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Biomarkers of immune dysfunction in HIV

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Abstract

Purpose of review—HIV infection is characterized by chronic immune system activation and inflammatory cytokine production. This review will highlight recent developments using plasma and cellular biomarkers of immune system activation and dysfunction to predict mortality and opportunistic disease in HIV-infected individuals.

Recent findings—HIV infection results in features characteristic of early aging of the immune system or ‘immune senescence’, driven by chronic antigen exposure and immune system activation. Microbial translocation of gut bacterial components is associated with chronic immune activation and possibly systemic inflammation. Antiretroviral therapy may not fully normalize this condition. Baseline elevations of certain biomarkers of inflammation or coagulopathy, notably interleukin-6 (IL-6), C-reactive protein (CRP), and D-dimer, have been associated with mortality or opportunistic disease, after adjustment for appropriate variables, in several large randomized clinical trials. It is not known if elevated IL-6 or CRP causes this morbidity and mortality or if they are simply surrogate markers of a global inflammatory state.

Summary—Several inflammatory biomarkers appear to add to our ability to predict mortality or opportunistic disease in HIV-infected individuals. Before biomarkers will be useful, it will be necessary to identify interventions that moderate biomarker levels, and then determine if this moderation attenuates disease outcomes.

Keywords

biomarker; C-reactive protein; D-dimer; HIV; immune activation; immune senescence; interleukin-6

Introduction

Highly active antiretroviral therapy (HAART) can suppress HIV replication for extended periods resulting in substantial reductions in mortality in HIV-infected patients. However, despite benefiting from significant reductions in opportunistic disease, HAART-treated persons living with HIV continue to experience a relatively high incidence of malignancy, cardiovascular disease (CVD), metabolic, bone, renal, and liver disease not unlike aging populations [1,2]. A large body of evidence supports the notion that inflammation plays a role in all of these conditions in the general population, which have become leading causes of morbidity and mortality in HIV-infected individuals in settings when HAART is routinely employed. Compared with the general population, high-sensitivity C-reactive protein (hs-

CRP), interleukin 6 (IL-6), D-dimer, and certain other inflammatory biomarkers are significantly elevated in the setting of HIV infection [3,4••,5••]. Also, HIV infection is associated with increased expression of cellular markers of T-cell activation and senescence. This review will highlight recent developments in using plasma and cellular biomarkers of immune system activation and dysfunction to predict outcomes, particularly opportunistic disease and mortality, in HIV-infected individuals.

Soluble markers of immune dysfunction

Several plasma or serum biomarkers that are clinically available, or would be readily adaptable to clinical labs, have been correlated to outcomes.

Markers of inflammation

Elevated levels of hs-CRP, IL-6, and other inflammatory biomarkers have previously been associated with HIV disease progression and mortality but not until recently has this been validated in several randomized clinical trials. In the Strategies for Management of Antiretroviral Therapy (SMART) study, all-cause mortality was higher for HIV-infected patients with CD4⁺ T-cell counts more than 350 cells/ μ l randomly assigned to CD4⁺ T-cell-guided interruption of HAART than continuous HAART [6]. To examine the role of inflammation in mortality, four inflammatory (hs-CRP, IL-6, amyloid A, and amyloid P) biomarkers were determined at baseline and the follow-up visit before death in 85 cases and 170 matched controls [7]. Biomarkers were also assessed for a random sample of 249 HAART interruption and 250 viral suppression patients at baseline and at the 1-month follow-up.

In the case-control study, elevated IL-6 at baseline was significantly [unadjusted odds ratio (OR) = 8.3; comparing highest and lowest quartiles; $P < 0.0001$] associated with mortality, as was hs-CRP (OR = 2.0; $P = 0.05$). When adjusted for typical HIV and CVD covariants, ORs were increased. Increases in IL-6 after treatment interruption were also associated with an increased risk of death. The most common underlying causes of death in SMART were non-AIDS defining malignancy and CVD; only 8% of deaths were due to opportunistic disease. Greater age and body mass index were associated with higher IL-6 but smoking was not.

Baseline or latest (before event onset) elevations of IL-6 or hs-CRP were also predictive of opportunistic disease ($n = 91$ cases) in SMART. Patients with a baseline plasma hsCRP greater than 5 μ g/ml had a 3.5 higher OR of opportunistic disease than did those with an hsCRP level less than 1 μ g/ml ($P = 0.003$) [8••]. Baseline IL-6 was increased in CVD cases in SMART [9].

Other nested case-control studies within randomized clinical trials have confirmed that baseline elevations of plasma IL-6 and hs-CRP levels can predict mortality and opportunistic disease. In the Flexible Initial Retrovirus Suppressive Therapies (FIRST) study, several biomarkers were determined at baseline for 63 cases of opportunistic disease or death occurring within 12 months of HAART initiation, along with 126 matched controls, in patients who were HAART naive at baseline [10]. Elevated IL-6 (unadjusted OR 2.4; $P < 0.01$) or hs-CRP (OR 2.1; $P < 0.01$) again predicted mortality. Interleukin-8 and IL-10 were marginally predictive but not when adjusted. Baseline tumor necrosis factor- α (TNF- α) and interferon- γ (IFN- γ) levels were not predictive of opportunistic disease or death. Examination of 187 cases and 374 matched controls from the South African Phidsa II trial also found that elevated IL-6 or CRP predicted mortality (OR 3.8; $P < 0.0001$ and 3.6; $P = 0.0003$ respectively, comparing highest and lowest quartiles) [11]. Note that CRP, which is produced by hepatocytes and other cells in response to IL-6, may not be as reliable a biomarker in the setting of liver disease [7,12]. Finally, higher baseline concentrations of

plasma IL-6 as well as soluble tumor necrosis factor receptor-1 (sTNFR-1), a marker of TNF receptor engagement, and soluble CD27 or soluble CD40 ligand, both markers of T-cell activation, predicted opportunistic disease or death in an examination of 41 cases and 111 controls from two clinical trials enrolling HAART-naive HIV-infected patients [13••].

It is not known if elevated IL-6 or CRP is a causative factor of the morbidity and mortality it is associated with in HIV or simply a surrogate marker of a globally enhanced inflammatory state. Interventions with agents that target specific inflammatory mediators, such as IL-6, will be necessary to show causality. IL-6 is secreted by cells of the innate immune system and by engaging the IL-6 receptor in numerous cell types, notably B cells, it results in activation of cellular proliferation and survival pathways involved in oncogenesis. Hong *et al.* [14] have reviewed IL-6 and its implications for translational therapeutics in neoplasia. Tissue-specific imbalances in IL-6 may also promote infection by antagonizing TNF- α and promoting intracellular replication of atypical mycobacterium or cytomegalovirus [15,16]. Elevated IL-6 levels are associated with aging and have been shown to be an independent predictor of mortality in the elderly [17]. Studies examining CVD and Alzheimer's disease identified a relationship between cytokine polymorphisms and longevity, suggesting that those individuals who were genetically predisposed to produce low levels of inflammatory cytokines (IL-6 and TNF- α) may be more likely to reach the extreme limit of human lifespan [18]. Especially relevant in the setting of HIV infection, aging with ongoing inflammation has been aptly described as 'inflamm-aging' [17].

Markers of coagulopathy

In addition to elevated inflammatory biomarkers in persons living with HIV, there is evidence of a significant coagulopathic process with decreased protein S, protein C, and antithrombin activities as well as an increase in plasma D-dimer [19,20]. Induced by a variety of microbial (viral and bacterial) components binding cell surface Toll-like receptors (TLRs), the procoagulant tissue factor (TF) initiates a coagulation cascade that results in thrombin activation, which then cleaves fibrinogen to fibrin to form a stable clot. Cleavage of fibrin by plasmin releases fibrin degradation products, one of which we measure is D-dimer as a late stage marker of ongoing coagulation. In HIV-infected individuals, monocyte TF expression is increased and correlates with HIV RNA and with plasma D-dimer [4••].

In the SMART case-control study, baseline levels of D-dimer and prothrombin fragment 1 + 2 were determined. Elevated D-dimer was strongly associated with mortality (OR = 12.4; $P < 0.0001$ comparing highest and lowest quartiles) and this association was enhanced (OR 41.2; $P < 0.0001$) after adjustment [7]. D-Dimer levels were positively associated with increased age, BMI, black race and HIV RNA. D-Dimer was negatively associated with HAART use and after treatment interruption plasma levels were significantly increased, with the increase associated with mortality. Elevated baseline D-dimer levels also predicted CVD but did not predict opportunistic disease in SMART [8••,9]. In FIRST and Phidsa II, elevated baseline D-dimer levels were again predictive of mortality (unadjusted OR 2.4; $P < 0.01$, and OR 2.6, when comparing highest and lowest quartiles, $P = 0.003$, respectively) [10,11].

Markers of microbial translocation

HIV infection results in rapid and severe gut-associated mucosal lymphoid tissue (GALT) CD4⁺ T-cell depletion and fibrosis that is only slowly improved by HAART [21,22,23••]. Disruption of gut mucosal and immunologic integrity results in elevated plasma levels of bioactive bacterial lipopolysaccharide (LPS) and bacterial DNA, specifically conserved sequences of DNA encoding the 16S rRNA subunit (16S DNA), that can be amplified by PCR. Both biomarkers correlate directly with quantity of cellular activation markers HLA-

DR and CD38 expressed on CD8⁺ T cells, as well as reduced CD4⁺ T-cell recovery after HAART initiation [24••,25,26]. Increased CD8⁺ T-cell activation is well known to positively correlate with rate of CD4⁺ T-cell depletion due to apoptosis [27,28•], although HIV contributes to CD4 T-cell depletion in other ways, including induction of thymic failure [29••,30]. Plasma bacterial 16s DNA levels declined progressively with years of HAART but generally did not normalize and interestingly, were significantly lower in HAART-treated viremic patients than non-HAART-treated patients with similar levels of viremia [24••]. As a marker of microbial translocation, 16s DNA may have an advantage over LPS in that it is not limited to measuring just the Gram-negative fraction of the gut microbiota, but further studies are required to validate this biomarker.

LPS is not only a potent immunostimulatory molecule associated with T-cell activation, but monocyte activation as well after it interacts with cell surface TLR-4. In response, soluble CD14 (sCD14) is secreted into plasma and serves as a biomarker of monocyte activation that is positively correlated with LPS and more easily standardized between labs [26,31–33]. Recently, elevated levels of sCD14 have been associated with CD8⁺ T-cell activation and with mortality in the setting of HIV [34,35]. Other microbial translocation biomarkers including LPS, bacterial 16s DNA, and anti-LPS-core antibodies, did not correlate with mortality, suggesting that monocyte responsiveness to microbial products may be the critical determinate [35]. Activated monocytes also respond to LPS by secreting inflammatory cytokines. Robust elevations of TNF- α and IL-6 are seen when volunteers are given LPS injections resulting in plasma levels as low as 14 pg/ml [36], well below LPS levels seen in HIV-infected individuals [26]. A modest positive correlation between plasma sCD14 and IL-6 has been observed [35] (D. Nixon, unpublished data). Plasma sCD14 levels have also been positively associated with IFN- γ and monocyte TF expression [4••,26].

Together, these data begin to link gut microbial translocation to a multifactorial systemic inflammatory response. Although suppression of viremia with HAART consistently reduces D-dimer [19,20,37,38•,39], it less consistently reduces IL-6 [37,38•,39,40] and does not appear to reduce hs-CRP significantly [37,38•,41]. Thus, these biomarkers often remain elevated in persons taking HAART, perhaps because microbial translocation can persist even when viremia is suppressed [24••,26]. It is also possible that HAART, particularly protease inhibitors, may perpetuate some portion of this residual inflammation by inducing cellular stress responses, coagulopathy, or disruption of intestinal epithelial barrier integrity, although clinical correlation studies are needed to validate these in-vitro data [42–44,45•, 46].

Cellular markers of immune dysfunction

Although somewhat more difficult to adapt to the clinical settings, carefully collected and prepared peripheral white blood cell specimens permit an examination of T-cell function.

Markers of immune activation and senescence

HIV infection shares numerous similarities with aging, including an increased incidence of CVD, malignancy, infection and chronic viral reactivation, osteoporosis, neurocognitive decline, and frailty [47•]. In addition to ‘inflamm-aging’ cytokine profiles, HIV infection and aging share cellular immunologic similarities as well, including reduced naïve T-cell generation and T-cell receptor diversity as well as expanded populations of memory T cells with reduced function and shortened telomeres. Early immune aging or ‘immune senescence’ as a result of constant antigen burden and persistent immune activation in the setting of HIV has been recently reviewed [29••]. Interestingly, other chronic viral infections particularly cytomegalovirus may contribute to this immune senescent T-cell phenotype which may be exacerbated in the setting of HIV [48•,49,50,51•].

In HIV infection as with aging, there is increased expression of activation marker CD38, expressed as either percentage or median fluorescence intensity, on both CD4⁺ and CD8⁺ T cells [28•,52]. There is a positive correlation between proportion of CD8⁺ T cells that are HLA-DR⁺/CD38⁺ with the rate of CD4⁺ T-cell decay and development of opportunistic disease [27,28•]. Persistent T-cell activation drives proliferation and T-cell differentiation, resulting in the accumulation of senescent, antigen-experienced memory T cells that have reduced expression of CD28 and increased expression of CD57 [53•]. CD57 expression has been linked to greater resistance to apoptosis in CD8⁺ T lymphocytes during HIV infection, facilitating accumulation [54•]. CD28 is a costimulatory molecule, and its loss on CD4⁺ and CD8⁺ T cells results in reduced B-cell function and restricted T-cell diversity. An elevated proportion of CD8⁺ T cells expressing CD57 has been observed in both aging and HIV infection and this senescent CD28⁻/CD57⁺ phenotype is characterized by reduced capacity to produce IL-2 and shortened telomeres [55,56].

A higher frequency of senescent CD8⁺ T cells (CD45RO⁺CD57⁺CD28⁻) and lower frequency of naïve CD4⁺ and CD8⁺ T cells (CD45RA⁺CD28⁺CCR7⁺) were found in HAART-treated HIV-infected individuals with suppressed viremia and high-CD4⁺ T-cell counts (median 724 cells) and older HIV-negative individuals as compared to HIV-negative younger controls [57]. CD8⁺ T-cell activation marker (HLA-DR⁺CD38⁺) expression was also higher in HIV-infected individuals than in older or younger HIV-negative individuals. Thus, HIV-infected individuals (median age, 56 years) with good immune reconstitution and viral suppression had immunologic similarities to much older (median age, 88 years) HIV-uninfected individuals. And as with increased CD8⁺ T-cell activation, increased senescence as determined by decreased CD28 median fluorescence intensity on CD8⁺ and CD4⁺ T cells has been associated with more rapid HIV disease progression [58•].

Conclusion

HIV infection shares numerous similarities with aging in the general population, among them, ‘inflamm-aging’ and immune senescence that is driven by chronic immune system activation. Gut microbial translocation is associated with chronic immune system activation and likely contributes to systemic inflammation, all of which may persist with HAART. Elevations of certain inflammatory or coagulopathic biomarkers, notably IL-6, hs-CRP, and D-dimer have been independently associated with mortality and other clinical endpoints in large randomized clinical trials (Table 1) [7,10,11]. As these biomarkers appear to add to the predictive value of traditional tests used in management of HIV (e.g., CD4⁺ T-cell counts and HIV RNA), they have the potential to help guide treatment decisions. Before ‘biomarker guided’ therapy can occur, it will be necessary to: identify interventions that reduce the biomarker; validate the value of the biomarker assay that would be the intervention threshold and deal with lab to lab variation issues; confirm that the intervention reduces both the biomarker and the clinical endpoint in the setting of HIV.

A large number of additional cytokine, chemokine, vascular endothelial, coagulopathy, tissue injury, microbial translocation, and cellular markers await to be examined in randomized clinical trials and correlated with outcomes. Markers of immune dysfunction for further evaluation in clinical outcome studies could include:

1. Soluble markers
 - a. Inflammation
 - i. tumor necrosis factor (TNF) α , soluble TNF receptor-1 and -2, IFN- γ , IL-1, -6, -8, -10, -17, and -23, monocyte chemotactic protein-1, macrophage inflammatory proteins -1 α and -1 β , C-reactive protein

b. Coagulation

- i.** α_2 -dimer, thrombin/anti-thrombin, soluble tissue factor

c. Microbial translocation and intestinal epithelial injury

- i.** Soluble CD14, lipopolysaccharide, anti LPS-core antibodies (EndoCab), bacterial 16s DNA, intestinal fatty acid binding protein

d. Soluble markers of T-cell activation

- i.** soluble CD27, soluble CD40 ligand

2. Cellular markers**a. Innate immune system (dendritic cells, natural killer cells)**

- i.** Activation: CD83, CD86, CD69

b. Adaptive immune system (CD4⁺ and CD8⁺ T cells)

- i.** Activation: HLA-DR, CD38, Ki67, Caspase 3
- ii.** T-cell exhaustion: programmed death-1 (PD-1) co-stimulatory receptor and ligand
- iii.** T-cell senescence: CD28, CD57

This should be a scientific priority. Of the markers examined thus far, α_2 -dimer may be the farthest along as it is clearly reduced by an intervention, HAART [19,20,37–39]. The Strategic Timing of Antiretroviral Therapy (START) study, which is currently randomizing HAART-naïve patients with CD4⁺ T-cell counts more than 500 cells to either immediate HAART or HAART initiated when the CD4 cell count reaches 350 cells/ μ l, may help validate the concept of ‘biomarker-guided’ HAART. In START, a baseline biomarker can be retrospectively determined and the outcomes of patients with ‘high’ levels of the biomarker assigned to either early or late HAART can be compared with patients with a ‘low’ level of the biomarker.

Beyond HAART, a number of other anti-inflammatory, anticoagulopathy, anti-immune activation interventions have been suggested and the most promising should be examined. Aspirin and statins for example have been shown to reduce α_2 -dimer, hsCRP, and certain inflammatory cytokines in several non-HIV clinical settings. Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) was a seminal inflammatory biomarker-guided therapy study that randomly assigned 17 802 healthy participants with normal LDL cholesterol and elevated hs-CRP levels (>2.0 mg/dl) to rosuvastatin or placebo and found a clear reduction in CVD and all cause mortality [59]. Additional studies are needed to confirm this result in the unique setting of HIV, but it is likely that interventions other than HAART will emerge to attenuate the adverse effects of HIV-associated immune dysfunction.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 561).

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Table 1

Inflammatory or coagulopathy biomarkers associated with mortality in randomized clinical trials of HIV-infected individuals

Biomarker	Mortality odds ratios ^a first vs. fourth quartile	Effect of antiretroviral therapy	Other known HIV disease associations
D-Dimer	12.4 [7], 2.4 [10], 2.6 [11]	Decreases [19,20,37,38•,39]	CVD
hs-CRP	2.0 [7], 2.1 [10], 3.6 [11]	No decrease [37,38•,41]	CVD, OD
IL-6	8.3 [7], 1.8 [10], 3.8 [11], 1.5 ^b [13••]	May decrease [37,38•,39,40]	CVD, OD
sCD14	6.0 [13••]	Unknown	Microbial translocation
sCD27	1.7 ^b [13••]	Unknown	
sCD40L	1.7 ^b [13••]	Unknown	
sTNFR-1	1.8 ^b [13••]	Unknown	

CVD, cardiovascular disease; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; OD, opportunistic disease; sCD14, soluble CD14; sCD27, soluble CD27; sCD40L, soluble CD40 ligand; sTNFR-1, soluble tumor necrosis factor receptor-1.

^a Odds ratios are unadjusted and in all cases in which adjustment is performed for covariants, the odds ratios are enhanced.

^b Odds ratio denotes incremental difference by 1 standard deviation in distribution of the variable for combined outcome of mortality and OD.