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Article

Association between soluble biomarkers - microbial translocation, inflammation and cardiovascular risk in HIV- infected individuals: a systematic review

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Microbial translocation is associated with the increased risk of cardiovascular disease in HIV-infected individuals. There is scarce information regarding the possible associations between the biomarkers of microbial translocation, inflammation and cardiovascular risk that can be evaluated in clinical laboratories using plasma or serum samples. This systematic review was conducted according to the PRISMA protocol in order to verify the most used soluble biomarkers of microbial translocation, inflammation and cardiovascular risk, as well as possible associations between them, in HIV-infected individuals. A search was performed using the Medline, Scopus and Web of Science databases to identify existing studies regarding the relationship between microbial translocation biomarkers, inflammation and cardiovascular risk in HIV-infected patients. Eleven articles that presented soluble biomarkers of microbial translocation (LPS, rDNA, sCD14, LBP and EndoCAb) were selected. The most frequently evaluated soluble biomarker was sCD14, followed by LPS; the latter were associated with some lipid profile parameters. This systematic review considered soluble blood biomarkers that can be utilized in laboratory diagnosis. The aim was to identify the interconnection between microbial translocation, inflammation and cardiovascular risk. Despite the fact that a large number of inflammation and cardiovascular risk biomarkers have been previously reported, it was noted that important markers involved in the pathophysiology of cardiovascular diseases need to be included in future research.

Keywords: Microbial translocation. Inflammation. Cardiovascular risk. HIV. Biomarkers.

INTRODUCTION

Cardiovascular disease is classified as a major cause of morbidity and mortality in HIV-infected individuals (Palella, Phair, 2011). Evidence indicates that systemic inflammation and chronic immune activation are related to increased cardiovascular risk (Grinspoon, 2014). Previous studies have suggested that these mechanisms can be triggered by the microbial translocation of products from the gut to the systemic circulation due to damage in the intestinal epithelium during the progression of HIV infection (Brenchley *et al*., 2006).

Microbial translocation can be evaluated in plasma by the direct quantification of bacterial products such as the presence of lipopolysaccharides (LPS) (components of gram-negative bacterial cell wall), peptidoglycans (components of gram-positive bacterial cell wall), or bacterial DNA fragments (such as rDNA - ribosomal bacterial DNA) (Lichtfuss *et al*., 2011).

In addition, the presence of LPS in plasma promotes the hepatic synthesis of the LPS-binding protein (LBP) responsible for the increased binding of LPS to the CD14 co-receptor, adjacent to the toll-like receptor 4 (TLR-4), which is expressed on the surface of monocytes and macrophages (Płóciennikowska *et al*., 2015). When

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the binding of LPS to the CD14 co-receptor occurs, these blood cells secrete soluble CD14 (sCD14) into the circulation (Plociennikowska *et al*., 2015). Therefore, the measurement of LBP and sCD14 can indirectly identify the effects of microbial translocation (Lichtfuss *et al*., 2011).

Another indirect biomarker of microbial translocation is related to the active protection of antibodies (such as EndoCAb/Endotoxin core antibodies) to neutralize LPS and its effects as a potent immunological activation molecule, thus limiting the effects of microbial translocation (Marchetti, Tincati, Silvestri, 2013).

After the activation of TLR-4 receptors by LPS binding, immune system cells trigger a signaling cascade, which leads to the production of proinflammatory cytokines (i.e. interleukin-1β, interleukin-6, tumor necrosis factor and type I interferons) (Meng, Lowell, 1997; Sandler *et al*., 2011; Zanoni, Granucci, 2013) and may induce chronic inflammatory conditions such as the development of atherosclerosis (Płóciennikowska *et al*., 2015).

Several studies have demonstrated the use of biomarkers of microbial translocation, inflammation and cardiovascular risk (Blodget *et al*., 2012; Yong *et al*., 2016; Ballegaard *et al*., 2017). However, information regarding possible associations between these biomarkers, which could be evaluated in clinical laboratories using plasma or serum samples, are scarce (Kelesidis *et al*., 2012). Therefore, a systematic review was conducted to verify the most used soluble biomarkers of microbial translocation, inflammation and cardiovascular risk, as well as the possible associations between them in HIV-infected individuals.

MATERIAL AND METHODS

Literature search

This systematic review was performed according to the Preferred reporting items for systematic reviews and meta-analyses - PRISMA (Liberati *et al*., 2009) checklist. We systematically searched electronic databases, including Medline, Scopus and Web of Science, to identify potential studies that examined the relationship between soluble biomarkers of microbial translocation, inflammation and cardiovascular risk in HIV-infected individuals. The following search terms: (HIV or human immunodeficiency virus or AIDS); and (microbial translocation); and (cardiovascular diseases) were used for the literature search. The search terms were limited to titles and abstracts. Publications were restricted to the following languages: English, Portuguese and Spanish. The databases were searched for studies published until December 2017.

Study selection

The following criteria were applied to identify eligible studies for this systematic review:

Firstly, the studies were screened on the basis of title and abstract. The following inclusion criteria were considered: i) case-control studies, cross-sectional cohort, longitudinal cohort and clinical trials; ii) studies that determined soluble biomarkers for microbial translocation, inflammation and cardiovascular risk (i.e. biomarkers that can be evaluated in plasma or serum). The following exclusion criteria were considered: i) review studies; ii) studies of HIV-infected individuals under the age of 18; iii) animal studies; and iv) *in vitro* studies.

Secondly, full-text articles were evaluated; those which did not present any relationship between soluble biomarkers of microbial translocation, inflammation and cardiovascular risk were excluded (Figure 1).

Data extraction

Using a standardized data extraction form, the following data were extracted from the retrieved full-text articles: country; year of publication; study design; sample size; gender; age; CD4+ T cell count; percentage of participants in antiretroviral therapy (ART); soluble biomarkers of microbial translocation, inflammation and cardiovascular risk assessed; laboratory methods for the biomarkers; and relationship between the biomarkers.

Critical analysis of the included studies

The selected articles were evaluated regarding the level of scientific evidence according to the Oxford Center for Evidence-Based Medicine Classification (Phillips *et al*., 2009). Two researchers independently performed all the stages of research and any discrepancies were discussed at a consensus meeting between two reviewers.

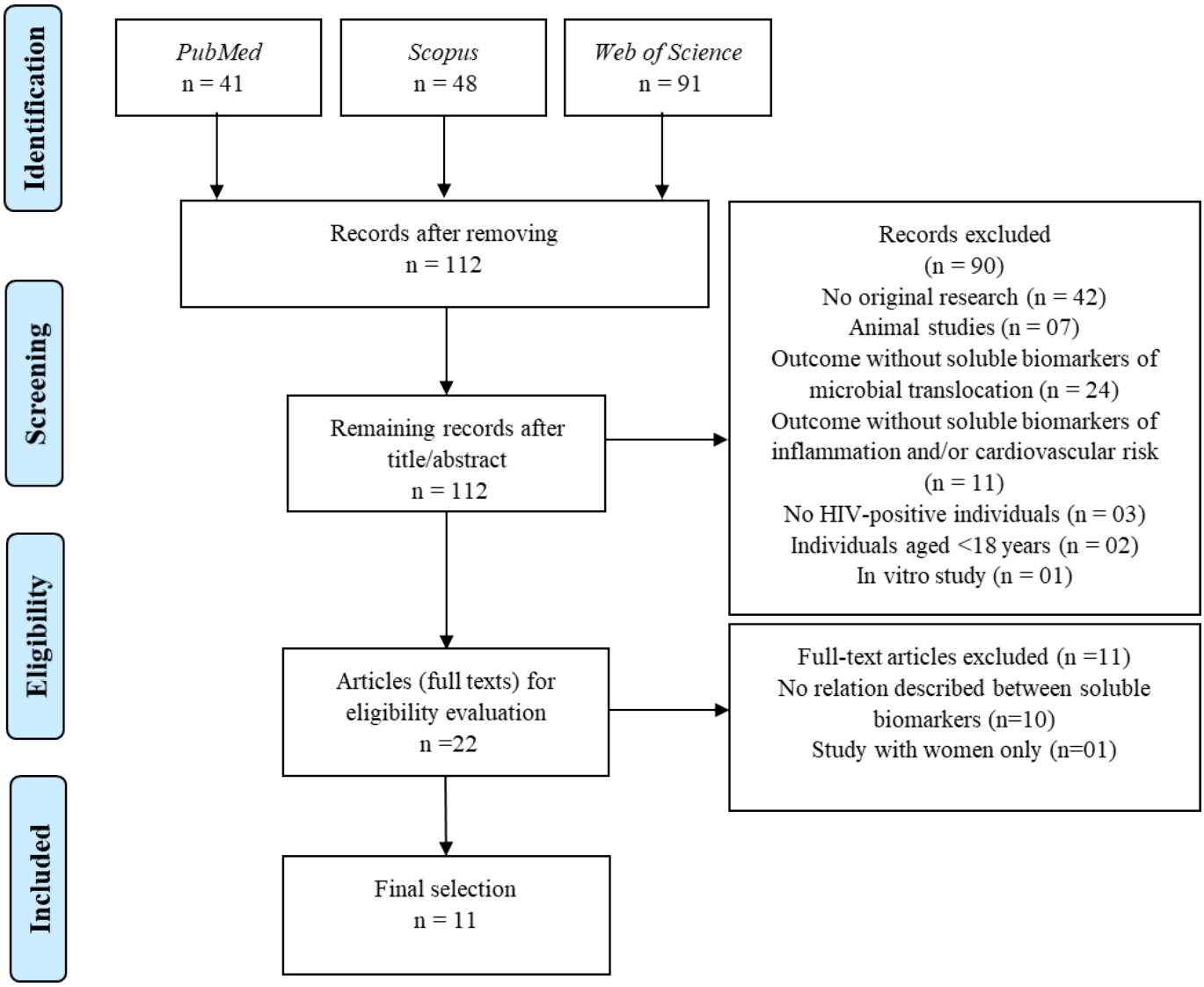

FIGURE 1 - Flowchart of study selection.

Analysis of results

Given the heterogeneity of the studies, especially the evaluation of the biomarkers in different groups and subgroups of HIV-infected individuals, the results were presented using descriptive statistics (mean ± standard deviation, number, and percentage).

The evaluations of possible associations between the biomarkers were divided into groups according to each microbial translocation biomarker identified in the studies. The extraction of the association results between the biomarkers was performed in the following order: i) multivariable regression analysis; ii) univariable regression analysis; and iii) correlation; if they were not reported, data were extracted from the relationship between the presence of microbial translocation and changes in biomarkers of inflammation and cardiovascular risk. The results were reported as follows: positive association; inverse association; and no association, or no data.

RESULTS AND DISCUSSION

The literature search produced 180 articles; 22 articles were screened using full-text, and 11 articles were eligible for data extraction (Figure 1). The characterization of the selected studies is set out in Table I. The results showed that most of the analyzed studies (45.5%) were conducted in the United States of America (Sandler *et al*., 2011, 2014; Kelesidis *et al*., 2012; Steele

et al., 2014; Timmons *et al*., 2014). Three studies (27.3%) were performed in Denmark (Pedersen *et al*., 2013, 2014; Haissman *et al*., 2017); two (18.2%) in Spain (Reus Bañuls *et al*., 2014; Leon *et al*., 2017); and one in the Netherlands (9.1%) (van den Dries *et al*., 2015). All the studies were published between 2011 and 2017.

The most frequent study design was cross-sectional cohort (54.6%) (Pedersen *et al*., 2013, 2014; Reus Bañuls *et al*., 2014; Steele *et al*., 2014; Timmons *et al*., 2014; Haissman *et al*., 2017), followed by longitudinal cohort (18.2%) (Kelesidis *et al*., 2012; van den Dries *et al*., 2015); case-control (18.2%) (Sandler *et al*., 2011; León *et al*., 2017); and only one clinical study (9.1%) (Sandler *et al*., 2014) (Table I). Therefore, the majority of studies (73%) were classified as Level 2 of scientific evidence (Kelesidis *et al*., 2012; Pedersen *et al*., 2013, 2014; Reus Bañuls *et al*., 2014; Steele *et al*., 2014; Timmons *et al*., 2014; van den Dries *et al*., 2015; Haissman *et al*., 2017) according to the Oxford Center for Evidence-Based Medicine Classification (Table I).

TABLE I - Metal mass concentrations, in $mg.g^{-1}$, of dried cell mass at the three groups: Zero-control, M-control, and Gd-Glu for incubation times of 30 and 50 min

Group Specification	Incubation time [min]	
	30	50
Zero-control	0.0 (*n.d*)	0.0 (*n.d*)
M-control	-	2780 ± 440 (Uc)
Gd-Glu group	13404 ± 2104	11347 ± 2742

A total of 1,769 HIV-infected individuals were included. The demographic characteristics (gender and age), clinical data regarding HIV infection (CD4 + T cell counts, and use of antiretrovirals), and information regarding the biomarkers of microbial translocation, inflammation and cardiovascular risk are set out in Table II.

The majority of the studies showed a prevalence of males; more than 50% of the population consisted of men, with a mean age of 46.50 ± 4.70 years (Table II). The mean CD4 + T cell count was 532 ± 107 cells/mm3 and the use of ART (antiretroviral therapy) was observed in 10 (90.9%) studies (Sandler *et al*., 2011; Kelesidis *et al*., 2012; Pedersen *et al*., 2013, 2014; Reus Bañuls *et al*., 2014; Steele *et al*., 2014; Timmons *et al*., 2014; van den Dries *et al*., 2015; Haissman *et al*., 2017; León *et al*., 2017) (Table II).

Figure 2 shows the number of studies that evaluated biomarkers of microbial translocation, inflammation, and cardiovascular risk. The soluble microbial translocation biomarkers that were studied were LPS, rDNA, sCD14, LBP and EndoCAb. The most frequently evaluated biomarker was sCD14, which was used in 10 (90.9%) studies (Sandler *et al*., 2011, 2014; Kelesidis *et al*., 2012; Pedersen *et al*., 2014; Reus Bañuls *et al*., 2014; Steele *et al*., 2014; Timmons *et al*., 2014; van den Dries *et al*., 2015; Haissman *et al*., 2017; León *et al*., 2017), followed by the detection of LPS in nine (81.8%) studies (Sandler *et al*., 2011, 2014; Kelesidis *et al*., 2012; Pedersen *et al*., 2013, 2014; Steele *et al*., 2014; Timmons *et al*., 2014; van den Dries *et al*., 2015; Haissman *et al*., 2017). The biomarker rDNA was reported in three (27.3%) studies (Sandler *et al*., 2011; Reus Bañuls *et al*., 2014; León *et al*., 2017), EndoCArb was reported in two (18.2%) studies (Sandler *et al*., 2011, 2014), and

TABLE II - Attributions of the *m*/*z* signals in the spectrum of the Gd-Glu solution, the relative abundance in ESI-MS, the specification of theoretical assignment of the chemical formula, nominal mass (a.m.u) estimation and possible ion species in the complex formation

m/*z*	Relative Abundance (%)	Theoretical assignment	MW (a.m.u)	Proposed species
516	26	$C_{12}H_{22}O_{12}Gd$	515.54	$[Gd(Glu)_2 - 2H]^+$
552	100	$C_{12}H_{23}O_{12}GdCl$	552.00	$[Gd(Glu)_2Cl - H]^+$
696	34	$C_{18}H_{34}O_{18}Gd$	695.69	$[Gd(Glu)_3 - 2H]^+$
923	12	$C_{18}H_{33}O_{18}Gd_2Cl_2$	922.84	$[Gd_2(Glu)_2Cl_2 - 3H]^+$

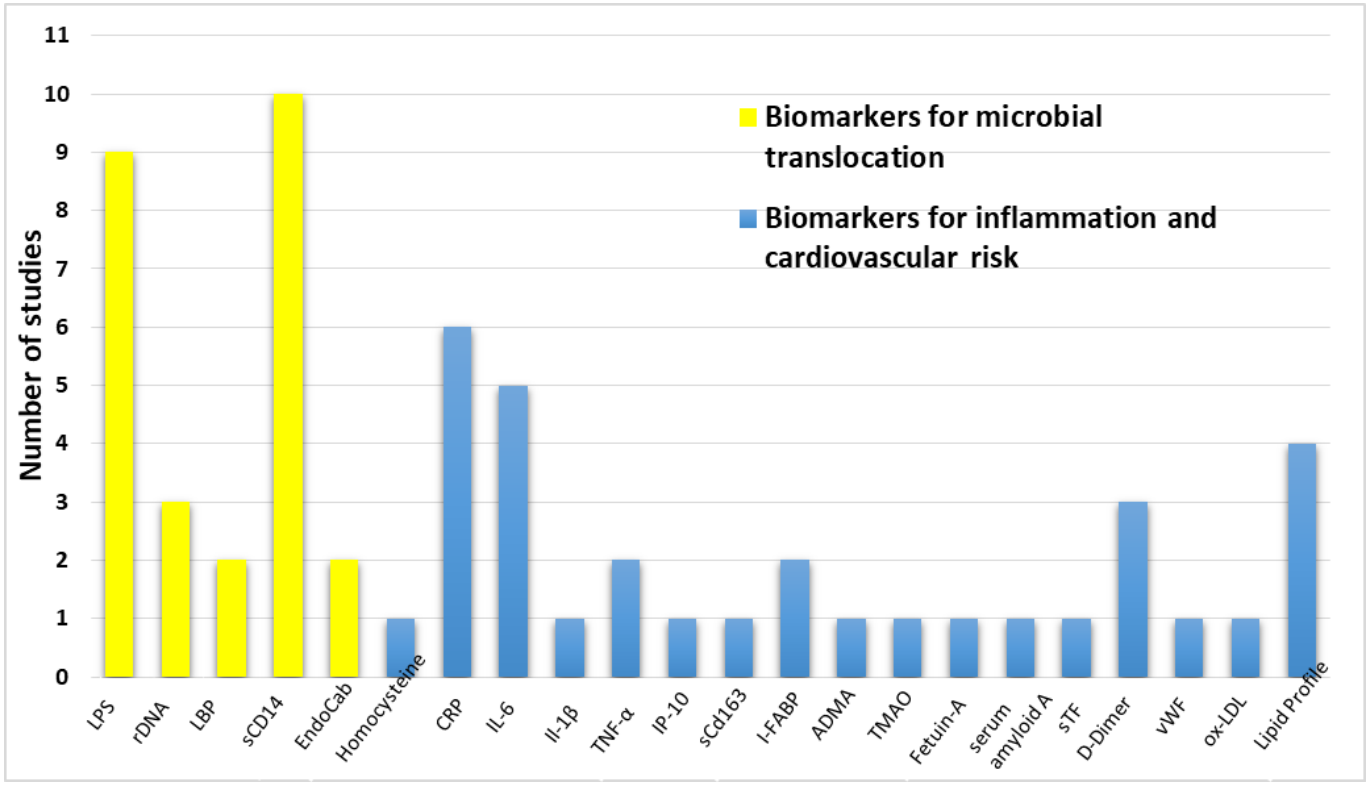

FIGURE 2 - Number of studies evaluating biomarkers of microbial translocation, inflammation and cardiovascular risk. LPS, lipopolysaccharides; rDNA, ribosomal bacterial DNA; LBP, LPS binding protein; sCD14, soluble CD14; EndoCAb, endotoxin nucleus antibody; CRP, C-reactive protein; IL-6, interleukin-6; IL-1β, interleukin 1β; TNF-α, tumor necrosis factor-alpha; IP-10, interferon-γ-inducible protein 10; sCD163, soluble CD163; I-FABP, intestinal fatty acid binding protein; ADMA, asymmetric dimethylarginine; TMAO, trimethylamine-N-oxide; sTF, soluble tissue factor; vWF, von Willebrand factor; oxLDL, oxidized LDL cholesterol.

LBP was reported in two (18.2%) studies (Sandler *et al*., 2014; van den Dries *et al*., 2015) (Figure 2).

A large number of biomarkers of inflammation and cardiovascular risk were used. The use of CRP (C-reactive protein) was prominent and it featured in six studies (54.5%) (Sandler *et al*., 2011, 2014; Kelesidis *et al*., 2012; Pedersen *et al*., 2014; Steele *et al*., 2014; Haissman *et al*., 2017). The use of IL-6 was reported in five (45.4%) studies (Sandler *et al*., 2011, 2014; Reus Bañuls *et al*., 2014; Steele *et al*., 2014; León *et al*., 2017).

The results of the present study are in accordance with a previous literature review that highlighted i) LPS and rDNA as the main biomarkers for microbial translocation; ii) LBP, sCD14 and EndoCAb for response to bacterial products; and iii) CRP, IL-6 and D-dimer for inflammation/activation immunity (Lichtfuss *et al*., 2011). However, the aforementioned review did not demonstrate any association between the biomarkers of microbial translocation, (Lichtfuss *et al*., 2011).

The associations between sCD14, inflammation biomarkers and cardiovascular risk are presented in Figure 3; sCD14 showed a more frequent positive association with CRP (Sandler *et al*., 2011, 2014; Kelesidis *et al*., 2012; Steele *et al*., 2014) and IL-6 (Sandler *et al*., 2011, 2014; Reus Bañuls *et al*., 2014; Steele *et al*., 2014) in four studies (36.4%), and with triglycerides in two studies (18.2%) (Kelesidis *et al*., 2012; Timmons *et al*., 2014). However, there was an inverse association with HDL-C in one study (9.1%) (Timmons *et al*., 2014) (Figure 3).

The association of sCD14 with CRP, IL-6 and triglycerides, demonstrates the interconnection between microbial translocation, inflammation and CVD, considering that sCD14 is produced by monocytes in response to lipopolysaccharide stimulation (Zanoni, Granucci, 2013); CRP and IL-6 are traditional biomarkers of systemic inflammation and cardiovascular risk (Ridker, 2003; Sarwar *et al*., 2012), and triglycerides

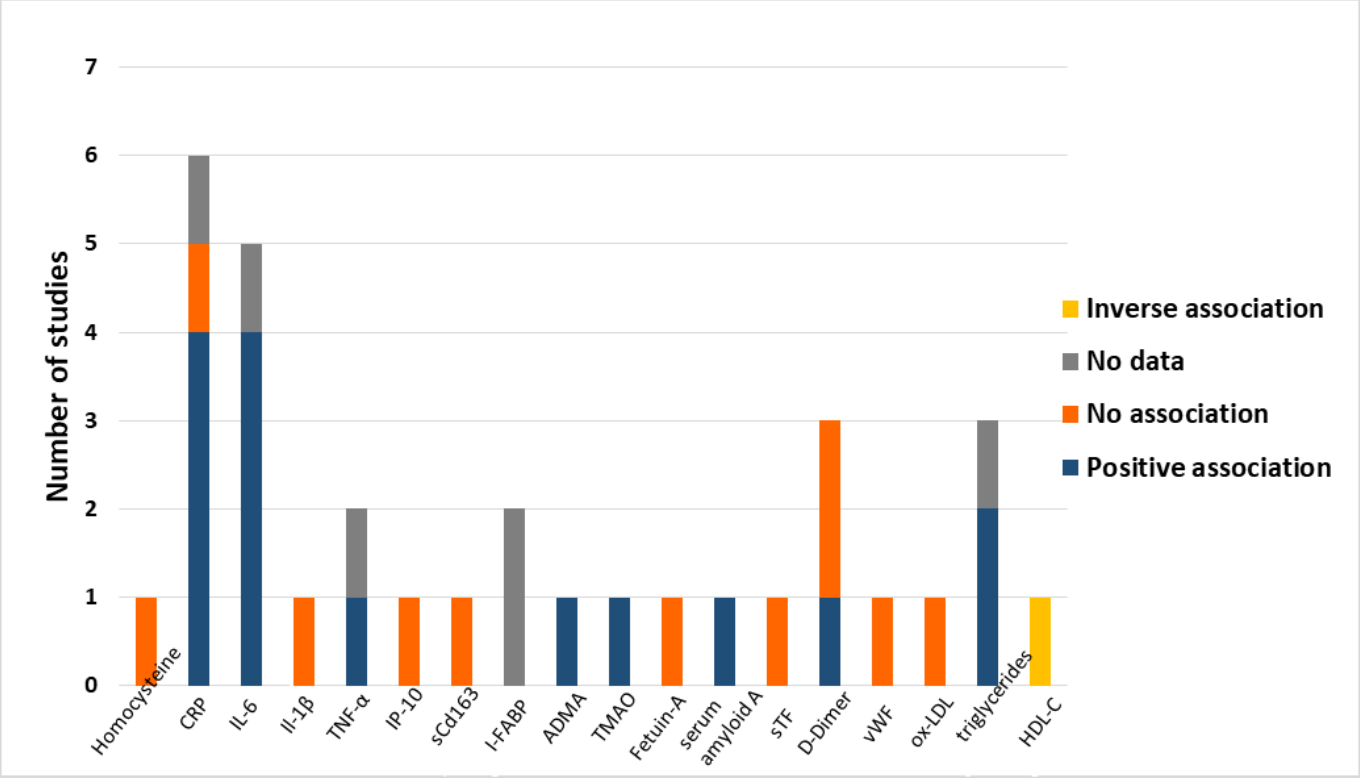

FIGURE 3 - Association between sCD14 microbial translocation biomarker and biomarkers of inflammation and cardiovascular risk. sCD14, soluble CD14; EndoCAb, endotoxin nucleus antibody; CRP, C-reactive protein; IL-6, interleukin-6; IL-1β, interleukin 1β; TNF-α, tumor necrosis factor-alpha; IP-10, interferon-γ-inducible protein 10; sCD163, soluble CD163; I-FABP, intestinal fatty acid binding protein; ADMA, asymmetric dimethylarginine; TMAO, trimethylamine-N-oxide; sTF, soluble tissue factor; vWF, von Willebrand factor; oxLDL, oxidized LDL cholesterol.

are a source of energy for macrophages (Kelesidis *et al*., 2012), as well as an independent risk factor for CVD (Cullen, 2000).

The associations between LPS and the biomarkers of inflammation and cardiovascular risk are presented in Figure 4. LPS was only positively associated with the lipid profile: triglycerides (18.2%) (Pedersen *et al*., 2013; Timmons *et al*., 2014); cholesterol (9.1%) (Pedersen *et al*., 2013); and LDL-C (9.1%) (Pedersen *et al*., 2013) (Figure 4).

Regarding rDNA (one of the microbial translocation biomarkers), there was a positive association with IL-6 in two (18.2%) studies (Reus Bañuls *et al*., 2014; León *et al*., 2017), and with TNF-α in one study (9.1%) (Reus Bañuls *et al*., 2014). LBP showed a positive association with CRP and IL-6 (Sandler *et al*., 2014), whereas EndoCAb had no association with biomarkers of inflammation and cardiovascular risk.

Microbial translocation biomarkers were also evaluated for associations with each other; only one study showed a correlation between LPS and sCD14 (van den Dries *et al*., 2015). In most of the studies there was no correlation between LPS and sCD4; LBP and sCD14; LPS and LBP; sCD14 and EndoCAb; and LPS and EndoCab (Kelesidis *et al*., 2012; Pedersen *et al*., 2014; Sandler *et al*., 2014; Steele *et al*., 2014; van den Dries *et al*., 2015).

With regard to the methodology that was employed, plasma levels of sCD14 were evaluated by ELISA (enzyme-linked immunosorbent assay) and LPS was evaluated by using limulus amebocyte lysate (LAL) (Table II). The most frequently used method to quantify LPS in plasma is LAL, which utilizes a series of enzymatic reactions that mimic the coagulation cascade (Lichtfuss *et al*., 2011). However, this assay can easily show high sensitivity and contamination during sample preparation (Lichtfuss *et al*., 2011). In addition, enzymatic reactions critically depend on time and temperature, and may be affected by numerous inhibitors that are present in plasma (Lichtfuss *et al*., 2011).

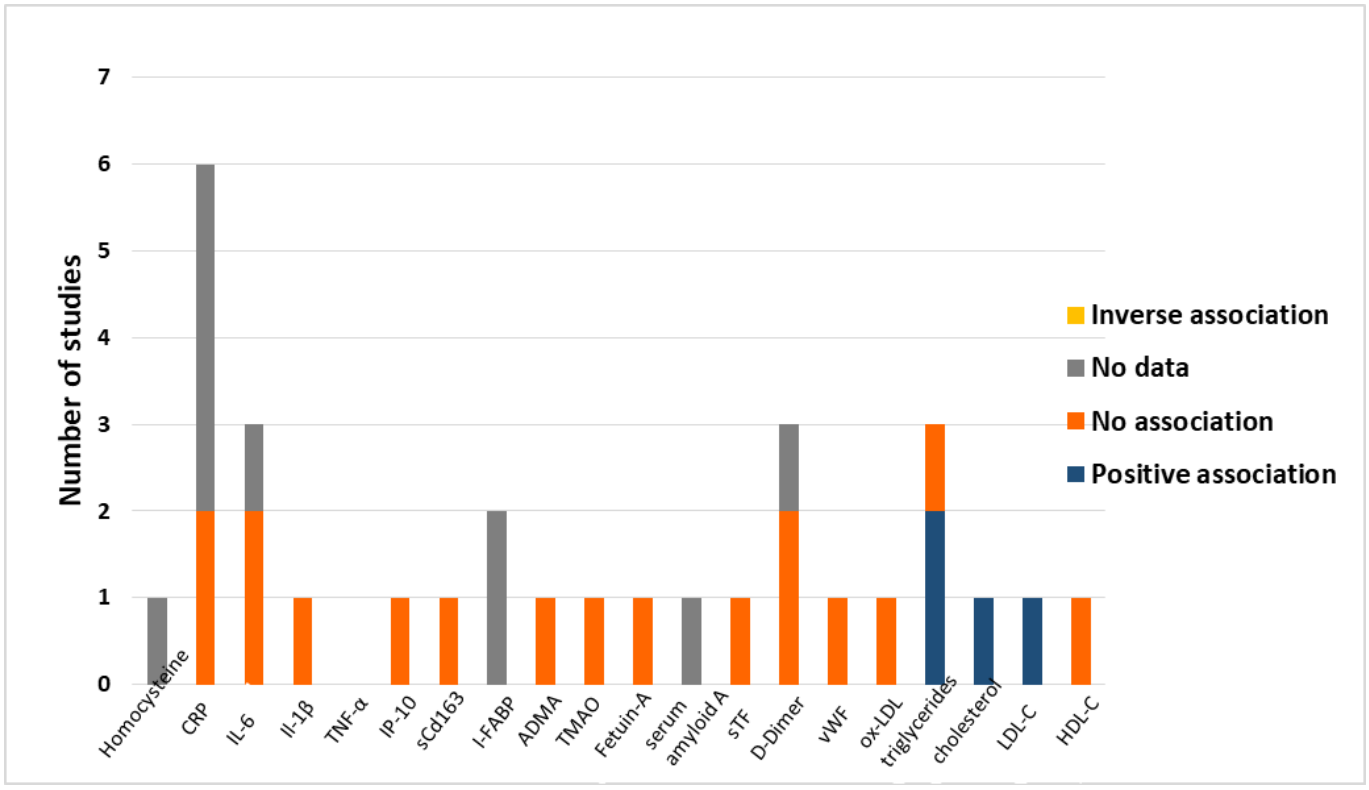

FIGURE 4 - Association between LPS microbial translocation biomarker and biomarkers of inflammation and cardiovascular risk. LPS, lipopolysaccharides; CRP, C-reactive protein; IL-6, interleukin-6; IL-1β, interleukin 1β; TNF-α, tumor necrosis factor-alpha; IP-10, interferon-γ-inducible protein 10; sCD163, soluble CD163; I-FABP, intestinal fatty acid binding protein; ADMA, asymmetric dimethylarginine; TMAO, trimethylamine-N-oxide; sTF, soluble tissue factor; vWF, von Willebrand factor; oxLDL, oxidized LDL cholesterol.

Furthermore, the heterogeneity present in the structure, solubility, physical state and bioactivity of LPS pose important limitations in the interpretation of plasma endotoxin tests (Munford, 2016). Thus, the association of plasma endotoxin levels with inflammatory markers has been inconsistent (Munford, 2016). Consequently, unlike LPS, which has significant limitations, the quantification of sCD14 is highly reproducible and can be used reliably (Lichtfuss *et al*., 2011).

Other biomarkers of inflammation and cardiovascular risk that were associated with sCD14 were: tumor necrosis factor-alpha (TNF-α) (Reus Bañuls *et al*., 2014); asymmetric dimethylarginine (ADMA) and its symmetrical dimethylarginine stereoisomer (SDMA) (Pedersen *et al*., 2014); trimethylamine-N-oxide (TMAO) (Haissman *et al*., 2017); and serum amyloid A and D-dimer (Sandler *et al*., 2011). However, these data were reported only once and should be interpreted with caution (Figure 3).

The only association found by van den Dries *et al*. (2015) was a correlation between the biomarkers of microbial translocation, sCD14 and LPS (r = 0.255; p = 0.003); however, there was a weak trend toward positive correlation between plasma levels of sCD14 and von Willebrand factor (vWF) (r = 0.184; p = 0.078), which is related to increased cardiovascular events (Kato *et al*., 2018). There was no correlation between the vWF and LPS; vWF and LBP; sCD14 and LBP; and LPS and LBP (van den Dries *et al*., 2015).

TMAO, which is a metabolite of the intestinal microbiota and is considered to be a biomarker for CVD risk independent of the traction risk factors (Bergeron *et al*., 2016), was evaluated by Haissman *et al*. (2017), who demonstrated the association of sCD14 with TMAO (univariable regression, r = 0.381, p = 0.008) in untreated HIV-infected individuals, but not in HIV-infected individuals on ART. However, no association between LPS and TMAO was found (Haissman *et al*., 2017). In the multivariate regression models, sCD14 remained an

independent predictor of TMAO after adjusting for age, gender, smoking and viral load (Haissman *et al*., 2017). It should be noted that a recent study demonstrated that TMAO was associated with increased risk of carotid plaques in HIV-infected patients, and also exhibited a correlation with the sCD14 biomarker (Shan *et al*., 2018).

Kelesidis *et al*. (2012) observed associations between the levels of sCD14 with hs-CRP levels ($p < 0.001$) and triglycerides ≥ 150 mg/dL ($p = 0.027$) using univariable regression analysis. The results for multivariate regression were similar. However, there was no change in LPS levels over time within the study groups, and there was no association with sCD14, which was probably due to the individual variability of the host response to LPS rather than the amount of LPS in the activation of macrophages (Kelesidis *et al*., 2012).

Nevertheless, when the aforementioned study evaluated atherosclerosis using ultrasonographic measurement of the carotid artery intima-media thickness, it was found that sCD14 and LPS biomarkers were associated with the progression of subclinical atherosclerosis, providing a potential unifying etiology for the increased risk of cardiovascular disease in HIV-infected individuals (Kelesidis *et al*., 2012).

Leon *et al*. (2017) used ribosomal bacterial DNA (rDNA) as a microbial translocation biomarker; they demonstrated that the relationship between the presence of rDNA and the level of IL-6. HIV-infected individuals with microbial translocation had significantly ($p = 0.001$) higher median values [34 (17 – 51) pg/mL] of IL-6 compared to individuals without microbial translocation [4.9 (2.4 – 6.2) pg/mL] (León *et al*., 2017).

Pedersen *et al*. (2013) presented the results for the biomarkers from 50 HIV-infected individuals divided into tertiles (17, 16 and 17 individuals, respectively) according to the level of LPS [46.1 (43.2 to 49.1) pg/mL, 62.4 (60.0 to 64.7) pg/mL and 84.7 (72.0 to 97.5) pg/mL, respectively]. The highest level of triglycerides was found in the third tertile compared to the first and second tertiles ($p = 0.006$ and $p = 0.046$, respectively) (Pedersen *et al*., 2013). In addition, total cholesterol was higher in the second and third tertiles compared to the first tertile ($p = 0.022$ and $p = 0.016$, respectively). LDL-C was higher in the second and third tertiles compared to the first tertile ($p = 0.009$ and $p = 0.002$, respectively) (Pedersen *et al*., 2013). There was no difference in the HDL-C concentrations. Significant associations between LPS and lipids were found: triglycerides ($r = 0.450$, $p = 0.001$); total cholesterol ($r = 0.147$, $p = 0.005$); and LDL-C ($r = 0.110$, $p = 0.018$) (Pedersen *et al*., 2013).

Another study by Pedersen *et al*. (2014) demonstrated a correlation between sCD14 and ADMA and SDMA ($r = 0.38$, $p = 0.008$ and $r = 0.51$, $p < 0.001$, respectively), which are biomarkers of endothelial dysfunction and contribute to the impairment of endothelial function by the inhibition of nitric oxide (NO) synthesis. In contrast, LPS did not correlate with ADMA or SDMA ($r = -0.02$, $p = 0.900$ and $r = 0.02$, $p = 0.889$, respectively) (Pedersen *et al*., 2014). Furthermore, this study did not demonstrate associations between sCD14, LPS, hs-CRP and D-dimer (Pedersen *et al*., 2014).

Reus Bañuls *et al*. (2014) demonstrated that HIV-infected individuals treated with ART, and with higher values of IL-6 and TNF-α inflammatory markers, had a higher frequency of bacterial translocation and a history of cardiovascular disease. In addition, the aforementioned study demonstrated a correlation between sCD14 and IL-6 and TNF-α ($r=0.28$; $p=0.01$; $r = 0.40$, $p < 0.001$; respectively). This article also demonstrated the association (multivariate and univariate regression model) of high inflammatory markers with rDNA, sCD14 and cardiovascular events (Reus Bañuls *et al*., 2014). However, in the multivariate analysis, the IL-6 and TNF-α values were only independently associated with the presence of bacterial DNA (Odds Ratio 62, $p = 0.0001$) and history of cardiovascular events (Odds Ratio 25, p = 0, 01) (Reus Bañuls *et al*., 2014).

A study by Sandeler *et al*. (2011) found that a high percentage of HIV-infected individuals undergoing ART presented an undetectable viral load, and the biomarker levels were studied by comparing individuals with HIV RNA levels ≤ 400 copies/mL (n = 420) with those with HIV RNA levels > 400 copies/mL (n=155). In the group of individuals with HIV RNA levels ≤ 400 copies/mL, sCD14 was correlated to IL-6 ($r = 0.18$, $p < 0.001$), serum amyloid A ($r = 0.16$, $p < 0.001$), hs-CRP ($r = 0.10$, $p = 0.04$) and D-dimer ($r = 0.11$, $p = 0.03$). In contrast, in the individuals with detectable levels of HIV RNA, sCD14 was correlated to IL-6 ($r = 0.26$, $p < 0.001$), serum amyloid A ($r = 0.18$, $p = 0.004$) and hs-CRP ($r = 0.18$, $p = 0.005$) (Sandler *et al*., 2011).

This study examined the association between microbial translocation markers and inflammation in the untreated subset (n = 117), with a correlation of sCD14 with IL-6 ($r = 0.35$, $p < 0.001$) and D-dimer ($r = 0.26$, $p = 0.006$) (Sandler *et al*., 2011). However, these results should be interpreted with caution because the

number of individuals evaluated for the biomarkers did not represent the sample of the total of individuals enrolled in the study. Furthermore, the effects of ART on intestinal permeability and immune response to microbial products are not well characterized.

Another study by Sandler *et al*. (2014) showed no correlation between the microbial translocation biomarkers LPS, sCD14, and EndoCAb. However, levels of the acute phase proteins LBP, IL-6 and CRP were significantly correlated with each other (Sandler *et al*., 2014).

Steele *et al*. (2014) presented associations between sCD14 and hs-CRP ($r = 0.292$, $p = 0.008$) and IL-6 ($r = 0.418$, $p < 0.0001$) in HIV-infected individuals. However, there was no association with the intestinal fatty acid binding protein (iFABP) ($r = -0.019$, $p = 0.870$) (Steele *et al*., 2014). Regarding the biomarker of LPS microbial translocation, there was a tendency for a positive correlation with hs-CRP ($r = 0.206$, $p = 0.076$) and IL-6 ($r = 0.209$, $p = 0.089$) but none reached statistical significance in this cohort (Steele *et al*., 2014). In addition, the LPS biomarker did not correlate with sCD14s ($r = 0.186$, $p = 0.112$) (Steele *et al*., 2014).

Timmons *et al*. (2014) evaluated the associations of biomarkers for all HIV-infected individuals included in the study. Moreover, they investigated the markers for each subgroup of the two cohorts that were evaluated. Regarding the total population of HIV-infected individuals, there was a positive correlation between LPS and triglycerides ($n = 167$, $r = 0.32$, $p < 0.01$), and a negative correlation between sCD14 and HDL-C ($n = 166$, $r = -0.21$, $p < 0.01$) (Timmons *et al*., 2014). The group of the AIDS clinical trial group cohort, before ($n = 81$, $r = 0.35$, $p < 0.01$) and after treatment ($n = 79$, $r = 0.81$, $p < 0,01$) presented a correlation between LPS and triglycerides (Timmons *et al*., 2014). However, the Indiana University cohort presented associations only for the ART group, with a positive correlation for LPS and sCD14 with triglycerides ($n = 40$, $r = 0.33$, $p = 0.04$; $n = 42$, $r = 0.35$, $p < 0.02$; respectively) and a negative correlation between sCD14 and HDL-C ($n = 41$, $r = -0.32$, $p < 0.04$) (Timmons *et al*., 2014). Although these results are relevant, they should be carefully considered because the number of individuals evaluated for the biomarkers did not represent the sample of the total individuals enrolled in each subgroup of the study.

This systematic review summarized the main biomarkers of microbial translocation, inflammation and cardiovascular risk that are currently used, as well as the possible associations between them in HIV infection. The strong point of this review for clinical practice was the finding that, of all the biomarkers of microbial translocation, sCD14 showed a greater association with the biomarkers of inflammation and cardiovascular risk.

However, it is necessary to consider some of the limitations of this systematic review, such as the methodological differences between the various studies, especially with respect to the assessment of the biomarkers in different groups and subgroups of HIV-infected individuals. It is important to note that the results of the associations between biomarkers may be influenced by differences in the populations of HIV-infected individuals evaluated in the studies. In addition, several studies did not evaluate the biomarkers relative to the total HIV-infected population included in the study.

This systematic review considered the soluble blood biomarkers that can be inserted in clinical laboratory routines for the purpose of diagnosis, as well as their connections with microbial translocation, inflammation and cardiovascular risk. Despite the fact that a large number of inflammation and cardiovascular risk biomarkers have been reported, especially CRP and IL-6, it is clear that important markers involved in the pathophysiology of cardiovascular diseases need to be included in future research.

REFERENCES

Ballegaard V, Ralfkiaer U, Pedersen KK, Hove M, Koplev S, Brændstrup P, et al. MicroRNA-210, microRNA-331, and microRNA-7 are differentially regulated in treated HIV-1-Infected individuals and are associated with markers of systemic inflammation. J Acquir Immune Defic Syndr. 2017;74(4):e104-13.

Bergeron N, Williams PT, Lamendella R, Faghihnia N, Grube A, Li X, et al. Diets high in resistant starch increase plasma levels of trimethylamine-N-oxide, a gut microbiome metabolite associated with CVD risk. Br J Nutr. 2016;116(12):2020-9.

Blodget E, Shen C, Aldrovandi G, Rollie A, Gupta SK, Stein JH, et al. Relationship between microbial translocation and endothelial function in HIV infected patients. PLoS One. 2012;7(8):e42624.

Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006;12(12):1365-71.

Cullen P. Evidence that triglycerides are an independent coronary heart disease risk factor. Am J Cardiol. 2000;86(9):943-9.

van den Dries LWJ, Gruters RA, Hövels-van der Borden SBC, Kruip MJHA, de Maat MPM, van Gorp ECM, et al. von Willebrand Factor is elevated in HIV patients with a history of thrombosis. Front Microbiol. 2015;6:180.

Grinspoon SK. Cardiovascular disease in HIV: Traditional and nontraditional risk factors. Top Antivir Med. 2014;22(4):676-9.

Haissman JM, Haugaard AK, Ostrowski SR, Berge RK, Hov JR, Trøseid M, et al. Microbiota-dependent metabolite and cardiovascular disease marker trimethylamine-N-oxide (TMAO) is associated with monocyte activation but not platelet function in untreated HIV infection. BMC Infect Dis. 2017;17(1):445.

Kato Y, Iwata A, Futami M, Yamashita M, Imaizumi S, Kuwano T, et al. Impact of von Willebrand factor on coronary plaque burden in coronary artery disease patients treated with statins. Medicine (Baltimore). 2018;97(17):e0589.

Kelesidis T, Kendall MA, Yang OO, Hodis HN, Currier JS. Biomarkers of microbial translocation and macrophage activation: association with progression of subclinical atherosclerosis in HIV-1 infection. J Infect Dis. 2012;206(10):1558-67.

León R, Reus S, López N, Portilla I, Sánchez-Payá J, Giner L, et al. Subclinical atherosclerosis in low Framingham risk HIV patients. Eur J Clin Invest. 2017;47(8):591-9.

Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. PLoS Med. 2009;6(7):e1000100.

Lichtfuss GF, Hoy J, Rajasuriar R, Kramski M, Crowe SM, Lewin SR. Biomarkers of immune dysfunction following combination antiretroviral therapy for HIV infection. Biomark Med. 2011;5(2):171-86.

Marchetti G, Tincati C, Silvestri G. Microbial translocation in the pathogenesis of HIV infection and AIDS. Clin Microbiol Rev. 2013;26(1):2-18.

Meng F, Lowell CA. Lipopolysaccharide (LPS)-induced macrophage activation and signal transduction in the absence of Src-family kinases Hck, Fgr, and Lyn. J Exp Med. 1997;185(9):1661-70.

Munford RS. Endotoxemia--menace, marker, or mistake? J Leukoc Biol. 2016;100(4):687-98.

Palella FJ, Phair JP. Cardiovascular disease in HIV infection. Curr Opin HIV AIDS. 2011;6(4):266-71.

Pedersen KK, Manner IW, Seljeflot I, Kvale D, Os I, Gerstoft J, et al. Monocyte activation, but not microbial translocation, is independently associated with markers of endovascular dysfunction in HIV-infected patients receiving cART. J Acquir Immune Defic Syndr. 2014;67(4):370-4.

Pedersen KK, Pedersen M, Trøseid M, Gaardbo JC, Lund TT, Thomsen C, et al. Microbial translocation in HIV infection is associated with dyslipidemia, insulin resistance, and risk of myocardial infarction. J Acquir Immune Defic Syndr. 2013;64(5):425-33.

Phillips B, Ball C, Sackett D, Badenoch D, Straus S, Haynes B DM. Oxford Centre for Evidence-based Medicine - Levels of Evidence. BJU Int [Internet]. 2009 [cited 2018 Jun 30];103(8):1147–1147. Available from: https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/.

Płóciennikowska A, Hromada-Judycka A, Borzęcka K, Kwiatkowska K. Co-operation of TLR4 and raft proteins in LPS-induced pro-inflammatory signaling. Cell Mol Life Sci. 2015;72(3):557-81.

Reus Bañuls S, Portilla Sogorb J, Sanchez-Paya J, Boix Martínez V, Giner Oncina L, Frances R, et al. Association between inflammatory markers and microbial translocation in patients with human immunodeficiency virus infection taking antiretroviral treatment | Asociación entre marcadores inflamatorios y traslocación bacteriana en pacientes con infección por el virus de la inmunodeficiencia humana en tratamiento antirretroviral. Med Clin (Barc). 2014;142(2):47-52.

Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation. 2003;107(3):363-9.

Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, et al. Plasma levels of soluble CD14 independently predict mortality in HIV infection. J Infect Dis. 2011;203(6):780-90.

Sandler NG, Zhang X, Bosch RJ, Funderburg NT, Choi AI, Robinson JK, et al. Sevelamer does not decrease lipopolysaccharide or soluble cd14 levels but decreases soluble tissue factor, low-density lipoprotein (ldl) cholesterol, and oxidized ldl cholesterol levels in individuals with untreated hiv infection. J Infect Dis. 2014;210(10):1549-54.

Sarwar N, Butterworth AS, Freitag DF, Gregson J, Willeit P, Gorman DN, et al. Interleukin-6 receptor pathways in coronary heart disease: A collaborative meta-analysis of 82 studies. Lancet. 2012;379(9822):1205-13.

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Shan Z, Clish CB, Hua S, Scott JM, Hanna DB, Burk RD, et al. Gut microbial-related choline metabolite Trimethylamine-N-oxide is associated with progression of carotid artery atherosclerosis in HIV Infection. J Infect Dis. 2018;218(9):1474-1479.

Steele AK, Lee EJ, Vestal B, Hecht D, Dong Z, Rapaport E, et al. Contribution of intestinal barrier damage, microbial translocation and HIV-1 infection status to an inflammaging signature. PLoS One. 2014;9(5):e97171.

Timmons T, Shen C, Aldrovandi G, Rollie A, Gupta SK, Stein JH, et al. Microbial translocation and metabolic and body composition measures in treated and untreated HIV infection. AIDS Res Hum Retroviruses. 2014;30(3):272-7.

Yong YK, Shankar EM, Westhorpe CL V, Maisa A, Spelman T, Kamarulzaman A, et al. Genetic polymorphisms in the CD14 gene are associated with monocyte activation and carotid intima-media thickness in HIV-infected patients on antiretroviral therapy. Medicine (Baltimore). 2016;95(31):e4477.

Zanoni I, Granucci F. Role of CD14 in host protection against infections and in metabolism regulation. Front Cell Infect Microbiol. 2013;3:32.

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