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Serum Biomarkers Following Induction Therapy for Cryptococcal Meningitis Predict Survival

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Abstract

Background: In the Cryptococcal Optimal ART Timing (COAT) trial, early ART initiation (7-11 days) after cryptococcal meningitis was associated with higher mortality compared with deferred (5 weeks) ART initiation. A significant proportion of deaths (70%, 49/70) occurred after completion of 14 days of amphotericin induction therapy. We hypothesized that serum biomarkers may enable risk stratification of short and long term mortality.

Methods: 177 COAT subjects were randomized in Kampala and Mbarara, Uganda and Cape Town, South Africa. 156 participants survived to the end of 14 days of amphotericin therapy of which 151 had 22 serum biomarkers profiled using ELISA or Luminex multiplex assay. We compared by logistic regression log_2-transformed biomarkers versus 5 week survival (i.e. time of outpatient clinic registration) and 46 week survival, adjusting for randomized arm (early vs. deferred ART).

Results: Of 151 persons who survived 14 days after cryptococcal meningitis diagnosis, 121 (80%) survived for 5 weeks until time of outpatient clinic registration and 104 (69%) survived >1 year. Twelve serum biomarkers were associated with increased risk of mortality. Odds Ratio (OR) for mortality for each two-fold increase in serum biomarker is presented in **Table 2**. Serum CRP was a consistent risk factor for short and long term mortality. Additionally, when CRP was measured at 21 days after diagnosis (2 weeks after randomization), CRP was associated with mortality between 3-5 weeks (OR 2.22 per 2-fold increase (95%CI: 1.35, 3.66, P=.0002) as well as mortality through one year (OR 1.45 per 2-fold increase, 95% CI: 1.08, 1.95; P=.01).

Conclusion: Serum biomarkers at the end of amphotericin induction therapy may enable risk stratification of short and long term mortality, most notably including CRP, a commonly available routine lab test. By enabling risk stratification, closer clinical follow up may be wise for high risk individuals.

Background

- Cryptococcal meningitis remains a significant cause of death amongst HIV-infected individuals in sub-Saharan Africa.[1,2]
- In the COAT trial, early ART initiation (7-11 days) after cryptococcal diagnosis was associated with higher mortality compared with deferred (5 weeks) ART initiation.[3]
- A significant proportion of cryptococcal deaths occur following induction therapy, and the results of COAT suggest that this late mortality may be an immune-driven process.
- We hypothesized that serum biomarkers may enable risk stratification of short and long term mortality.

Study Population

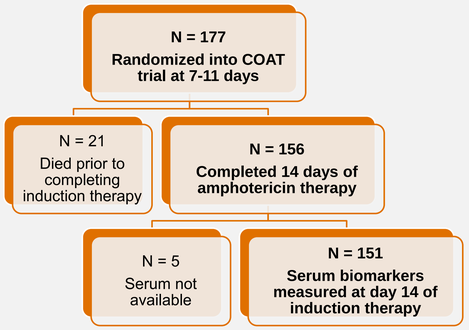


Figure 1: Analysis Cohort:

- 177 HIV-infected, ART naïve adults with cryptococcal meningitis enrolled into COAT study in Kampala, Uganda, in Mbarara, Uganda, and in Cape Town, South Africa.
- 151 survived 14 days of amphotericin 0.7-1.0 mg/kg/day with fluconazole 800mg/day induction therapy and were analyzed.

References

1. Park BJ et al. AIDS. 2009 Feb 19;23(4):525–30.
2. Jarvis JN et al. AIDS. 2009 May 31;23(9):1182–3.
3. Cryptococcal Optimal ART Timing Trial. CROI 2013 Abstract: S-104

Methods

- Blood was collected upon completion of 14 days of amphotericin therapy and excess serum was stored at -80°C and shipped on dry ice.
- Available cryopreserved serum samples were thawed and analyzed in batches.
- Concentrations of 22 biomarkers were measured using Luminex magnetic bead technology (Bio-Rad) or commercial ELISA kits (R&D Systems) according to manufacturers instructions.
- Logistic regression models were used to compare log_2-transformed biomarkers versus:
  1. 5-week survival
  2. 46-week survival
- Regression model included COAT trial randomization arm (early vs deferred ART)

COAT Trial Participant Baseline Characteristics

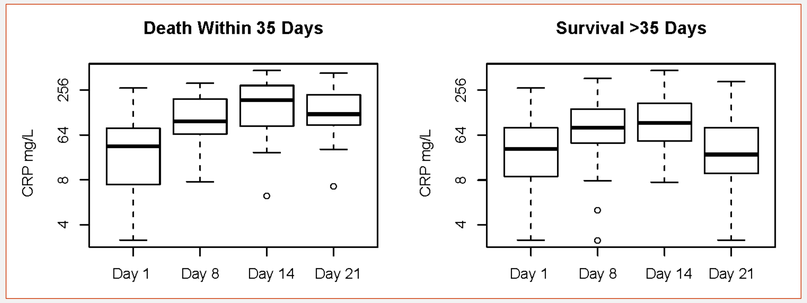
	Early ART (N=88)	Deferred ART (N=89)
Age (years)	35 (28, 40)	35 (30, 40)
Male, N (%)	46 (52%)	47 (53%)
Weight (kg)	53.0 (46.5, 58.8)	54.1 (48.3, 59.0)
Reduced GCS (<15) N (%)	21 (24%)	28 (30%)
Duration of headache (days)	14 (7, 28)	14 (7, 25)
CSF Opening Pressure (mmH_20)	280 (190, 360)	260 (180, 410)
CSF Quantitative Culture (log_{10}CFU/mL)	5.3 (4.1, 5.7)	4.8 (3.7, 5.5)
CSF White Cells (cells/μL)	9 (4, 85)	25 (4, 110)
On TB treatment	17 (19%)	15 (17%)
CD4 Count, cells/μL	19 (9, 69)	28 (11, 76)
HIV RNA, log_{10}copies/mL	5.5 (5.2, 5.8)	5.5 (5.3, 5.8)

Table 1. Baseline Characteristics By Exposure Status. Medians (IQR) and N (%).

Results

- Of 151 persons who survived 14 days after cryptococcal meningitis diagnosis:
  - 121 (80%) survived for 5 weeks (time of outpatient clinic registration)
  - 104 (69%) survived >1 year.
- Twelve serum biomarkers were associated with increased risk of mortality when measured at completion of amphotericin therapy (**Table 2**).
- Serum CRP was a particularly consistent risk factor for short and long term mortality.
- When measured at 21 days after diagnosis, CRP was associated with:
  - Mortality at 3-5 weeks (OR 2.22 per 2-fold increase (95%CI: 1.35, 3.66, P=.0002)
  - Mortality through 1 year (OR 1.45 per 2-fold increase, 95% CI: 1.08, 1.95; P=.01).

Figure 2. Serum CRP over Time by Survival Status



- Upon completion of 14 days of amphotericin-based induction therapy:
  - CRP levels remained high in subjects that died within 35 days.
  - CRP levels were overall lower and trended down sooner in >35 day survivors.

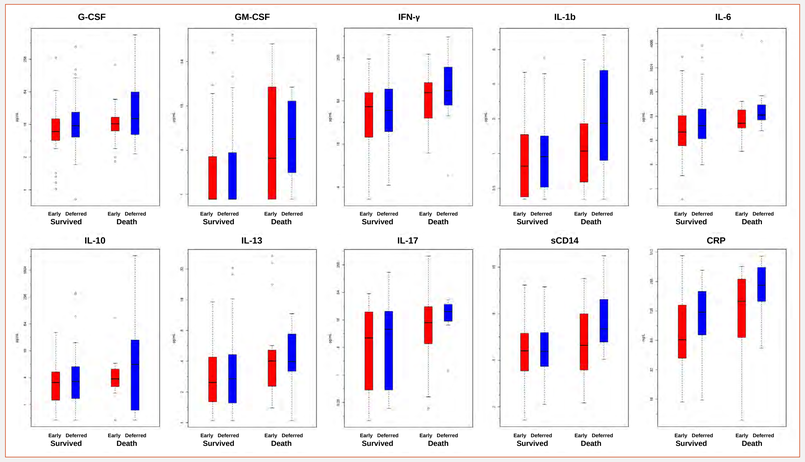
Serum Biomarkers Predicting Mortality

Biomarker (Serum)	5-week mortality Odds Ratio (95% CI) (per 2-fold increase in serum level)	P-value	1 year mortality Odds Ratio (95% CI) (per 2-fold increase in serum level)	P-value
G-CSF	1.31 (0.97, 1.75)	0.07	**1.30 (1.02, 1.66)**	**0.03**
GM-CSF	**1.37 (1.13, 1.68)**	**0.002**	**1.24 (1.05, 1.46)**	**0.01**
IFN-γ	**1.49 (1.10, 2.02)**	**0.01**	1.18 (0.97, 1.44)	0.11
IL-1β	**1.60 (1.08, 2.36)**	**0.02**	**1.44 (1.05, 1.97)**	**0.02**
IL-6	**1.27 (1.04, 1.55)**	**0.02**	**1.24 (1.04, 1.46)**	**0.02**
IL-8	1.23 (0.90, 1.69)	0.2	**1.32 (1.01, 1.71)**	**0.04**
IL-10	**1.29 (1.04, 1.59)**	**0.02**	**1.22 (1.02, 1.46)**	**0.03**
IL-13	**1.54 (1.09, 2.17)**	**0.01**	1.21 (0.92, 1.58)	0.16
IL-17	**1.21 (1.03, 1.43)**	**0.02**	**1.16 (1.02, 1.31)**	**0.02**
MCP-1 (CCL2)	1.25 (0.97, 1.60)	0.08	**1.25 (1.01, 1.55)**	**0.04**
sCD14	**2.43 (1.21, 4.85)**	**0.01**	1.71 (0.98, 2.97)	0.06
CRP	**1.84 (1.23, 2.77)**	**0.003**	**1.53 (1.12, 2.10)**	**0.008**

Table 2. Biomarkers Associated with Mortality through 5 Weeks and 1 Year

- 6 biomarkers (GM-CSF, IL-10, IL-17, IL-6, IL-1b, and CRP) predicted mortality at both 5 weeks and through 1 year. *Odds Ratio are per 2-fold increase in serum level.*
- 3 biomarkers (IFN-γ, IL-13 and CD14) predicted mortality through 5 weeks.
- 3 biomarkers (G-CSF, IL-8 and MCP-1) predicted mortality between 5 weeks to 1 year.

Figure 3. Plots for Biomarkers at Completion of Amphotericin Induction Associated with 5-Week Survival



Boxplots displayed by survival status (35 day) and randomization arm (early ART vs deferred ART).

Conclusions

- Several serum biomarkers following amphotericin induction therapy for *Cryptococcus* are predictive of survival and may enable risk stratification of short or long term mortality.
- CRP, a commonly available routine lab test, appears to be a particularly suitable marker for predicting survival, and could have immediate clinical utility. CRP previously has been shown to be associated with future risk of IRIS.
- By enabling risk stratification with serum biomarkers, closer clinical follow up may be advised for high risk individuals.

Acknowledgements

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