CSF Cytokines And Chemokines Associated With Mortality In Cryptococcal Meningitis

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Modified Abstract:

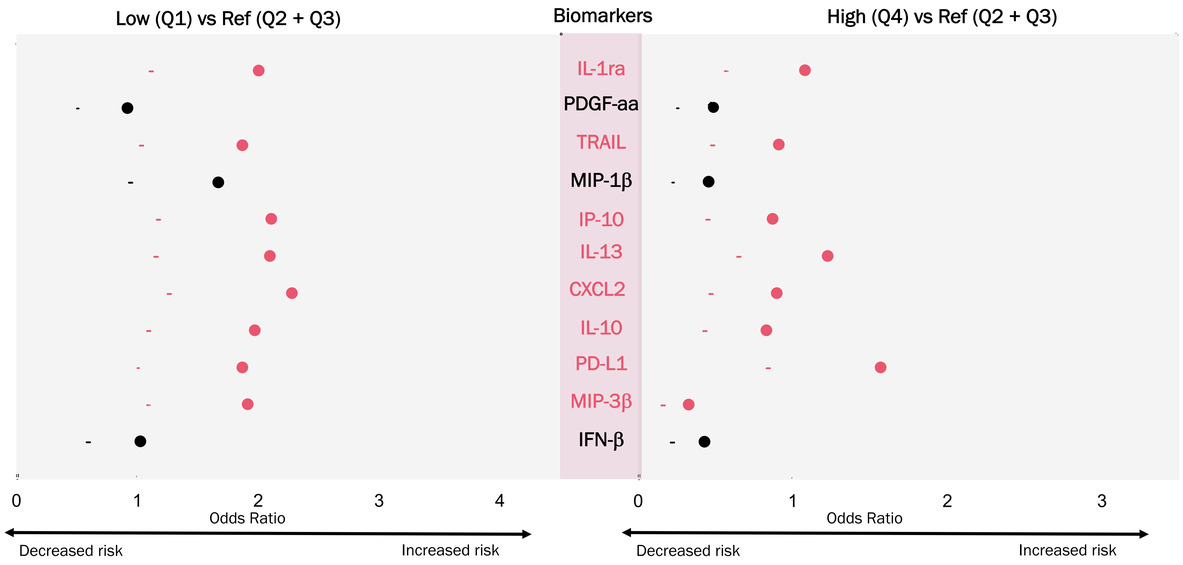
Background: Cryptococcal meningitis causes substantial mortality globally, with a one-year mortality rate of 70% in Sub-Saharan Africa.[1] Our understanding of the role of the host immune system in patient outcomes is limited. Induction therapy of antifungal treatment is a crucial period for decreasing fungal burden and decreasing mortality. We investigated the cytokine and chemokine environment at the site of infection, the CNS, to better understand the impact of immune cell activation and tissue inflammation on 14-day mortality.

Methods: We prospectively enrolled Ugandans presenting with first episode cryptococcal meningitis from March 2015 to May 2017 , as part of a larger study. [2] We analyzed the CSF of 337 subjects at diagnosis for soluble biomarkers by Luminex assay. Biomarkers were analyzed as a continuous variable (log_2 transformed) via logistical regression to determine association with 14-day mortality. We compared by quartiles, Low (Q1) and High (Q4) to the reference group (Q2+Q3). Significant values are displayed in modified forest plots after adjusting for Glasgow coma score (GCS) and *Cryptococcus* quantitative culture. Significance was set at a p-value of 0.05.

Results: Biomarker analysis as a continuous variable revealed that low levels of several pro-inflammatory and cytotoxicity associated molecules increased the risk of 14-day mortality. Further quartile analysis revealed that participants with Low (Q1) levels of markers of cytotoxicity associated molecules such as TRAIL and IP-10 had significantly increased risk of 14-day mortality compared to levels of the reference group (Q2+Q3). Participants with Low (Q1) levels of markers associated with naïve T-cell polarization and recruitment such as IL-13, IL-10, CXCL2, PDL1, and MIP-3b also had increased risk of 14-day mortality. Inflammatory mediators such as TNF-α, IFN-γ, IL-6, and IL-1β were not associated with 14-day mortality in quartile analysis.

Conclusion: There is a crucial role for inflammation, through both innate and adaptive immune responses, and cytotoxic cell populations in human cryptococcal outcomes. Further research efforts should include: characterizing the role of cytotoxic cells in *Cryptococcus* clearance, activating stimuli to induce cytotoxicity, and T-cell function in activation of the adaptive immune response in humans with cryptococcal meningitis.

Results: Modified Forest Plot of Biomarkers by Quartile (adjusted for GCS and CSF)



Results: Baseline CSF Biomarkers Predicting 14-day Mortality

Biomarker	OR (95% CI)	P-value	Biomarker	OR (95% CI)	P-value	Biomarker	OR (95% CI)	P-value
Innate Immune Response			Adaptive Immune Response			Hemostasis and Proliferation		
IL-6	**0.90 (0.83, 0.98)**	**0.015**	**IL-12**	**0.85 (0.76, 0.97)**	**0.012**	TGF-a	0.95 (0.83, 1.07)	0.381
MCP-1	0.96 (0.80, 1.14)	0.630	IL-15	1.09 (0.91, 1.31)	0.336	EGF	0.99 (0.90, 1.09)	0.819
MIP-1a	0.88 (0.75, 1.03)	0.125	IFN-g	0.93 (0.84, 1.04)	0.200	PDGFaa	0.91 (0.74, 1.12)	0.356
MIP-1b	**0.81 (0.66, 0.98)**	**0.030**	**TNF-a**	**0.88 (0.79, 0.99)**	**0.030**	PDGFab	0.96 (0.89, 1.03)	0.259
IL-8	0.91 (0.81, 1.02)	0.112	IL-4	0.97 (0.89, 1.06)	0.526	VEGF	1.09 (0.89, 1.33)	0.426
MIP-3a	0.92 (0.80, 1.05)	0.205	IL-2	0.89 (0.78, 1.03)	0.110	FGFbasic	0.96 (0.85, 1.09)	0.520
GM-CSF	0.86 (0.73, 1.03)	0.105	**IL-5**	**0.89 (0.80, 0.98)**	**0.022**	Flt3Ligand	0.98 (0.78, 1.24)	0.886
G-CSF	0.96 (0.86, 1.08)	0.520	IL-10	0.86 (0.70, 1.04)	0.125			
IL-1b	0.90 (0.80, 1.02)	0.099	IL-13	0.87 (0.75, 1.01)	0.064			
MIP-3b	**0.84 (0.74, 0.96)**	**0.009**	IL-33	0.97 (0.91, 1.03)	0.319			
IFN-a	0.94 (0.85, 1.06)	0.319	**Granzyme B**	**0.88 (0.81, 0.97)**	**0.007**			
IFN-b	**0.89 (0.80, 0.98)**	**0.020**	TRAIL	0.91 (0.82, 1.01)	0.077			
Fractalkine	0.91 (0.79, 1.06)	0.248	**IL-17a**	**0.90 (0.83, 0.99)**	**0.026**			
IL-25	0.93 (0.85, 1.02)	0.113	IL-7	1.05 (0.91, 1.21)	0.527			
IL-1ra	0.89 (0.78, 1.01)	0.067	PDL1	0.91 (0.79, 1.05)	0.200			
IL-1a	**0.87 (0.78, 0.98)**	**0.017**	IL-3	1.03 (0.95, 1.12)	0.492			
Eoxtaxin	0.88 (0.76, 1.00)	0.056	RANTES	0.92 (0.82, 1.04)	0.166			
CXCL1	0.92 (0.83, 1.02)	0.099	**IP-10**	**0.84 (0.73, 0.97)**	**0.017**			
CXCL2	0.92 (0.82, 1.03)	0.126	CD40L	0.91 (0.82, 1.02)	0.102			

Description: Raw biomarker levels were log transformed and then analyzed by logistical regression as a continuous variable. Table displays Odds Ratio (OR) where highlighted (pink) cytokines and chemokines were significantly associated with risk of 14-day mortality. Interleukin (IL), Interferon (IFN), Tumor- necrosis factor (TNF), Granulocyte-colony stimulating factor (G-CSF), Granulocyte-macrophage colony stimulating factor (GM-CSF), Monocyte chemoattractant protein-1/CCL2 (MCP-1), Inflammatory protein (IP), and Macrophage inflammatory protein (MIP)

Focused Demographic Data:

- A total of 253 subjects (75.1%) survived 1st episode CM at 14-days, while 84 subjects (24.9%) died within 14-days of diagnosis.
- 132 subjects (39.2%) were female and the median age was 35 years in subjects who survived at 14-days and those that died within 14-days of diagnosis.
- Subjects who died within 14-days of diagnosis had a significantly lower hemoglobin (p=0.01) along with a significantly higher CSF quantitative *Cryptococcus* culture (p=0.02) and GCS (p<0.01) compared to those who survived through 14-days.
- There was no significant difference in opening intracranial pressure, total CSF white blood cells count, ART usage at CM diagnosis, or CD4 count between those who survived and those who died within 14-days of CM diagnosis.

Future Directions:

- Characterizing the activating stimuli and targeted killing capacity of cytotoxic cells (CD8 T-cells and Natural Killer cells) against *Cryptococcus in vitro* and with *in vivo* mouse models.
- Determine exhaustion state of cytotoxic cells and identify potential immunomodulating agents to restore cytotoxic function.
- Determine exhaustion state of cryptococcal specific T-cell, by tetramer pull down, involved in releasing inflammatory mediators.

Notes:

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Conflict of interest: No relevant conflict of interest – all authors.

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