Biomarkers of Aging in HIV: Inflammation and the Microbiome

Camilla Tincati[1], Esther Merlini[1] Giuseppe Ancona[1] and Giulia Marchetti[1]

[1]Department of Health Sciences, Clinic of Infectious Diseases, ASST Santi Paolo e Carlo, University of Milan, Italy

Correspondence to: Camilla Tincati, M.D., Ph.D.

Address: Clinic of Infectious Diseases, Department of Health Sciences, University of Milan, San Paolo Hospital, Milan, Italy, Via di Rudini' 8, 20142, Milan, Italy

Phone: +390281843046; Fax: +390281843054; Email: camilla.tincati@unimi.it

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Abstract

Purpose

HIV-infected subjects present increased levels of inflammatory cytokines and T-cell activation in the peripheral blood despite suppressive combination antiretroviral therapy which renders them at increased risk of premature aging. The purpose of the present work is to review existing evidence on the ways in which the anatomical and microbiological abnormalities of the gastrointestinal tract can represent a major cause of organ disease in HIV infection.

Methods

We conducted a systematic review of the Pubmed database for articles published from 2014 to 2018. We included studies on inflammatory/activation biomarkers associated with cardiovascular and bone disease, neurocognitive impairment and serious non-AIDS events in HIV-infected subjects. We also included researches which linked peripheral inflammation/activation to the anatomical, immune and microbiological alterations of the gastrointestinal tract.

Results

Recent literature data confirm the association between non-infectious comorbidities and inflammation in HIV infection which may be driven by gastrointestinal tract abnormalities, specifically microbial translocation and dysbiosis. Furthermore, there is mounting evidence on the possible role of metabolic functions of the microbiota in the pathogenesis of premature aging in the HIV-infected population

Conclusions

Biomarkers need to be validated for their use in the management of HIV infection. Compounds which counteract microbial translocation, inflammation and dysbiosis have been investigated as alternative therapeutic strategies in viro-suppressed HIV-infected individuals, but appear to have limited efficacy, probably due to the multifactorial pathogenesis of non-infectious comorbidities in this setting.

Keywords

HIV, inflammation, comorbidities, microbial translocation, microbiome, metabolome

HIV, inflammation and end-organ disease

Infection with the Human Immunodeficiency Virus (HIV) can be considered an inflammatory disease [1]. If left untreated, viral replication leads to the loss of CD4+ T-cell counts and HIV infection progresses to the Acquired Immunodeficiency Syndrome (AIDS). Combination antiretroviral therapy (cART), i.e. the combined use of three, effective antiviral drugs which impede HIV replication, has drastically changed the natural course of infection by restoring CD4+ T-cell counts and hampering advancement of HIV-infected subjects to AIDS [2]. Literature has consistently shown, however, that inflammation and activation persist in the course of viro-suppressive treatment, albeit at lower levels than those observed when the infection remains untreated [1]. Specifically, HIV-infected subjects on cART feature activation of innate (sCD14, sCD163) and adaptive (CD38+HLADR+) immunity, senescent immune phenotypes (low expression levels of CD28, elevated levels of CD57 and PD-1), impaired thymic output, persistence of HIV reservoirs, reduced antigen-specific responses and heightened levels of pro-inflammatory and coagulation markers (IL-6, d-dimer, high-sensitivity C Reactive Protein, CRP) [1]. Interestingly, such chronic, low-grade inflammation presents immune abnormalities that characterize physiologic aging [3]. The so called “inflammaging” signature of viro-suppressed, HIV-infected subjects renders this population at risk of premature aging (cardiovascular, bone and renal disease as well as neurocognitive impairment) and death from non-infectious comorbidities [3].

Inflammatory and activation biomarkers have been object of numerous studies for their exploitation in clinical practice in order to aid in the identification and management of HIV-infected subjects at risk of non-communicable diseases [4, 5]. Yet, despite these efforts, the lack of validation standards for the majority of biomarkers investigated and the multifactorial process behind it [6] are causing a dearth of parameters to support clinicians in their decisions.

Microbial translocation from the gut lumen to the systemic circulation has been widely investigated as one of the possible causes of activation/inflammation linked to premature aging in HIV infection

[6]; furthermore, it triggered studies on the gastrointestinal tract as a major site of HIV pathogenesis and shed light on possible mechanisms to target for the elaboration of alternative therapeutic interventions [1].

The purpose of the present work is to review existing evidence on the ways in which the anatomical and microbiological abnormalities of the gastrointestinal tract can represent a major cause of organ disease in HIV infection.

Microbial translocation-induced immune activation and inflammation are linked to non-infectious comorbidities in HIV infection

Microbial translocation, i.e. the passage of bacteria and microbial bioproducts (e.g. lipopolysaccharide, LPS, of Gram-negative microrganisms) through the gastrointestinal tract to the peripheral circulation, is a major cause of immune activation in HIV infection.

Over a decade go, a seminal paper by Brenchley et al. described increased plasma levels of LPS in HIV-infected subjects and demonstrated their positive correlation with markers of innate and adaptive immunity [7]. Subsequent literature observations confirmed such finding and demonstrated high levels of microbial-induced immune activation in subjects with poor CD4+ T-cell recovery on cART [8, 9] as well as non-infectious comorbidities.

In this respect, microbial translocation, inflammation and immune activation have been invariably associated with cardiovascular risk in the HIV-infected population [10]. In particular, various parameters have been linked to dyslipidemia [11-13], insulin insensitivity [11, 12], endothelial dysfunction [14], visceral fat accumulation, higher Framingham risk [11, 12, 15], subclinical artery disease [16-20] and biomarkers of cardiovascular disease in this setting [12], highlighting the possible common pathways that cardiovascular disease and HIV share in their pathogenesis.

Similarly, osteopenia and osteoporosis in HIV-positive subjects may be driven by the inflamed and activated immune milieu which features infection [21-25]. While some reports confirm the association between immune biomarkers and impaired osteogenesis [25, 26]/mineral density [27,

28], others have however failed to detect such a link [29, 30]. Future studies are thus warranted to explore the true effects of activation/inflammation on bone mineral density and fractures in the HIV-infected population.

More recently, inflammatory biomarkers have also been studied in the context of HIV-related neurocognitive disorders. A growing body of evidence shows that immune-mediated brain injury may contribute to cognitive impairment and structural brain abnormalities [31-36]. The clinical significance of these findings remains yet an open matter, as central nervous system activation and inflammation also feature asymptomatic individuals [37-39].

Finally, large cohort studies confirmed the above-mentioned findings by demonstrating that markers of microbial translocation (LPS,), immune activation (sCD14), inflammation (IL-6, sTNF-RI, sTNF-RII, high-sensitivity CRP) and coagulation (d-dimer and sICAM-1) are independent predictors of mortality as well as AIDS and non-AIDS related morbidity in HIV infection [40-45].
The most recent findings on biomarkers predicting non-infectious comorbidities, serious non-AIDS events (SNAEs) and death in cART-treated subjects are summarized in Table 1.

The gut as a source of inflammation in HIV infection

Suggestive evidence of microbial translocation being a cause of immune activation prompted the in-depth study of the gut as a pathogenic site of HIV infection.

At the very beginning of the epidemic HIV-related enteropathy with malabsorption and steathorrea in untreated individuals was described in literature and featured both jejunal (partial villus atrophy with crypt hyperplasia) and colonic abnormalities (focal cell degeneration near the crypt base) [46]. More recently, studies have shed light on the underlying mechanisms of HIV-related enteropathy by demonstrating that exposure of genital and intestinal epithelial cells to HIV can directly breach the integrity of mucosal epithelial barrier allowing for microbial translocation [47]. Furthermore, in the context of untreated infection, microbial-induced immune activation, measured by the levels of

soluble CD14 (sCD14) correlated with markers of intestinal damage, thus pointing to the gut as a possible source of systemic inflammation [48].

A solid bulk of evidence demonstrated that the anatomical defects of HIV-related enteropathy persist on cART. Indeed, a reduced expression of ileum and colonic gut junctional complex proteins (cadherins, claudins, occludins) features long-term treated subjects [49-51] and is linked to increased gut permeability and persistent levels of microbial translocation [51]. Moreover, the negative correlation between LPS-dependent immune activation (sCD14 plasma levels, peripheral $CD8^+CD38^+$ T-cell frequencies) and gut junctional proteins [49, 51] further highlights that GI tract defects may contribute to inflammation. In line with this finding, a cohort study showed that peripheral blood levels of intestinal fatty acid binding protein, marker of intestinal damage, and zonulin-1 are independent predictors of mortality in cART-treated subjects [52].

Another important alteration that occurs in the gastrointestinal tract which may fuel microbial translocation is the impairment of mucosal immunity: early studies highlighted the massive depletion of CD4+ T-cell at mucosal sites in all phases of HIV infection and their partial reconstitution in the course of treatment [46]. More recently, studies have focused on cell populations which safeguard mucosal surfaces, namely IL-17-producing subsets: indeed, IL-17 is a crucial cytokine for protection against infectious microbes [53] and epithelial maintenance occurs through wound repair [54-56]. Both innate ($\gamma\delta$T) and adaptive (Th) IL-17 producing cells are lost in the natural course of HIV infection [57, 58]; while their frequencies may be restored by the introduction of early treatment, their function invariably remains impaired [46], thus possibly explaining the persistence of microbial translocation and structural defects of the gastrointestinal tract despite effective therapy. Many reports have indeed confirmed the inverse relationship between IL-17-producing cell frequencies and inflammation in untreated disease [46], yet whether functional defects of mucosal populations directly drive inflammation and activation in the periphery of subjects on cART has yet to be demonstrated.

Findings on impaired gut structure and mucosal immunity in the setting of HIV infection have prompted the study of the gut microbiota, given its role in the maturation of the gut barrier and control of immune homeostasis at mucosal sites [57].

Early reports in naïve subjects identified an outgrowth of fecal *P.aeurginosa* and *C. albicans* as well as a reduction of bifidobacteria and lactobacilli; these alterations were linked to increased fecal calprotectin levels, marker of intestinal inflammation [46]. Subsequent studies confirmed a dysbiotic fecal microbiota in the course of HIV infection featuring the enrichment for *Proteobacteria* and skewing of the *Bacteroidetes* phylum with a *Prevotella*-rich/*Bacteroides*-poor profile [59-64], which is only partially restored by cART [65]. Importantly, HIV-related dysbiosis correlates with heightened T-cell and myeloid dendritic cell activation, lower mucosal IL-17/IL-22 secretion, as well as inflammatory markers [59, 61]. In the attempt to demonstrate the mechanisms whereby the fecal microbiota causes peripheral T-cell activation and inflammation, a very recent study reported greater production of pro-inflammatory cytokines in monocyte cultures (TNF-α, IL-6, IFN-γ) and the selective activation of peripheral T-cells following culture stimulation with fecal bacterial communities in HIV infection [66].

Taken together, literature studies convene that HIV-related dysbiosis may drive peripheral blood inflammation and may be thus implicated in organ disease.

The metabolic pathways of a dysbiotic microbiome may drive inflammation and organ disease in HIV infection

Technical advances have not only shed light on the composition of resident bacteria in HIV infection but also on the functional activity of the microbiota, which is critical to human health and disease [67].

An early study showed differences between the relative abundance of several imputed metagenomic functions between untreated HIV-infected subjects and healthy controls, suggesting the impairment of the functional activity of the microbiota in HIV infection [68]. In line with this observation,

researches that followed showed that the microbiota is depleted in genes belonging to main energetic processes (pyruvate metabolism, glycolysis and gluconeogenesis) and amino acid metabolism [63] which could impact on cART-mediated reconstitution of CD4+ T-cells [69]. In addition, bacterial genera enriched in HIV-infected, untreated subjects were found to encode enzymes involved in tryptophan catabolism [70, 71], an independent predictor of non-AIDS comorbidities and death [52, 72], thus entailing that the dysbiotic bacteria in HIV infection may directly contribute to immunoactive tryptophan catabolism in HIV and negatively impact on overall mortality [70].

Stemming from these findings, other intestinal microbiota-generated metabolites known to cause organ disease were explored in the setting of non-infectious comorbidties in HIV infection. In particular, trimethylamine-N-oxide (TMAO), a metabolite of phosphatidylcholine, was linked to plaque burden [73, 74], myocardial perfusion defects [75], coronary stenosis [76] in HIV-infected subjects. Although TMAO was independently associated with microbial translocation markers (LPS) [77] and macrophage activation (sCD14, CD163) [74, 77], a recent report failed to find a relationship between the metabolite and inflammation [78], suggesting its limited use as clinical marker of cardiovascular risk in HIV infection given possible confounders.

Significant evidence exists in literature on the gut-brain axis, i.e. the bidirectional communication between the central and the enteric nervous system, linking emotional and cognitive centers of the brain with peripheral intestinal functions [79]. The production of bacterial metabolites represents a possible mechanism by which the intestinal microbiota interacts with the brain. In the setting of HIV infection, increased choline and N-acetyl aspartate were recently found in the brain of subjects with neurocognitive impairment [80]; with evidence currently lacking on the source of such metabolites, future studies are critical for addressing whether microbial-dependent metabolites play a role in the pathogenesis of neurocognitive disorders in HIV infection.

In line with the findings of a link between the microbiota and the central nervous system, studies in the clinical setting of rheumatoid arthritis and inflammatory bowel disease have demonstrated the

existence of a gut-immune-skeletal axis in which alteration of the gut microbione induce an activated immune phenotype and inflammatory milieu which associates with bone loss [81]. Given the pathogenic and clinical features of HIV disease, further research should explore the effects of the microbiome/metabolome in HIV-relates osteopenia and osteoporosis.

Open issues and future directions

The present review focused on microbial translocation-induced activation and inflammation as a cause of premature aging in HIV disease. We highlighted the role of the gastrointestinal tract as a driver of inflammation: alterations of the gut epithelial barrier, mucosal immune populations and microbiome/metabolome all play a key role in causing and perpetuating low-grade inflammation which features the inflammaging phenotype of viro-suppressed HIV-infected individuals.

Clinical questions remain as to whether any on the inflammatory biomarkers examined in the literature may be used as a screening tool to identify subjects at risk of developing non-infectious comorbidities. Inflammatory biomarkers lack validation standards and are not routinely measured in HIV-infected individuals; this leaves clinicians with the CD4+ T-cell count as the only reliable marker to assess subjects' immune status, which nonetheless does not capture the "inflammaging" signature of cART-treated disease. Studies focusing on longitudinal cohorts with powered clinical endpoints are needed in order to exploit biomarkers of premature aging in the clinical practice.

Another relevant aspect is how to turn-off microbial-driven inflammation. In this respect, work has been carried out in the attempt to reverse microbial translocation and dysbiosis. In humans, LPS-antagonizing compounds (sevelamer), anti-inflammatory agents (mesalamine) and antibiotics (rifaximin) have lead to little/no effects on microbial translocation, inflammation and T-cell activation [46]. Similarly, the administration of pre/probiotics did not result in significant changes in inflammation/activation [46] and composition of the microbiome [82]. A possible explanation for the failure of these treatments is that they target a limited set of causes underlying a multifactorial process (i.e. microbial translocation, reservoirs, CMV co-infection) [6]. Future studies should aim at

combining several approaches in order to counteract the complex phenomenon of aging in HIV infection.

Table 1: Biomarkers linked to non-infectious comorbidities, serious non-AIDS events and death in cART-treated, HIV-infected subjects

Clinical Event	Study design	Patient Population	Aim	Markers	Main findings	Reference
CVD	Cross-sectional	61 cART-treated	Investigate whether microbial translocation and monocyte activation associate with endovascular dysfunction, inflammation and altered coagulation.	LPS, sCD14, hs-CRP, d-dimer	sCD14 associates with endovascular dysfunction in HIV.	[14]
CVD	Cross-sectional	147 cART-treated	Explore the relationship between inflammation, T-cell/monocyte activation and coronary calcification/subclinical vascular disease.	sCD14, sCD163, hs-CRP, IL-6, sTNFR-II, sVCAM-1, OPG, RANKL	sCD14 independently associates with coronary artery calcification.	[19]
CVD	Cross-sectional	252 cART-treated	Explore the association of soluble biomarkers with patient demographics, HIV progression, components of the metabolic syndrome, viral co-infections, Framingham risk score and the VACS Index.	IL-6, cystatin C, hs-CRP, TNF-α, d-dimer	Elevated inflammation and coagulation associate with higher scores on risk stratification indices.	[15]
CVD	Cross-sectional	242 cART-treated and 348 uninfected controls	Investigate the association of inflammatory markers with subclinical coronary artery disease.	IL-6, sICAM-1, fibrinogen, d-dimer, hs-CRP, sTNFR I and II	Higher inflammatory marker levels associated with greater prevalence of coronary stenosis.	[16]
CVD		149 cART-treated	Determine the associations of markers of immune activation with atherosclerosis and mortality.	IL-6, hs-CRP, sCD14, sCD163, d-dimer, T cell activation (HLA-DR/CD38) and senescence (CD57/CD28), monocytes subsets (CCR2/CX3CR1 /CCR5)	Monocyte CCR5 expression and plasma IL-6 associate with atherosclerosis. IL-6 and carotid artery IMT associate with all-cause mortality.	[17]
CVD/Bone disorders	Cross-sectional	94 cART-treated and 41 uninfected controls	Investigate the role of traditional factors, T-cell phenotype and osteoprotegerin in bone and cardiovascular disease.	OPG, T cell activation (HLA-DR/CD38) and senescence (CD57/CD28)	OPG and T-cell activation/senescence linked to bone and cardiovascular disease.	[28]
Bone disorders	Cross-sectional	142 cART-treated	Determine the association between	RANKL, OPG,	Weak correlation	[29]

			bone health and inflammation, T-cell activation, and monocyte activation.	sVCAM-1, sICAM-1, IL-6, TNF-α, sTNFR I and II, hs-CRP, d-dimer, sCD14, sCD163	between BMD and markers of inflammation/immune activation . Bone resorption associated with ICAM-1	
Neurogognitive impairment	Longitudinal	99 cART-treated	Examine the relationship between mild HAND and CSF NFL/neopterin.	Neopterin, NFL	Mild HAND was associates with increased intrathecal immune activation.	[35]
Neurogognitive impairment	Cross-sectional	41 cART-treated	Investigate the role of cell-free mitochondrial DNA in the CSF.	mitochondrialDNA, sCD14, TNF-α, IL-6, IL-8, MCP-1, IP-10, NFL	Higher cell-free mitochondrial DNA is associates with inflammation	[39]
Neurogognitive Impairment	Longitudinal	51 acutely-infected, cART-treated and 18 uninfected controls	Explore whether early cART impacts CD163 shedding, with possible implications on the CNS.	sCD163	Early treatment reduces sCD163 which is linked to neuropsychological perfomances	[32]
Neurogognitive Impairment	Cross-sectional	90 cART-treated, 94 uninfected controls	Assess the impact of HIV on complex motor performances.	d-dimer, IL-6, MCP-1, sCD14, TNF-α	Inflammation accounts for worse complex motor skills.	[36]
Death	Case-control	64 cases - 128 controls (LCOSA cohort) 27 cases – 54 controls (SCOPE cohort)	Assess immunologic predictors of mortality.	IL-6, I-FABP, sCD14, hs-CRP, zonulin-1, sTNFR-1, d-dimer, KT ratio, T cell activation (CD38/HLA-DR), exhaustion (PD1), senescence (CD57/CD28)	Gut epithelial barrier dysfunction, innate immune activation, inflammation, independently predict mortality	[52]
SNAEs/death	Case-control; longitudinal	cART-treated: 143 cases (non-AIDS events) 315 controls	Assess the role of biomarkers on disease outcomes.	IL-6, IFN- γ, sCD14, IP10, sTNFR-1, sTNFR-II, d-dimer T cell activation (CD38/HLA-DR), exhaustion (PD1) senescence (CD57/CD28)	IL-6, sTNFR-I and II, sCD14,, plasma KT ratio, and d-dimer level associate with a higher risk of non–AIDS-related morbidity and mortality.	[72]
SNAEs	Case-control;	cART-treated:	Investigate whether unchanged or	sCD14, sCD163,	Unchanged or slow rates	[83]

	longitudinal	39 cases (non-AIDS events) 39 controls	smaller decreases in systemic inflammation predict SNAEs.	IL-6	of decrease of sCD14 and IL-6, levels predict SNAEs.	
SNAEs	Longitudinal	249 cART-treated	Examine the longitudinal changes in biomarkers following cART their association with future non-AIDS events.	IL-6, d-dimer	Persistent elevation of d-dimer levels associate with an increased risk of non-AIDS events.	[43]
SNAEs/death	Cross-sectional	3756 cART-treated: 1748 SMART, 1446 ESPRIT, 572 SILCAAT cohorts	Examine the associations of biomarkers with SNAEs/death in 3 large cohorts.	IL-6, d-dimer, hs-CRP	IL-6 and d-dimer are independently associatee with SNAEs.	[42]

Legend:
CVD, cardiovascular disease; cART, combination antiretroviral therapy; LPS, lipopolysaccharide; sCD14, soluble sCD14; hs-CRP, high-sensitivity C Reactive Protein; sCD163, soluble 163; sTNFR, soluble Tumor Necrosis Factor Receptor; sVCAM-1, vascular cell adhesion molecule-1; OPG, Osteoprotegerin; RANKL, RANK Ligand; TNF-α, Tumor Necorsi Factor-α ; IL-, Interleukin; sICAM-1, intracellular adhesion molecule-1; IMT, Intima Media Thickness; CSF, Cerebro-spinal Fluid; NFL, Neurofilament Light protein; HAND, HIV-Associated Neurocognitive Disorder; MCP-1, Monocyte chemoattractant protein-1; IP-10, Interferon- γ-induced protein 10; IFN- γ, Interferon-γ; I-FABP, Intestinal fatty-acid binding protein; KT, Kynurenine tryptophan; SNAE, Serious Non-AIDS Event.

Conflict of Interests Statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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