Comparison of clinical and biological characteristics of HIV-infected patients presenting *Cryptococcus neorformans* versus *C. curvatus/C. laurentii* meningitis

B. ZONO MD[1, 5], M. MOUTSCHEN PhD[2], H. SITUAKIBANZA PhD[3], R. SACHELI PhD[4, 5], P. KABUTUTU PhD[1], G. MVUMBI PhD[1], MP. HAYETTE PhD[4,5]

[1]Molecular Biology Service, Basic Sciences, Faculty of Medicine, University of Kinshasa, Kinshasa, DRC; [2]Infectious Diseases and General Internal Medicine Department, University Hospital Center of Liege, Liege, Belgium; [3]Infectious Diseases Service, Internal Medicine/Tropical Medicine, Faculty of Medicine, University of Kinshasa, Kinshasa, DRC; [4]National Reference Center for Mycosis, University Hospital Center of Liege, Liege, Belgium; [5]Center for Interdisciplinary Research on Medicines, University of Liege, Liege, Belgium.

Background

- Cryptococcal meningitis (CM) is mainly caused by *Cryptococcus neoformans/C. gattii* complex. [1]
- Long been considered as saprophytic and non-pathogenic to humans, non-*neoformans* and non-*gattii* *Cryptococcus* species have been associated to cryptococcal infections in recent years. [2-3]
- The interest of *Cryptococcus spp.* identification at the species level is based on the fact that some species, such as *C. gattii*, cause infections that require a more intensive therapeutic approach for their management. [4]

Research hypothesis and objectives

- The clinical and biological characteristics of HIV-infected patients (HIVIP) with meningitis due to *C. neoformans* (*Cn)* could be different from those of patients infected by *C. curvatus/C. laurentii* (*Cc/Cl*).
- The objective of this study was to compare strains molecular identification, biological and clinical characteristics, and antifungal susceptibility profile of isolates from HIVIP with *Cn* versus *Cc/Cl* meningitis.

Design and Methods

- This is a comparative analytical study.
- Patients were drawn from a cross-sectional study conducted in three Kinshasa hospitals (DRC) between 2019 and 2020, having included HIVIP with meningeal syndrome.
- Apart from patients' clinical data, the following analysis were performed and the results were compared in both groups :

○ CSF first comparative analysis

Fig.1: Cryptococcal antigen test

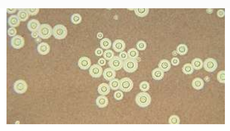

Fig.2: India ink staining

Fig,3: Culture on SDA-C

○ Characterization analysis

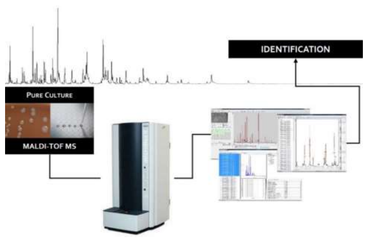

Fig.4: MALDI-TOF MS

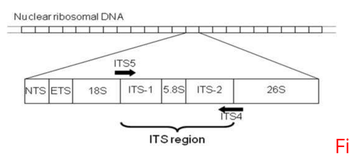

Fig.5: ITS region sequencing

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Fig.6: In vitro antifungal susceptibility procedure used

- The " *Cryptococcus* species group identified " was the mean outcome variable and was compared to other variables of the same type using the Pearson chi-square test or the Fisher exact test.
- Ethics Committee approval number : ESP/CE/071/2019 (Public Health School of the Faculty of Medicine of the University of Kinshasa, DRC).

Results

- Out of 29 HIVIP included for cryptococcal meningitis with CSF positive culture, six had *Cc/Cl* meningitis (20.7%, 95% CI: 6.9 - 34.5%).
- Among them, five were infected by *Cc*, and one *Cl* infection.

Fig.7: *C. Curvatus* beige mucoid colonies with reddish hues on SDA-C after 48-hour incubation

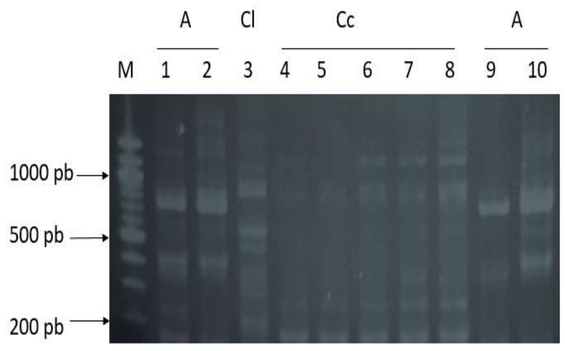

Fig.8: Atypical DNA fragments of serotype A *Cn*, and *Cc/Cl* isolates from multiplex PCR amplification of LAC1 and CAP64 genes

Fig.9: One of the HIVIP with CM included in the study, in the induction phase of antifungal treatment.

Tab: Clinical Characteristics and microbiological identification of HIVIP with *Cn versus Cs/Cl* meningitis

Variable	Cryptococcal species*		*p*
	C. neoformans **n** (%) ***	***C. curvatus/C. laurentii*** **n** (%) ***	
Demographic characteristics			
Age, mean (year) ± SD	36.7 ± 11.3	38.3 ± 9.1	0.1
Female sex	15 (65.2)	4 (66.7)	1
WHO HIV clinical stage IV	21 (100)	4 (80.0)	0.19
Clinical signs			
Headache	23 (100)	3 (50.0)	**0.005**
Meningeal signs	7 (30.4)	0	0.28
Visual disturbances	0	2 (33.3)	**0.03**
Microbiological analysis			
MALDI-TOF MS *Cryptococcus spp.*	23 (100)	4 (80)	0·1
Multiplex PCR serotype A	23 (100)	0	**<0.0001**
Positive India ink on CSF	18 (78.3)	2 (33.3)	0.06
CrAg present on CSF	22 (95.7)	4 (66.7)	0.09
Mean Glycorrachia (mg/dl) ± SD	46.7 ± 11·2	66.1 ± 22.5	**0.04**

*ITS sequencing identification
** with available data
***percentage of columns calculated for each group

- A total of 23 *Cn* meningitis cases versus 6 *Cc/Cl* meningitis were diagnosed using ITS sequencing.
- A higher proportion of *Cn* strains (91.3%) showed a low Minimum Inhibitory Concentration (MIC) (<8mg/l) for fluconazole compared to *Cc/Cl* strains (66.7%).
- *Cc/Cl* strains resistant to 5-flucytosine and amphotericin B were found in 16.7% of cases for each of the two antifungal agents.

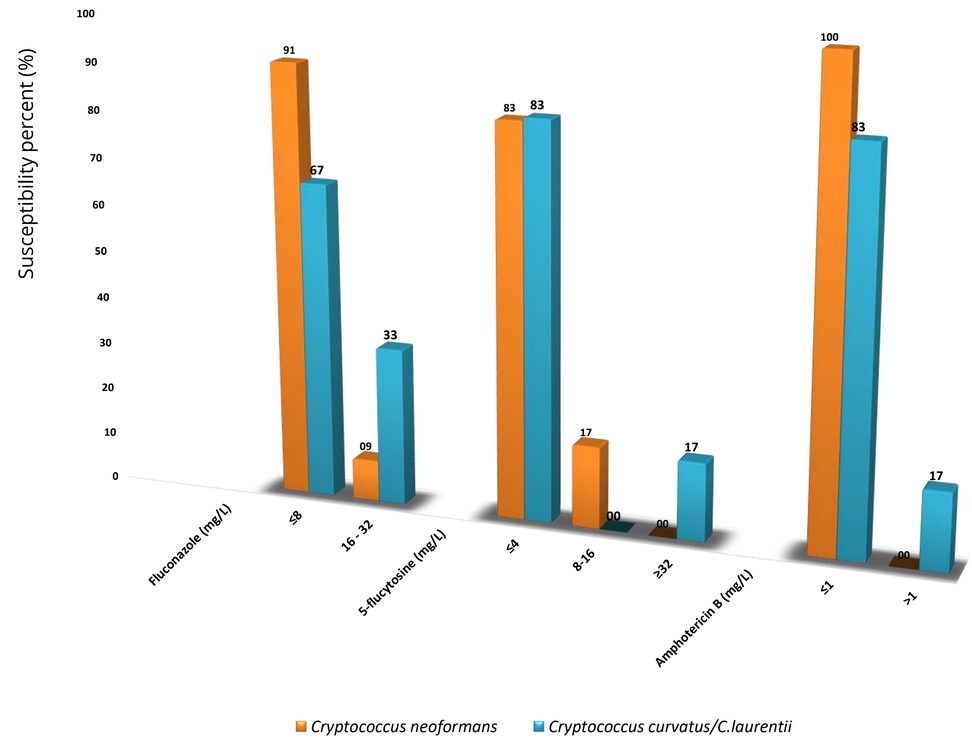

Fig.10: Comparative susceptibility of *Cn* versus *Cc/Cl* strains to usual antifungal agents by EUCAST procedure

- After treatment with amphotericin B, 5-flucytosine and fluconazole in both groups, the outcome was similar.

Conclusion

- Clinical presentation of *Cn* meningitis is certainly more severe than that of *Cc/Cl* meningitis, but *Cc/Cl* infection should be considered in the management of HIVIP with meningeal syndrome because of the diagnostic difficulty and the high MICs of antifungal agents required for the treatment of meningitis due to these cryptococcal species.

For more information: bive.zono@unikin.ac.cd

References

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