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Aspergillus and aspergillosis in patients in an intensive care unit with mechanical ventilation

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Objectives: Invasive aspergillosis (IA) is a frequent complication in patients with hematological disease. Besides them, studies have been showing that critically ill patients in intensive care units (ICU), mainly those infected by respiratory viruses such as SARS-CoV-2 are at risk to be colonized by Aspergillus and developing IPS.

Methods: All samples of tracheal aspirate (TA) from patients admitted to the ICU and in mechanical ventilation from July 2020 to June 2021 were included in the study. We performed these different tests to detect Aspergillus spp.: (1) serological culture in Sabouraud Agar, detection by microscope and macroscopic evaluation of the colonies to the identification of Aspergillus section, (2) lateral flow for the detection of Aspergillus Galactomannan (GMB) performed with the cube reader (IMMY Diagnostics, OK, USA), using a cut-off of 1.4 units; (3) quantitative polymerase chain reaction (qPCR) with GoExi Probe qPCR (Promega, Wisconsin, EUA) to amplify the small subunit ribosomal RNA target using the forward (5' FGGTGGAGTGGCTTGTGTGC) and reverse (5' TCTAAGGATGCAGAAGGTG) primers, and the probe (5' TCGGCGCTAAGGCGGCTGGCTGG). Samples presenting the cycle threshold (CT) < 40 were considered positive; DNA obtained from an Aspergillus isolate was used as positive control, and DNA-free water as negative control.

Results: A total of 54 patients were included in the study. Causes of ICU admissions were acute complications (n = 11), COVID-19 (n = 9), severe acute kidney disease (n = 6), tuberuloses (n = 1), and other reasons, including post-surgery, septic shock, severe acute respiratory syndrome, and cardiac problems (n = 4). Aspergillus spp. was isolated in culture of the TA in 50% of the patients (17/34), being 12 Aspergillus section Flavicomp, three Aspergillus section Nigri, and two Aspergillus section Nigri. Eighty-eight patients were positive for GM, and five patients had a positive result in the qPCR assay. Positive aspergillosis was confirmed in 20.6% (7/34), being these patients positive in culture and GM, and three in culture and qPCR. One patient was positive in the three tests. COVID-19-associated aspergillosis (CAPA) corresponded to two of these seven cases. The outcome was death in 13/34 patients, 4 of them (31%) had probable aspergillosis. The other three patients, also diagnosed with probable aspergillosis, were treated with amphotericin B, desoximazole plus micafungin, and survived. The mortality rate was 37.5% (4/11) in the group with and without probable aspergillosis, respectively.

Conclusion: These partial results suggest that aspergillosis can have an important impact in critically ill patients in the intensive care unit of our hospital.

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Massive parallel fungal sequencing on formalin-fixed tissues: development and contribution in integrated histo-molecular diagnosis

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Objectives: The gold standard for distinguishing between colonization and fungal infection, but it does not provide a precise diagnosis of fungal species. Hence, if the culture is negative or if no-specimen is sent to the Microbiology laboratory, the specific species sent to the Pathology department is available. Formalin fixation and paraffin embedding (FFPE) cause DNA damage, making it difficult to perform molecular techniques.

Methods: Pathological review of all cases was performed. DNA extraction from FFPE tissues was optimized by: (1) microdissecting the fungal-rich area on the paraffin block; (2) comparing the efficiency of two DNA extraction kits (Qiagen DNeasy-kit, QIAamp; Maxwell 16 LEVNA-FFPE Purification Kit, Promega, optimized for RNA and DNA extraction), by comparison of Aspergillus fumigatus and Mucor spp. specific PCR results for 50 cases. For 124 other cases, the sensitivity of two primer pairs (ITS4/5 & MITS2A/B) was tested for identification by Sanger sequencing and then MPS. Finally, a histomolecular comparison was performed. This work was funded by the Société Pathologique inter-Fédération de Langue Française (SPLIF).

Results: To optimize extraction, DNA was extracted by both kit from samples of 16 mucormycoses and 14 A. fumigatus infections. PCR sensitivity was better with the QIAamp extraction kit [100% (10/10)] compared to the Promega kit [86.7% (26/30)].

PCR amplification of fungal DNA from an additional 124 FFPE tissues was performed. The primer pair ITS5/4 and MITS2A/B, allowed: (1) identification by Sanger sequencing-histopathological analysis in 58.7% (40/68) of the cases in total, and more specifically 33.3% (40/124) of the cases with the ITS4/5 primers and 52.3% (40/124) of cases with the MITS2A/B primers, and (2) identification by aggregated SNP-histopathological analysis in 77.5% (95/124) of all cases (primers ITS4/5 and MITS2A/B), and more specifically 66.1% (62/94) for ITS4/5 and 62.1% (77/124) for MITS2A/B (both primer pairs did not discriminate the same fungal genera/species). The combination of all results from Sanger sequencing and MPS led to fungal identification in 79.8% (95/124) of cases. In total, the addition of NGS to Sanger sequencing increased the diagnostic proportion by 36.3% [95/124, P < 0.001]. Example of integrated histomolecular diagnosis (Fig. 1): patient with a pseudomembranous presentation of pulmonary invasive aspergillosis (b: thoracic CT scan, b: macroscopic examination of lesion after formalin fixation; C, D, E: Histology slides paraffin, x20: b: observation of a necrotic mass caused by hyalohyphomycetes; F: Electron microscopy, x4000: b: no culture or molecular identification available on fresh tissue. In contrast, identification by MPS on FFPE tissue was compatible with morphological analysis: Aspergillus section Flavus, leading to the integrated histomolecular diagnosis of invasive pulmonary aspergillosis.

Conclusion: The development of the fungal MPS on FFPE tissues is innovative and unprecedented for the advancement of an integrated histomolecular diagnosis in fungal pathology. It increases significantly the diagnostic proportion by 36.3%. This strategy can be used in hospitals and could improve patient management, especially when no sample is sent to the Mycology laboratory or when the culture is negative.
Identification of Cryptic species of Aspergillus using Beta-tubulin gene in a tertiary care center in South India

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Objectives: To identify cryptic species of the genus Aspergillus using Beta-tubulin gene by sequence typing.

Methods: Aspergillus genomes from various clinical samples (Ear swab, Bronchial wash, Endotracheal Aspiration, Paramaxil sinus, BAL, Sputum) were subcultured on Sabouraud’s Dextrose Agar/Tween.0.9% Agar.

DNA Extraction: Using phenol-chloroform method. DNA was extracted and purified directly from 4-day-old cultures and used as a template for polymerase chain reaction (PCR) amplification.

DNA Amplification: A fragment of the target gene were amplified using PCR. The primer pairs used for Beta-tubulin gene were Bt2a (Forward primer) and Bt2b (Reverse primer).

Bt2a F: GGAACCAATCGTGCTGCTTTC
Bt2b R: ACCCTCGTGTGCTGACCCCTTGGC

DNA Purification: The PCR product was purified with multi-screen filter plates.

DNA Sequencing: The purified DNA product was subjected to agarose gel electrophoresis and specific band formation was observed for the species of Aspergillus.

DNA Sequencing: The purified product will be used as a template for sequencing. An applied biotechnology 3730 sequencer will be used to obtain DNA sequences.

Phylogenetic Analysis: Parsimony analysis of individual and combined matrices will be conducted using PAUP version 4.0b10 software. Parsimony analysis by the maximum-likelihood (ML) and Neighbor-joining methods.

Results: 40 Aspergillus isolates were collected and identified using Phenotypic methods.

The DNA extraction and PCR amplification were done and sent for Sequencing. The results of Sequence typing are awaited.

Conclusion: Cryptic species are morphologically indistinguishable forms of Aspergillus and their identifications can be confirmed exclusively by using molecular techniques which have led to the description of previously unknown or rare species among different Aspergillus species complex. Using Phylogenetic analysis of individual and combined matrices will be conducted using PAUP version 4.0b10 software.

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DeFungi: direct mycological examination of microscopic fungi images

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Objectives: To classify five fungal types using two different deep learning approaches and three different convolutional neural network models: VGG16, Inception V3, and ResNet50.

Method: A mycological laboratory in Colombia donated the images used for the development of this research work. They were manually labeled into five classes and curated with subject matter expert assistance. The images were later cropped and modified with automated scaling routines to produce the final dataset.

Results: We present experimental results classifying five types of fungi using different deep learning approaches and three different convolutional neural network models: VGG16, Inception V3, and ResNet50. The first approach benchmarks the classification performance of the models trained from scratch, while the second approach benchmarks the classification performance using pre-trained models based on the ImageNet dataset. Using 4-fold cross-validation testing on the 5-class dataset, the best performing model trained from scratch was Inception V3, reporting 73.5% accuracy. Likewise, the best performing model using transfer learning was VGG16, with 85.04% accuracy.

Conclusion: The statistics provided by the two approaches create an initial benchmark to encourage future research work to improve classification performance. Furthermore, the dataset built is published on Kaggle and GitHub to encourage future research.