



Serum Inflammatory Biomarkers Predict HIV Immune Reconstitution Inflammatory Syndrome and Death after Cryptococcal Meningitis



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Abstract:

Background: HIV immune reconstitution inflammatory syndrome (IRIS) is an exaggerated inflammatory reaction occurring as the damaged immune system recovers with antiretroviral therapy (ART). Cryptococcal meningitis (CM) is the second most frequent AIDS-defining illness in Sub-Saharan Africa, and IRIS frequently occurs after CM on ART.

Methods: We prospectively evaluated 115 ART-naïve Ugandan subjects with AIDS for 12 months after initiation of ART. 65 subjects had recent CM and 50 subjects had no known active OI. The 50 subjects without an OI were used as a control. Serum cytokines were measured serially over 24 weeks of ART using Luminex multiplex assays.

Results: In subjects with recent CM, IRIS manifested as paradoxical worsening occurred in 51% (38/73). The median time of CM-IRIS was 6 weeks (IQR 4-19; max 29). Eighteen CM subjects died (26%). CM-IRIS was independently associated with death (Odds Ratio (OR)=7.1, 95% CI: 1.7-29.2, P=.006) in a multivariate analysis including CM-status and CD4 count. At the time of IRIS events, levels of CRP, d-dimer, IL-6, IL-1ra, IL-10 were increased in IRIS subjects compared to time-matched controls (P<.05). Elevated pre-ART CRP levels of >32 mg/L (highest-quartile) were independently associated with future death (OR=6.2, 95% CI: 1.8-20.7, P=.003) or IRIS (OR=3.6, 95% CI: 1.1-11.7, P=.03). Higher pre-ART levels of interleukin (IL)-17, C-reactive protein (CRP), and lower TNF-α levels were associated with future IRIS (P<.05). A predictive model of these three biomarkers had 67% accuracy in predicting future IRIS or non-IRIS (sensitivity 64%; specificity 72%).

Conclusions: IRIS is a common early complication of ART in resource-limited regions. Serum inflammatory biomarkers can predict risk for development of cryptococcal IRIS.

This abstract is revised based on continued accrual of data through 1 Feb 2009.

Background:

Immune Reconstitution Inflammatory Syndrome (IRIS) is a paradoxical inflammatory response after initiating HIV antiretroviral therapy (ART) evident by clinical deterioration of active, latent, or previous infections due to an improvement in immune function. Common IRIS scenarios include the "unmasking" of an occult opportunistic infection or the paradoxical symptomatic relapse of a prior infection despite microbiologic treatment success.

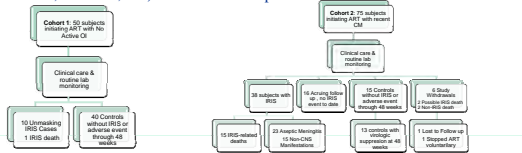
A variety of microorganisms including bacteria, mycobacteria, fungi, and viruses can trigger the inflammatory response of IRIS. Cryptococcal IRIS is particularly problematic due to high mortality. Prediction of IRIS is presently not possible.

Objectives:

1. To discover predictive biomarkers of IRIS in a prospective cohort
2. To discover objective biomarkers for diagnosis of IRIS in a prospective cohort

Methods: Two prospective cohorts of 125 ART-naïve patients in total were evaluated for 48 weeks after initiating ART. Two distinct groups were recruited.

- Cohort 1: 50 Subjects without an active OI were recruited at the Infectious Disease Institute (IDI) clinic in Kampala, Uganda from May 16, 2005 to June 1, 2008.
- Cohort 2: 75 Subjects with recent cryptococcal meningitis (CM) were recruited at Mulago hospital at time of meningitis, initiating ART a median of 5 weeks after CM (Range 3-8 weeks).
- Serial sera was tested for 27 cytokines using a Luminex 100 system (Human 27-Plex Panel (171-A11127) Bio-Rad, Hercules, CA) at the serial time points.



Paradoxical Cryptococcal IRIS Working INSHI Case Definition

1. Prior Cryptococcal disease with clinical improvement after appropriate antifungal therapy and prior to IRIS.
2. Response to anti-retroviral therapy by:
 - a. Receiving HIV anti-retroviral therapy and;
 - b. Virologic response with >1 log₁₀ copies/mL decrease in HIV RNA (if available).
3. Clinical deterioration with an inflammatory condition, such as:
 - a. CNS manifestations: recurrent meningitis or intra-parenchymal cryptococcomas evident by histopathology or imaging.
 - b. Non-CNS manifestations: pneumonitis, lymphadenopathy, or cutaneous or soft tissue lesion(s).
4. Symptoms cannot be explained by:
 - a. Cryptococcal relapse (i.e. positive culture with <4-fold decrease in quantitative culture or CRAG titer)
 - b. Alternative active infection
 - c. Significant ART or antifungal non-compliance

Potential Predictive Biomarkers for future Cryptococcal IRIS

Serum Biomarker	Univariate Odds Ratio	Univariate P-value	Multivariate Odds Ratio	Multivariate P-value
IL-17	1.05 (.90 -1.21)	.51	1.35 (1.02 to 1.80)	.034
CRP	1.30 (1.01-1.68)	.039	1.42 (1.002 to 2.02)	.048
TNF-α	0.78 (.61 -1.003)	.053	0.47 (.28 to .79)	.003
G-CSF	1.18 (.77 - 1.80)	.44	1.70 (.95 to 3.06)	.07

Odds calculated per 2-fold change in cytokine values, log_e transformed. Odds for CRP are per 10mg/L CRP increase.

Predictive Model of pre-ART Serum CRP, IL17, TNF-α, and G-CSF

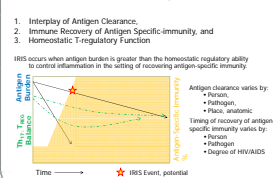
$$y = -1.274 + .353 * CRP + .301 * \log_2(IL17) - .747 * \log_2(TNF-\alpha) + 0.535 * \log_2(G-CSF)$$

Predictive Performance: (y > 0.5 is positive)

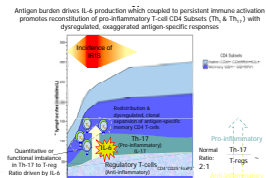
Sensitivity 64%; Positive Predictive Value 78%; Accuracy 67%
Specificity 75%; Negative Predictive Value 68% R² = .40

IRIS Pathogenesis Hypothesis:

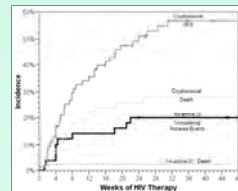
Role of Pathogen



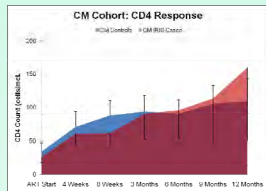
Role of Host



Incidence of IRIS and Death



CD4+ response to ART



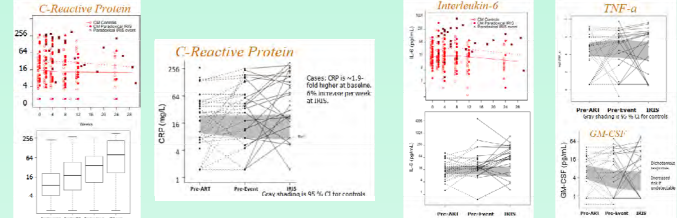
Results:

- IRIS events occurred in 51% (38/75) of persons with prior CM (95% CI: 32-57%).
- 18 CM subjects died (26%), and one subject in the No OI cohort. CM-IRIS was independently associated with death (Odds Ratio (OR)=7.1, 95% CI: 1.7-29.2, P=.006) in a multivariate analysis including CM-status and CD4 count. Mortality: 15/38 with IRIS; 4/37 without definite IRIS.
- The simplest predictive marker for IRIS and death was a pre-ART CRP >32 mg/L (4-fold elevation).
 - Of those with pre-ART CRP > 32mg/L, 74% (14/19) developed IRIS and 53% (10/19) died.
- At time of CM-IRIS events, serum biomarkers of inflammation were elevated in almost all patients, most frequently elevated cytokine at time of IRIS events was IL-6.
- A variety of cytokines were frequently increased by ≥3 SD over time matched controls [table below]; however in modeling over time, fewer cytokines increased at time of IRIS.
- IL-6, CRP, and TNF-α increased at time of IRIS. IL-8 and IP-10 were decreased at IRIS.

Increase Serum Cytokines at time of Cryptococcal paradoxical IRIS Events compared to Time-Matched CM Controls

Biomarker	Controls Time-Matched Geometric Mean pg/mL	IRIS Event Geometric Mean pg/mL	Relative Increase	95% CI %	P-value	Relative Increase prior to IRIS & pg/mL/week (95% CI)	P-value	Summary
CRP (mg/L)	9.2	59.5	548%	530-566	<.0001*	1.06 (1.02-1.09)	.0001*	↑ pre-ART, ↑ IRIS
IL-6	9.8	63.5	543%	527-559	<.0001*	1.04 (1.01-1.08)	.003*	↑ pre-ART, ↑ IRIS
G-CSF	31.1	60.1	93%	90-96	.0008*	1.02 (.99 - 1.04)	.11	↑ pre-ART
d-dimer (µg/mL)	1.9	2.6	54%	20-88	.002*	.99 (.97 - 1.02)	.73	↓ controls 8-24 wks
IL-10	7.4	15.7	110%	84-127	.002*	1.01 (.97-1.03)	.388	
IL-1ra	90.2	294	226%	224-229	.002*	1.04 (1.0 - 1.08)	.07	
IL-9	14.7	43.6	196%	180-213	.005	1.04 (1.0 - 1.08)	.054	
IL-13	6.6	12.4	88%	69-107	.006	1.01 (.99-1.04)	.45	↑ pre, ↑ cases <8 wk
IL-7	14.2	21.9	55%	48-61	.011	1.02 (.99-1.04)	.25	
GM-CSF	3.2	7.6	140%	70-210	.02	1.03 (.99 - 1.07)	.12	
IL-8	17.4	29.6	70%	63-77	.02	.97 (.94 - .99)	.01	↓
IL-2	1.4	2.6	81%	-53-215	.07			~45% undetectable IL-2
IP-10	1460	1775	22%	-28-63	.4	1.06 (1.02-1.11)	.004	↓ IRIS
TNF-α	21.5	22.2	3%	-9-15	.9	1.05 (1.02-1.11)	.004	↓ pre-ART, ↑ IRIS
IL-17	34.1	36.5	7%	-10-24	.9	1.03 (.97 - 1.09)	.38	

The table displays serum cytokines at time of IRIS event compared to cohort time match controls from weeks 4-12 of HIV therapy, corresponding to the interquartile range of the timing of IRIS events. Statistical comparison is via an independent Hotelling test assuming unequal sample variances. All displayed data are based on log_e transformation, and the geometric mean is presented. Relative geometric increase is calculated per the relative risk reduction formula = ((A/Case-Control)/Control). P-value remains statistically significant with Holm-Bonferroni adjustment for multiple comparisons (p<.27). *Relative increase prior to IRIS* is adjusted for CD4 counts. This is calculated via linear mixed effects regression model with a random effect for each subject to account for repeated measures. A join point at 8 weeks allowed different slopes prior to and after 8 weeks. This is the change from the pre-IRIS event specimen to IRIS event.



Conclusions:

- IRIS events following Cryptococcal meningitis occur in ~50% of Ugandans.
- Cytokine profiles at time of IRIS events are varied; however, IL-6 and CRP are commonly increased at time of IRIS events.
- In utilizing IL-17, TNF-α, G-CSF, and CRP, moderate predictive ability may exist for cryptococcal IRIS. These 4 biomarkers correctly predicted 2/3 of patient outcomes.
- ART causes dynamic changes in inflammatory markers. Cytokine analysis is complicated.
- CRP may be a simple objective marker of IRIS and adverse ART outcome.

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