Immunologic Discrimination of Cryptococcal IRIS from Culture-positive Relapse

David R Boulware,[1] Joshua Rhein,[1,2] Edward N Janoff,[3] Andrew Kambugu,[1,2] Kate Birkenkamp,[1] Abdu Musubire,[1,2] Paul R. Bohjanen,[1,2] David B. Meya [1,2]

[1]Division of Infectious Diseases and International Medicine, Department of Medicine, University of Minnesota, Minneapolis, Minnesota; [2] Infectious Disease Institute, Makerere University, Kampala, Uganda; [3] University of Colorado at Denver, Denver, Colorado.

Background:

Immune Reconstitution Inflammatory Syndrome (IRIS) may complicate antiretroviral therapy (ART). Whereas ART promotes HIV viral suppression and CD4+ T cell increases on a population level, the utility of these measurements as objective diagnostic criteria for IRIS remains unclear for an individual. We determined whether CD4, plasma HIV RNA, or CSF cytokines measurements are diagnostically useful to help differentiate paradoxical IRIS from culture-positive relapse.

Methods:

- In prospective cohorts of HIV-infected Ugandans with cryptococcal meningitis from 2006-2012, 70 persons had 75 recurrent symptomatic meningitis episodes, due to paradoxical IRIS (n=62) or culture-positive relapse (n=13).
- IRIS was defined as per the INSHI consensus case definition, with CSF culture status being the primary factor distinguishing culture-negative paradoxical IRIS from culture-positive relapse.
- We measured CSF cytokines using Luminex cytokine assays (Bio-Rad), per manufacturer instructions.
- We compared CD4 count change, plasma HIV viral load change, and CSF cytokines (n=50) between IRIS vs relapse.

Paradoxical IRIS Consensus Case Definition:

(A) Antecedent requirements
- Taking antiretroviral therapy
- Cryptococcal disease diagnosed pre-ART by positive culture or typical clinical features plus positive India ink staining or antigen detection
- Initial clinical response to antifungal therapy with: (1) partial or complete resolution of neurologic symptoms or signs, fever, or other lesions, or (2) reduction in CSF cryptococcal antigen or quantitative culture

(B) Clinical criteria
- Event occurs within 12 months of ART initiation
- Clinical deterioration with one of the following inflammatory manifestations of cryptococcosis (see text for possible rarer manifestations):
  1. Meningitis
  2. Lymphadenopathy
  3. Intracranial space-occupying lesion/s
  4. Multifocal disease
  5. Cutaneous / soft tissue lesions
  6. Pneumonitis or lung nodules

(C) Other explanations for clinical deterioration to be excluded:
- Non-adherence to antifungal therapy
- Suboptimal antifungal therapy, indicated by positive cryptococcal culture after 3 months
- Other infection or malignancy in the affected site
- Failure of ART (if possible, e.g. failure to achieve ≥1 $\log_{10}$ copies/mL decrease in VL by 8 weeks of ART)

as published in Haddow *et al*. *Lancet Infect Dis* 2010; 10: 791 – 802.

Figure 1. CSF Biomarkers at Time of IRIS vs. Relapse

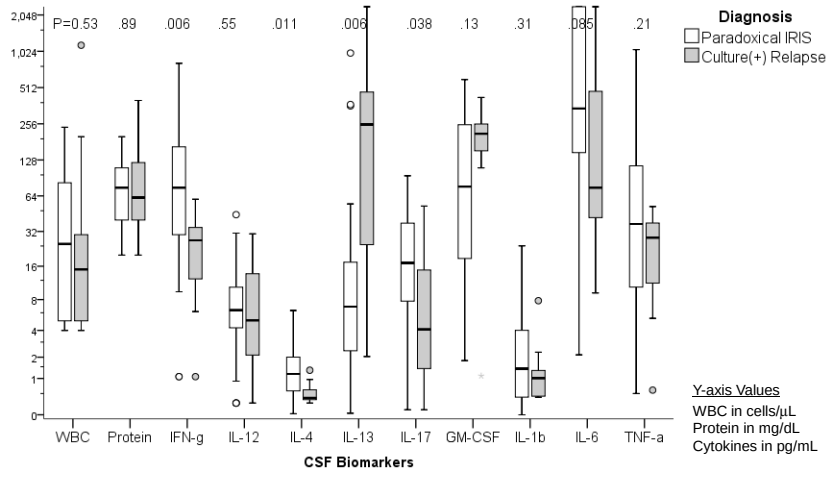


Table 1. Change in CD4 T cell count to first IRIS Event

CD4 Change cells/μL	IRIS Cases	ART Weeks (mean ± SD)
Negative	16 (26%)	6.7 ± 6.9
< +25	8 (13%)	6.4 ± 6.1
+25 to +50	11 (18%)	18.5 ± 12.0
> +50	27 (43%)	18.2 ± 11.9

Figure 2. HIV Viral Load: IRIS vs. Relapse

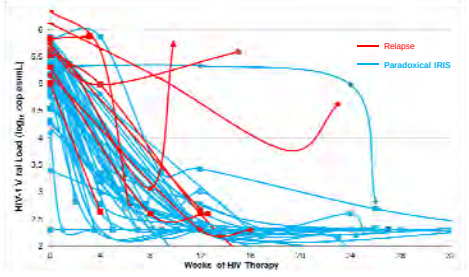


Table 2. HIV and CD4 Parameters in persons with Microbiologic Relapse

CSF Culture, CFU/mL	ART Weeks	CSF WBC cells/μL	CD4+ T cell count cells/μL			HIV viral load /mL	
			Pre-ART	At Relapse	Change	Pre-ART	Relapse
33,900	12	<5	21	56	+25	5.2 $\log_{10}$	<400
38,000	25	15	4	21	+17	5.3 $\log_{10}$	375,112
30,000	15	<5	61	34	-27	5.0 $\log_{10}$	<400
5000	34	<5	30	72	+42	5.7 $\log_{10}$	<400
700	2	30	--	63	N/A	--	835
10	26	<5	3	54	+51	5.3 $\log_{10}$	<400
97,000	15	50	13	13	0	4.9 $\log_{10}$	564,555
366,000	None (+4)	<5	78	72	-6	5.8 $\log_{10}$	>750,000
800	17	200	4	20	+16	5.3 $\log_{10}$	<400
221,000	12	<5	51	119	+68	5.6 $\log_{10}$	390,433
299.000	27	30	4	33	+29	5.5 $\log_{10}$	<20
281,000	6	125	75	46	-29	6.3 $\log_{10}$	427,284
265	18	1150	202	239	+37	5.0 $\log_{10}$	41,360

Results:

- 62 paradoxical cryptococcal-IRIS events did not consistently present with substantial reconstitution of CD4+ T cell counts (61% increased by ≥25 cells/mL)
- CD4 counts did not distinguish IRIS from 12 culture-positive relapses (50% with increases of ≥25 cells/μL).
- All IRIS cases (100%) had effective, appropriate virologic responses compared with only 50% (6/12) of culture-positive relapse (P<0.001).
- The median CSF WBC counts did not differ at time of cryptococcal-IRIS event (median 25; IQR, 5 to 85, max 240 cells/mcL) versus relapse (median 15; IQR, <5 to 40, max 1150 cells/mcL) (P=.53).
- CSF CrAg Titers were higher in relapse, but overlapped
  - IRIS: median titer: 1:128 (IQR 16-500)
  - Relapse: median titer: 1:1600 (IQR, 256-2048)
- Soluble cytokines which distinguished IRIS at event included 3-4 fold greater CSF levels of:
  - Th1 cytokine interferon (IFN)-g (P=.006),
  - Th17 pro-inflammatory IL-17 (P=.038).
- Culture-positive relapse had higher IL-13 (P=.006):
  - Relapse: median 253 (IQR, 63-471) pg/mL vs.
  - IRIS: median 6.9 (IQR, 2.4-17) pg/mL.

Conclusions:

- CD4 counts, plasma HIV RNA, nor CSF WBCs at event did not discriminate IRIS from relapse.
- Distinct immunologic signatures did distinguish IRIS from relapse, including 35-fold higher CSF IL-13 levels in relapse, a Th2 cytokine which is non-protective with uncontrolled cryptococcal infection in mouse models.
- HIV viral load testing should be targeted on those with CSF culture-positive cryptococcal relapse as 50% did not have viral suppression.

This research was made possible through support from the National Institute of Allergy and Infectious Diseases (U01AI089244, T32AI055433, K23AI073192) and Fogarty International Center and National Institute of Neurologic Diseases and Stroke (R21NS065713, R01NS086312, R25TW009345).

UNIVERSITY OF MINNESOTA
Driven to Discover℠