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D-dimer and CRP levels are elevated prior to antiretroviral treatment in patients who develop IRIS

Brian O. Porter, M.D., Ph.D., M.P.H.,
National Institutes of Health (NIH), National Institute of Allergy & Infectious Diseases (NIAID)

G. Laissa Ouedraogo, M.D., M.P.H.&T.M.,
SAIC, National Institute of Allergy & Infectious Diseases (NIAID)

Jessica Hodge, B.S.,
National Institutes of Health (NIH), National Institute of Allergy & Infectious Diseases (NIAID)

Margo Smith, M.D.,
WHC

Alice Pau, Pharm.D.,
National Institutes of Health (NIH), National Institute of Allergy & Infectious Diseases (NIAID)

Gregg Roby, R.N.,
National Institutes of Health (NIH), National Institute of Allergy & Infectious Diseases (NIAID)

Richard Kwan, P.A.,
National Institutes of Health (NIH), Clinical Center

Rachel Bishop, M.D.,
National Institutes of Health (NIH), NEI

Catherine Rehm,
National Institutes of Health (NIH), National Institute of Allergy & Infectious Diseases (NIAID)

JoAnn Mican, M.D., and
National Institutes of Health (NIH), National Institute of Allergy & Infectious Diseases (NIAID)

Irini Sereti, M.D., M.H.S.
National Institutes of Health (NIH), National Institute of Allergy & Infectious Diseases (NIAID)

Abstract

Biomarkers could be useful in evaluating immune reconstitution inflammatory syndrome (IRIS). A cohort of 45 HIV-1-infected, antiretroviral treatment (ART)-naïve patients with baseline CD4 T cell counts ≤100 cells/μL who were started on ART, suppressed HIV-RNA to <50 copies/mL, and seen every 1-3 months for 1 year were retrospectively evaluated for suspected or confirmed IRIS. D-dimer, C-reactive protein (CRP), and selected autoantibodies were analyzed at baseline, 1 and 3 months

Corresponding Author: Dr. Irini Sereti, National Institutes of Health, Building 10, Clinical Center, Room 11B07A, 10 Center Drive, Bethesda, MD, 20892, Phone: (301) 496-5533, Fax: (301) 480-9978, isereti@niaid.nih.gov.

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post-ART in cryopreserved plasma. Median differences between cases and controls were compared with Mann-Whitney and Fischer's exact tests. Sixteen patients (35.6%) developed IRIS (median of 35 days post-ART initiation): unmasking=8, paradoxical=7, autoimmune=1. Pre-ART D-dimer and CRP were higher in IRIS cases versus controls (D-dimer: 0.89mg/L versus 0.66mg/L, p=0.037; CRP: 0.74mg/L versus 0.39mg/L, p=0.022), while D-dimer was higher in unmasking cases at IRIS onset (2.04mg/L versus 0.36mg/L, p=0.05). These biomarkers may be useful in identifying patients at risk for IRIS.

Keywords

immune reconstitution inflammatory syndrome; immune restoration disease; antiretroviral therapy; unmasking IRIS; paradoxical IRIS; biomarker; D-dimer; C-reactive protein

Introduction

Immune reconstitution inflammatory syndrome (IRIS) is recognized as an important sequela of antiretroviral treatment (ART) in advanced HIV-infection, as the restoration of an impaired immune system may lead to dysregulated, pathologic inflammatory reactions [1;2]. However, laboratory markers commonly available in clinical settings have yet to be identified, which distinguish IRIS cases from non-IRIS controls. In particular, few studies have examined the potential for inflammatory markers to serve as either baseline predictors or proximate indicators of an IRIS event [3]. Such biomarkers would be useful in the identification of patients at high risk for IRIS upon ART initiation, as well as in the diagnosis of IRIS.

Patients who develop IRIS experience an inappropriate and exuberant immune response to either foreign (infectious) or self (autoimmune) antigens following treatment of their HIV infection, which results in either localized or generalized pathologic inflammation [4]. As the immunopathogenesis of this process remains unclear, many studies have sought to characterize this process in specific forms of IRIS limited to individual infectious organisms, such as *Mycobacteria* [5;6;7;8;9] or *Cryptococcus* [10;11;12;13;14]. However, in clinical practice, most HIV specialists encounter a wide spectrum of IRIS episodes, including bacterial, viral, and fungal associated events. As the immunopathogenesis of these forms of IRIS may differ, readily accessible laboratory markers have yet to be identified, which are applicable to IRIS cases involving different types of infectious organisms and differing patterns of onset i.e., unmasking IRIS wherein previously unrecognized opportunistic infection emerges post-ART initiation versus paradoxical IRIS in which infections previously improving with pathogen-specific treatment demonstrate symptomatic worsening following the commencement of ART. Such markers could be useful in the differential diagnosis of this potentially life-threatening condition or in the closer follow-up of a subgroup of patients who may be at increased risk of developing IRIS after initiating ART.

In this pilot cohort study of treatment-naïve patients with CD4 T cell counts of 100 cells/μL or less, we sought to identify laboratory markers for IRIS cases, focusing on plasma biomarkers, given their utility in the outpatient setting. In addition, the presence of autoantibodies was examined to evaluate the possibility of a break in self-tolerance as a potential immune mechanism of IRIS.

Materials and Methods

Study design and participants

A retrospective review of all patients who received their primary HIV care at the National Institutes of Health from February of 1995 to February of 2009 was conducted. Patients were

included in the study if, at the time of presentation, they: 1) were ART-naïve or had interrupted highly active ART (HAART) for at least 1 year with viral rebound of > 10,000 copies/mL, 2) had a baseline CD4 T cell count of ≤ 100 cells/μL), 3) had suppressed HIV-1 viral load to < 50 copies/mL after at least 1 year on ART, and 4) had cryopreserved plasma available from pre-ART and post-ART time points. A cohort of 45 HIV-1-infected adults was included in the analysis. Participants were enrolled in National Institute of Allergy and Infectious Diseases Institutional Review Board-approved protocols, and all patients signed informed consent prior to participation.

Participants were evaluated at baseline pre-ART, at 2, 4, 8, and 12 weeks post-ART initiation, and every 3 months after that for up to a year. At each visit, a thorough clinical history and physical examination were completed, as well as serum chemistries with hepatorenal function tests and a complete blood count with differential, CD4 T cell count, and HIV-1 viral load (bDNA, version 3, Chiron, lower detection limit of 50 copies/mL, with one exception for a patient tested with an earlier version of the assay with a lower detection limit of 500 copies/mL). Human leukocyte antigen (HLA) genotype analysis was also completed via molecular genotyping at the following loci: HLA-A, HLA-B, HLA-Cw, HLA-DQ, and HLA-DRB1.

Plasma biomarker measurements

Cryopreserved plasma samples were analyzed retrospectively for D-dimer (Liatest latex agglutination, Diagnostica Stago; detection limit of 0.20 mg/L; normal range 0.00 - 0.50 μg/mL), C-Reactive Protein (CRP immunonephelometry, Beckman Coulter; detection limit of 0.1 mg/dL; normal range < 0.80 mg/dL), anti-thyroglobulin (Captia Thyroglobulin ELISA, Trinity Biochem, detection limit of 0.067 Index value) and anti-thyroperoxidase (Captia Microsomal ELISA, Trinity Biochem, detection limit of 0.08 Index value) antibodies, anti-cardiolipin IgM (Quanta Lite ACA IgM III ELISA, INOVA Diagnostics, detection limit of 4.0 MPL) and IgG (Quanta Lite ACA IgG III ELISA, INOVA Diagnostics, detection limit of 4.0 GPL) antibodies, and lupus anticoagulant (Lupus Staclot LA phospholipid neutralization, Diagnostica Stago, qualitative report unit as negative or positive) at baseline prior to ART initiation, between 4-8 weeks post-ART (Month 1), and between 12-16 weeks post-ART (Month 3). For a subset of participants (7 IRIS and 18 non-IRIS patients), we also measured plasma levels of lipopolysaccharide (Limulus Amebocyte Lysate, Lonza, detection limit of 30 pg/mL) and soluble CD14 (Quantikine Human sCD14 Immunoassay, R & D Systems, detection limit of 125 pg/mL) as indicators of bacterial translocation, as previously described [15].

Case definitions

Participants were categorized as non-IRIS (controls), confirmed IRIS or suspected IRIS cases, using ACTG case definition criteria (AIDS Clinical Trials Group, revised May 24th, 2005) [1]: 1) initiation or reintroduction of ART, 2) CD4 T cell rise from baseline to IRIS onset ≥ 2-fold or ≥ 50 cells/mL and/or a fall in HIV viral load of > 0.5 $\log_{10}$, 3) symptoms or signs of an infectious/inflammatory condition, 4) such condition cannot be explained by a newly acquired infection, the expected clinical course of a previously recognized infection, or side effects of ART, and 5) identification of a specific pathogen or condition to which this presentation may be attributed. Standardized case report forms for all suspected IRIS cases were reviewed by the study team. Confirmed cases met all five criteria, while suspected cases lacked either criterion 4 or 5, but not both. All other cases were classified as non-IRIS controls.

Statistical methods

Non-parametric comparative inferential statistics were conducted for IRIS (all, paradoxical or unmasking) cases, grouped as either confirmed plus suspected or confirmed cases alone, versus non-IRIS controls. Continuous and ordinal variables were compared using the Mann-Whitney U test, while the distribution of categorical variables was analyzed using either the Fisher's

exact or Chi-Square tests for binary or multi-level variables, respectively. Clinical and laboratory indices were compared cross-sectionally at baseline, Month 1, and Month 3. Changes in continuous variables from baseline to Month 1 or Month 3 were similarly evaluated. Given the exploratory nature of this study, no adjustments were made for multiple comparisons and unadjusted two-sided p values were considered significant at $p \leq 0.05$.

Results

Cohort description

Demographics and baseline immune characteristics for the 16 IRIS cases and 29 non-IRIS controls are presented in Table 1. No significant differences were observed pre-ART initiation between IRIS cases (confirmed plus suspected) and non-IRIS controls in any of these indices, except for baseline median white blood cell (WBC) count, which was greater in subjects who eventually developed IRIS (4380 cells/μL, interquartile range (IQR) = 2853 to 5698 cells/μL in IRIS versus 3200 cells/μL, IQR = 2500 to 3820 cells/μL in controls, p = 0.041). Of note, lower baseline median hemoglobin levels were observed in paradoxical (confirmed plus suspected) IRIS cases compared to controls (10 gm/dL, IQR = 9.6 to 11.0 gm/dL in IRIS versus 11.7 gm/dL, IQR = 10.2 to 13.2 gm/dL in controls, p = 0.025). However, no other significant differences in baseline variables were seen between either confirmed unmasking or paradoxical IRIS cases versus non-IRIS controls (data not shown). Exclusion of the single autoimmune IRIS case in the cohort did not alter any of these results (data not shown).

IRIS events

Table 2 presents a clinical summary of each of the observed IRIS cases (11 confirmed and 5 suspected), which developed within 12 months of ART initiation. Seventeen IRIS episodes occurred in 16 patients, as one subject experienced both a flare of transaminemia associated with underlying chronic hepatitis B at 2 months post-ART and severe worsening of human papilloma virus-related anogenital condylomata at 1 year post-ART, which required surgical resection. Eight unmasking and 7 paradoxical IRIS cases were observed, with the most common causative pathogens being viruses (44%; 7 of 16 cases) and Mycobacteria (31%; 5 of 16 cases). The median time to onset of first IRIS event was 35 days (32 and 42 days from ART initiation for confirmed and suspected IRIS cases, respectively). Moreover, 69% of IRIS cases developed IRIS within the first 60 days post-ART. No IRIS-attributable mortality was observed.

Biomarkers in IRIS patients at baseline (pre-ART)

As depicted in Figure 1, median D-dimer (Panel A) and CRP (Panel B) levels were significantly increased when measured pre-ART in IRIS cases versus non-IRIS controls (D-dimer: 0.89 mg/L, IQR = 0.79 to 2.19 mg/L in IRIS versus 0.66 mg/L, IQR = 0.44 to 1.08 mg/L in controls, p = 0.037; CRP: 0.74 mg/L, IQR = 0.45 to 2.54 mg/L in IRIS versus 0.39 mg/L, IQR = 0.39 to 0.81 mg/L in controls, p = 0.022). Statistically significant differences were not seen when confirmed IRIS cases alone were compared to non-IRIS controls, although median values remained higher (D-dimer: 0.88 mg/L, IQR = 0.57 to 1.62 mg/L in IRIS, p = 0.183; CRP: 0.66 mg/L, IQR = 0.39 to 0.81 mg/L in IRIS, p = 0.150). When analyzed separately, baseline D-dimer was significantly elevated in unmasking IRIS cases compared to non-IRIS controls (D-dimer: 1.07 mg/L, IQR = 0.81 to 2.35 mg/L, versus 0.66 mg/L, IQR = 0.44 to 1.08 mg/L in controls, p = 0.039), while CRP was significantly elevated in paradoxical IRIS cases versus controls (CRP: 0.81 mg/L, IQR = 0.63 to 2.92 mg/L versus 0.39 mg/L, IQR = 0.39 to 0.81 mg/L in controls, p = 0.022).

Comparative HLA genotypic analyses revealed the overall distribution of alleles at the HLA-A genetic locus was significantly different in confirmed IRIS cases versus non-IRIS controls

(Fisher's exact test; p=0.028), with an under-representation of allele HLA-A02 (IRIS: 1 of 22 alleles or 4.5%; non-IRIS: 19 of 58 alleles or 33%) and an over-representation of HLA-A03 and HLA-A29 (IRIS: 3 of 22 alleles or 13.6%; non-IRIS: 2 of 58 alleles or 3.4%). By contrast, no differences were observed in plasma lipopolysaccharide or soluble CD14 levels at any time point in a subset of IRIS patients tested compared to non-IRIS controls, although these markers decreased over time in both groups following ART initiation (data not shown).

Biomarkers at IRIS events

As shown in Figure 2, when confirmed plus suspected IRIS cases were analyzed collectively (Panels A and B) or separately as unmasking or paradoxical IRIS cases (Panels C and D), neither D-dimer nor CRP was significantly elevated at Month 1 or Month 3 post-ART, compared to non-IRIS controls. Moreover, no significant differences were observed between IRIS and non-IRIS patients in terms of the magnitude of change in D-dimer or CRP from baseline to either Month 1 or Month 3 (data not shown), despite lower median D-dimer levels in non-IRIS controls at all time points. In addition, as shown in Panels A and C, D-dimer levels in both IRIS and non-IRIS cases decreased over time following the initiation of ART. However, when only confirmed IRIS cases (either unmasking or paradoxical) were compared separately to non-IRIS controls, plasma D-dimer concentration was significantly greater at Month 1 in confirmed unmasking IRIS cases (2.04 mg/L, IQR = 0.50 to 3.67 mg/L in IRIS versus 0.36 mg/L, IQR = 0.23 to 0.74 mg/L in controls, p = 0.05), as depicted in Figure 3. Because 75% of subjects with unmasking IRIS episodes developed symptoms within 42 days of ART initiation, this increase in D-dimer approximated the onset of unmasking IRIS.

With regard to plasma markers of autoreactivity, the proportion of lupus anticoagulant, anti-cardiolipin IgM and IgG, and anti-microsomal thyroid peroxidase antibody positivity did not differ between IRIS cases and non-IRIS controls at any time point (data not shown). The proportion of anti-thyroglobulin antibody positivity was significantly greater at Month 1 in both total (19%, p = 0.040) and confirmed (27%, p = 0.017) IRIS cases versus non-IRIS controls, which were uniformly negative. However, when the single autoimmune IRIS subject (who was anti-thyroglobulin positive) was excluded from analysis, no significant differences were observed.

In terms of clinical laboratory variables, neither absolute count nor percentage of CD4 or CD8 T cells differed between total IRIS cases and non-IRIS controls at either Month 1 or Month 3 post-ART (data not shown). However, total WBC count was significantly elevated at Month 3 in confirmed IRIS cases compared to non-IRIS controls (IRIS = 6990 cells/μL, IQR = 4200 to 9630 cells/μL; non-IRIS = 4230 cells/μL, IQR = 2945 to 5340 cells/μL; p = 0.011), although no differences were seen in absolute lymphocyte or monocyte counts. Subset analyses revealed the difference in WBC count was only seen in confirmed paradoxical IRIS cases (IRIS = 7190 cells/μL, IQR = 4313 to 10,770 cells/μL; p = 0.016). Of note, 2 patients with confirmed paradoxical IRIS and 1 with confirmed unmasking IRIS received prednisone during their IRIS episodes.

Discussion

IRIS has been recognized as a potentially serious, albeit rarely fatal complication of ART initiation during advanced stages of AIDS. To date, no biomarkers have been identified to either predict the future development of IRIS or aid in its diagnosis. In this study we have identified significant increases in the plasma inflammatory markers D-dimer and CRP prior to the initiation of ART in subjects who eventually developed a broad range of infectious IRIS manifestations (both unmasking and paradoxical) within 12 months of ART initiation. In addition, we further identified elevated levels of D-dimer one month after HAART initiation in subjects who developed unmasking IRIS events, compared to non-IRIS controls. Given that

the median time to onset of IRIS from the point of HAART initiation was 35 days for IRIS cases overall, with nearly 70% manifesting within the first 2 months post-ART, these findings suggest that pre-ART D-dimer levels may serve as a potential indicator of patients at risk for the development of IRIS soon after ART initiation, as well as aid in the diagnosis of unmasking IRIS.

Lymphopenia has been demonstrated by others to be sufficient for a break in self-tolerance and the development of autoimmunity [16]. Moreover, several groups have described autoimmune phenomena as IRIS manifestations, including Graves disease [17;18], autoimmune thyroiditis [19], and sarcoidosis [20;21]. However, the increases in inflammatory biomarkers seen in our study did not appear to be reflective of an overall break in self-tolerance, as a limited panel of autoimmune markers including lupus anticoagulant, anti-cardiolipin IgM and IgG, and anti-microsomal thyroid peroxidase antibodies failed to reveal any differences between IRIS cases compared to non-IRIS controls. Thus, these data highlight the importance of foreign (microbial) antigen-specific reactivity in the immunopathogenesis of IRIS, rather than dysregulated autoreactivity, supporting the notion that IRIS results from an imbalance of immunoregulatory pathways and inflammatory responses to foreign antigen.

To explore whether genetic differences in the capacity to display and recognize foreign antigen may contribute to the subsequent development of IRIS, we performed HLA genotypic analysis on our cohort, which revealed an under-representation of the HLA-A02 allele and an over-representation of HLA-A03 and HLA-A29 in IRIS patients. Interestingly, French et al. demonstrated that HLA-A02 (in conjunction with HLA-B44 and HLA-DR4) was associated with herpesvirus-related immune restoration disease, particularly cytomegalovirus [22;23]. Given our limited sample size and wide spectrum of associated infectious agents, it remains to be seen whether these genotypic associations will remain evident upon analysis of larger, prospective IRIS cohorts.

Our findings did not confirm previously identified predictors of IRIS such as lower pre-ART CD4 T cell counts [6;24;25;26] and greater declines in HIV viral load post-ART initiation [26;27]. This could be related to the pre-selected group of patients with low CD4 T cell counts. Baseline hemoglobin was lower specifically in subjects who developed paradoxical IRIS, while higher WBC counts at baseline and Month 3 post-ART were associated with IRIS development in general. This latter finding may have reflected the greater inflammatory response in patients who developed IRIS or corticosteroid-induced neutrophil demargination in a limited number of patients. However, these findings might also suggest that higher pre-ART leukocyte counts may reflect a greater microbial load predicting a greater risk of IRIS.

In terms of diagnostic utility, the use of inflammatory biomarkers to either predict treatment sequelae or serve as indicators of active disease in HIV infection has gained wide interest in recent years. Results from the SMART trial revealed that levels of D-dimer and high-sensitivity CRP, among other markers, were elevated both at baseline and during acute clinical episodes in HIV-infected subjects who interrupted ART. Moreover, these same patients were at greater risk of non-HIV-associated morbidity and mortality [28]. In addition, several studies have established a link between D-dimer and CRP levels and a range of pathogenic inflammatory conditions in HIV infection, including cardiovascular disease and endothelial dysfunction [29;30]. It is therefore of interest to confirm whether an easily accessible soluble plasma marker such as D-dimer or CRP would be useful in raising clinical suspicion for patients at risk of developing ART-associated immune restoration disease, as well as assist in the early identification of unmasking forms of IRIS. Although the distribution of biomarker levels in this study appears to overlap between IRIS and non-IRIS groups, prospective studies on a larger cohort could potentially determine useful cut-off points for the purposes of predicting future IRIS events.

In summary, we demonstrated that both D-dimer and CRP were higher at baseline in patients who developed IRIS. The importance of such inflammatory markers in this study is consistent with the extent of HIV disease progression and concurrent opportunistic infections present in the patients comprising this cohort, in contrast to participants in the SMART study who were virally suppressed at baseline [31]. In turn, our present findings may be helpful in identifying a reliable biomarker of IRIS in patients with advanced AIDS, as the present cohort experienced a wide spectrum of both paradoxical and unmasking IRIS events of varied infectious etiologies. Validation of both CRP and D-dimer as biomarkers of IRIS will be needed in larger, prospective patient cohorts.

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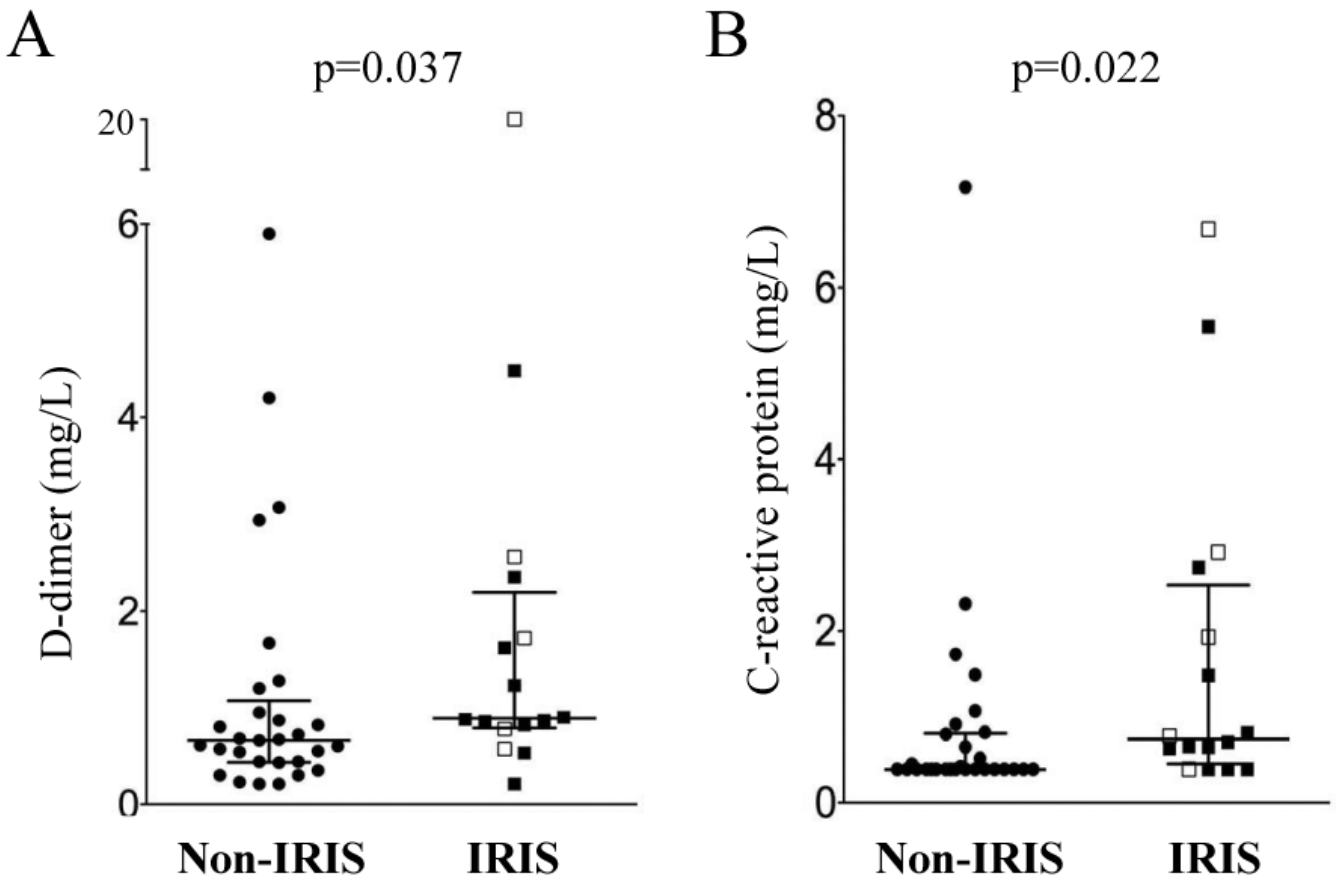

Figure 1. Baseline plasma inflammatory marker levels in IRIS cases versus non-IRIS controls

Median values and interquartile ranges are shown for A) D-dimer and B) C-reactive protein in non-IRIS controls (closed circles) versus confirmed (closed squares) plus suspected (open squares) **IRIS** cases. Intergroup comparisons were done with the Mann-Whitney U test.

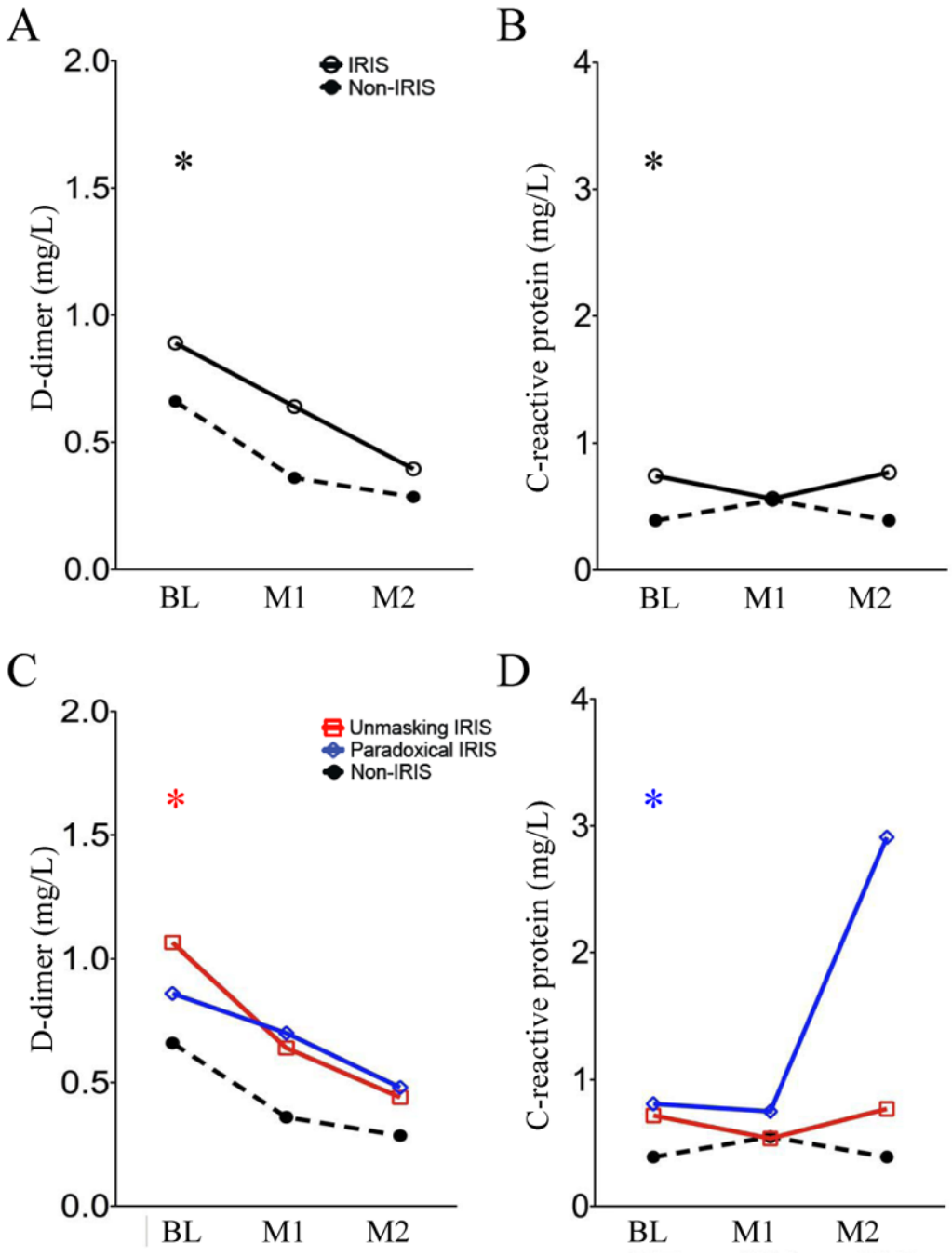

Figure 2. Longitudinal changes in plasma inflammatory markers among confirmed plus suspected IRIS cases versus non-IRIS controls

Median values are shown at baseline (BL), Month 1 (M1), and Month 3 (M3) for D-dimer (A and C) and C-reactive protein (B and D) in non-IRIS controls (closed circles and dashed line) versus confirmed plus suspected total IRIS cases (open circles and solid line) in Panels A and B or unmasking IRIS cases (open red squares and solid red line) and paradoxical IRIS cases (open blue diamonds and solid blue line) in Panels C and D. Intergroup comparisons versus non-IRIS controls were done with the Mann-Whitney U test. *$p < 0.05$

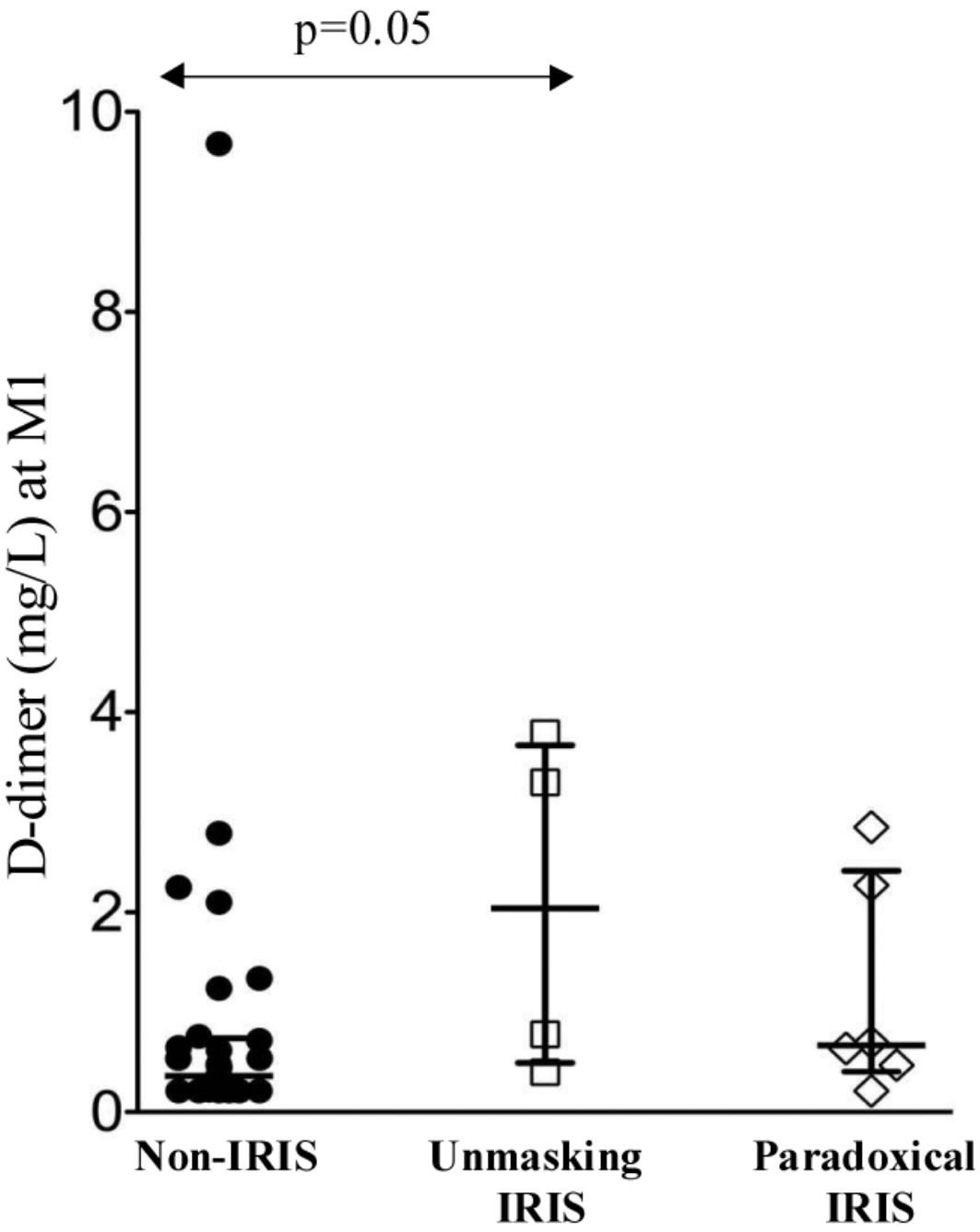

Figure 3. D-dimer at Month 1 among confirmed unmasking and paradoxical IRIS cases versus non-IRIS controls

Median values and interquartile ranges are shown for non-IRIS control (closed circles), unmasking IRIS (open squares), and paradoxical IRIS (open diamonds) cases at Month 1 (M1). Intergroup comparisons versus non-IRIS controls were done with the Mann-Whitney U test.

Table 1

Baseline demographic and immunologic characteristics of IRIS cases versus non-IRIS controls

Median and interquartile range or proportional values are shown, which were analyzed via Mann-Whitney U, Chi-square, or Fisher's exact tests.

Baseline Characteristic	Total IRIS Cases	Non-IRIS Controls
N	16	29
Age at ART initiation (years)	38 (30 – 44)	37 (32 – 47)
Gender (%)	M=63 TF=6 F=31	M=83 F=17
Race (%)	B=50 L=25 W=12.5 O=12.5	B=38 L=14 W=17 O=31
BMI (kg/m^2)	24.6 (21.1 – 26.2)	22.3 (20.8 – 24)
ART Base (%)	PI=31 NNRTI=69	PI=55 NNRTI=45
Nadir CD4 T cells/μL	19 (5 – 40)	19 (6 – 50)
Days from nadir CD4 to ART	9 (0 – 18)	15 (4 – 23)
OI within 3 months pre-ART	94%	83%
Distribution of lifetime pre-ART AIDS-defining illnesses [+]	0=38% 1=38% 2=25% 3=0%	0=31% 1=41% 2=24% 3=4%
Days of pre-ART OI treatment	27 (12 – 37)	40 (14 – 67)
Hemoglobin (g/dL)	11.2 (9.7 – 12.6)	11.7 (10.2 – 13.2)
HIV-RNA Log_{10} (copies/ml)	5.11 (4.94 – 5.46)	5.31 (4.80 – 5.56)
CD4 T cells/μL	20 (6 – 51)	27 (8 – 80)
CD4 T cell percentage (%)	2 (1 – 8)	4 (2 – 8)
CD8 T cells/μL	482 (273 – 667)	334 (232 – 526)
CD8 T cell percentage (%)	57 (49 – 74)	58 (47 – 67)
White blood cells/μL	[*]4380 (2853 – 5698)	3200 (2500 – 3820)
Lymphocyte count (cells/μL)	807 (454 – 1103)	657 (486 – 1063)
Lymphocyte percentage (%)	22 (9 – 28)	24 (15 – 34)
Monocyte count (cells/μL)	314 (126 – 546)	318 (240 – 359)
Monocyte percentage (%)	7 (4 – 13)	10 (7 – 13)
Neutrophil count (cells/μL)	2213 (1323 – 3613)	1680 (1376 – 2112)
Neutrophil percentage (%)	58 (47 – 72)	53 (49 – 65)

M=male, F=female, TF=transgender female, B=black, L=Latino, W=white, O=other, BMI=body mass index, ART=antiretroviral treatment, PI=protease inhibitor, NNRTI=non-nucleoside reverse transcriptase inhibitor, OI=opportunistic infection,

[+] Percentages represent the proportion of patients with the indicated number of lifetime AIDS-defining illnesses prior to ART initiation,

[*] $p < 0.05$

Table 2

Clinical description of confirmed and suspected IRIS cases observed within 12 months of antiretroviral therapy initiation

Diagnostic Class	IRIS Type	Gender Age (yr) Ethnicity	IRIS Etiologic Agent	IRIS Manifestations	ADIs within 3 months pre-ART Initiation	Days of pre-ART OI Therapy	Days post-ART to IRIS	Treatment and Outcome
Confirmed	Unmasking	M 47 B	MAC diffuse lymphadenopathy	Abdominal pain, fever, hypotension, chills, & hypoxia with reticulonodular lung disease, and lymphadenitis; negative blood cultures for MAC	1) Candidal Esophagitis 2) CMV Enterocolitis	1) 12 2) 6	99	Short ICU stay, requiring oral prednisone treatment for over 1 year
Confirmed	Unmasking	M 43 B	Pulmonary MAC	Fevers, increasing hilar lymph nodes, bilateral pulmonary infiltrates and 3cm lung cavitation; negative blood cultures for MAC	None	N/A	35	Improved with 12 months of MAC therapy
Confirmed	Unmasking	F 27 B	HHV8 Kaposi's Sarcoma	Tender, enlarged femoral lymphadenitis	1) PCP 2) HSV Laryngitis	1) 24 2) 4	14	None: improved spontaneously after one month
Confirmed	Unmasking	TF 31 O	CMV	Unilateral retinitis	None	N/A	28	Improved with valganciclovir/ocular ganciclovir implant
Confirmed	Paradoxical	F 41 B	VZV	Unilateral progressive outer retinal necrosis (PORN) with retinal detachment, despite reduction in vitreous fluid VZV viral load from 120,000 to 1700 copies/ml by PCR	VZV PORN	12	15	Required retinal tamponade, foscarnet, and ganciclovir
Confirmed	Paradoxical	M 29 L	MTB lymphadenitis	Recurrent fever with emergence of reactive PPD (25 mm)	MTB Lymphadenitis	35	28	Self-limited: improved after one week
Confirmed	Paradoxical	M 39 W	HHV8 Kaposi's Sarcoma	Increased size and number of skin lesions	HHV8 Kaposi's Sarcoma	N/A: no pre-ART treatment	32	Lesions regressed with doxorubicin
Confirmed	Paradoxical	M 29 O	Cryptococcal Meningitis	Recurrent headache, decreased cognition, and increased intracranial pressure with negative CSF cultures post-ART	Cryptococcal meningitis	40	118	Improved with amphotericin and maintenance fluconazole, serial lumbar punctures, and prednisone; IRIS flares during prednisone taper

Diagnostic Class	IRIS Type	Gender Age (yr) Ethnicity	IRIS Etiologic Agent	IRIS Manifestations	ADIs within 3 months pre-ART Initiation	Days of pre-ART OI Therapy	Days post-ART to IRIS	Treatment and Outcome
Confirmed	Paradoxical	F 35 B	1) Hepatitis B 2) HPV	1) Transaminemia with undetectable hepatitis B viral load post-ART 2) Worsening bulky hemorrhagic genital condylomata	None	N/A	1) 58 2) 343	1) Self-limited: improved after two months 2) Stabilized after extensive surgical resection
Confirmed	Paradoxical	M 45 B	MTB Pulmonary	Worsening lung cavitations and nodules, hypoxia, and hypotension	MTB Pulmonary	37	9	Short ICU admission requiring prednisone; subsequent IRIS flares during weaning of prednisone
Confirmed	Autoimmune	M 30 W	Self-reactive	Alopecia Totalis	None	N/A	89	Self-limited
Suspected	Unmasking	M 41 L	Pulmonary Cryptococcosis	Worsening right-sided lung mass with indeterminate serum CRAG	None	N/A	42	Improved after two weeks of oral fluconazole; CRAG negative after 13 months
Suspected	Unmasking	F 51 B	EBV Hodgkin's Lymphoma	B symptoms (fever, night sweats, rigors) with worsening hepatosplenic lesions	None	N/A	35	Splenectomy and chemotherapy for Hodgkin's lymphoma
Suspected	Unmasking	M 26 L	PML (JC virus)	New-onset seizures with leptomeningeal enhancement and worsening ring-enhancing cavitary lesions on head MRI; CSF PCR positive for JC virus	1) Toxoplasmosis 2) Histoplasmosis	1) 33 2) 27	110	Improved with levetiracetam and adherence to ART regimen
Suspected	Unmasking	M 43 L	Strongyloides	Chronic urticarial rash on face/torso	None	N/A	28	Improved with ivermectin two months after onset of rash
Suspected	Paradoxical	F 33 B	MAC	Nausea, vomiting, and necrotic inflamed abdominal lymphadenopathy; negative blood cultures for MAC at worsening	1) MAC 2) PCP	1) N/A: 10 months of pre-ART treatment 2) 39	197	Hospitalization requiring broad-spectrum IV antibiotics and intensification of MAC therapy

ADIs=AIDS-defining illnesses, OI=opportunistic infection, ART=antiretroviral treatment, M=male, F=female, TF=transgender female, B=black, W=white, L=Latino, O=other, MAC=Mycobacterium avium complex, HHV8 = human herpes virus 8, PCP=Pneumocystis jirovecii pneumonia, HSV=herpes simplex virus, CMV=cytomegalovirus, VZV=varicella zoster virus, MTB=Mycobacterium tuberculosis, EBV=Epstein-Barr virus, PML=progressive multifocal leukoencephalopathy, CRAG=*cryptococcal antigen*