We recruited 41 FLD patients and 43 HEC from five university hospital pneumology departments in France and Switzerland. *L. corymbifera* proteins were extracted from the reference strain BBCM/IHEM 3809 isolated from FLD-linked hay. Proteins were separated by two-dimensional electrophoresis and subjected to western blotting, with sera from FLD patients ($n = 7$) or controls ($n = 9$). FLD-specific proteins were identified by mass spectrometry (LC-MS/MS) and were produced as recombinant antigens as previously described[1]. The diagnostic performance of ELISA tests using the recombinant antigens was assessed with all the sera from FLD patients and controls.

Result When compared western blot membranes revealed by FLD and HEC serum, 25 spots were considered as FLD specific. The 25 FLD specific spots were cut from the gel and analyzed by LC-MS/MS. Sixty-nine different proteins were identified from the 25 spots. Six proteins were selected to be produced as recombinant antigens: acylCoAdehydrogenase, proteasome alpha, pyruvate kinase, malate dehydrogenase, ATP synthase alpha, dihydrolipoyl dehydrogenase.

ELISA tests were performed using each recombinant antigen, with all the sera from FLD patients ($n = 41$) and controls ($n = 43$). Dihydrolipoyl dehydrogenase was the most effective recombinant antigens for discriminating FLD patients from controls, with AUC = 0.82 and with sensitivity and specificity of 81% and 77%, respectively. ELISA using proteasome a also showed AUC above 0.80, with sensitivity of 88%, but sensitivity was only 65%.

Conclusion Involvement of *L. corymbifera* in FLD has been described mainly in East of France and Finland. Combining recombinant antigens from *L corymbifera* with recombinant antigens from other micro-organisms (*Saccharopolyspora rectivirgula, Aspergillus*) involved in FLD would be probably helpful to produce a standardized ELISA kit effective for diagnosing FLD whatever the geographic location of the patient. A prospective study, using such a test combining most effective recombinant antigens from *S rectivirgula*[1]and from *Aspergillus2* with dihydrolipoyl dehydrogenase and proteasome alpha from *L. corymbifera,* is ongoing in our lab to assess diagnosis performance with patients from different geographic origins.

1. Barrera C, Millon L, et al. Immunoreactive proteins of *Saccharopolyspora rectivirgula* for farmer's lung serodiagnosis Proteomics Clin Appl. 2014: 8(11–12):971–81

2. Millon, Roussel et al. *Aspergillus* recombinant antigens for serodiagnosis of farmer's lung disease J Allergy Clin Immunol, 2012, 130(3):803–805.

P471

Variation in the polysaccharide capsule size interferes with identification of *Cryptococcus neoformans* and *C. gattii* by MALDI-TOF mass spectrometry

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Objectives to investigate the interference of *Cryptococcus*'s capsule size in MALDI-TOF MS species identification.

Methods four *C. neoformans* reference strains, WM 148 (VNI), WM 626 (VNII), WM 628 (VNIII), WM 629 (VNIV), and four *C. gattii*, WM 179 (VGI), WM 178 (VGII), WM 161 (VGIII), and WM 779 (VGIV) were used for capsule growth experiments. Initially the yeast cells of each strain were incubated overnight in acid Sabouraud broth at 30 °C with shaking. After centrifugation, the pellets were incubated in the capsule growth inducing medium (Sabouraud broth diluted 10 times, pH 7.3) at 37 °C with shaking. The capsule induction was carried out replacing medium every 48 h during a period of 20 days. To reduce the size of the capsules, the yeast cells were then seeded in acid Sabouraud agar plus 2.9% NaCl, and incubated at 30 °C for 48 h. For each strain, the capsule size was measured in light microscope using the software Axiovision. At least ten different fields of the slides were randomly chosen and 40 to 50 capsule cells were measured. Subsequently, the strains were analyzed by MALDI-TOF mass spectrometry after standard extraction protocol with ethanol 70% and formic acid 70%. Then, the supernatants were placed in MALDI target plate wells, dried at room temperature and overlaid with the matrix containing a saturated solution of HCCA. Mass spectra (MS) were generated by the Autoflex MALDI-TOF mass spectrometer and compared to main spectra of both *C. neoformans* and *C. gattii* from the database Biotyper 3.1. To ensure the reproducibility of spectra each strain was tested in quadruplicate in eight different experiments. Results were expressed as log score of 1.700–1.999 indicating a probable genus identification and log score above 2.000 indicating secure genus and species identification.

Results the strains of *C. gattii* developed larger capsule sizes in comparison with *C. neoformans* strains (mean 16.8 μm × 5.6 μm, $P = 0.002$) when incubated in the induction medium. Capsule size was inversely related to MS quality. All replicates with capsule size above 10 μm had unreliable species identification. The mean capsule size of *Cryptococcus* strains with correct species identification was 2.18 μm, whereas for those assigned with log score below 2.000 was 5.77 μm ($P = 0.03$).

Conclusion The capsule of *Cryptococcus* negatively interfered with the performance of MALDI-TOF MS species identification. Capsule size reduction is recommended to achieve a reliable laboratory identification of *Cryptococcus* isolates by this technology.

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Dermatophyte infections in the Lisbon and Tagus valley

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Objective A retrospective study was done in the Portuguese National Institute of Health, in order to establish the prevalence and characterize dermatophytic infections within the NUTSII region of Lisbon and Tagus Valley.

Material and methods This retrospective study included 4193 biological samples from patients with medical suspicion of fungal infection, collected from 2004 to 2013. Samples were obtained by extracting hairs and scraping skin and nails using a sterile curette over the affected areas. Samples were microscopically examined after 30% W/V for 20 min potassium hydroxide preparation and cultured on Sabouraud dextrose agar with cicloheximide. Species were identified by the observation of morphological features that included colony pigmentation, texture, growth rate and distinctive microscopic structures. Physiological tests (urea, vitamin and amino acid test agars) were used whenever necessary.

Results The average frequency of dermatophyte infections was 21%, ranging from 18% to 26%; 841 individuals (425 female and 410 male) had positive cultures. *Tinea capitis* was confirmed in 236 (28%) patients and was more prevalent in children from the group of 1–9 years old. In scalp dermatomycosis, *Microsporum audouinii* was the most frequently isolated species ($N = 120$, 51%), followed by *T. soudanense* ($N = 44$, 19%). Males were more affected (58%) than females (42%).

Onychomycosis caused by dermatophytes were confirmed in 385 cases (46%). Other fungi recognized as cause of onychomycosis were not considered for this report. Skin samples were positive in 220 cases (26%).

The most prevalent dermatophytes isolated in nail and skin samples were *T. rubrum* (206 and 93 isolates, respectively) and *T. mentagrophytes* with (49 and 39 isolates respectively).

E. floccosum was the species less frequently found in skin samples (1%).

Conclusion The frequency of infections by dermatophytes has revealed itself steady for the last 10 years. The spectrum of dermatophytes is similar to those reported in other studies for *tinea corporis* and onychomycosis, being *T. rubrum* the main aetiological agent.

However, our results differed from several studies that describe a raise, during the recent years, in the number of cases of *tinea capitis* caused by *M. canis*, especially in other Mediterranean countries. Our results showed a high prevalence of anthropophilic species (*M. audouinii* and *T. soudanense)* in the region of Lisbon and Tagus valley, where the number of foreign citizens from African countries is higher.

Like in other studies the number of infections by *E. floccosum* was very low suggesting that there was a replacement of this aetiological agent by other dermatophytes like *T. rubrum*.

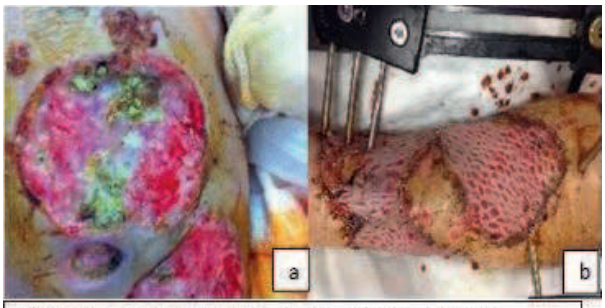

Figure 1a; Before treatment, 1b; After treatment

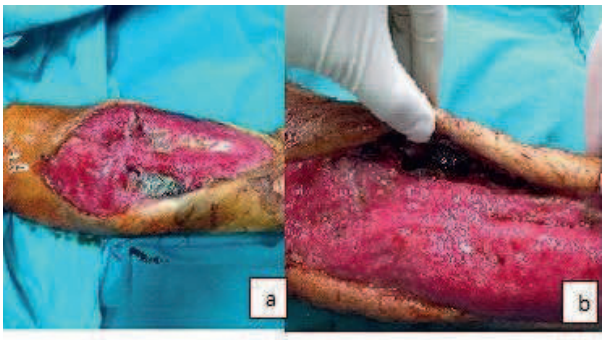

Figure 2a; Before treatment, 1b; After treatment

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In treatment of primary cutaneous Aspergillosis, systemic antifungal therapy and surgical management; Presentation of two cases

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Objectives Aspergillus skin involvement occurs by hematogenous spread or local inoculation. Primary cutaneous aspergillosis that appears with local inoculation is rarely determined on immunocompetent patient. However, it is an important cause of morbidity and mortality in surgical patients. Treatment is controversial and different medical and/or surgical approaches can be applied. Here, two cases of primary cutaneous aspergillosis diagnosed with the growth of *Aspergillus flavus* on tissue samples are presented. In the treatment of patients, voriconazole and surgical treatment was applied together. Different methods were used for the wound care.

Methods Patient 1: 53 years old female, who had a motorcycle accident resulted with left leg injury. She had a wound with a large tissue defect on her left cruris with tibia and fibula fracture. Together with fracture treatment repeated surgical debridement were applied on her wounds that had wide tissue defects. But in 3 weeks of surgical treatment due to the appearance of infected necrotic tissue, the wounds were debrided again for the tissue cultures. *A. flavus* has grown in culture. For the distinction of colonisation and infection, tissue cultures were repeated for twice. *A. flavus* has also grown in both. Fungal spores and hyphae structure branching with narrow angle were demonstrated. Because of having necrotic areas with the continuing infected appearance and growing of *A. flavus* in 3 consequent tissue cultures she was treated with recurrent surgical debridement and iv voriconazole. Antibacterial absorbent polymer dressing - Sorbact absorption dressing; SB was also used for the wound care. After third week of the treatment there was no fungus growth in cultures. Reconstruction was made with the skin graft taken from lateral side of right thigh. Voriconazole therapy was completed in 12 weeks (Figure 1).

Patient 2: 39-years old male patient applied to emergency service with burn and carbon monoxide intoxication. Because of the development of right forearm, right thigh and cruris compartment syndrome, fasciotomies were performed for both extremities. He stayed in ICU for 16 days. Infected necrotic wounds were debrided for twice. *A. flavus* has grown in the tissue culture. He was treated with voriconazole and tigecycline. Vacuum-assisted closure (VAC) therapy was used for wound care. There was not any growth in the tissue cultures taken in the second week of the antifungal treatment. Following negativity in the tissue cultures, wound was primarily sutured. He was treated successfully with voriconazole for 8 weeks (Figure 2).

Discussion Primary cutaneous aspergillosis may develop at intravenous catheter insertion sites and under adhesive tape dressings and bandages or in cases whose skin integrity is impared. Both of the reported cases are primary cutaneous aspergillosis. It has been thought that slow progressive massive necrosis might be guiding for the opportunistic fungus infection in both cases. In treatment of cases, repeated debridement/ different wound care and systemic voriconazole treatment was administered together. In slow but progressively destructive post-traumatic wound infections, primary cutaneous aspergillosis should be considered in differential diagnosis.

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Fusarium solani isolated from patients with onychomycosis: nail infection potential and biofilm formation ability

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Objective The aim of the present study was to perform an epidemiological study, evaluate the ability of *Fusarium solani* to use the human nail as a single source of nutrients and its potential for biofilm formation.

Methods We first performed an epidemiological study to determine the frequency of *F. solani* in patients with onychomycosis. The study included data from all patients who attended the Teaching and Research Laboratory of Clinical Analysis (LEPAC), Division of Mycology, Universidade Estadual de Maringá (UEM), between January and December 2013 with suspected onychomycosis. This descriptive, retrospective, cross-sectional, observational study was