Clinical and immunologic predictors of *Mycobacterium avium* complex immune reconstitution inflammatory syndrome in a contemporary cohort of patients with HIV

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40 words summary: Clinical and immunologic measurements were tested for prediction of MAC-IRIS in patients with a histologic and/or microbiologic confirmed MAC diagnosis, within a prospective study of 206 ART-naïve patients with CD4 counts <100 cells/μL followed up to 96 weeks post-ART initiation.

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ABSTRACT

Background: Patients with HIV (PWH) can present with new or worsening symptoms associated with *Mycobacterium avium* complex (MAC) infection shortly after antiretroviral therapy (ART) initiation as MAC immune reconstitution inflammatory syndrome (MAC-IRIS). In this study, we assessed the utility of several laboratory tests as predictors of MAC-IRIS.

Methods: PWH with clinical and histologic and/or microbiologic evidence of MAC-IRIS were identified and followed up to 96 weeks post-ART initiation within a prospective study of 206 ART-naïve patients with CD4 <100 cells/µL.

Results: Fifteen (7.3%) patients presented with MAC-IRIS within a median interval of 26 days after ART initiation. Patients who developed MAC-IRIS had lower BMI, lower hemoglobin levels, a higher alkaline phosphatase and increased CD38 frequency and MFI on $CD8^+$ T-cells, at the time of ART initiation compared to non-MAC IRIS patients. A decision tree inference model revealed that stratifying patients based on levels of alkaline phosphatase and D-dimer could predict the likelihood of MAC-IRIS. A binary logistic regression demonstrated that higher levels of alkaline phosphatase at baseline were associated with increased risk of MAC-IRIS development.

Conclusions: High alkaline phosphatase levels and increased $CD8^+$ T-cell activation with low CD4 counts at ART initiation should warrant suspicion for subsequent development of MAC-IRIS.

Keywords: HIV; MAC; mycobacterium; IRIS; biomarker; CRP; D-dimer; alkaline phosphatase

INTRODUCTION

Mycobacterium avium complex (MAC) is comprised of slow-growing mycobacteria that include both *Mycobacterium avium* and *Mycobacterium intracellulare,* as well as lesser known species. These environmental pathogens are present in soil, animals, and water and infections have been described after exposure to infected water systems [1, 2]. Before the onset of the HIV epidemic, MAC infections were rare and typically manifested with significant structural lung disease in immunocompetent persons [3] .

Beginning in 1981, reports of MAC infections in what would later be known as HIV/AIDS emerged [4]. In contrast to MAC infections in immunocompetent individuals, patients with HIV (PWH) presented with disseminated infection and non-specific constitutional symptoms including fever, weight loss, and night sweats [5, 6]. In the following years, MAC infections contributed significantly to morbidity and mortality in PWH [7], particularly in patients with CD4 counts ≤ 50 cells/μL) [6, 8]. Although the widespread use of combination anti-retroviral therapy (ART) has been associated with a 2.4 to 25.8 fold decrease in MAC incidence [9], MAC infection remains a significant pathogen for PWH with severe immune suppression. Unfortunately, despite tremendous improvements in access to ART worldwide, a significant proportion of PWH continue to be diagnosed late in the course of HIV infection, after having already developed HIV/AIDS [10-12].

Initiation of ART is essential for the survival of severely immunosuppressed PWH, however, patients with underlying MAC infection are at risk for an abrupt onset or worsening of MAC manifestations after initiation of ART called immune reconstitution inflammatory syndrome (IRIS) [3, 13-15]. Approximately 10-25% of PWH who initiate ART develop IRIS [16, 17], which is called unmasking when occurring in the context of a previously unrecognized opportunistic infection (OI) and paradoxical when the OI was previously recognized and on treatment.

MAC-IRIS frequently causes significant morbidity, commonly presenting with fever, lymphadenitis, pulmonary and/or intra-abdominal disease [3, 13-15, 18, 19]. Because the differential diagnosis for these clinical presentations is broad, including other serious conditions such as lymphoma and tuberculosis, the diagnostic work-up to establish a diagnosis of MAC-IRIS can be time-consuming, challenging, and burdensome for the patient [3]. To improve the clinical management of PWH with severe immune suppression, clinicians need tools to suspect and efficiently recognize MAC-IRIS. In this study, we describe the clinical and immunologic characteristics of patients with MAC-IRIS and assess the utility of several laboratory tests as predictors of MAC-IRIS.

METHODS

Study Design and Population

Within a previously described prospective observational 96-week study of ART-naïve PWH with CD4 <100 cells/μL initiating ART (NCT00286767) [20], we identified MAC-IRIS events that occurred in the 206 patients at the NIH Clinical Center site (Bethesda, Maryland, USA). All participants signed informed consent before any study procedures. MAC infection was diagnosed prospectively on the basis of clinical and histologic and/or microbiologic evidence of MAC. Clinical signs and symptoms considered suspicious for MAC included persistent fever, weight loss, cough, lymphadenopathy, and radiologic evidence of disease. Microbial evidence of MAC included growth of a MAC species in culture from a bronchoalveolar lavage (BAL), sputum sample, blood sample, or fine needle aspirate (FNA) from a lymph node. Histologic evidence of MAC included granulomas on biopsy.

The clinical team prospectively identified MAC-IRIS events and presented clinical and diagnostic data to an endpoint review committee that adjudicated whether the events were consistent with IRIS using the ACTG IRIS Definition Criteria, as previously described [20, 21]. One patient developed an

IRIS event due to either cytomegalovirus (CMV) or *Strongyloides stercoralis* after 10 days post-ART initiation but had a positive sputum culture for MAC at week 28. This patient was determined not to have had MAC-IRIS by an independent committee and was excluded from analysis.

MAC Treatment

Patients diagnosed with MAC were treated as per standard of care, which generally included azithromycin, ethambutol, and moxifloxacin (if $CD4^+$ <50 T cells/μL) [3, 22] . For patients with pulmonary MAC, antimicrobial treatment was continued until the participant was culture negative for one year [3]. Participants with disseminated MAC infections were continued on antimicrobial treatment for at least 12 months and until symptoms abated with sustained immune recovery ($CD4^+$ > 100 T cells/μL) [3].

Laboratory Methods

C-reactive protein (CRP), D-dimer, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactic dehydrogenase (LDH) were measured in a Clinical Laboratory Improvement Act (CLIA)-certified laboratory. Peripheral blood immunophenotyping was performed according to the manufacturer's instructions, with a modified version of the Centers for Disease Control and Prevention guidelines in a CLIA-certified laboratory. Using whole blood phenotyping was performed with monoclonal antibodies from BD Biosciences (San Jose, CA) then the cells were lysed after staining with Optilyse C (Beckman Coulter, Hialeah, FL), washed twice, and resuspended in 500μL of phosphate-buffered saline (Cambrex, Walkersville, MD). Sample analysis occurred immediately on a Becton Dickinson FacsCanto flow cytometer (BD Biosciences, San Jose, CA). The monoclonal antibodies used were PD-1 (MIH4), HLA-DR (L243), CD38 (HB7), CD3 (UCHT1), CD8 (SK1), CD4 (RPA-T4) and Ki-67 (B56). Data were analyzed with Facs Diva software version 6.1.3.

Statistical analysis

The median values, with interquartile ranges (IQR) or 95% confidence intervals (CI) were used as measures of central tendency and dispersion, respectively. Pearson's chi-square test was used to compare frequencies between the study groups whereas continuous variables were compared using the Mann-Whitney *U* test (unpaired comparisons) or Wilcoxon-signed rank test (paired comparisons). Two models of binary multivariable logistic regressions were performed as indicated in the Results section, with the odds ratio (OR) and 95% confidence interval (CI) of the association between values of indicated parameters and MAC-IRIS occurrence. The variables included in the models were the biomarkers levels at indicated timepoints that displayed a P-value <0.3 in univariate comparisons. A machine-learning based conditional tree including the values of all the biomarkers was designed to identify the best biomarker or combination of markers that were able to discriminate MAC-IRIS from controls. All analyses were pre-specified. A P-value below 0.05 was considered statistically significant. The analyses were performed using GraphPad Prism version 8.0 (GraphPad Software, Inc., San Diego, CA), JMP 14 (SAS Institute Inc., Cary, North Carolina), and the party library in R software.

RESULTS

MAC-IRIS incidence and clinical presentation

Fifteen (7.3%) of the 206 participants experienced MAC-IRIS, representing 31% of all IRIS events [20]. Among those 191 participants who did not develop MAC-IRIS, 17% (33 patients) presented with IRIS due to other opportunistic pathogens, mainly varicella zoster virus (Supplementary Table 1). Additionally, 7 participants were diagnosed with MAC but did not develop MAC-IRIS, including 4 who were diagnosed with MAC before and 3 after ART. The median duration from ART initiation to onset of MAC-IRIS symptoms was 26 days. Table 1 provides a detailed summary of the 15 cases (3 paradoxical and 12 unmasking MAC-IRIS).

The participants who experienced paradoxical MAC-IRIS had been diagnosed with HIV with extremely low $CD4^+$ T-cell counts (3, 2, and 5 cells/μL) and MAC (before ART) after found to have MAC bacteremia. One participant's AFB blood culture had been prompted by the presence of pancytopenia, high alkaline phosphatase, and diffuse adenopathy on physical exam, whereas the other 2 participants' AFB blood cultures were ordered to evaluate weight loss and fever. Antimycobacterial therapy (azithromycin, ethambutol +/- moxifloxacin) was started in all prior to ART. With paradoxical MAC-IRIS (after ART), 1 participant developed cervical lymphadenopathy, 1 participant (with baseline abdominal lymphadenopathy) developed diarrhea, abdominal pain, and weight loss, and 1 participant experienced fever, fatigue, and extensive adenopathy most notable in the axillary, epicardic, mesenteric, and retroperitoneal regions.

Of the 12 participants who developed unmasking MAC-IRIS, 7 presented with pulmonary MAC-IRIS, 4 presented with adenopathy, and 1 presented with symptoms consistent with sepsis (high fever, tachycardia, and hypotension) and was found to have MAC bacteremia. Median baseline CD4 T cell counts among unmasking MAC-IRIS participants was 27 cells/μL (range: 1-86 $CD4^+$ T-cells/μL).

Many of the patients who developed MAC-IRIS were coinfected with other opportunistic pathogens or presented with HIV-related malignancies at the time of IRIS: oral/ esophageal candidiasis (n = 7), genital/rectal herpes simplex virus (HSV; n = 6), *Pneumocystis jirovecii* pneumonia (n = 5), cytomegalovirus (n = 4), diarrheal illness associated with HIV (n = 3), HIV-related lymphoma (n = 3), *Strongyloides stercoralis* (n = 2), human papillomavirus-related malignancy (n = 1), or cryptococcal meningitis (n = 1) (Table 1).

MAC-IRIS management and morbidity

Triple antibiotic therapy, with azithromycin, ethambutol, and moxifloxacin, was implemented in the majority of participants (n = 11) because of the low CD4 count and severity of disease. The median duration of treatment for patients who completed therapy was 418 days for azithromycin (n = 10), 599 days for ethambutol (n = 11), and 513 days for moxifloxacin (n = 9).

Ten participants (66%) were hospitalized for the diagnostic work-up and/or symptom management of MAC-IRIS events. Three participants required corticosteroids for management of persistent systemic symptoms such as painful adenopathy, respiratory distress, or intractable fevers. The median duration of time between onset of IRIS to resolution of symptoms was 7.6 weeks (range: 1.7 to 23.6 weeks). One participant who experienced resolution of MAC-IRIS symptoms after a prolonged course of corticosteroids (approximately 4 months) subsequently presented with a MAC-IRIS flare more than two years later despite continued ART and MAC treatment. One participant (6.7%) died of sudden cardiac death 890 days after starting ART following multiple, prolonged hospitalizations secondary to MAC-IRIS, lymphoma, and failure-to-thrive.

Risk factors for MAC-IRIS

Participants who developed MAC-IRIS did not differ in terms of sex and age from those who did not (Supplementary Table 1). Laboratory and clinical evaluations, as well as the immunophenotyping of lymphocytes, were performed at study baseline (pre-ART) and after two weeks of ART initiation (Figure 1and Supplementary Table 2). Importantly, plasma HIV viremia (5.32 log copies/mL vs. 5.11 log copies/mL, P = 0.26) and $CD4^+$ T-cell count (8.0 vs. 19.5 $CD4^+$ T-cells/µL, P = 0.11) were not different between the clinical groups before ART initiation (Supplementary Table 2). Patients who developed MAC-IRIS during the follow up had a lower median BMI (21.1 Kg/m^2 vs. 23.4 Kg/m^2, P = 0.03) and hemoglobin values (9.9 g/dL vs. 11.0 g/dL, P = 0.03) at the time of ART initiation than did

non-MAC-IRIS patients (Figure 1A). Furthermore, pre-ART levels of alkaline phosphatase (178.0 units/L vs. 86.0 units/L, P = 0.001), AST (41.0 UI/L vs. 33.0 UI/L, P=0.04) and ALT (61.0 UI/L vs. 41.0 UI/L, P=0.021) were higher in MAC-IRIS participants than in non-IRIS controls (Figure 1A).

After two weeks on ART, values of alkaline phosphatase (P = 0.002), D-dimer (P = 0.011) and CRP (P = 0.013) were higher in those with MAC-IRIS, with persistent lower levels of hemoglobin (P = 0.003) compared to non-IRIS controls (Figure 1A). When variation in values of these biomarkers between pre-ART and week 2 were examined within each clinical group, we observed an overall decrease in levels of all measurements in both groups, except for CRP values, which tended to increase mainly in those who developed MAC-IRIS, with marginal statistical significance (P = 0.05) (Figure 1B).

The results presented above suggested that increased systemic inflammation can characterize MAC-IRIS participants; therefore, we investigated the implications of such scenario in T cell activation (Figure 2 and Supplementary Table 2). Because other studies have reported that IRIS is closely related to expansion and activation of $CD4^+$ T-cells, that is frequently associated with activation of other cells such as $CD8^+$ T lymphocytes [23], we hypothesized that differential activation profile of these cell types could characterize MAC-IRIS patients. To test this hypothesis we examined frequencies of $CD4^+$ or $CD8^+$ T-cells expressing CD38, Ki-67, and/or PD-1 at pre-ART and 2 weeks after initiation. Although the expression of these activation markers in $CD4^+$ T-cells of MAC-IRIS patients was indistinguishable from non-IRIS controls, we observed a higher proportion of $CD8^+$ T-cells expressing $CD38^+$ (97% vs. 93%; P=0.005) and higher mean fluorescence intensity (MFI) of $CD8^+CD38^+$ T-cells (360 AU [arbitrary unit] vs. 197.2 AU; P = 0.009) compared to non-MAC-IRIS controls both at baseline and at week 2 after therapy commencement (Figure 2A and Supplementary Table 2). Additionally, we observed higher proportions of $CD8^+$ T-cells expressing Ki67 at 2 weeks on ART in those with MAC-IRIS compared to non-IRIS (16% vs. 9.5%; P = 0.01) (Figure 2A and

Supplementary Table 2). Furthermore, we found that the proportion of $CD8^+$ T-cells expressing PD-1, and the MFI CD38 on $CD8^+$ T-cells substantially decreased at week 2 in non-IRIS patients but did not change significantly in those who developed MAC-IRIS (Figure 2B).

Predicting MAC-IRIS

We further extended our analyses to test whether biomarkers measured in peripheral blood could be used to predict MAC-IRIS. We first employed a multivariate regression model (Figure 3) using the biomarkers that displayed P-values < 0.3 in the univariate models presented in Supplementary Table 2. After adjustment for baseline levels of BMI, hemoglobin, HIV viral load, $CD4^+$ and $CD8^+$ T-cell counts, we found that higher pre-ART levels of alkaline phosphatase were independently associated with increased odds ratio of MAC-IRIS occurrence (OR [95%CI]: 1.007 [1.001-1.012]; P = 0.013), whereas levels of D-dimer (OR [95%CI]: 1.123 [0.846-1.489]; P = 0.42), CRP (OR [95%CI]: 0.981 [0.940-1.025]; P = 0.39) and $CD8^+$ T-cell activation markers were not independently associated with risk of MAC-IRIS (Figure 3). Of note, applying the same model to values obtained in week 2, we found that higher levels of CRP (OR [95%CI]:1.015 [1.00-1.030]; P = 0.043), increased frequency of $CD8^+CD38^+$ T-cells (OR [95%CI]:1.159 [1.009-1332]; P = 0.036) and higher CD38 MFI values on $CD8^+$ T-cells (OR [95%CI]:1.005 [1.001-1.009]; P = 0.023) were independently associated with MAC-IRIS occurrence.

A machine-learning conditional tree inference model incorporating values of all the clinical laboratory parameters assessed at the two distinct study time points was designed with two main objectives: (i) to identify a combination of biomarkers that could best identify MAC-IRIS cases; (ii) to establish cut-off values of the markers that could be used to differentiate between cases and non-cases. This approach identified three significant splits in the decision tree, including two distinct values of alkaline phosphatase at baseline and of D-dimer at week 2 of ART (Figure 4). To identify MAC-IRIS patients, the first split in the decision tree was composed by pre-ART alkaline phosphatase levels greater than

248 units/L, which alone could identify the majority of the cases (Figure 4A and 4B). A second split using D-dimer levels at week 2 on ART above 3.15 mg/L was useful in prediction of MAC-IRIS. Among those who exhibited D-dimer levels ≤ 3.15mg/L at week 2 of ART, a final split using alkaline phosphatase > 165 units/L at baseline could identify the remaining MAC-IRIS cases, except for 4 who were misclassified using this system (Figure 4A and 4B). We next employed a second model of multivariate regression, using the variables identified in the decision tree and categorizing the study participants according to the cut-off values established (Figure 4C). The variables used for adjustment were: BMI (kg/m^2), CD4$^+$ T-cell count (cells/μL), hemoglobin (g/dL) and CRP (mg/L) at baseline (for adjustment of alkaline phosphatase) or week 2 (for adjustment of D-dimer). This analysis revealed that a patient presenting with alkaline phosphatase levels greater than 248 units/L pre-ART, exhibited 15 times increased odds of developing MAC-IRIS than those who had lower levels (OR: 15.1; IQR: 1.2-188.1, P=0.035). Using this same approach, levels of D-dimer higher than 3.15 mg/L at week 2 of ART were associated with almost 4 times higher odds of MAC-IRIS than those who had lower levels (P < 0.01), however such association was lost in the adjusted model (P = 0.107). These observations demonstrate that increased risk of MAC-IRIS is strongly associated with higher concentrations of alkaline phosphatase in plasma.

DISCUSSION

Although the increased availability of ART has led to a steep decline in MAC incidence, PWH who are diagnosed with HIV after having already developed severe immunosuppression remain at risk of MAC and MAC-IRIS in the weeks following ART initiation. Given the significant morbidity associated with MAC-IRIS and the diagnostic challenges it poses, clinicians need tools to predict and efficiently recognize MAC-IRIS. Prompt initiation of anti-mycobacterial therapy, which could be started empirically in patients with a high clinical suspicion of MAC-IRIS even before results of cultures are available, could abrogate the morbidity associated with MAC-IRIS. With the goal of

identifying higher clinical risk persons, we conducted this investigation focused on the incidence, clinical presentation, risks factors, and predictive markers for MAC-IRIS.

The incidence of MAC-IRIS in immunosuppressed PWH is 2.6%-3.5% [14, 24]. Of note in our study, 7.3% of ART-naïve patients with $CD4^+$ T-cell counts <100 cells/μL starting ART developed MAC-IRIS, demonstrating that MAC-IRIS remains an important concern for severely immunosuppressed patients initiating ART. Apart from 3 participants with paradoxical IRIS, the majority of patients experienced unmasking MAC-IRIS, developing signs and symptoms of MAC infection only after initiating ART. These data suggest that clinicians should have a high clinical suspicion for MAC infection in PWH with severe immunosuppression, both before and immediately after the initiation of ART. Our results also show that MAC-IRIS causes a significant burden to patients, often necessitating hospitalization and the administration of corticosteroids for symptomatic management. In some cases, MAC-IRIS can persist for months and/or recur despite continued ART, HIV viral suppression, and continued MAC therapy.

Previous studies of MAC-IRIS in PWH have described lymphadenopathy as the primary manifestation of this syndrome [3]. Importantly, despite a high incidence of lymph-node diseases in our cohort, pulmonary symptoms represented the most common manifestation of MAC-IRIS overall in our study. This important finding highlights that clinicians should consider the possibility of pulmonary MAC-IRIS in the differential diagnosis of patients who present with fever and respiratory symptoms soon after initiating HIV therapy.

We found that lower BMI, lower hemoglobin, higher alkaline phosphatase, and increased expression of CD38 (frequency of cells or MFI) by $CD8^+$ T-cells at the time of ART initiation were risk factors

for the development of MAC-IRIS, in univariate analysis. In adjusted analysis, elevated alkaline phosphatase levels before ART initiation remained an independent predictor of MAC-IRIS infection.

Lower BMI and hemoglobin may indicate more severe immunosuppression and, thus, higher risk for opportunistic infections like MAC, whereas elevations in alkaline phosphatase may be due to MAC infiltration into the liver and possibly the bone marrow prior to immune reconstitution. CD38 expression on $CD8^+$ T-cells may be a marker of baseline inflammation and has importantly been described as an independent predictor or mortality in the pre-ART era in advanced disease [25, 26].

We sought to create a clinical predictive tool using circulating levels of laboratory parameters at the time of ART initiation. We found that elevated alkaline phosphatase (>248 units/L at ART initiation), D-dimer (>3.15 mg/L at week 2 of ART) supported the diagnosis of MAC-IRIS in this cohort. Our data suggest that in the absence of these elevated biomarkers, development of MAC-IRIS is unlikely. Further studies exploring prospectively the use of these markers as predictors of MAC-IRIS should be considered.

Improved understanding of the pathogenesis of MAC-IRIS is necessary to create targeted strategies to control the inflammatory response in these patients. Although the baseline immunophenotypes of $CD4^+$ T-cells of patients who develop MAC-IRIS were similar to those patients who would not develop disease, the $CD8^+$ T cell profiles were different. CD38 is a marker of cell activation and $CD8^+CD38^+$ lymphocytes have been shown to be an independent predictor of mortality in HIV patients in the pre-HAART era [27]. Given that the high mortality associated with HIV infection in the pre-HAART era was largely due to opportunistic infections including MAC, the activation of $CD8^+$ T cells previously associated with mortality could have been due to underlying opportunistic infections. Ki67 is a marker of cell cycling and, therefore, an increase in $CD8^+Ki67^+$ T cells in

patients who would develop MAC-IRIS could indicate that these cells are highly activated due to underlying infections or a higher HIV antigen load. Importantly, here we demonstrated that higher expression of CD38 by $CD8^+$ T-cells after 2 weeks on ART was associated with MAC-IRIS occurrence after adjustment for confounding variables. Nevertheless, T cell activation measurements were not identified as relevant to distinguish MAC-IRIS from controls in our machine learning model.

Our study has two important limitations. First, the focus was on PWH starting ART with low CD4 counts and may not be generalizable to persons starting ART at higher CD4 counts. Additionally, our study included only 15 MAC-IRIS events, which likely does not reflect the entire breadth of potential MAC-IRIS presentations.

MAC infection and MAC-IRIS continue to cause significant morbidity among PWH with severe immunosuppression initiating ART and our findings indicate that clinicians should suspect MAC-IRIS in patients with low BMI and hemoglobin who present with lymphadenopathy or respiratory symptoms in the weeks that follow ART initiation, especially in those with a high alkaline phosphatase value at ART initiation.

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Figure 1. Differences in plasma biomarkers in MAC-IRIS. (A) Circulating levels of indicated laboratory biomarkers pre-ART (baseline, bsl) and week 2 (wk2) of ART within each group of patients, red symbols for MAC-IRIS and light grey for no MAC-IRIS. The Mann-Whitney *U* test was used. (B) Distributions of the indicated measurements were compared between MAC-IRIS and no MAC-IRIS controls at each study timepoint. Comparisons were performed using the Wilcoxon-signed rank paired test.

Figure 2. Immunophenotyping of $CD8^+$ T-cells in MAC-IRIS. (A) Dynamics of markers associated with $CD8^+$ T-cell activation between pre-ART (baseline, bsl) and week 2 (wk2) of ART within each group of patients stratified according to occurrence of MAC-IRIS. The Mann-Whitney *U* test was used. (B) Distributions of the indicated parameters were compared between MAC-IRIS and no MAC-IRIS controls at each study timepoint. Comparisons were performed using the Wilcoxon-signed rank paired test.

Figure 3: Multivariate regression analysis to evaluate association between plasma levels of laboratory parameters and MAC-IRIS

The multivariate regression model included the variables shown in Table 1 which displayed univariate P-value ≤ 0.3. Odds ratios represent the 95% confidence interval (CI) increase in concentrations of each biomarker. Target variables for comparisons at study baseline (pre-ART) and week 2 on ART: alkaline phosphatase (units/L), D-dimer (mg/L), CRP (mg/L), percent of cells expressing CD38 and HLA-DR, percent of CD8 expressing CD38, MFI of CD38, percent of cells expressing Ki67 and percent of cells expressing PD-1. Variables used for adjustment in both timepoints: baseline levels of BMI (Kg/m^2), $CD4^+$ T-cell count (cells/µL), $CD8^+$ T-cell count, hemoglobin (g/dL) and viral load HIV-RNA (log copies/mL). Significant associations are highlighted in red and significant p-values are shown in bold type-font.

Figure 4: Machine learning decision tree model to predict MAC-IRIS.

All variables included in Table 1 were included in the model. Variables included: BMI (kg/m2), plasma HIV viral load (log copies/mL), $CD4^+$ T-cell count (cells/µL), $CD8^+$ T-cell count (cells/µL), hemoglobin (g/dL), alkaline phosphatase (units/L), D-dimer (mg/L), CRP (mg/L), from pre-ART and week 2 when indicated. Only alkaline phosphatase and D-dimer were shown to be informative in the system to segregate MAC-IRIS cases from no MAC-IRIS patients. **(A)** Three splits were identified in the decision tree. The first split marks alkaline phosphatase at the time of ART initiation (week 0). The second split is comprised of D-dimer levels at week 2. The final split is alkaline phosphatase at the time of ART initiation. **(B)** Box plot of distribution of individual according to levels of markers used in (A). Dashed lines represent the cut-off values used in the conditional model. **(C)** Multivariable regression model of variables (categorized by cutoff values) shown to be significant in the decision tree model. The variables used for adjustment were BMI (kg/m^2), $CD4^+$ T-cell count (cells/µL), hemoglobin (g/dL) and CRP (mg/L) at baseline (for adjustment of alkaline phosphatase) or week 2 (for adjustment of D-dimer). Alkaline phosp.: Alkaline phosphatase, CRP: C-reactive protein, OR: odds ratio, CI: confidence interval.

Table 1: Case descriptions of MAC-IRIS

Age and gender	$CD4^+$ T cell count at baseline (at IRIS)	ART regimen	Other OIs (before or at the time of MAC dx)	Time to IRIS from ART initiation (Days)	MAC diagnosis	MAC IRIS Presentation	MAC therapy (days)	Corticosteroids for MAC treatment	Hospitalizations and outcome
47yo M	7 (97)	ABC + 3TC, ATV	Esophageal candidiasis, oral candidiasis, CMV enterocolitis, Cryptosporidiosis	99	Liver, lymph node aspirates grew MAC	Lymphadenopathy (Unmasking IRIS)	Started 107 days after ART: • Azithromycin (1461) • Ethambutol (1461)	Prednisone	Hospitalized for 12 days for diagnostic work-up and supportive care for presenting MAC-IRIS symptoms (fever, diarrhea, and vomiting). MAC-IRIS resolved after treatment with corticosteroids.
27yo M	40 (108)	FTC + TDF, ZDV, ATV + RTV	*Pneumocystis carinii* pneumonia, HSV (perianal) Kaposi's sarcoma, oral candidiasis, oral hairy leukoplakia	38	Biopsy of abdominal lymph node positive for MAI	Lymphadenopathy (Unmasking IRIS)	Started 60 days after ART: • Clarithromycin (965) • Ethambutol (965)	None	Hospitalized for 3 days for diagnostic work-up and supportive care for presenting MAC-IRIS symptoms (fevers, chills, myalgia, and vomiting). MAC-IRIS resolved without corticosteroids.
48yo F	1 (48)	EFV + FTC + TDF	*Pneumocystis carinii* pneumonia, microsporidia, cryptosporidium, oral candidiasis, genital/oral HSV	18	Acid Fast Bacilli (AFB) blood culture grew MAC	Disseminated; high fever, hypotension, and tachycardia (Unmasking IRIS)	Started 35 days after ART: • Azithromycin (141) • Ethambutol (141) • Moxifloxacin (140)	None	Hospitalized in ICU for 2 days for diagnostic work-up of presenting MAC-IRIS symptoms (hypotension, tachycardia, fever). MAC-IRIS resolved without corticosteroids.
25yo M	86 (488)	EFV + FTC + TDF	Diffuse Large B-Cell Lymphoma, oral candidiasis	54	Sputum [positive for MAC by Acid Fast Stain, culture, and SecA1 PCR/sequencing	Pulmonary (Unmasking IRIS)	Started 65 days after ART: • Azithromycin (495) • Ethambutol (495) Moxifloxacin (495)	None	Briefly hospitalized for diagnostic work-up of presenting MAC-IRIS symptoms (cough in the setting of new pulmonary lesions). MAC-IRIS resolved without corticosteroids.
32yo F	5 (12)	ATV, FTC + TDF	Disseminated histoplasmosis, Strongyloides, Toxoplasmosis, Herpes simplex infection (rectal), CMV viremia, baseline MAC	44	AFB blood culture grew MAC	Cervical lymphadenopathy (Paradoxical IRIS)	Started 3 days before ART • Azithromycin (172) • Ethambutol (837) • Moxifloxacin (837)	Prednisone	Hospitalized for 3 days for diagnostic work-up of presenting MAC-IRIS symptoms (cervical adenopathy and fever). MAC-IRIS resolved following

									approximately 4 months of corticosteroid treatment.
49yo M	2 (13)	FTC + TDF, ATV	CMV retinitis, HSV (perirectal), oral candidiasis, candida esophagitis, baseline MAC	93	AFB blood culture grew MAC	Extensive abdominal lymphadenopathy (Paradoxical IRIS)	Started 4 days before ART: • Azithromycin (341) • Ethambutol (808) • Moxifloxacin (467)	None	Briefly hospitalized for MAC-IRIS symptom management (abdominal pain and fever). MAC IRIS resolved without corticosteroids.
45yo M	37 (124)	EFV + FTC + TDF	Oral candidiasis, CNS lymphoma	14	Bronchoalveolar Lavage (BAL) fluid grew MAC	Pulmonary (Unmasking IRIS)	Started 19 days after ART: • Azithromycin (513) • Ethambutol (513) • Moxifloxacin (513)	Prednisolone	Hospitalized for 13 weeks for CNS lymphoma, MAC-IRIS, and failure-to-thrive. Patient experienced sufficient recovery to allow for hospital discharge but died of sudden cardiac death at home 890 days after ART initiation.
31yo M	4 (26)	ATV, FTC + TDF, RTV	*Pneumocystis carinii* pneumonia, HSV (rectal)	14	Fine needle aspiration (FNA) of lymph node positive by AFB smear, BAL fluid grew MAC	Lymphadenopathy (Unmasking IRIS)	Started 42 days after ART: • Azithromycin (736), • Ethambutol (736), • Moxifloxacin (725)	Prednisone	Not hospitalized; diagnostic work-up and MAC-IRIS symptoms monitored through frequent outpatient visits. MAC-IRIS recurred more than 2 years after initial diagnosis despite continuous ART and prolonged MAC treatment.
44yo M	5 (119)	EFV + FTC + TDF	*Pneumocystis carinii* pneumonia	21	FNA of paratracheal lymph node positive for AFB by Fite stain, AFB culture of lymph node biopsy material rew MAC	Pulmonary (Unmasking IRIS)	Started 48 days after ART: • Azithromycin (599) • Ethambutol (599) • Moxifloxacin (584)	None	Hospitalized for 3 days for diagnostic work-up of presenting MAC-IRIS symptoms (fever, dry cough, and night sweats). MAC-IRIS resolved without corticosteroids.
47yo F	3 (14)	EFV + FTC + TDF	Oral candidiasis, baseline MAC	14	AFB blood culture grew MAC	Extensive lymphadenopathy (Paradoxical IRIS)	Started 42 days after ART: • Azithromycin (91) • Ethambutol (550) • Moxifloxacin (550)	None	Not hospitalized; diagnostic work-up and supportive care for presenting MAC-IRIS symptoms (fever, adenopathy, and tachycardia) provided through frequent outpatient visits. MAC-IRIS resolved

									without corticosteroids.
50yo M	25 (119)	EFV + FTC + TDF	Cryptococcal meningitis	9	AFB smear of material collected from FNA of abdominal mass positive; AFB culture e of the fluid also grew MAC	Extensive mesenteric lymphadenopathy (Unmasking IRIS)	Started 99 days after ART: • Azithromycin (86) • Ethambutol (72) • Rifabutin (86)	None	Extensive work-up of MAC-IRIS presenting symptoms (weight loss with new abdominal mass on imaging) conducted primarily in the outpatient setting, with 2 brief hospitalizations. Diagnosis ultimately made following fine needle aspiration of abdominal mass for AFB culture. MAC-IRIS resolved without corticosteroids.
29yo M	29 (157)	RAL, FTC + TDF	None	56	AFB culture of BAL fluid grew MAC	Pulmonary (Unmasking IRIS)	Started 62 days after ART: • Azithromycin (611) • Ethambutol (611) • Moxifloxacin (611)	None	Not hospitalized; diagnostic work-up and supportive care for presenting MAC-IRIS symptom (pleuritic chest pain) provided with frequent outpatient visits. MAC-IRIS resolved without corticosteroids.
39yo M	44 (178)	DRV, RAL, FTC + TDF	Strongyloides	30	Histologic diagnosis by granulomas on lung biopsy	Pulmonary (Unmasking IRIS)	Started 71 days after ART: • Azithromycin (363) • Ethambutol (363) • Moxifloxacin (126)	None	Not hospitalized. Patient did not have clinical symptoms of MAC-IRIS; diagnostic work-up revealing unmasking MAC-IRIS was prompted by newly positive PPD 4 weeks after ART initiation.
41yo M	35 (154)	EFV + FTC + TDF, RAL	*Pneumocystis carinii* pneumonia, CMV pneumonia	32	AFB culture of BAL fluid grew MAC	Pulmonary (Unmasking IRIS)	Started 42 days after ART: • Azithromycin (579) • Ethambutol (579)	None	Not hospitalized; diagnostic work-up and MAC-IRIS symptoms monitored through frequent outpatient visits. MAC-IRIS resolved without corticosteroids.
37yo F	8 (11)	EFV + FTC + TDF	Diffuse Large B-Cell Lymphoma, HSV, oral candidiasis	11	AFB culture of BAL fluid grew MAC	Pulmonary (Unmasking IRIS)	Started 17 days after ART: • Azithromycin (736) • Ethambutol (736) • Moxifloxacin (736)	Prednisone	Hospitalized for 10 days for diagnostic work-up and management of MAC-IRIS symptoms in the

	setting of recent cancer treatment and neutropenia. MAC IRIS resolved.

Efavirenz = EFV; emtricitabine = FTC; tenofovir = TDF; Abacavir = ABC; lamivudine = 3TC; Atazanavir = ATV; Ritonavir = RTV; Raltegravir = RAL; Zidovudine = ZDV; Darunavir = DRV.

Figure 1

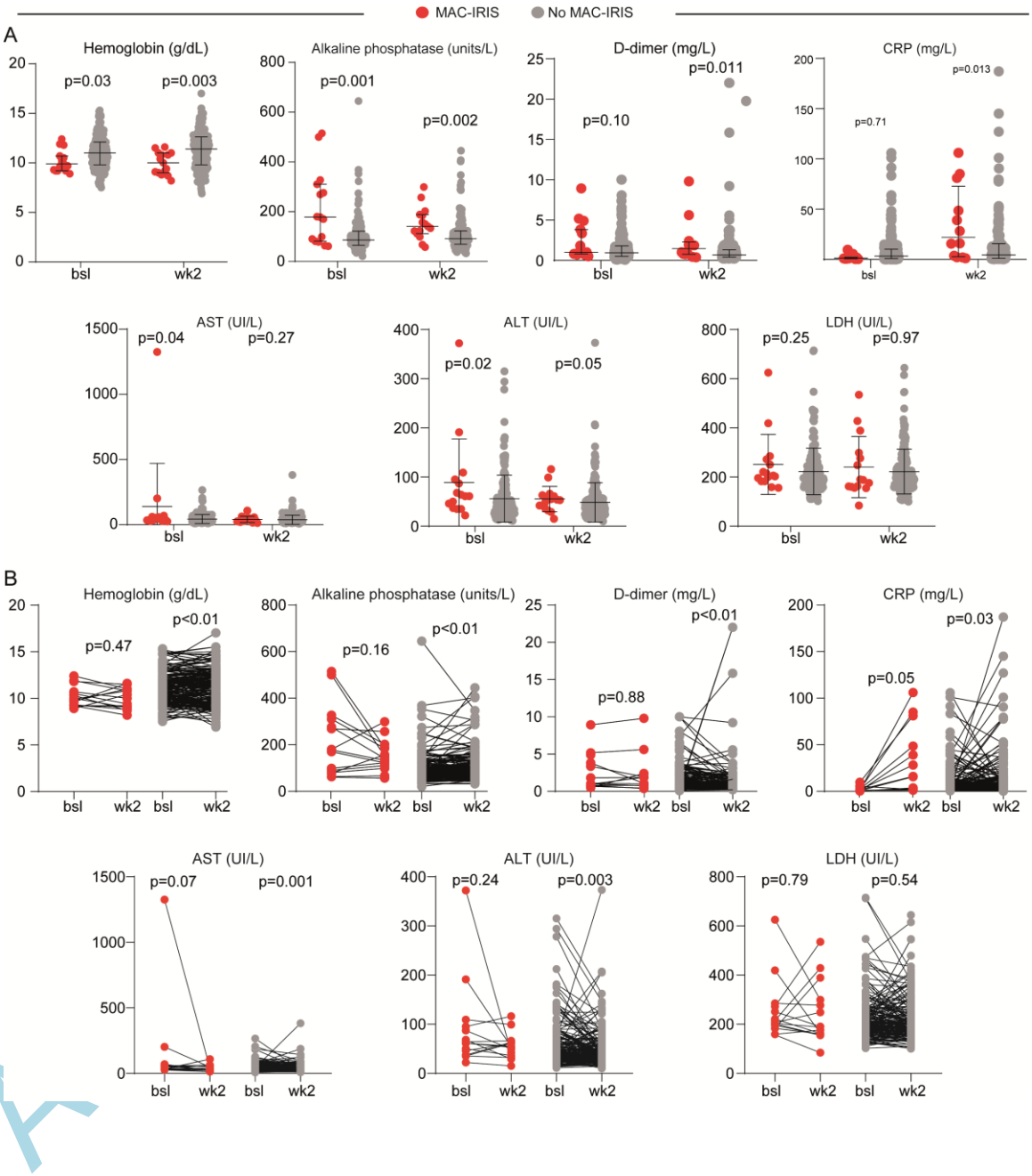

Figure 2

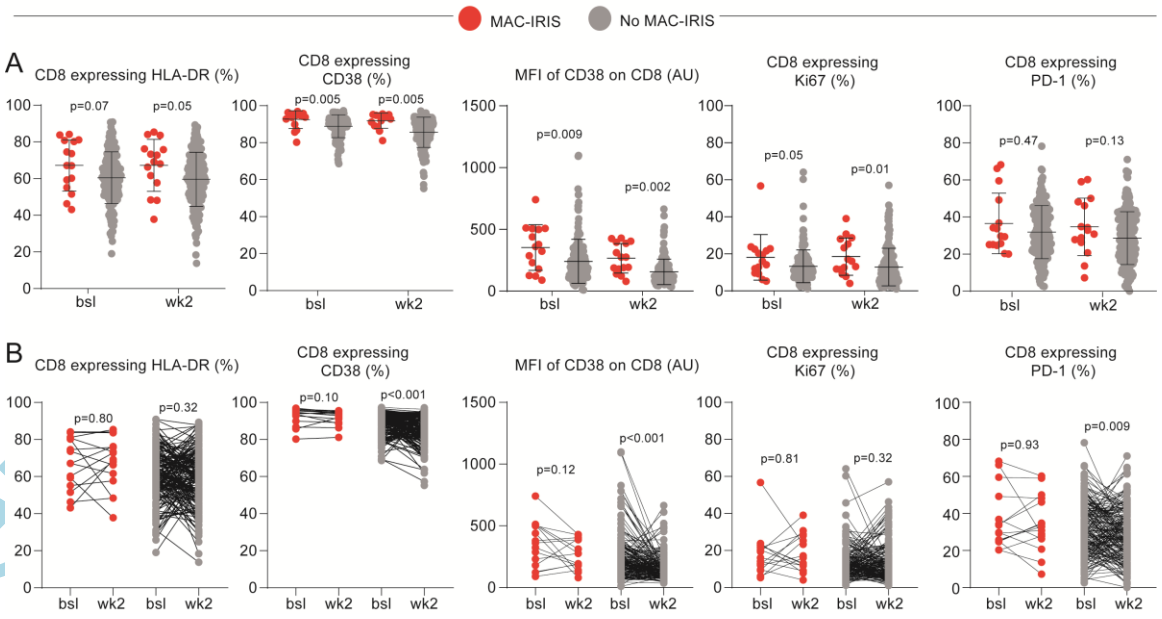

Figure 3

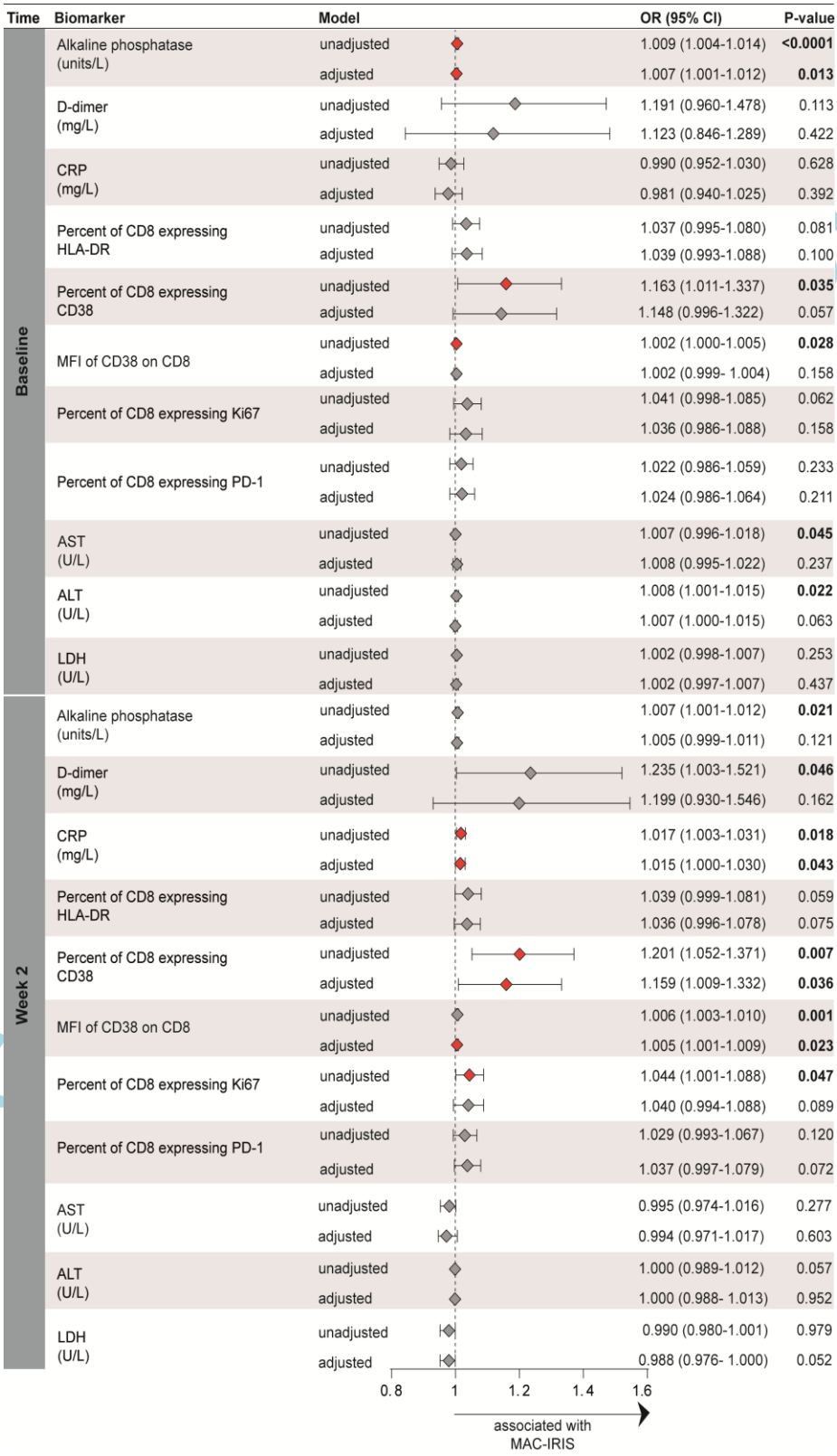

Figure 4