EBioMedicine 4 (2016) 115–123

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

# Vitamin D, D-dimer, Interferon γ, and sCD14 Levels are Independently Associated with Immune Reconstitution Inflammatory Syndrome: A Prospective, International Study☆

Laura W. Musselwhite [a,b], Bruno B. Andrade [c,d,e], Susan S. Ellenberg [f], Ann Tierney [f], Pablo F. Belaunzaran-Zamudio [g,h], Adam Rupert [i], Michael M. Lederman [j], Ian Sanne [k], Juan G. Sierra Madero [g], Irini Sereti [a,*]

[a] Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), 10 Center Drive, Bethesda, MD 20892, USA
[b] Duke Hubert Yeargan Center for Global Health, Duke University, 310 Trent Drive, Duke Box 90518, Durham, NC 27708, USA
[c] Laboratory of Parasitic Diseases, NIAID, NIH, 50 South Drive, Bethesda, MD 20892, USA
[d] Unidade de Medicina Investigativa, Laboratório Integrado de Microbiologia e Imunorregulação (LIMI), Centro de Pesquisas Gonçalo Moniz (CPqGM), Fundação Oswaldo Cruz (FIOCRUZ), Salvador 40296-710, Brazil
[e] Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER Initiative), Brazilian Institute for Tuberculosis Research, José Silveira Foundation, Salvador, Brazil
[f] Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, 611 Blockley Hall, 423 Guardian Drive, Philadelphia, PA 19104-602, USA
[g] Departamento de Infectología, Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán", México City, México
[h] División de Investigación, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, México
[i] Leidos Biomedical Inc., 11951 Freedom Drive, Reston, VA 20190, USA
[j] Case Western Reserve University School of Medicine, 2061 Cornell Rd, Cleveland, OH 44106, USA
[k] University of Witwatersrand, Johannesburg, South Africa

ARTICLE INFO

*Article history:*
Received 14 October 2015
Received in revised form 10 January 2016
Accepted 12 January 2016
Available online 14 January 2016

*Keywords:*
IRIS
HIV
Biomarker
Vitamin D
D-Dimer
Inflammatory cytokine

ABSTRACT

To determine the immunological profile most important for IRIS prediction, we evaluated 20 baseline plasma biomarkers in Acquired Immunodeficiency Syndrome (AIDS) patients initiating antiretroviral therapy (ART). Patients were enrolled in a randomized, placebo-controlled ART initiation trial in South Africa and Mexico to test whether maraviroc could prevent IRIS. Participants were classified prospectively as having IRIS within 6 months of ART initiation. Twenty plasma biomarkers were measured at study enrollment for 267 participants. Biomarkers were tested for predicting IRIS with adjustment for covariates chosen through forward stepwise selection. Sixty-two participants developed IRIS and of these 19 were tuberculosis (TB)-IRIS. Baseline levels of vitamin D and higher D-dimer, interferon gamma (IFNγ), and sCD14 were independently associated with risk of IRIS in multivariate analyses. TB-IRIS cases exhibited a distinct biosignature from IRIS related to other pathogens, with increased levels of C-reactive protein (CRP), sCD14, IFNγ, and lower levels of Hb that could be captured by a composite risk score. Elevated markers of Type 1 T helper (Th1) response, monocyte activation, coagulation and low vitamin D were independently associated with IRIS risk. Interventions that decrease immune activation and increase vitamin D levels warrant further study.



## 1. Introduction

Immune reconstitution inflammatory syndrome manifests as paradoxical worsening or uncovering of infection or malignancy following ART initiation, despite successful suppression of HIV replication and effective microbiologic control of underlying infection in cases of paradoxical IRIS. Among patients with HIV infection in resource-limited settings, IRIS usually occurs within the first few weeks and up to six months after start of therapy; in these settings, resource utilization and mortality can be high (Hoyo-Ulloa et al., 2011; Muller et al., 2010). Despite a substantial global disease burden, diagnostic criteria are ill defined, molecular mechanisms accounting for pathogenesis are unknown, and effective therapies to mitigate risk are needed (Sereti et al., 2010).

In an earlier retrospective study increased baseline plasma levels of CRP, D-dimer, interleukin-6 (IL-6), and hyaluronic acid (HA) predicted IRIS/death within the first year of ART (Boulware et al., 2011). It is uncertain whether the same markers would have clinical utility when

☆ Preliminary results of this study were presented at the 22nd Conference on Retroviruses and Opportunistic Infections (CROI 2015) in Seattle, February 23–26, 2015. Abstract 308.

* Corresponding author at: Clinical and Molecular Retrovirology Section, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 10, Room 11B-07A, Bethesda, MD 20892, USA.
*E-mail address:* isereti@niaid.nih.gov (I. Sereti).

http://dx.doi.org/10.1016/j.ebiom.2016.01.016

applied prospectively to a population at higher risk due to lower CD4 count at ART initiation and higher prevalence of TB (Boulware et al., 2011).

Recent attention has focused on the role of vitamin D in infectious (de Haan et al., 2014) and autoimmune disease, including tuberculosis (Yang et al., 2013). In resource-limited settings, which have the largest burden of advanced HIV disease, mycobacteria are the most common pathogen involved in the development of IRIS (Conesa-Botella et al., 2009). Vitamin D deficiency is also prevalent and associated with AIDS progression (Van Den Bout-Van Den Beukel et al., 2008). A recent randomized, placebo-controlled trial of vitamin D supplementation in patients with pulmonary tuberculosis demonstrated more rapid clinical recovery than was seen in placebo recipients, although, further investigation of vitamin D for the prevention or reactivation of tuberculosis infection is needed (Salahuddin et al., 2013). Indeed, mounting evidence indicates a strong role for vitamin D in the regulation of the human immune response (Modlin, 2007) and resolution of TB-induced inflammation (Coussens et al., 2012). Multiple in vitro studies have shown that vitamin D suppresses the stimulation of cell-mediated immunity (Coussens et al., 2012). Furthermore, a prominent role for monocyte activation in paradoxical TB-IRIS was highlighted recently (Andrade et al., 2014). Biomarkers that indicate monocyte and myeloid cell activation may improve prediction of IRIS and suggest new pathways of exploration for preventive and therapeutic strategies.

As an adjunctive study to a large randomized controlled trial of antiretroviral treatment (ART) plus maraviroc or ART alone in treatment-naïve individuals in South Africa and Mexico, we tested the hypothesis that pro-inflammatory cytokine levels, myeloid cell activation, coagulation and fibrosis markers were associated with IRIS risk prior to starting ART. We further speculated that high levels of vitamin D might protect against IRIS. Our findings suggest that T-cell and monocyte activation, inflammation and low vitamin D levels are independently associated with IRIS risk.

## 2. Methods

### 2.1. Study Outline

Between 2009 and 2012, the C-C Chemokine Receptor 5 (CCR5) Antagonist to Decrease the Occurrence of Immune Reconstitution Inflammatory Syndrome in HIV-Infection (CADIRIS) trial randomized and followed 276 ART-naïve HIV-infected patients for six months to test the utility of the CCR5 antagonist maraviroc as an adjuvant to a standard ART regimen to reduce the occurrence of IRIS (Sierra-Madero et al., 2014; Mendonca et al., 2013). Participants received maraviroc 600 mg twice daily or placebo added to an ART regimen that included tenofovir, emtricitabine, and efavirenz for 48 weeks. The primary endpoint was an IRIS diagnosis within 6 months of ART initiation. Clinical data were prospectively collected by health care providers at the clinical sites. The study was sponsored by the Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran. The main clinical trial was sponsored by Pfizer Inc. This study was approved by the Ministry of Health and Federal Commission for Sanitary Risks Protection of Mexico, and the Medicines Control Council and Human Research Ethics Committee of South Africa. The ClinicalTrials.gov registration number is NCT00988780. Results of the main clinical trial were published in the Lancet HIV (Sierra-Madero et al., 2014).

### 2.2. Study Participants

Eligible subjects in the CADIRIS Trial were HIV-infected, at least 18 years-old, had a CD4 cell count < 100 μL and had not received steroids within two weeks of randomization. We first evaluated IRIS and mortality according to baseline levels of selected biomarkers in the entire CADIRIS population. IRIS cases were reviewed by a central adjudication committee and identified as those who had an event during the first 24 weeks of ART (Sierra-Madero et al., 2014). IRIS events were predefined as symptoms consistent with an infectious or inflammatory condition, temporally related to ART initiation and associated with an increase in CD4 count, a decrease in viral load, or both, not explained by a new infection, the expected clinical course of a previously diagnosed infection, or side effects of ART according to the ACTG IRIS criteria. On-site clinicians utilized the above criteria to make a preliminary diagnosis of IRIS, and documented criteria in an electronic data management system. To capture all possible IRIS cases, the study coordination center in both countries actively monitored case report forms and electronic data management system of all patient visits. The central adjudication committee of four experts not involved in study execution or data collection reviewed all preliminary cases and ultimately determined the classification of IRIS events by consensus.

Opportunistic infections occurring in IRIS and non-IRIS cases are described in the original publication of the related CADIRIS Trial in electronic Tables 3 and 4.

### 2.3. Biomarker Measurement

All plasma samples were obtained at study enrollment, prior to ART initiation, and were stored at -80°. Biomarkers were measured in duplicate after a single freeze–thaw cycle in batched assays. Coagulation markers were measured in plasma collected in citrate tubes and the remaining biomarkers were measured in plasma collected in Ethylenediaminetetraacetic acid (EDTA).

Interferon-γ, interleukin (IL)-1b, IL-6, IL-8, IL-10, IL-12p70, IL-17 and tumor necrosis factor-α (TNFα), CRP, serum amyloid A (SAA), P-selectin, interferon-inducible protein (IP)-10 were measured by electrochemiluminescence (Mesoscale Discovery, Rockville MD). Leukotriene B4 (LTB4), soluble (s) CD14, sCD40 ligand, sCD163, Von Willebrand Factor (vWF) activity, fibrinogen levels, proteins C and S, and HA were assessed with the use of standardized enzyme-linked immunosorbent assays (ELISAs) (R&D Systems, AdipoBioscience, Zymutest, and Corgenix). D-Dimer was measured with the use of an enzyme-linked fluorescence assay on a VIDAS instrument (Biomerieux). 25 hydroxyvitamin D is the most abundant of all circulating vitamin D metabolites and is generally accepted as the best indicator of vitamin D supply (Aloia et al., 2008). Therefore, the plasma concentration of 25 hydroxyvitamin D was measured by a standard ELISA assay (ALPCO).

### 2.4. Statistical Analysis

In this study, all participants who developed IRIS were included as IRIS cases and participants who did not develop IRIS were controls. Descriptive statistics were used to compare baseline demographics, laboratory test results, and biomarker measurements between the groups. Results of laboratory tests were analyzed as continuous variables and variables not normally distributed were $\log_{10}$-transformed prior to comparisons. We used Fisher's Exact test to evaluate the association between categorical variables and IRIS.

We used logistic regression to examine the association between biomarker levels and IRIS. We first performed univariate analyses to assess the potential impact of baseline variables, selecting those with a two-sided p value of $<0.10$ for inclusion in a forward stepwise regression analysis to determine which were independently associated with the development of IRIS. Separate analyses were performed for all-cause IRIS, TB-IRIS and viral IRIS. Next, we performed univariate analyses of the biomarkers, adjusting for the covariates above, and selected those significantly associated with all-cause IRIS ($p < 0.10$) for inclusion in a forward stepwise regression analysis to identify those markers appearing independently associated with all-cause IRIS, TB-IRIS, and viral IRIS.

Further sub-analyses were performed by IRIS type (no-IRIS, TB-IRIS and other IRIS) utilizing Kruskal–Wallis tests with Dunn's multiple comparisons post-test because most variables in the clinical subgroups were not normally distributed even after logarithmic transformation.

The inferential networks (described here as host interactome) were generated from Spearman correlation matrices containing values of