Fibrosis in the presence or absence of *Aspergillus* infection or ABPA

	Total N=807(%)	**Non-*Aspergillus* / ABPA group N=654(%)**	***Aspergillus* /ABPA group N=153(%)**	**Adjusted[&] OR (95% CI)**	**Adjusted [&] p-value[1]**
COVID Severity					
Community treated	612 (77.08)	510 (79.19)	102 (68.00)	0.56(0.35 - 0.84)	0.005
Hospital admission	182 (22.92)	134 (20.81)	48 (32.00)	1.79(1.19 - 2.85)	0.005
ICU admission	30 (3.72)	26 (3.98)	4 (2.61)	0.65(0.20 - 1.96)	0.534
Oxygen requirement	92 (11.54)	77 (11.96)	15 (9.80)	0.80(0.34 - 1.23)	0.214
Non-invasive ventilation	23 (3.11)	18 (2.80)	5/153(3.27)	1.25(0.34 - 2.92)	0.876
invasive ventilation	17 (2.30)	13/603 (2.16)	4/135 (2.96)	1.39(0.39 - 4.80)	0.521
Complications during COVID					
Sepsis*	8/482 (1.66)	4/424 (0.94)	4/58 (6.90)	7.78(1.78 - 49.43)	0.008
Multiorgan failure	5 (0.62)	4 (0.61)	1 (0.66)		
CF pulmonary exacerbation*	103/806 (14.65)	78/653 (11.95)	25/153 (16.34)	1.62(0.84 - 2.43)	0.167
ARDS	7/480 (1.46)	7/422 (1.66)	0/59 (0.0)		

Bacterial Pneumonia*	14/480 (2.92)	11/422 (2.61)	3/58 (5.17)	2.04(0.45 - 8.02)	0.282
Outcome of COVID					
Death	11 (1.61)	10 (1.53)	3 (1.95)	1.29(0.30 - 5.38)	0.619

All variables: Numbers (%). *If missing data is ≥10% for specific variable, total number is specified. [&]Odds ratio with 95% confidence interval and p-value$_1$ adjusted for age at SARS-CoV-2 infection, pre-infection value of percentage predicted FEV$_1$, status of lung transplant. ICU; intensive care unit, ARDS; acute respiratory distress syndrome.

S12.6

Increasing triazole resistance rate in invasive aspergillosis in Belgium

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Objectives: A national prospective surveillance study on invasive aspergillosis (IA) in Belgium dates from 2011 and revealed a triazole resistance rate of 4.6%. The aim of this study is to evaluate if there is a change in the epidemiology of invasive aspergillosis between the years 2011/2012 and 2022/2023.

Materials & Methods: All Belgian laboratories were invited to send *Aspergillus species* complex isolates cultured from clinical samples collected in the one-year study period (April 2022 to March 2023) to the National Reference Center for Mycosis at UZ Leuven (Belgium). Isolates were included in the study when judged clinically relevant according to consensus definitions on IA (EORTC/MSGERC, (modified) *Asp*ICU, expert case definitions for IAPA and COVID ECMM/ISHAM criteria). To prevent selection bias, laboratories were asked to send all clinically relevant isolates, accompanied by information on the patient, isolate, underlying disease and antifungal treatment. Triazole-resistance screening was performed using the commercially available 4-well agar plate dilution method VIPCheck®. In case of growth in any traziole-containing well, the presence of resistance was evaluated using the EUCAST broth microdilution reference method. *Aspergillus fumigatus* complex isolates with decreased triazole susceptibility were processed with CYP51A sequencing to detail the resistance mechanism. All isolates were identified to the species level by MALDI-TOF mass spectrometry or by sequencing of the β-tubulin gene.

Results: A total of 322 isolates were included in the study, contributed by 29 clinical laboratories located across Belgium. For the current evaluation, 22 additional isolates were not included as insufficient information was available to correctly classify their clinical relevance. The median age of the patients was 66 years and 60.9% was male. *Aspergillus species* were isolated from a variety of sample types, with BAL being the most common (43.5%), followed by bronchial aspirate (23.3%) and sputum (20.8%). The 322 evaluated isolates were categorized as proven IA (4.0%) probable IA (55.6%), putative IA (15.8%), CAPA (17.7%), and IAPA (6.8%). While identification to species level is still ongoing, the predominance of *Aspergillus fumigatus* complex (90.4% of 291/322) isolates was confirmed by microscopy, followed by *Aspergillus niger* complex (4.3%) and *Aspergillus flavus* complex (2.8%) isolates. Azole resistance screening by VIPCheck® of these 291 *Aspergillus fumigatus* complex isolates showed a triazole-resistance rate of 9.6% (28/291), more than double the prevalence of 4.6% that was reported for the Belgian study conducted in 2011 (Vermeulen *et al*. 2015). Azole resistance was confirmed for all 28 *Aspergillus fumigatus* complex isolates by the EUCAST reference method, for which CYP51A sequencing showed that the majority of isolates (73.1%) were characterized by the resistance mechanism TR34/L98H, while 23.1% carried the profile TR46/Y121F/T289A.

Conclusions: A higher triazole resistance prevalence of 9.6% was observed for *Aspergillus fumigatus* complex isolates from patients diagnosed with invasive aspergillosis in Belgium for 2022-2023, roughly

double the rate compared to the prevalence of 4.6% for IA that was reported in 2011. These data strenghten the need for national and international surveillance data to guide decisions on emprical antifungal treatment regimens.

S13.4

Cophylogenetic analysis of Cryptococcus neoformans and Cryptococcus gattii with their homeothermic hosts

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Objectives:

The host range of a pathogen is determined by the evolutionary histories of the pathogen and its potential host. *Cryptococcus* has a broad host range, starting from protists to higher vertebrates. Several families of higher vertebrates have been reported to be infected by *Cryptococcus* spp. We hypothesize that host affinity, host specificity, and host selection in pathogenic species of *Cryptococcus*- *C. neoformans* and *C. gattii* are due to the coevolution of these basidiomycetous yeasts with their hosts.

Methods:

The nucleotide sequences of the cytochrome C oxidase subunit I of forty-eight homeotherms hosts and ten sequences of *C. neoformans* and *C. gattii* isolates from mammalian hosts were retrieved from NCBI GenBank and were subjected to host-pathogen association analysis using Matrix. The phylogenetic trees of host and pathogen were constructed using the Phylip webserver. The cophylogenetic analysis was performed by the PACo programme by constructing a Procrustean superimposition plot and tanglegram. Thereafter, host-*Cryptococcus* links were determined using the ParaFit test.

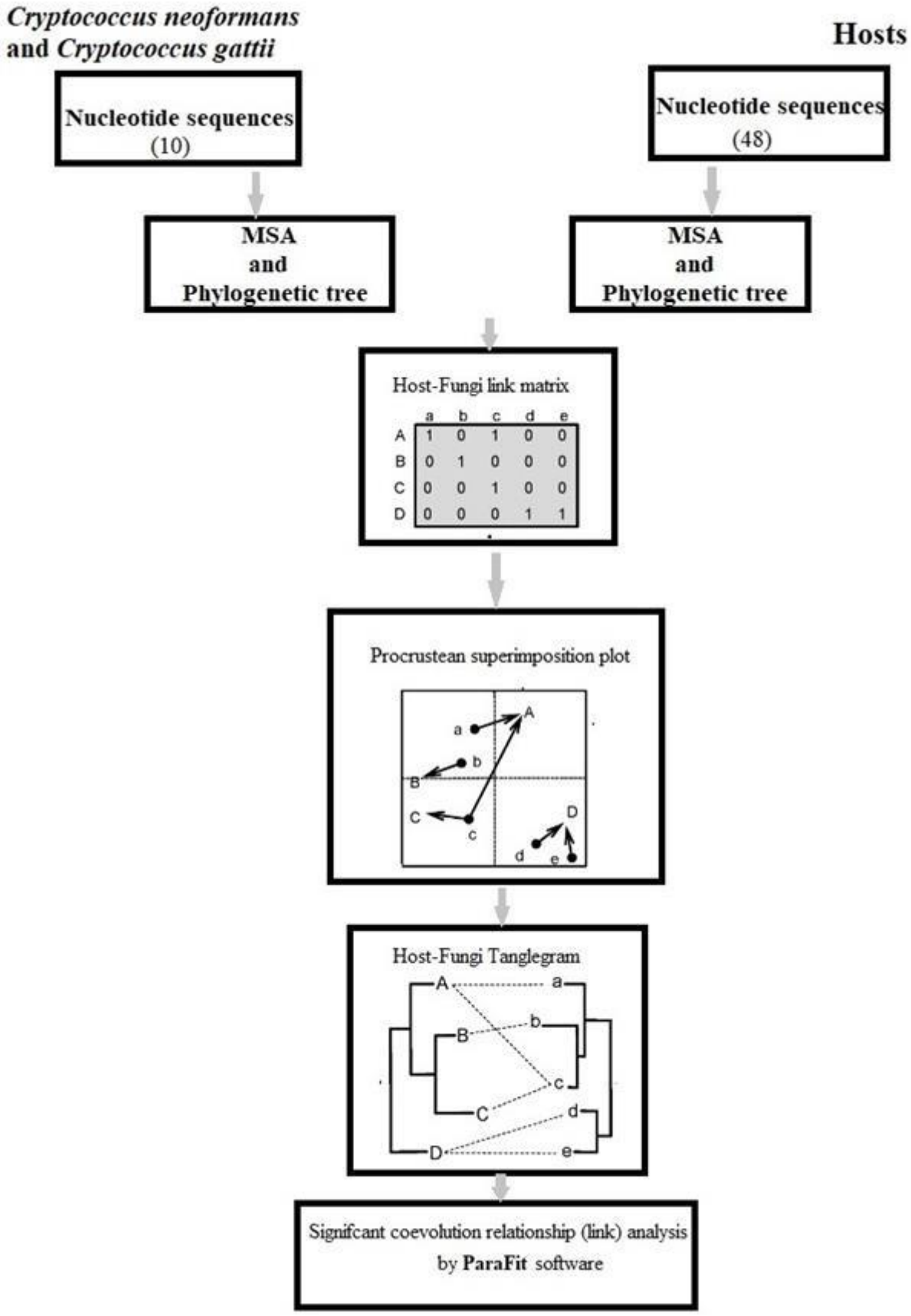
Results:

1. Two hundred and sixty links in the host-*Cryptococcus* interaction matrix were created among ten sequences of *Cryptococcus* (five *C. neoformans* and five *C. gattii*) and forty-eight warm-blooded vertebrate hosts.
2. Five isolates of *Cryptococcus neoformans* var. *neoformans* (Accession no. JN939495.1, KP835567.1, OW988632.1, NG_064879.1, and OW986477.1) showed interaction with thirty vertebrate hosts.
3. *Felis catus*, *Ovis aries*, *Cervus canadensis*, and *Canis lupus* showed interactions with all screened sequences of *Cryptococcus*.

4. *Streptopelia turtur* showed the most significant interaction with *Cryptococcus neoformans* in terms of the host-pathogen link, followed by *Alauda arvensis*, *Sturnus vulgaris*, *Passer domesticus*, and *Gymnorhina tibicen*.
5. Procrustes plot displayed the overlapping evolution of *Cryptococcus* with respect to its host. Multiple strains of *Cryptococcus* residing in the same host evolved similarly.
6. The length of the arrows in the Procrustes plot corresponded to their position in the phylogenetic tree.

Conclusions:

Although in the cophylogenetic analysis, all the screened isolates of *C. neoformans* and *C. gattii* exhibited affinity towards *Felis catus*, *Ovis aries*, *Cervus canadensis*, and *Canis lupus. C. neoformans*, however, follows the coevolutionary pattern with its avian hosts, most significantly *Streptopelia turtur*. This study will provide a better understanding of the host specificity and host affinity of *C. neoformans* and *C. gattii*, which may be helpful in predicting the transmission of cryptococcosis among homeotherms. Moreover, the coevolutionary adaptation studies may provide insight into the development of therapeutics against cryptococcal infections.


Cryptococcus neoformans and *Cryptococcus gattii*
Hosts
Nucleotide sequences (10)
Nucleotide sequences (48)
MSA and Phylogenetic tree
MSA and Phylogenetic tree
Host-Fungi link matrix
a b c d e
A 1 0 1 0 0
B 0 1 0 0 0
C 0 0 1 0 0
D 0 0 0 1 1
Procrustean superimposition plot
Host-Fungi Tanglegram
Signifcant coevolution relationship (link) analysis by **ParaFit** software

S13.5A

Cryptococcus gattii with reduced susceptibility to azoles harbors the substitution R258L in a substrate recognition site of the lanosterol 14-alpha-demethylase

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Objectives. This study aimed to establish the amino acid composition of the lanosterol 14-alpha-demethylase (ERG11) of Colombian clinical isolates of *Cryptococcus neoformans* and *Cryptococcus gattii* and to correlate any possible substitution with the *in vitro* susceptibility profile of the isolates to fluconazole, voriconazole and itraconazole. *C. neoformans* and *C. gattii* cause cryptococcosis, a life-threatening fungal infection, affecting mostly immunocompromised patients. Because of long-term azole therapies to treat this mycosis, resistance to fluconazole has long been reported in both fungal species, leading to treatment failure and poor prognosis. Among the mechanisms implied in resistance to azoles, mutations in the *ERG11* gene, encoding the azole target enzyme ERG11, have been described.

Materials & Methods. The minimal inhibitory concentration (MIC) to fluconazole, voriconazole and itraconazole, of 31 and 19 clinical isolates of *C. neoformans* and *C. gattii*, respectively, was determined using broth microdilution, and following the Clinical and Laboratory Standards Institute guidelines. Amplification of the *ERG11* gene was done, followed by DNA alignments, annotation and translation to amino acids. ERG11 substitutions previously reported to be related with azole resistance in *Cryptococcus*, other yeasts and filamentous fungi, as well as new substitutions in ERG11 were sought for. Structural modelling of ERG11 of all amino acid sequences found among the studied isolates, was carried out.

Results. Two and four different ERG11 sequences were identified among the *C. neoformans* and *C. gattii* isolates, respectively. Antifungal susceptibility testing showed that *C. gattii* isolates are less susceptible to azoles than *C. neoformans* isolates, which could correlate with differences in the amino acid composition and structure of ERG11 of each species. In addition, in a *C. gattii* isolate with high MICs to both fluconazole (64 µg/ml) and voriconazole (1 µg/ml), a G973T mutation in the *ERG11* gene resulting in the amino acid substitution R258L, was identified. This non-conservative substitution is located in the substrate recognition site 3 of ERG11.

Conclusions. This study shows differential susceptibility to azoles among cryptococcal species, with some isolates presenting high MICs to the assessed drugs, which underscores the need for *in vitro* susceptibility testing of clinical isolates in order to assist patient management. Our findings also suggest the association of a newly reported substitution, R258L, with the azole resistant phenotype in *C. gattii*. Further investigations are needed to determine the exact role that R258L plays in the decreased susceptibility to fluconazole and voriconazole, as well as to determine the participation of additional mechanisms of resistance to azole drugs.

S13.6A

Development of a Candida auris gene editing system reveals multiple zinc cluster transcription factors which significantly impact antifungal resistance

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Objectives:
Candida auris is an emerging multidrug-resistant fungal pathogen, recognized by the Center for Disease Control and Prevention and the World Health Organization as an urgent threat to public health. Approximately 90% of *C. auris* isolated from patients are found to be resistant to at least one class of antifungal agents, and numerous cases of isolates resistant to all three major classes of available antifungals have been reported. A promising strategy to enhance and extend the activity of existing antifungal agents is the co-targeting of regulators and mediators of antifungal stress response. The **overall objective** of this study is to identify and characterize the *C. auris* zinc-cluster transcription factor (ZCF) genes which influence antifungal stress response, characterize their impact on antifungal efficacy, and delineate the regulatory network of ZCF representing molecular vulnerabilities which might be therapeutically exploited.

Materials & Methods:

A novel and recyclable *C. auris*-optimized Episomal Plasmid Induced Cas9 (EPIC) gene-editing system was developed and verified to be funtional in all five known genetic clades of *C. auris.* Individual *C. auris* ZCF genes were disrupted in a pan-antifungal drug-resistant clinical isolate of *C. auris* using the EPIC system without the integration of dominant or auxotrophic markers. All strains were confirmed by PCR and Sanger sequencing. ZCF disruption strains were then assessed for changes in antifungal susceptibility and stress response by measuring minimum inhibitory concentrations (MIC) by broth microdiliution and Etest using CLSI methodology, and antifungal fungicidal activity was assessed by time-kill assay using pharmacologically-relevant concentrations. The transcriptional network of ZCF strains observed to significantly alter antifungal suscpetibility were then examined by transcriptional profiling using RNAseq.

Results:
Whole genome sequencing revealed strains created using the EPIC system to have no unintended geneomic variations in coding or non-coding regions. Disruption of numerous *C. auris* ZCF genes was observed to positively or negatively impact susceptibility to antifungal drugs from all three major classes of clinically available antifungal drugs (triazoles, amphotericin B, and echinocandins). Notably, disruption of either of two *C. auris* ZCF (B9J08_000592 and B9J08_004819) resulted in significant elevations in echionocandin MIC (≥8-fold). Conversley, disruption of either B9J08_000270 or B9J08_004820 resulted in stark decreases in MIC for all triazole tested. Furthermore, disruption of B9J08_000270 confered a potent and rapid fungicidal activity to triazoles, which are classically limited to fungistatic activity againts species of *Candida,* at pharmacologically relevant concentrations. Transcriptional profiling revealed 833 genes differentially expressed (>2-fold change and FDR <0.01) between the parental clinical isolate and the B9J08_000270 disruption strain following triazole treatment. Differentialy expressed genes included both genes predicted to be involved in drug efflux and ergosterol biosynthesis.

Conclusions:
These data demonstrate both the power of the *C. auris*-optimized EPIC gene editing system and that multiple *C. auris* ZCF impact clinical antifungal susceptibility. Notably, the B9J08_000270 regulatory network may hold the key to unlocking fungicidal activity for the triazoles against *C. auris*. Further characterization of *C. auris* ZCF transcriptional networks and how they influence antifungal susceptibility is greatly needed.

S14.4

Assessment of genetic diversity among Histoplasma capsulatum isolates recovered in France from patients originating from Africa, America, Asia: preliminary results

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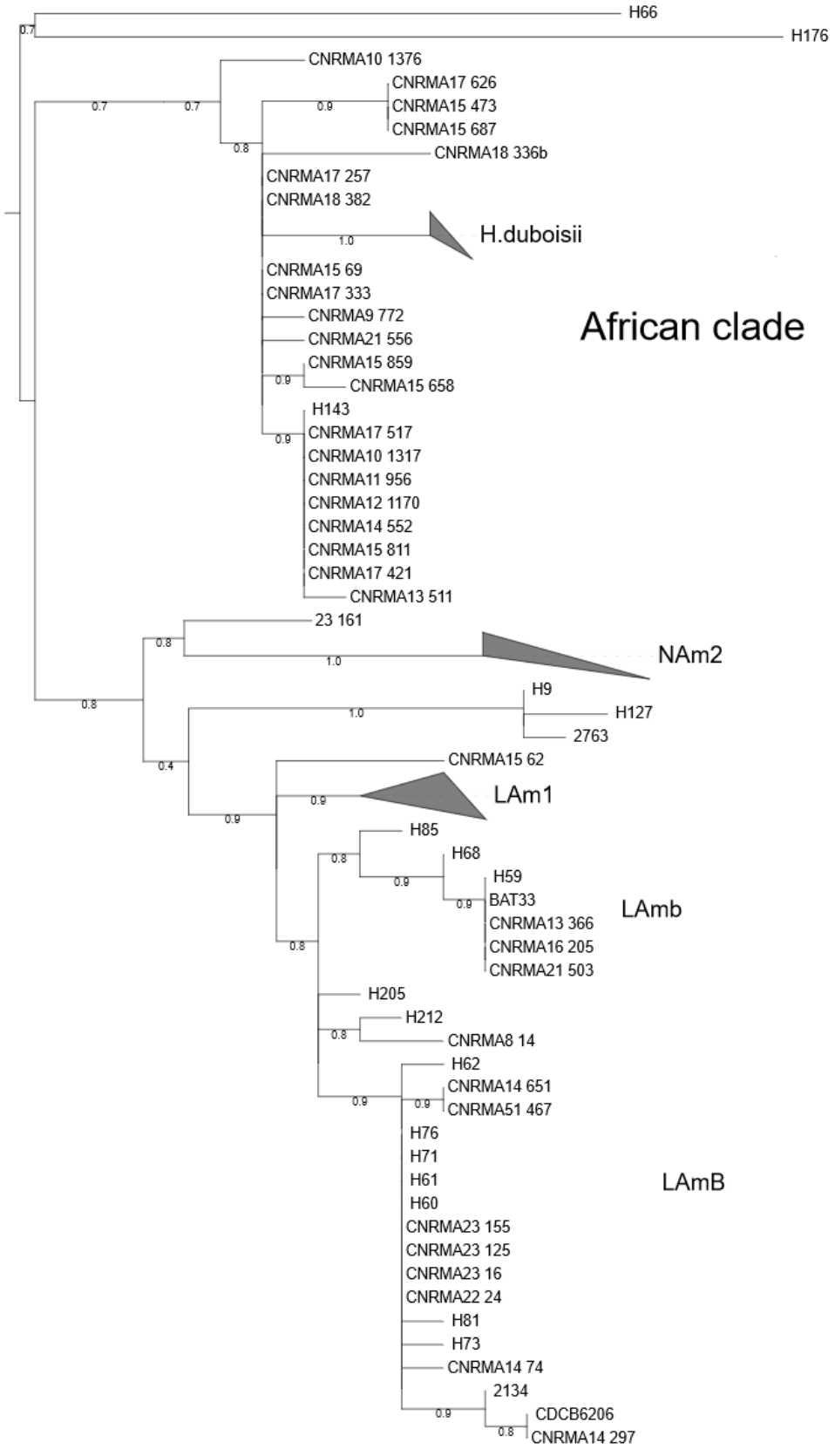
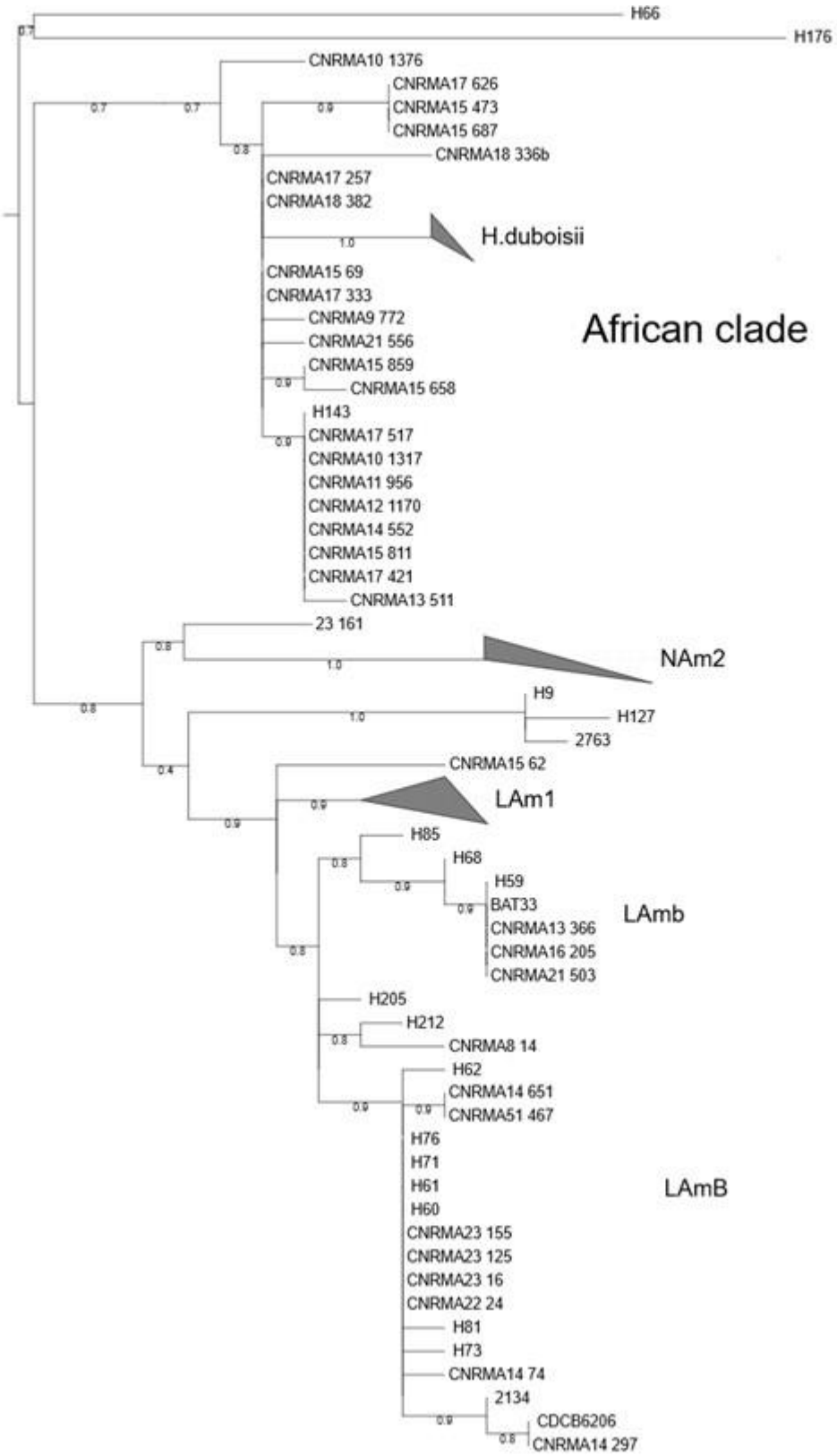
Background/Objectives *Histoplasma capsulatum* is a thermally dimorphic soil-based fungus belonging to the Ajellomycetaceae family. It is found worldwide but the most important endemic pockets are found in the Southern & Central United States and various regions in Latin America. Historically, *Histoplasma capsulatum* included three varieties: *H*. *capsulatum* var. *capsulatum*, *H*. *capsulatum* var. *duboisii* and *H*. *capsulatum* var.*farciminosum*. During the last decade, multiple molecular analyses have considerably modified the taxonomy of this genus. In 2003, Kasuga et al. using a four protein-coding gene sequencing scenario, proposed seven cryptic species belonging to the *H*. *capsulatum* complex and corresponding to clades of distinct geographical origin. In 2013, Cordeiro et al. explored the phylogenetic relationship of PRP8 inteins among isolates of *H. capsulatum* suggesting the suitability of this marker to differentiate most of the cryptic species. Later, Sépulveda and collaborators in 2017, proposed 4 phylogenetic species (*H. capsulatum* sensu stricto, *H*. *mississippiense*, *H*. *ohiense*, *H*. *suramericanum*) based on whole genome approach. The “well-defined species” status for the variety *H*. *capsulatum* var. *duboisii* (African clade) is still pending.
Here we characterize by a polyphasic approach, *Histoplasma capsulatum* complex isolates received at the National Reference Center for Invasive Mycoses & Antifungals (NRCMA) since 2005. The aim of this work is to investigate the genetic diversity of *Histoplasma* isolates in France.
Materials & Methods For the initial analysis, a total of 54 isolates previously identified as *H. capsulatum* complex were used. They were mostly recovered from respiratory tract, blood, ganglia and skin. Phenotypic characterization by morphological methods (in containment level 3 laboratory) was performed. The internal region of the PRP8 intein was amplified with primers P129-P131 (Cordeiro et al.). DNA intein sequences retrieved from GenBank were included in the multiple alignments along with the clinical strains
Results/Perspectives In addition to collecting the strains at the NRCMA we have the clinical information including the geographic origin of patients. Our preliminary results showed that the intein marker was suitable to distinguish the different geographic clades. We were indeed able to highlight the link to the origin of the patients. We identified 25 isolates (42%) that fell in the African clade ie. two of them showed 100% sequence identity to H91(*H*. *duboisii*) and eight isolates had 100% identity with sequence H143 (H. *capsulatum* from South Africa). Ten isolates (17%) belonged to the LAmA (Latin American), and we found strains belonging to the LAmB and Nam2 (North American) clades. Data analysis and sequencing of additional isolates is ongoing. These preliminary results will allow us to investigate this particular “African cluster”

also in terms of differences in clinical presentation, disease location, severity and patients' risk factors.




H66
H176
CNRMA10 1376
CNRMA17 626
CNRMA15 473
CNRMA15 687
CNRMA18 336b
CNRMA17 257
CNRMA18 382
H.duboisii
CNRMA15 69
CNRMA17 333
CNRMA9 772
CNRMA21 556
CNRMA15 859
CNRMA15 658
African clade
H143
CNRMA17 517
CNRMA10 1317
CNRMA11 956
CNRMA12 1170
CNRMA14 552
CNRMA15 811
CNRMA17 421
CNRMA13 511
23 161
NAm2
H9
H127
2763
CNRMA15 62
LAm1
H85
H68
H59
BAT33
CNRMA13 366
CNRMA16 205
CNRMA21 503
LAmb
H205
H212
CNRMA8 14
H62
CNRMA14 651
CNRMA51 467
H76
H71
H61
H60
CNRMA23 155
CNRMA23 125
CNRMA23 16
CNRMA22 24
LAmB
H81
H73
CNRMA14 74
2134
CDCB6206
CNRMA14 297

S14.5

Latin American Aspergillus fumigatus Environmental Azole Resistance Survey 2021-2023 in 12 Countries/26 Laboratories - an interim report

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Objectives: Aspergillosis is caused by 4 out of more than 250 *Aspergillus* species described so far (*Aspergillus fumigatus*, *A. flavus*, *A. terreus* and *A. niger*), and is one of the most prevalent invasive fungal diseases, with a death rate of over 50%. Most of those infections are coursed by *A. fumigatus*. Aspergillosis is primarily treated with azoles, with voriconazole being the drug of choice for treatment. However, over the last decade increasing rates of azole antifungal drug resistance are reported from many parts of the world. As infections are typically caused via inhalation of fungal spores from the environment, the increasing use of azole fungicides in agriculture, which are closely resemble those used to treat human disease, is thought to be a major cause for the emergence of resistant strains. To close the knowledge-gap a survey of azole resistance *A. fumigatus* throughout Latin America, using the existing Latin American Medical Mycology Network has been carried out using a citizen science approach from 2021-2023.

Materials and Methods: Air sampling was performed by ~700 volunteers in Chile, Colombia, Costa Rica, Ecuador, Guatemala, Mexico, Paraguay, Peru, Uruguay, Venezuela, and Antarctica, resulting in 3840 air samples.

Results: 1103 possible *A. fumigatus* strains where isolated. The results of the ID verification by MALDI-TOF or *βTUB* sequencing, the azole resistance screening and detailed MIC testing, the screening for the presence of *CYP51A* mutations in resistant isolates and the genetic relatedness via whole genome sequencings will be presented. **Conclusions:** This study is the first covering most of Latin America, indicating a relatively low prevalence of environmental resistance in the context of widespread usage of agricultural azoles.

S14.6

Resistance to fluconazole in Candida parapsilosis is associated with deffects in filamentation, biofilm formation and invasion

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Objectives: *Candida parapsilosis* is one of the most frequent causes of candidemia worldwide. Its incidence is associated with nosocomial infections in patients with medical implants due to its ability to form biofilms on biotic and abiotic surfaces. In Spain, and since 2020, the national reference laboratory noticed a great increase in the incidence of fluconazole-non susceptible (FNS) C. parapsilosis isolates, which raised a great concern about its clinical impact and in particular, about their ability to colonize the hospital and cause clinical oubreaks of difficult management. Most of these isolates were grouped in specific genotypes and carried the Y132F substitution in the *ERG11* gene. The objective of this work is to characterize some of the most important virulence traits from susceptible and FNS *C. parapsilosis* strains with the aim to find a correlation with the preferial selection of the resistant strains.
Materials & Methods: We investigated the ability of susceptible and FNS C. parapsilosis to develop pseudohyphae by real time microscopy in RPMI medium. We also investigated the ability to form biofilm on plastic surfaces (microdilution plates) by XTT metabolization. These studies were also complemented by observing biofilm formation in a microfluidics systems where medium was effluxed during the whole experiment. Finally, we investigated the ability to invade solid agar medium
Results: Although the results in the sensitive strains were variable, most of them were filamentous, formed biofilms (both statically and dynamically), and invaded the agar. Regarding the resistant strains, the results were opposite and almost all of the strains did not suffer any morphological change; they did not invade the agar; and they hardly formed a biofilm in a static way. Interestingly, under dynamic conditions, resistant yeasts were able to adhere, but the biofilm produced was unstable and no longer adhered to the surface over time.
Conclusions: we conclude that the resistance to azoles in the analyzed strains is associated with a filamentation defect and the formation of unstable biofilms. We believe that these findings may explain why these strains are more easily dispersed and spread in the hospital environment.

Funding. This work has been funded by a non-restricted grant by Gilead.

S16.4

Risk factors and outcome of COVID-19-associated pulmonary mucormycosis: a multicenter experience from India

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BACKGROUND AND OBJECTIVES: Most data on coronavirus disease (COVID-19)-associated mucormycosis is on COVID-19-associated rhino-orbital mucormycosis (CAROM). There is little information on the risk factors and outcome of COVID-19-associated pulmonary mucormycosis (CAPM).

Our primary objective was to compare the demographics, predisposing factors, treatment practices, and outcomes of CAROM and CAPM. Our secondary objective was to evaluate the factors associated with CAPPM in COVID-19 patients. The tertiary objective was identifying the factors associated with 12-week mortality in CAPM.

METHODS: We enrolled 1724 CAM (CAPM=122, CAROM=102) subjects from the multicenter Indian study (Mucovi2; from 25 participating centers). We collected information on the demography, predisposing factors for mucormycosis (diabetes mellitus, renal transplantation, and others), details of COVID-19 illness (hypoxemia, ICU or hospital admission during COVID-19, the glucocorticoid dose, and the use of zinc for COVID-19). For the primary objective, we performed a univariate analysis comparing the characteristics of CAROM and CAPM. For the secondary objective, we compared 122 CAPM subjects (cases) with 3911 COVID-19 subjects without mucormycosis (controls). The primary exposure evaluated for the risk factor for CAPM among COVID-19 was COVID-19 severity (defined by the presence of hypoxemia). For the secondary and tertiary objectives, we performed multivariable logistic regression analysis and reported the strength of association as an adjusted odds ratio (aOR) with 95% confidence interval (CI).

RESULTS: The study participants' mean age (52.5 years) was similar in CAPM and CAROM. CAPM more frequently affected men (83.6%; 102/122) than CAROM (73.3%; 1175/1602). Diabetes mellitus was the most common risk factor for both CAPM (75.4%) and CAROM (81.5%). More subjects in the CAPM group had renal transplantation (4.9% vs. 1.6%; p=0.007) or hematological malignancy (1.6% vs. 0.2%; p=0.01) than in the CAROM group. Hypoxemia during COVID-19 (64% vs. 44.8%; p=0.0001) and admission to the ICU (45.1% vs. 18.5%; p=0.0001) or the need for mechanical ventilation (18.6% vs. 8.8%; p=0.001) was significantly higher among subjects developing CAPM than CAROM.

Comparing 3,911 COVID-19 controls and 122 CAPM cases, hypoxemia during COVID-19 was not associated with CAPM (OR 1.06, 95% CI [0.58-1.94]). Instead, we identified diabetes mellitus (OR 4.87, 95% CI [2.52-9.40]), renal transplantation (OR 7.88, 95% CI [1.62-38.23]), cumulative dose of dexamethasone (OR 1.002, 95% CI [1.001-1.004]), and zinc supplementation for COVID-19 (OR 2.02, 95% CI [1.18-3.46]) independently associated with CAPM development after adjusting for age, sex, and hypoxemia during COVID-19 illness.

CAPM had a significantly higher 12-week mortality than CAROM (52.3% vs. 30.5%; p=0.0001). On multivariate analysis, hypoxemia during COVID-19 illness was associated with higher odds of death (OR 3.29; 95% CI [1.11-9.72], p=0.03) among CAPM, while surgery for CAPM (OR 0.11, 95% CI (0.04-0.29); p=0.0001) was associated with lesser odds of death at 12 weeks, after adjusting for age, sex, presence of comorbid illness and risk factors predisposing to mucormycosis.

CONCLUSION: COVID-19-associated pulmonary mucormycosis is a distinct entity with a higher mortality than rhino-orbital mucormycosis. We identified diabetes mellitus, renal transplantation, cumulative dexamethasone, and zinc supplementation during COVID-19 to be associated with CAPM.

Keywords: pulmonary mucormycosis, epidemiology, COVID-19, glucocorticoids, invasive mold infection

S16.5

A Prospective Cohort Study to Evaluate Performance of MycoMEIA – Aspergillus:

A New Urine Diagnostic for Aspergillosis

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Objectives: The Myco*MEIA* urine diagnostic was developed as an enzyme immunoassay, by Johns Hopkins (JH) and Pearl Diagnostics. In a retrospective study of 310 people, the assay was 90.5% (95% CI 70-99) sensitive and 89.2% (95% CI 82-94) specific. Here, we report results of a prospective study performed at JH Hospital (JHH).

Methods: People with suspected invasive aspergillosis (IA) were identified by screening of diagnostic tests sent by clinicians at JHH. Urines from consented subjects were tested in the clinical mycology lab within 8 hrs of collection. Results were not used to inform care. Established diagnoses were adjudicated by clinicians blinded to lab results, using consensus criteria. Performance was analyzed in evaluable subjects who had CTs within 2 wks of suspected IA. Findings supporting proven or probable IA, or no infection defined cases and controls, respectively. Myco*MEIA* results in subjects with possible IA, mixed infections, or other infections (non-IA) were evaluated descriptively.

Results: 107 urines were tested from 72 subjects with suspected IA. 15 subjects had >1 samples tested. Subjects were mostly male (50, 69%), with median age of 51 (range 7 – 87 yrs). IA was suspected during treatment for heme malignancies (59, 82%), solid organ transplant (9), cancer (5), and rheumatologic disease (1). Of 72 subjects enrolled, 30 had possible IA (42%), 30 no infection (42%), 4 probable IA (6%), 4 mixed infections (6%) and 4 other infections, including bacterial abscess (1), fusariosis (1), fungal sinusitis (1) and otitis externa (1). The assay was 100% sensitive (95% CI 76–98) and 93% specific (95% CI 79–99) for IA, with likelihood ratio of 15. Five of 30 (16.7%) people with possible IA and 1 with fusariosis had positive Myco*MEIA* urine tests.

Conclusions: In prospective testing by the clinical lab, performance of the urine Myco*MEIA* – *Aspergillus* test appears consistent with retrospective findings and favorable compared to current blood assays. The proportion of lab confirmed diagnoses using current commercial assays was low; high negative predictive value with urine testing may support restriction of empirical antifungals. Cross reactivity amongst pathogenic Ascomycetes is biologically anticipated and requires further study. The study remains ongoing.

S16.6

Increased incidence of COVID-19-associated pulmonary aspergillosis (CAPA) in the vaccination era

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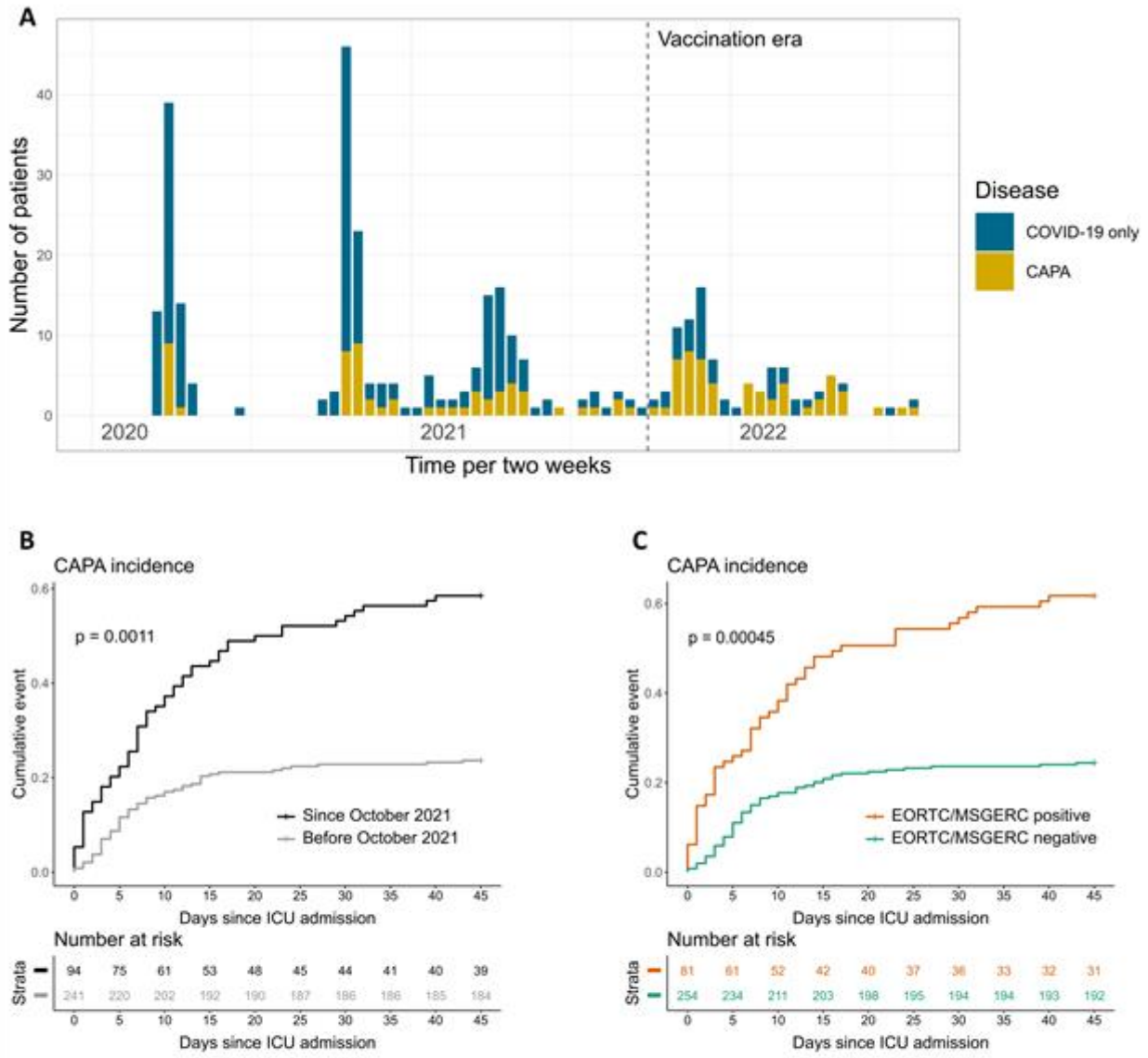
[1]University Hospitals Leuven, Leuven, Belgium, [2]KU Leuven, Leuven, Belgium, [3]Medical University of Graz, Graz, Austria, [4]Bio TechMed, Graz, Austria

Objectives:
COVID-19-associated pulmonary aspergillosis (CAPA) is a superinfection frequently encountered in critically ill COVID-19 patients. CAPA is associated with worse outcome, including increased mortality. Currently, our knowledge on CAPA is limited to studies completely or largely conducted during the pre-vaccination era. As, since the widespread implementation of vaccination, severe COVID-19 patients who require mechanical ventilation are in proportion increasingly immunocompromised, we studied the incidence and impact of CAPA in the vaccination era.

Materials & Methods:
We performed a monocentric, retrospective, observational study in the ICU of University Hospitals Leuven, a tertiary referral centre. We collected data from adult patients with severe COVID-19 requiring mechanical ventilation admitted between 1st March 2020 and 14th November 2022. The 2020 ECMM/ISHAM criteria were used to diagnose probable or proven CAPA. Beginning of the vaccination era was seen as October 2021, as that month >80% of the inhabitants of the Flemish region (where University Hospitals Leuven operates) had completed vaccination against SARS-CoV-2.

Results:
We included 335 mechanically ventilated COVID-19 patients, of whom 300 (90%) received at least one bronchoalveolar lavage (BAL) sampling during ICU stay. A diagnosis of CAPA was made in 112 (33%) patients (Figure 1A). CAPA incidence was particularly high in EORTC/MSGERC host factor positive patients compared to host factor negative patients (62% (50/81) versus 24% (62/254)) (Figure 1B). As presence of EORTC/MSGERC host factors increased from 10% (25/241) to 60% (56/94) in the vaccination era, CAPA incidence increased from 24% (57/241) to 59% (55/94) in patients admitted to ICU before and since October 2021 respectively (Figure 1C). Multivariable analyses including binary logistic regression analysis and a Fine and Gray model correcting for competing events (extubation or death) corroborated the independent association between CAPA development and EORTC/MSGERC host factors, and ICU admission in the vaccination era. Importantly, using a Cox multivariable analysis, CAPA remained strongly associated with 90-day mortality in the vaccination era.

Conclusions:
Classical EORTC/MSGERC host factors for invasive mold disease are associated with increased CAPA incidence and worse outcome parameters, and are the main drivers for the significantly higher incidence of CAPA in the vaccination era. Our findings warrant further prospective and multicentre studies, and investigation of the potential of antifungal prophylaxis in severe COVID-19.



Panel A: Visualization of CAPA incidence per two weeks within the timeframe of inclusion for this study. Panels B-C: Cumulative incidence curves depicting the 45-day incidence of CAPA. Stratification by admission before or since the vaccination era (before or since October 2021) in Panel B, and stratification by presence of EORTC/MSGERC host factors for invasive mold infection in Panel C. P-values as obtained by a Fine and Gray model correcting for competing risks (extubation or death).

S17.4

Exploring the clinical features and risk factors for children tinea capitis complicated with allergic diseases

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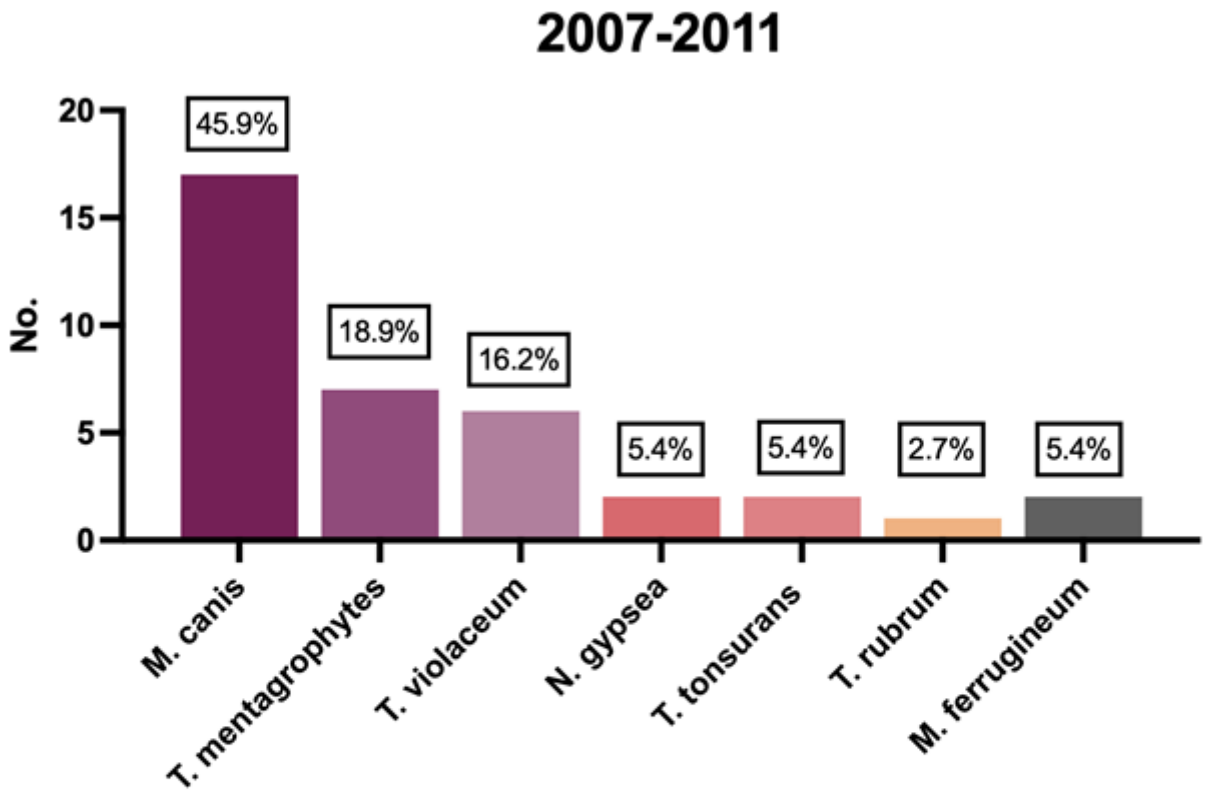
Background:
Tinea capitis, atopic dermatitis and allergic rhinitis are the most common disorders endured by prepubescent children. Dermatophyte infections have been linked to allergic disorders, such as increased sensitivity to dermatophytes in patients with atopic dermatitis.

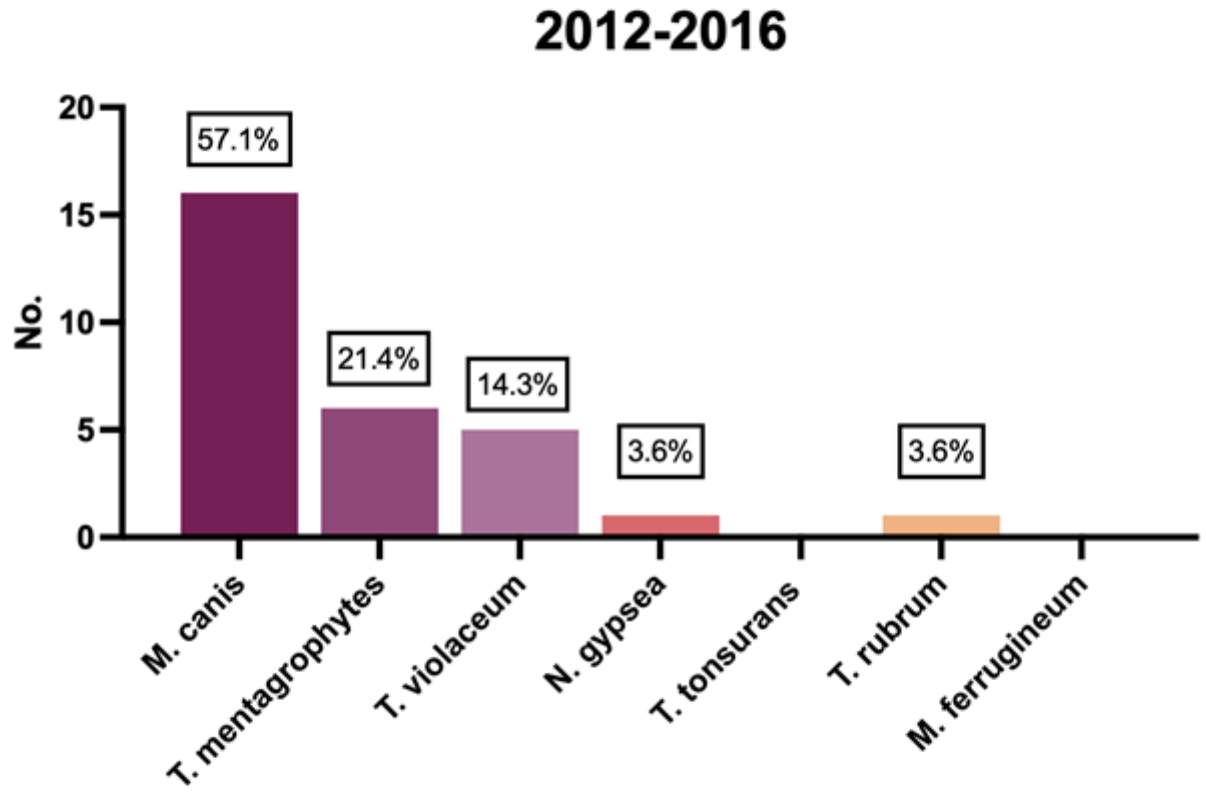
Objectives:
To explore the correlation between *tinea capitis* and allergic diseases in children and to analyse their risk factors.

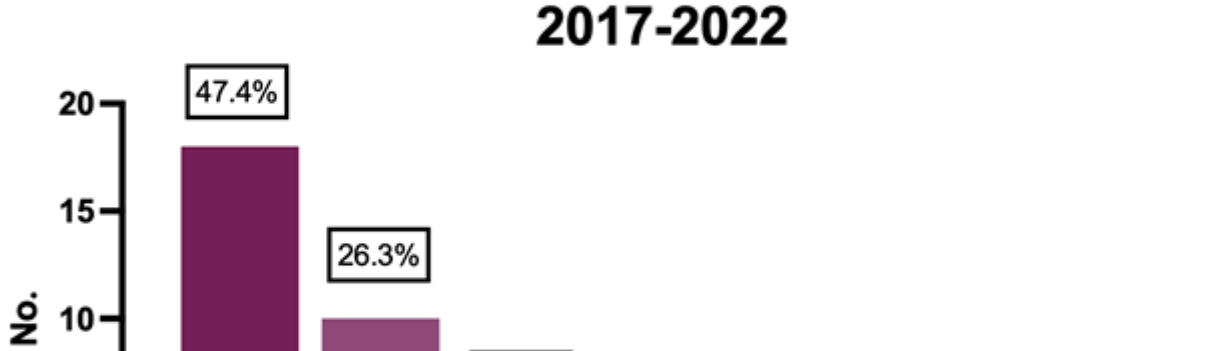
Methods:
This study monitored epidemiological changes in childhood *tinea capitis* and risk factors for whom with allergic disease in a single centre in three consecutives five year intervals by reviewing clinical data and multivariate logistic data analysis ().

Results:
Between 2007 and 2022, there were 127 children patients with *tinea capitis*, the mean age was 4.83 years, and the male-to-female ratio was 1.76:1. Zoophilic *Microsporum canis* and *Trichophyton mentagrophytes* were the most prevalent pathogens, and the proportions remained relatively constant every 5 years (fig1). There were 34 (26.8%) children with *tinea capitis* complicated with allergic disease, among them 14 children with atopic dermatitis/eczema, 13 with allergic rhinitis, 8 urticaria, 6 food allergies and 1 allergic asthma. Male, kerion, zoophilic species infections and animal contact history were prevalent features in allergic disease combined with *tinea capitis*. Patients with *tinea capitis* plus allergic disease mostly had a family history with similar complications (fig2).

Conclusion:
M. canis and *T. mentagrophytes* were the most prevalent pathogens of *tinea capitis* in the last 15 years; atopic dermatitis/eczema and allergic rhinitis were the most frequently associated allergic diseases. Male, kerion, zoophilic pathogen and animal contact history are risk factors.


2007-2011
No.
20
15
10
5
0
45.9%
18.9%
16.2%
5.4%
5.4%
2.7%
5.4%
M. canis
T. mentagrophytes
T. violaceum
N. gypsea
T. tonsurans
T. rubrum
M. ferrugineum


2012-2016
No.
20
15
10
5
0
57.1%
21.4%
14.3%
3.6%
3.6%
M. canis
T. mentagrophytes
T. violaceum
N. gypsea
T. tonsurans
T. rubrum
M. ferrugineum


2017-2022
No.
20
15
10
47.4%
26.3%

Epidemiological factors	With allergic disease ($n = 34$)		Without allergic disease ($n = 69$)		p-Value chi-square test	p-Value logistic regression
	n	%	n	%		
Age (year)	5.23		4.24		.4807	
Gender						
Male	25	73.5	43	62.3	.2586	.3987
Female	9	36.5	26	37.7		
Type						
Kerion	14	41.2	20	29	.216	.8765
Gary patch	15	44.1	42	60.9	.1078	
Black dot	4	14.7	7	8.7	.8024	
Id reaction	9	26.5	11	15.9	.204	
Animal contact	19	55.9	18	26	.003	.0084
Family history						
Tinea	4	11.8	2	2.9	.0708	
Allergic history	9	26.5	0	0	<.0001	
Pathogens						
Zoophilic	27	79.4	47	68.1	.1067	.4238
Anthropophilic	7	0.20588	17	24.6	.3622	
Geophilic	0	0	5	7.2	.1076	

S17.5

Safety and Efficacy Data for Isavuconazonium Sulfate in Children: Combined Data from Two Pediatric Trials

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Objectives: Isavuconazonium sulfate (ISAV) has been available for the treatment of invasive aspergillosis (IA) and invasive mucormycosis (IM) in adults since 2015. While these life-threatening invasive fungal diseases (IFDs) also occur in critically ill and/or immunocompromised pediatric patients, the available treatment options have limitations in this vulnerable and clinically unique patient population. This analysis summarizes the safety, efficacy, and pharmacokinetics (PK) for ISAV in pediatric patients with IFDs.

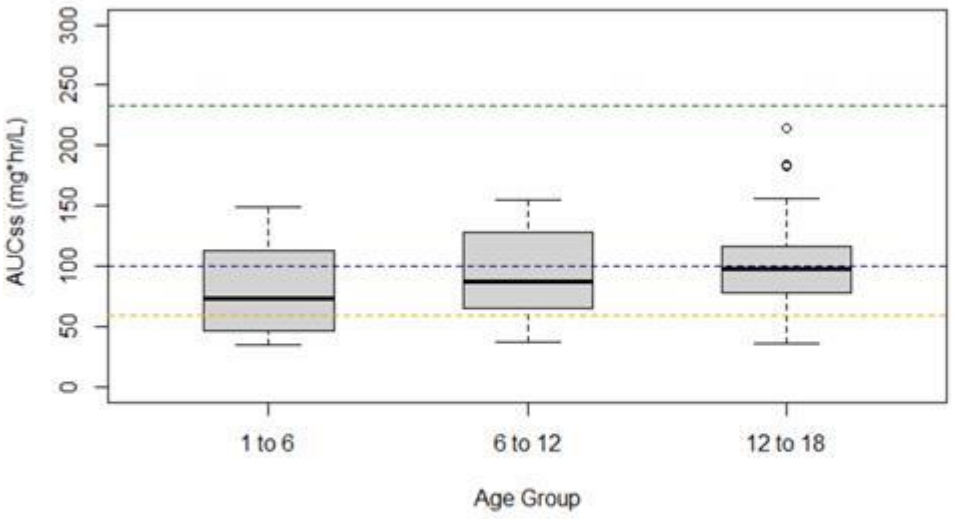
Materials & Methods: Safety data from two clinical studies of ISAV (Phase 1 study: NCT03241550, and Phase 2 study: NCT03816176) were combined. Patients aged 1 to <18 years received ISAV as prophylaxis for IFDs (Phase 1 study), or ISAV for the treatment of possible, probable or proven IA or IM (Phase 2 study). ISAV (intravenous or oral) was dosed at 10 mg/kg (maximum 372 mg) every 8 hours on Days 1 and 2, and once-daily thereafter. Treatment-emergent adverse events (TEAEs) were summarized. Efficacy endpoints (Phase 2 study) included all-cause mortality through Day 42 and overall response at end of treatment (EOT; assessed by an independent adjudication committee). The pediatric population PK (PPK) model was constructed from the 2 clinical studies; area under the curve (AUC) was derived to evaluate for the proportion of patients who achieved exposures within the target range (60–230 mg·hr/L) with the administered dose. Data were summarized descriptively.

Results: Seventy-seven patients with a mean (standard deviation) age of 10.1 (4.7) years were enrolled in the 2 clinical studies; 54.5% were female and 67.5% were White. ISAV was administered for a median (range) duration of 15 (1–181) days. TEAEs occurred in 72 (93.5%) patients, and treatment was withdrawn in 9 patients due to TEAEs. Twenty-nine (37.7%) patients experienced drug-related TEAEs, and 7 (9,1%) patients withdrew due to drug-related TEAEs. Serious TEAEs occurred in 38 (49.4%) patients and were assessed as drug-related by the investigator in 3 patients (3.9%). In the Phase 2 study, all-cause mortality through Day 42 was 6.5% (2/31), and successful overall response was 54.8% (17/31) at

EOT. In the PPK model, derived AUCs ranged from 35.6–215.5 mg·hr/L, and 80% of the patients fell within the target range (Figure 1). Mean AUCs in patients aged 1 to <6 years were numerically lower (84.6 mg·hr/L) than in the older age groups (6 to <12 years: 97.5 mg·hr/L, and 12 to <18 years: 104.0 mg·hr/L).

Conclusions: Critically ill and/or immunocompromised pediatric patients are at considerable risk for IFDs. With limited treatment options available, new treatments are urgently needed. In this analysis, treatment with ISAV was well tolerated in pediatric patients at risk for, or with, IFDs; only a small proportion of patients had serious drug-related TEAEs. In the Phase 2 study, successful overall response was observed in more than half of patients at EOT, and all-cause mortality was low through Day 42. Derived AUCs were within the target range in the majority of patients, and were lower in younger versus older patients, as seen with other azoles.

Figure 1. Box plot of derived AUC values from pediatric participants to established exposure range



Box-and-whisker plots of drug exposure AUCss in pediatric age groups (1 to < 6, 6 to < 12, 12 to <18 years). Boxes represent the median and 25th and 75th percentiles, whiskers represent the range of maximum and minimum values within 1.5× the interquartile range, and outliers are shown as circles. Dashed blue line is the mean AUCss (100 mg·hr/L) from SECURE study. Dashed green line is the minimum (233 mg·hr/L) AUC_{24} value in a high dose adult study (1116 mg) with increased toxicity. Dashed orange line is the lowest targeted value (25th percentile, with AUCss of 60 mg·hr/L) based on exposures from SECURE study. AUC_{24}, area under the concentration–time curve for 0–24 hours; AUCss, area under the concentration–time curve at steady state.

S17.6

Antifungal policies and diagnostic capacities in European paediatric and neonatal units

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Objectives: To obtain insight of antifungal policies, antifungal stewardship programs, use of diagnostic tests and biomarkers for fungal infections in European paediatric and neonatal units.

Methods: A point prevalence study (PPS) was organized in paediatric and neonatal units throughout Europe. Data on number of beds, presence of antifungal stewardship programs, antifungal prophylactic policies, use of fungal biomarkers were recorded at a single time in all participating units and entered in an online database (REDCAP). Descriptive statistics were applied.

Results: A total of 130 hospital units in 12 European countries participated: 27 neonatal units (NUs), 18 Pediatric Intensive Care Units (PICUs), 21 Paediatric Hematology-Oncology (PONCOs), 10 Paediatric BM/Solid Organ Transplantation Units (P-BM/SOT), 19 General Paediatric Units (GPs), 13 Paediatric Surgery Units (PSs) and 22 other paediatric units. Of those, 49% followed antifungal prophylaxis practices (NUs 55.6%, PICUs 44.4%, all PONCOs and P-BM/SOT, GPs 5.3%, PSs 7.7% and other paediatric units 36.4%). The most common reasons for prophylaxis were prematurity and low birth weight in neonates, central venous catheters, high risk oncology and transplant patients and patients on long term parenteral nutrition or long term broad-spectrum antibiotic administration. A variety of antifungal agents were used for prophylaxis. Fluconazole (67%) was most commonly prescribed, followed by other antifungals (55%, liposomal amphotericin B or caspofungin), posaconazole (30%) and voriconazole (25%). Antifungal stewardship programs were in place in 47% of units; 44.4% of NUs, 38.9% of PICUs, 76% of PONCOs, 90% of P-BM/SOT, 22.7% of GPs, 46.2% of PSs and 27.3% of other paediatric units. The majority of units (68%) reported using fungal biomarkers, 98% of them performed identification testing (95% culture, 83% histology, 79% MALDI-TOF and 77% PCR) and antifungal susceptibility testing (97%, 57% EUCAST microdilution, 67% E-test, 25% automated, 30% other). Almost half of the units used EUCAST guidelines and the rest CLSI for antifungal susceptibility testing. The most common fungal biomarkers available were galactomannan (96%), cryptococcal antigen (75%), beta-D glucan (62%), while T2candida and lateral flow device (LFD) were used in only 20% and 6% of units, respectively.

Conclusions: Almost half of European paediatric and neonatal units reported using antifungal prophylaxis, mostly in high-risk patients and having antifungal stewardship programs established. Although, availability of diagnostic tools for fungal infections was reported in most units, few of them had access to newer non-culture-based biomarkers such as T2candida and LFD.

S19.5

Multi drug Resistance in Candida parapsilosis; An Impending Threat?

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Objectives: *Candida parapsilosis* has been reported as an increasing cause of Candidemia in neonatal intensive care units (NICUs). Fluconazole prophylaxis has been the method to prevent *Candida parapsilosis* infections, but this has driven resistance. However, there has been reports of fluconazole resistance among *Candida parapsilosis* isolates.
We examined isolates of *Candida parapsilosis* which were gotten from samples of preterm neonates in the neonatal unit of Lagos University Teaching Hospital. These antifungal susceptibility patterns of these isolates were determined using Biomerieux Vitek® 2 Compact.
Methods and Materials: Serial swabs were collected from the mouth, rectum, and umbilicus of preterm neonates within 24 hours of birth, 72 hours, day 7 and for 4 weeks or until discharge/death, in the Neonatal Intensive care Unit of the Lagos University Teaching Hospital, Lagos. The swab samples were cultured on Saboraud Dextrose agar slants at 37°C for 24 to 72 hours. Identification of yeast isolates was carried out using the Biomerieux Vitek® 2 Compact, and data analysis was done using IBM SPSS statistics version 26
Results: A total of 63 *Candida parapsilosis* isolates were identified from the mouth, umbilicus, and rectum of preterm babies in this study. Of the 63 isolates, 14(22.2%) were resistant to Fluconazole, 16(25 4%) were resistant to Caspofungin, 15(23.8%) were resistant to Amphotericin B, and 25(39.7%) were resistant to Flucytosine. All the isolates were susceptible to Voriconazole. Ten (15.9%) isolates were multidrug resistant and were resistant to Caspofungin, Fluconazole, Amphotericin B and Flucytosine
Conclusion: Multidrug resistance in *Candida parapsilosis* is an emerging threat that requires urgent attention from healthcare professionals, researchers, and policymakers. There is a need for better surveillance, diagnostics, and effective treatment strategies to manage this issue. The global surveillance of MDR C. parapsilosis is also necessary to understand the scope of the problem and guide future interventions. Future research should focus on understanding the mechanisms of resistance, the epidemiology of resistant strains, and perhaps the development of new antifungal agents.

Table 1: Resistance pattern of *Candida parapsilosis*

ANTIFUNGAL	R	S	I	SDD	TRM	Total
FLUCONAZOLE	14	47	-	1	1	63
VORICONAZOLE	-	63	-	-	-	63
CASPOFUNGIN	13	46	3	-	1	63
MICAFUNGIN	-	62	-	-	1	63
AMPHOTERICIN B	15	48	-	-	-	63
FLUCYTOSINE	13	39	10	-	1	63

Key: R- Resistant, S- Susceptible, I- Intermediate, SDD- Susceptible Dose Dependent, TRM- Terminated result

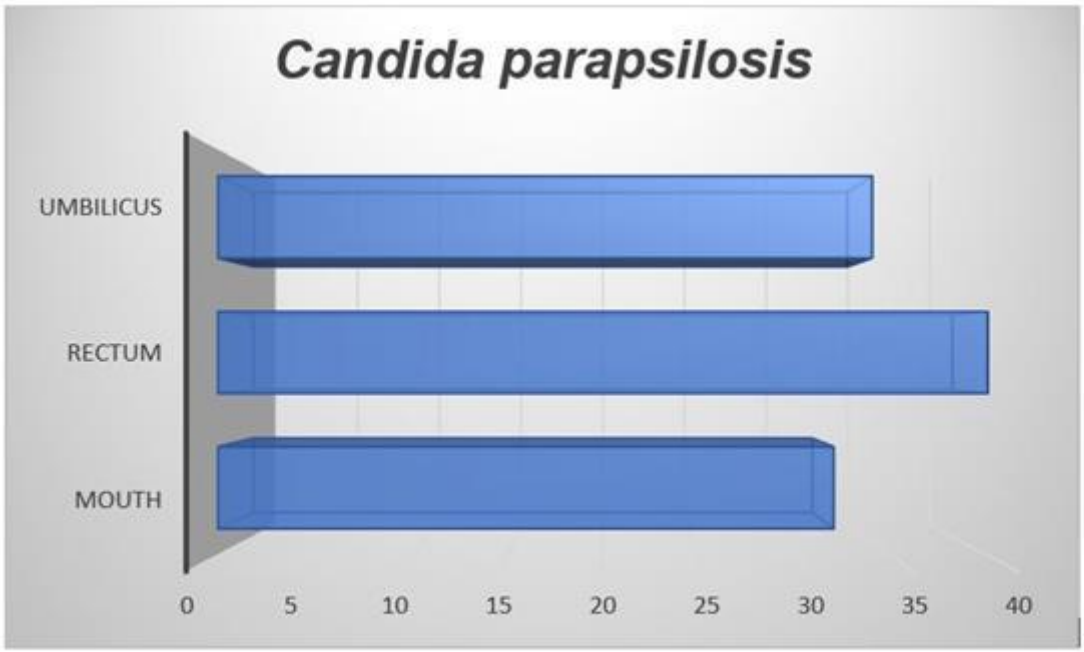

Figure 1: Distribution of *Candida parapsilosis* isolates in the clinical samples.

S20.4

Geotrichum infections in the hematologic patient

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Objectives: Rare yeast infections account for <1% of all invasive yeast infections (IYI) and include yeasts in the *Geotrichum* "spectrum" (*G. candidum*, *Saprochaete clavata* and *Magnusiomyces capitatus*); the host is usually immunocompromised, often within the context of an acute leukemia. Approximately 85% of cases of *M. capitatus* IYI have been reported in Europe (~85% of which in Italy and Spain).

Due to their rarity and restricted geographic distribution, clinical experience is limited, and hinders the development of treatment guidelines; we aim to contribute to the current knowledge with our robust clinical series.

Materials & Methods: We reviewed all positive fungal identifications for *Geotrichum* "spectrum" infections in our Adult Hematology Department over a 12-year period from April 2011 to April 2023. "Diagnosis" is the date of collection of the positive sample.

Results: We identified 45 patients (58% male) with "*Geotrichum*" isolates (91% *M. capitatus* and 9% *S. clavata)*. The average number of yearly cases was 3.4 (range: 1-6), with a peak in 2014-2015 coinciding with construction work in our hospital. The median age at IFI-Diagnosis was 62.7 years.

The majority of patients had an acute leukemia (60% myeloid and 11% lymphoblastic), 4% had a myelodysplastic syndrome (MDS), 2% had aplastic anemia, and the remainder had chronic lymphoid-lineage neoplasms.

Two-thirds (n=31) had fungemia in peripheral (PB) or central venous (CV) cultures (33%, n=15, in both); 24% (n=11) were positive in either sputum or bronchial aspirate (n=1 in both); 7% had urinary isolates and one patient each had positive cultures in a wound exudate and in abscess material.

The median OS after IFI-Diagnosis was 16 days; 32% (n=14) died within a week of Diagnosis, half of whom within the first 3 days, often before fungal identification; IYI was considered to be their cause of death.

A further 36% of patients (n=16) died from a week to a month after Diagnosis; the direct cause of death in this group could not be established in the absence of autopsy, and the infection and the underlying cancer were considered equally likely causes.

A final 20% of patients (n=9) died between one month and one year after the Diagnosis of IYI, due to progressive hematologic disease. Five patients (11%) are still alive, with no evidence of recurring IYI, and in complete hematologic remission. While one patient had fungemia (in both PB and CV), the other four surviving patients had no evidence of fungemia and had positive cultures in sputum (n=3) and abscess fluid (n=1).

Discussion: We present a very large unicentric clinical series, overtaking the largest published multicentric series. Here, the acute leukemias, together with High-Risk MDS, account for 3/4 of cases. Approximately 1/3 of patients died of active infection in the acute phase of IYI, while 1/5 died of progressive hemato-oncologic disease; in 1/3 of patients the cause of death (IFI vs progressive disease) was not established. Only 1/9 of the cohort currently survives; this subset of patients is relevant for the relative absence of fungemia – present in only 20%, vs 66.7% of the full cohort.

S20.5

Potential implication of azole persistence in the treatment failure of haematological patients infected with Aspergillus fumigatus.

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Objectives:

Due to the immune deficiency caused by both the underlying disease and the applied immunosuppressive therapies, haematological patients are at high risk of developing severe infections, being invasive aspergillosis (IA) one of the most common and devastating. Azoles are the first-line therapy for both pre-emptive and evidence-based treatment of aspergillosis diseases. Worryingly, there has been a surge and spread of resistance to azoles, and it has been described that infection with azole resistant isolates associates with higher mortality. Nevertheless, IA mortality is also elevated in patients infected with azole susceptible isolates, even in those with correct diagnosis and under appropriate antifungal treatment. There are several non-mutually exclusive reasons that can account for mortality in these cases, as inadequate levels of azoles in blood or poor penetration at the foci of infection. In addition, antimicrobial tolerance and persistence have been implicated in treatment failure in bacterial infections, and fluconazole tolerance has already been suggested to correlate with therapeutic failure in candidemia. In this work we investigated if azole persistence could be implicated in treatment failure of two IA patients that were not infected with resistant isolates.

Materials & Methods:

Sequential isolates from two IA patients were typed using the TRESPERG methodology and characterised for their susceptibility profile (according to the EUCAST E.Def. 9.3 instructions), and their persistence potential, by measuring fungal viability after 72 hours of incubation in the presence of 8 µg/mL of voriconazole (VCZ) or isavuconazole (IVZ). IVZ blod levels were monitored using a liquid chromatographic method coupled with UV detection.

Results:

Two haematological patients at the Asturias University Hospital were diagnosed with proven IA. The patients were treated initially with VCZ, which was changed quickly to IVZ. Isavuconazole levels were monitored and confirmed to be in therapeutic range (>2 mg/L) throughout treatment. A total of 26 *A. fumigatus* isolates (as verified by partial sequencing of β-tubulin) were recovered from patient 1 (12 isolates) and 2 (14 isolates) throughout the treatment period.

Patient 1 carried up to three different strains, all of them susceptible to all azoles and to amphotericin-B. Isolates 2 and 3 were only recovered at one point during treatment, whilst isolate 1 was recovered throughout the entire period. Strain 1 showed a variable persister phenotype, which several isolates characterised as persister.

Patient 2 carried up to five different strains. The first four isolated were susceptible to all azoles and to amphotericin-B. Of those, the first two were non-persisters and were rapidly eradicated by treatment (i.e. non-recovered again). In contrast, strains 3 and 4 were persisters to VCZ and IVZ and were recovered in multiple occasions throughout treatment. Finally, isolate 5 (recovered very late in the infection course) was resistant due to a TR34/L98H mutation in *cyp51A*.

Conclusions:

Despite adequate therapy and good drug levels, infection could not be cleared and the patients finally reached *exitus*. Several of the recovered isolates were characterised as azole persisters, which likely prevented their efficient eradication by the action of the antifungal and caused a sustained infection. Therefore, we propose that azole persistence has contributed to therapeutic failure in these patients.

S23.4

Serial Cryptococcal Antigen is not Predictive of 12-week Mortality in Patients without HIV Infection

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Objectives:
Cryptococcosis is an invasive mycosis associated with significant mortality and morbidity that typically presents with pulmonary involvement, fungemia and/or meningoencephalitis. It has been shown that monitoring of serial cryptococcal antigen (CrAg) titers is not predictive of clinical outcomes in the HIV population. Evidence is lacking in patients without HIV. The purpose of our study was to explore the association between serial antigen testing and mortality in patients without HIV infection.

Materials & Methods:
This was a retrospective cohort study conducted at the 3 Mayo Clinic sites. We identified patients diagnosed with cryptococcosis based on risk factors, clinical findings, and positive CrAg in the serum and/or cerebrospinal fluid (CSF). We included patients who had at least two serial CrAg values with the second value obtained 2-12 weeks after initiation of antifungal treatment. We divided them into the following groups: (i) CrAg titer decrease by at least 2 dilutions, (ii) stable or increasing CrAg titer. Patients with more than two CrAg values were included if the trend was confirmed by subsequent testing.

The primary outcome was 12-week mortality. Secondary outcomes were CSF culture sterilization and radiographic improvement on chest CT imaging. The comparison was performed using chi-square, Fisher exact and Mann-Whitney tests, as appropriate. The study was approved by the Mayo Clinic IRB.

Results:
We identified 93 patients with positive serum CrAg and 33 patients with positive CSF CrAg. The median serum CrAg value was 1:16 (IQR 1:8-1:256, LA) and 480 (IQR 1:40-1:2560, LFA). Median CSF CrAg value was 1:4 (IQR 1:2-1:160, LA), 1280 (IQR 1:160-1:2560, LFA). Demographic and clinical characteristics are shown in Table 1. 27 (29.03%) patients had a decrease in serum CrAg titer, and 66 (70.96%) had stable or increasing titer. 23 (69.70%) patients had a decrease in CSF CrAg titer, and 10 (30.30%) had stable or increasing titer. The association between a high baseline CrAg (defined as ≥ 1:256 for LA and ≥1:320 for LFA) and the CNS disease was statistically significant (25/40 *vs* 9/53 p=0.0001). No association with high serum titer and 12-week mortality was found (5/40 *vs* 4/49 p=0.495).

We found no statistically significant difference in 12-week mortality both for serum (1/27 for decreasing titer *vs* 8/66 for stable/increasing titer, p= 0.276) and CSF CrAg trend (3/23 *vs* 1/10, p=1). Similarly, CSF sterilization and radiographic improvement did not correlate with CrAg trend (CSF culture sterilization serum CrAg: 6/6 *vs* 11/15 p=0.281, CSF CrAg: 15/17 *vs* 7/8 p=1; radiographic improvement 7/11 *vs* 4/21 p=0.151).

Conclusions:
In this cohort, serum and CSF CrAg titer trend was not predictive of mortality, CSF sterilization or radiographic improvement among patients without HIV infection. The study is limited by sample size. We further note that approximately 2/3 of patients did not have follow-up imaging. We found that the CrAg trend is not predictive of clinical outcomes similar to patients with HIV/AIDS. Further studies are needed to evaluate the utility of serial antigen testing in making decisions on the transition from induction to consolidation and maintenance therapy.

Age, years (median, IQR)	65.2 (57.1-71.2)	62 (57.1-72.8)	65 (56.4-70)	0.84
Race (n, %)				
Black or African American	7 (7.54)	0	7 (8.3)	
BMI (median, IQR)	26.1 (23.14-29.6)	28.1 (24.2-31.8)	26.3 (23.3-29.8)	0.98
CNS infection	34 (36.6)	4 (44.4)	30 (35.7)	0.720
BSI	19 (20.4)	4 (44.4)	15 (17.9)	0.081
Others	8 (8.6)	2 (22.2)	6 (7.1)	0.171
Comorbidities (n, %)				
Chronic pulmonary disease	10 (10.8)	1 (11.1)	9 (10.7)	1
Solid tumor	1 (1.1)	1 (11.1)	0	0.097
HSCT	3 (3.2)	1 (11.1)	2 (2.4)	0.266
Steroids use	49 (52.7)	4 (44.4)	45 (53.6)	0.731
Other immunosuppressive conditions	17 (18.27)	0	17 (20.2)	0.203
CrAg serum				
2	58 (62.37)			
4	13 (13.98)			
6	2 (2.15)			

Table 2. Primary and secondary outcomes of the study

	Decreasing CrAg Titer	Stable or Increasing CrAg Titer	p-value
SERUM CrAg			
Primary outcome			
Mortality	n=27	n=66	0.276
Death by 12 weeks	1	8	
Alive at 12 weeks	26	58	
Secondary outcomes			
CSF sterilization	n=6	n=15	0.281
CSF culture negative	6	11	
CSF culture positive	0	4	
			0.151
CT chest follow up	n=11	n=21	
Improvement	7	4	
No improvement	4	17	
CSF CrAg			
Primary outcome			
Mortality	n=23	n=10	1
Death by 12 weeks	3	1	
Alive at 12 weeks	20	9	
Secondary outcome			
CSF sterilization	n=17	n=8	1
CSF culture negative	15	7	
CSF culture positive	2	1	

S23.5

Once-weekly rezafungin versus daily caspofungin to treat intra-abdominal/peritoneal invasive candidiasis: ReSTORE and STRIVE pooled subgroup analysis

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Objectives:

Invasive candidiasis at deep visceral sites is associated with high mortality.[1] While echinocandins are recommended first-line for invasive candidiasis, improvements are needed to optimize efficacy in clinically challenging situations and prevent treatment resistance.[2] Rezafungin is a new FDA-approved echinocandin to treat candidaemia and invasive candidiasis (IC), and is in development to prevent invasive fungal diseases caused by *Candida*, *Aspergillus*, and *Pneumocystis* spp after blood and marrow transplantation. In contrast to current echinocandins (administered daily [QD]), the long-acting pharmacokinetic profile of rezafungin enables once weekly (QW) administration with front-loaded dosing to facilitate faster fungicidal activity. This pooled analysis of STRIVE and ReSTORE clinical trials was conducted to assess outcomes for patients with intra-abdominal/peritoneal IC.

Materials & Methods:

Phase 2 STRIVE (NCT02734862) and Phase 3 ReSTORE (NCT03667690) are double-blind randomized clinical trials of similar design.[3,4] This pooled analysis included participants from both studies with intra-abdominal/peritoneal IC (with or without candidaemia) who received rezafungin QW (Week 1: 400 mg; 200 mg thereafter) and caspofungin QD (Day 1 70 mg; 50 mg thereafter [weight and hepatic impairment adjusted]) for ≥14 days (up to 4 weeks). Efficacy endpoints included Day 14 Global response rate (comprising clinical cure [STRIVE: investigator assessed, ReSTORE: data review committee assessed], radiological cure [ReSTORE only], and mycological eradication; primary [European Medicines Agency]), 30-day all-cause mortality (ACM; primary [US FDA]), and mycological eradication at Days 5 and 14 (secondary).

Results:

Across ReSTORE and STRIVE, 66.7% (26/39) and 70% (28/40) of patients with IC who received rezafungin QW and caspofungin QD, respectively, had intra-abdominal/peritoneal infections. Demographic and disease characteristics were similar across the treatment arms (mean [SD] age: 58.2 [14.4] and 61.1 [14.5] y; mean weight: 85.0 [22.6] and 74.3 [23.8] kg; male: 61.5 and 64.3%; APACHE II score <20: 92.3 and 88.9%).

Efficacy outcomes for rezafungin QW vs caspofungin QD, including Day 14 Global response rate (61.5 vs 57.1%; difference [95% CI] 4.4 [-21.6, 29.7]), 30-day ACM (11.5 vs 10.7%; difference 0.8 [-17.9, 20.3]), and mycological eradication on Day 5 (42.3 vs 50.0%; difference -7.7 [-33.0, 18.7]) and Day 14 (61.5 vs 60.7%; difference 0.8 [-24.8, 26.2]) were comparable in patients with intra-abdominal/peritoneal IC (Table 1).
Treatment-emergent adverse events (TEAEs) occurred in 84.6 vs 76.7% (11.5 vs 10.0% were considered treatment related) of patients in the rezafungin QW vs caspofungin QD arm. Serious AEs occurred in 50.0 vs 50.0% (3.8 vs 3.3% were considered treatment related), respectively. Treatment-related TEAEs are summarized in Table 2.

Conclusions:

In this small population with intra-abdominal/peritoneal IC, once-weekly rezafungin was comparable to daily caspofungin for multiple efficacy measures including Day 14 Global response, 30-day ACM, and mycological erradication (Days 5 and 14), and had a similar safety profile to caspofungin QD. Rezafugin provides a new treatment option for patients with IC.

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Table 1. Efficacy outcomes in patients with intra-abdominal/peritoneal IC (mITT population[a])

n (%)	Rezafungin QW (N=26)	Caspofungin QD (N=28)
Day 14 Global response rate[b]	16 (61.5)	16 (57.1)
Difference (95% CI)[c]	4.4 (-21.6, 29.7)	
30-day all-cause mortality	3 (11.5)	3 (10.7)
Difference (95% CI)[c]	0.8 (-17.9, 20.3)	
Mycological eradication		
Day 5	11 (42.3)	14 (50.0)
Difference (95% CI)[c]	-7.7 (-33.0, 18.7)	
Day 14	16 (61.5)	17 (60.7)
Difference (95% CI)[c]	0.8 (-24.8, 26.2)	

CI, confidence interval; IC, invasive candidiasis; mITT, modified intent-to-treat population, QD, once daily; QW, once weekly
[a]Participants with documented *Candida* infection ≤4 days prior to randomization who received ≥1 dose of study medication
[b]Global response comprised clinical cure (per investigator [STRIVE] or Data Review Committee [ReSTORE] assessment), radiological cure (ReSTORE, only), and mycological eradication (ReSTORE and STRIVE)
[c]Difference is rezafungin minus caspofungin (analysis not adjusted for study due to low patient numbers)

Table 2. Treatment-related TEAE in patients with intra-abdominal/peritoneal IC (safety population[a])

n (%)	Rezafungin QW (N=26)	Caspofungin QD (N=30)
Any treatment-related TEAE[b]	3 (11.5)	3 (10.0)
Anaemia	0	1 (3.3)
Diarrhoea	0	1 (3.3)
Hepatocellular injury	0	1 (3.3)
Hypertransaminasaemia	0	1 (3.3)[e]
Infusion-related reaction	1 (3.8)[c]	0
Tremor	1 (3.8)	0
Urticaria	1 (3.8)[d,e]	0

IC, invasive candidiasis; QD, once daily; QW, once weekly; SAE, serious adverse event; TEAE, treatment-emergent adverse event
[a]Patients who received any amount of study drug
[b]>1 TEAE may occur in one patient
[c]Occurred on Day 3 infusion of saline placebo
[d]Associated with administration of oral placebo study drug
[e]Event considered an SAE

S23.6

In-Vitro Synergy of Amphotericin-B and the Novel Antifungal Drug Ibrexafungerp: A Potential New Paradigm in the Therapeutic Approach against Talaromycosis

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Objectives:
Talaromyces marneffei (Tm), a thermally dimorphic fungus endemic to Southeast Asia, causes a life-threatening fungal infection in immunocompromised hosts. Amphotericin B works by extracting ergosterol from the fungal cell membrane leading to cell death and is the most potent antifungal drug against Tm. However, the molecular mimicry between fungal ergosterol and human cholesterol makes Amphotericin B substantially toxic to human cells, and the mortality on Amphotericin B therapy remains as high as 30%. The novel triterpenoid antifungal drug Ibrexafungerp works by inhibiting the production of (1,3)-β-d-glucan – an essential element of the fungal cell wall – has excellent antifungal activity against *Candida* spps and has demonstrated full *in vitro* and *in vivo* synergy with Isavuconazole against *Aspergillus* spp. Here we explore the potential synergy between Amphotericin B and Ibrexafungerp in combating talaromycosis.

Materials & Methods:
We assessed the *in vitro* interaction between Amphotericin B and Ibrexafungerp in 30 randomly selected Tm clinical isolates using the standard checkerboard assay. For antifungal susceptibility testing, we employed our lab's novel colorimetric alamarBlue assay developed specifically for Tm, which enables precise quantification of Tm growth reduction as measured by optical density or fluorescence intensity relative to the positive control. An inoculum of 1–5 x 10^3 CFU/mL Tm yeast was added to microplates containing Amphotericin and Ibrexafungerp in two-fold serial concentrations ranging from 0.03 to 2 µg/mL and 0.25 to 16 µg/mL, respectively. The minimum inhibitory concentration (MIC) was defined as the lowest drug concentration that inhibits at least 95% of fungal growth. To describe drug interactions, we calculated the fractional inhibitory concentration index (FICI) - where FICI ≤ 0.5 indicating full synergy, 0.5 < FIC ≤ 1 indicating partial synergy, 1 < FICI ≤ 4 indicating indifference, and FICI > 4 indicating antagonism - according to the Loewe additivity theory.

Results:
The geometric mean (GM) of the MICs of Amphotericin B and Ibrexafungerp acting alone against the 30 Tm strains were 0.89 µg/mL (range: 0.50 – 1.00) and 4.95 µg/mL (range: 4.00 - 8.00), respectively. The GM of the MICs when tested in combination – 0.16 µg/mL (range: 0.016 – 0.50) for Amphotericin, and 0.98 µg/mL for Ibrexafungerp (range: 0.25 – 2.00) – were significantly lower than the MICs of the two drugs tested alone *(P* < 0.0001 for Amphotericin; *P* < 0.0001 for Ibrexafungerp; Mann-Whitney tests). Full synergy (i.e., at least four-fold reductions in MICs for each drug in combination) was observed in 25 of 30 (83.3%) strains whereas the remaining 5 (16.7%) strains showed partial synergy. Indifference or antagonism was not observed in any strains.

Conclusions:
Our study documents for the first time full *in vitro* synergy between Amphotericin B and Ibrexafungerp against Tm. This discovery adds to the growing data on the synergy between these two drugs for invasive mycoses and offers new hope in enhancing fungicidal activity, improving treatment outcomes, and reducing mortality while minimizing total body exposure to Amphotericin B.

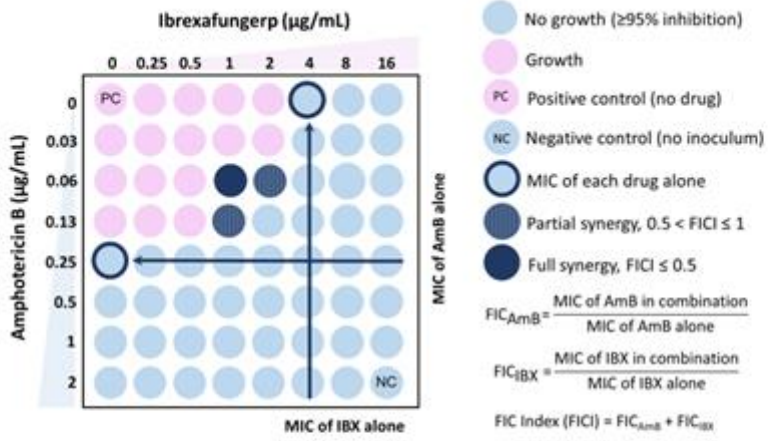

Figure 1 Checkerboard method to assess the interaction between Amphotericin B and Ibrexafungerp against *Talaromyces marneffei*. The positive control well (pink, noted "PC") contains no drug and allows for uninhibited growth. The negative control well (blue, noted "NC") contains no inoculum. Full synergy (solid dark blue well) is observed when the MIC of the drugs testing in combination is at least four-fold lower than the MICs of Amphotericin B and Ibrexafungerp alone (wells indicated by the arrows). Partial synergy (shaded dark blue well) is observed when the MIC of the drugs testing in combination is at least two-fold lower than the MICs of each drug alone.

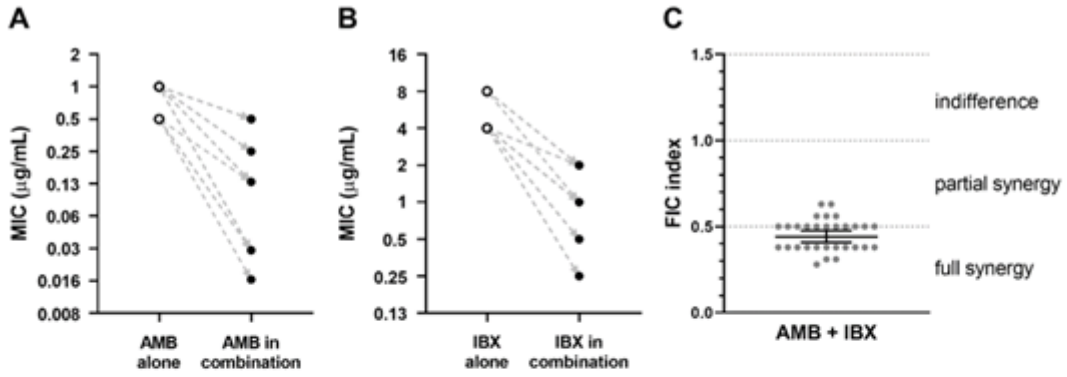

Figure 2. Change in the minimum inhibitory concentration (MIC) of Amphotericin B and Ibrexafungerp when testing alone versus in combination and classification of interactions in 30 *Talaromyces marneffei* clinical isolates. (A) Minimum inhibitory concentration (MIC) of Amphotericin B tested alone (GM: 0.89 µg/mL, range: 0.50 – 1.00) and in combination with Ibrexafungerp (GM: 0.16 µg/mL, range: 0.016 – 0.50) against 30 *Talaromyces marneffei* clinical isolates. Mann-Whitney test yielded $P < 0.0001$. **(B)** MIC of Ibrexafungerp tested alone (GM: 4.95 µg/mL, range: 4.00 – 8.00) and in combination with Amphotericin B (GM: 0.98 µg/mL, range: 0.25 – 2.00) against 30 *T. marneffei* isolates. Mann-Whitney test yielded $p < 0.0001$. **(C)** Fractional inhibitory concentration (FIC) index plotted for 30 *T. marneffei* strains tested against the Amphotericin B and Ibrexafungerp in combination (Full synergy in 25/30 strains, partial synergy in 5/30 strains). Geometric mean and error bars are denoted.