Semi-quantitative cryptococcal antigen rapid test (CryptPS, Bionyx®) for cryptococcal meningitis in patients living with HIV in Sub-Saharan Africa: prospective multicenter diagnostic accuracy study (DREAMM)

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Background: Cryptococcal meningitis (CM) remains a leading cause of HIV-related meningitis in Africans low- and middle-income countries (LMICs), causing 15%-20% of HIV-related deaths. Rapid Diagnostic Tests (RDTs) are powerful tools and key to speeding-up the diagnosis at the bedside, allowing for rapid and targeted treatment, especially in LMICs. For the past 10 years, Cryptococcal Antigen (CrAg) RDTs have a major role in CM management.

During Reduced ARV-Meaning-Exephalomyie Mortality (DREAMM) was a multicenter implementation science study and a capacity-building project to reduce the mortality of HIV-related central nervous system infections (CNS). One of the main DREAMM approaches was to improve the diagnosis of CNS infections at the bedside and in parallel in local laboratories.

Within DREAMM, HIV-infected adult people living with HIV (>18 years old) with suspected CNS infections were recruited in five hospital sites in Cameroon, Malawi, and Tanzania.

Objective: Our objective was to evaluate the implementation of CrAg CryptPS (Bionyx, Billich-Graffenstaden, France), a new semi-quantitative RDT, in routine case settings in Sub-Saharan Africa.

Methods: All CrAg CryptPS performed were compared to the reference CrAg lateral flow assay (InnoTest®). The evaluation was done by the local research teams in four DREAMM laboratories sites. CrAg CryptPS implementation was evaluated in 301 plasma samples and 258 cerebrospinal fluid (CSF) samples from 520 participants (patients diagnosed with cerebral toxoplasmosis did not have a lumbar puncture). In this analysis, the results will be considered in a binary way (positive/negative).

Results: Between January 2018 and March 2021, 316 participants were prospectively enrolled with suspected HIV-related CNS infections, including CM, tuberculosis meningitis, central toxoplasmosis, and bacterial meningitis cases. Cryptococcal meningitis was the leading cause of CNS infections in Malawi and Tanzania with 44.3% (138/316) and 59.6% (190/316) cases respectively, and the second cause of Cysticercus with 40.1% (127/316) cases after cerebral toxoplasmosis.

In plasma, CryptPS sensitivity was 99.23% (95% CI, 0.98–1.00) and specificity was 94.15% (95% CI, 0.91–0.98), positive and negative predictive values were 92.8% and 99.4%, respectively. In CSF, the sensitivity and specificity of CryptPS were 100% (95% CI, 0.00–1.00), and 99.26% (95% CI, 0.98–1.00), respectively, positive and negative predictive values were both 100%. A low number of false-positives were observed (<4% in plasma and <0.1% in CSF).

Conclusion: CryptPS was evaluated in a context of hospitalized patients within a project including all causes of HIV-related CNS infections, not only CM. The sensitivity and specificity of CryptPS calculated in these preliminary results are promising. Semi-quantitative CryptPS has the potential to be used to tailor antifungal therapy but further optimizations need to be done prior to large-scale implementation in African LMICs. In addition, further work to determine CrAg antigen titres is planned, in the perspective to optimize treatment of CrAg positive cases who decline lumbar puncture.

PM2

Estimation of the detection limit of extracted Candida DNA from spiked EDTA blood sample

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Background: Candidaemia caused by genus Candida, are opportunistic pathogens with life-threatening infection in immune-compromised individuals with a mortality rate being 40%-64% in patients. Blood culture is not a reliable and rapid method for the diagnosis of candidaemia as its sensitivity is as low as 50%. Molecular diagnosis is an alternative to conventional culture for early detection of candidemia. An appropriate DNA isolation technique is needed to obtain high purity DNA from blood specimen and improve the sensitivity of the polymerase chain reaction (PCR). In this study, two different methods were used to extract Candida DNA from spiked EDTA blood specimens, the In-house phenol-chloroform and potassium acetate method.

Objectives:
DNA extraction from the spiked blood samples
Quantification of the Candida DNA extracted from the spiked sample
Comparison of the extracted DNA by phenol-chloroform and potassium acetate method

Methods: A total of 5 ml of EDTA Blood samples from healthy volunteers were spiked with 104-106 Candida albicans cells (ATCC 9026) to determine the detection limit of our extraction method. DNA was extracted from whole blood using phenol-chloroform and the potassium acetate methods which involves precipitation with phenol-chloroform, isopropanol and bung fiss buffer. DNA from C. albican were amplified using ITS1 and ITS4 based primers PCR products were visualized agarose gel electrophoresis.

Results: The spiked Candida DNA samples were from 104-105 yeast cells. The total extracted DNA by phenol-chloroform extraction method ranged from 38.29 to 51.44 ng/µl respectively. Whereas the total extracted DNA from potassium acetate extraction method ranged from 35.58 to 52.30 ng/µl respectively. The presence of a 535 base pair (bp) product was considered positive (Fig. 1 and 2). The lower detection limit of PCR has been determined at 156-164 Candida cells for our spiked samples.

Conclusion: In our study, till date, the extracted quantity of DNA was found to be higher by phenol-chloroform method as compared to the potassium acetate method. Greater number tests and processing are required to obtain conclusive data.
Potassium Acetate

DNA ladder

535 bp

10^7

10^8

PC

NC

P453
Aspergillus and aspergillosis in patients in an intensive care unit with mechanical ventilation

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Intensive Pulmonary Aspergillosis (IPA) is a relevant opportunistic disease among neutropenic patients with hematological disease. Besides them, makes have been showing that critically ill patients in intensive care units (ICU), mainly those infected by respiratory viruses such as SARS-CoV-2 are also at risk to be colonized by Aspergillus and developing IPA.

Objectives: We aimed to evaluate the detection of Aspergillus in tracheal samples from patients in an ICU on mechanical ventilation from a tertiary hospital from Southern Brazil, and its relationship with their outcomes.

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 three different tests to detect Aspergillus spp.: (1) cyto- logical culture in Sabouraud Agar Dextrose, with macrom and microscopic evaluation of the colonies to the identification of Aspergillus section; (2) lateral flow test for the detection of Aspergillus Galactomannan (GM) performed with the cube reader (IMMY® Diagnostics, OK, USA), using a cut-off of 2.4 (units); (3) quantitative polymerase chain reaction (qPCR) with GoqPCR Probe qPCR (Promega, Wisconsin, USA) to amplify the small subunit ribosomal RNA target using the forward (5′-TGTGGTGGGTGATGGTTCTG-3′) and reverse (5′-TCTAGGGAATACAGACAC-3′) primers, and the probe (5′-TGGGCTCTAATAAAGGCTGCG-3′). 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. Probable aspergillosis was defined in these cases that presented positive results to at least, two of these two tests.

Results: A total of 34 patients were included in the study. Causes of ICU admissions were admission complications (n = 11), COVID-19 (n = 9), severe acute kidney disease (n = 6), sepsis (n = 5), 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 Fumigati, three Aspergillus section Flavi, and two Aspergillus section Nigri. Six out of eight patients were positive for GM, and five patients had a positive result in the qPCR assay. Probable aspergillosis was confirmed in 26.6% (9/34), 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 (ASA) corresponded to two of the seven cases.

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

P454
Massive parallel fungal sequencing on formalin-fixed tissues: development and contribution in integrated histo-molecular diagnose

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

The objective was to develop and evaluate the contribution of massive parallel sequencing (MPS) to FFPE tissues.

Methods: Histo-pathological review of all cases was performed. Then, DNA extraction from FFPE tissues was optimized by: (1) macrodissection of the fungal-rich area on the paraffin block; (2) comparing the efficiency of two DNA extraction kits (QiHarry, DNAcleaner-kit, QIAGEN; Maxwell 16 LEVYNA FFPE Purification kit, Promega) for RNA and DNA extraction, by comparison of Aspergillus fumigatus and Mucor specie PCR results for 30 cases. For 124 other cases, the sensitivity of two primer pairs (ITS5/4 and MITS2A/B) was tested for identification by Sangar sequencing and then MPS. Finally, a histomolecular comparison was performed. The work was funded by the Société Pathologique Internationale de Langue Francaise (SPIFL) .

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

PCR amplification of fungal DNA from an additional 124 FFPE samples was performed. The primer pairs ITS5/4 and MITS2A/B, allowed (1) identification by Sangar sequencing histopathological analysis in 58.7% [49/84] of the cases in total, and more specifically 33% (40/124) of cases with the ITS5/4 primers and 32.3% (40/124) of cases with the MITS2A/B primary, and (2) identification by integrated NGS histopathological analysis in 75% [95/124] of all cases (primers ITS5/4 and MITS2A/B), and more specifically 66.5% (82/124) for ITS5/4 and 62.1% (77/124) for MITS2A/B (both primer pairs did not decouple the same fungal genera/species). The combination of all results from Sangar sequencing and MPS led to fungal identification in 79.8% (98/124) of cases was total. In the addition of NGS to Sangar sequencing increased the diagnostic proportion by 36.3% (49/124, P < 0.001). Example of integrated histomolecular diagnostic (Fig. 1): patient with a pseudomembranous presentation of pulmonary invasive aspergillosis (b: thoracic CT scan), F. monocytogenes examination of lesion after formalin fixation; C, D, E. Histoplasma capsulata, a200 and w400: observation of a necrotic mass caused by histoplasmosis, F. Kryptos, a500 with no culture or molecular identification available on fresh tissue. In contrast, identification by MPS on FFPE tissue was compatible with histological analysis: Aspergillus section Fumigati, leading to the integrated histomolecular diagnosis of invasive pulmonary aspergillosis.

Conclusion: The development of the fungal MPS on FFPE tissues is innovative and unexplored 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.