



Soluble biomarkers of HIV transmission, disease progression and comorbidities

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Purpose of review

The purpose of this study is to survey and synthesize recent progress in soluble biomarkers relevant to HIV-1 disease stages, progression and comorbidities.

Recent findings

Soluble biomarkers in plasma and other body fluids provide insight into many aspects of HIV-1 disease. Chemokines and defensins in breast milk and cervicovaginal secretions have been associated with HIV-1 susceptibility and transmission. Acute infection plasma cytokine storm components, including serum amyloid A, IFN γ -induced protein 10, interleukin (IL)-12, interferon-gamma (IFN γ), IL-7 and IL-15, may help predict viral load set-point and the subsequent disease progression. During chronic infection, IL-6, soluble (s)CD14, sCD163, high-sensitivity C-reactive protein, D-dimer, fibrin and hyaluronic acid can help predict comorbidities, and to some extent disease progression and mortality, in patients both on and off antiretroviral therapy. Furthermore, recent results suggest that assessment of combinations of soluble biomarkers may prove more powerful than the single factors alone in predicting disease.

Summary

Soluble biomarkers help us understand HIV-1 immunopathogenesis. Integration of many biomarkers derived from a single plasma sample might become a powerful tool to optimize and individualize treatment and care.

Keywords

chemokines, cytokines, HIV, microbial translocation, soluble biomarkers

INTRODUCTION

Chronic diseases are associated with changes in the expression of various endogenous cell-bound or soluble proteins. Such factors may be directly involved in pathogenic processes or be mere byproducts of such processes. Irrespective of causative relationships, however, proteins that display altered expression during disease can prove useful as biomarkers to facilitate the understanding of pathogenic processes. Ultimately, some of these biomarkers can make their way into the clinical immunology laboratory and help predict a patient's clinical course or response to therapy. Chronic uncontrolled viral infections, such as HIV-1 infection, are associated with persistent ongoing immune responses as well as progressive immunopathology. These processes give rise to soluble markers related to innate and adaptive immune activation, as well as cell death and tissue destruction [1,2]. The goal of the present review is to cover recent progress in our understanding of soluble

biomarkers in plasma and other bodily fluids during HIV-1 infection. We will aim to briefly cover recently published data on putative biomarkers during transmission, in acute infection, during chronic progressive infection, as well as biomarkers relevant for comorbidities.

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KEY POINTS

- Components of the acute HIV infection plasma cytokine storm, such as serum amyloid A, IP-10, IL-12, IFN γ , IL-7 and IL-15, may help predict viral set-point variation and disease progression.
- Systemic sCD14 levels are likely influenced by the rate of microbial translocation, the status of the liver and the type I interferon response.
- IL-6, sCD14, sCD163, hsCRP, D-dimer, fibrin and hyaluronic acid can be clinically useful biomarkers in predicting comorbidities in HIV-1-infected patients both on and off antiretroviral therapy.
- Integration of many biomarkers derived from a single plasma sample might become a powerful tool in clinical practice to optimize and individualize treatment and care.

SOLUBLE BIOMARKERS DURING TRANSMISSION AND ACUTE INFECTION

Soluble markers in plasma and other body fluids reflect the individual's immunological status and may therefore be helpful in assessing risk of HIV-1 transmission, acquisition, as well as early-stage disease progression in those who become infected.

VERTICAL TRANSMISSION

Breast feeding accounts for a large fraction of mother-to-child transmissions, particularly in sub-Saharan Africa. Bosire *et al.* [3] reported that HIV-1-infected women in Kenya have elevated levels of the chemokines regulated upon activation normal T cell expressed and presumably secreted (RANTES) and macrophage inflammatory protein-1 β (MIP-1 β) in breast milk at 10 days postpartum, and that the RANTES levels are significantly higher in milk from mothers who transmit infection to their infant. Together with the recent results indicating that MIP-1 β levels, as well as T cell interferon-gamma (IFN γ) responses against HIV-1 Gag, in breast milk are associated with lower odds of transmission [4], we can begin to understand which biomarkers might inform us about the risk for vertical transmission. Amniotic fluid from HIV-negative women was recently found to inhibit HIV-1 replication [5], although the factors involved are yet to be identified. Relationships between factors in cervicovaginal lavage (CVL) and perinatal infection remain to be determined.

SEXUAL TRANSMISSION

Dezzutti *et al.* [6^{*}] identified markers in CVL predictive of sexual transmission in a cohort of

high-risk women and found that seroconverters had higher *Escherichia coli* bactericidal activity in their preseroconversion CVL. Higher levels of human β defensin-2 (H β D-2) were found in the vaginal swabs of seroconverters prior to HIV acquisition [6^{*}]. In this case, H β D-2 may be a biomarker of environmental stimuli that predisposes to a higher risk of infection, and this might explain the seemingly contradicting results in the study by Ghosh *et al.* [7] suggesting H β D-2 in CVL having an antiviral activity. Ghosh *et al.* [7] also found that Elafin and secretory leukocyte protease inhibitor (SLPI) were both positively associated with viral load in the CVL, while MIP-3 α correlated with CVL antiviral activity. It would be interesting to investigate these soluble CVL factors in terms of susceptibility to infection. Furthermore, two recent studies [8,9^{*}] have tracked cytokines and chemokines in the CVL of HIV-1-infected women and link changes in a range of factors to detectable viral load in CVL. These may, however, represent responses to infection rather than innate protective factors. In contrast, Naranbhai *et al.* [10] recently reported in a nested case-control study of immunological risk factors that the systemic cytokine signature in women who acquired HIV-1 was more pro-inflammatory than in women who did not get infected.

Levels of cytokines and chemokines in semen are difficult to link to susceptibility. However, enrichment of certain cytokines has been observed in semen and this may indicate a constitutive innate activation state, which may be supportive of HIV-1 replication [11]. The cytokine profile in semen was broadened in infected individuals. Whether these soluble components can be used to identify individuals carrying a higher risk of transmission remains to be elucidated.

ACUTE INFECTION

Events during acute HIV-1 infection, the period before establishment of the viral load set-point, are likely to be important for the subsequent course of disease. In a proteomics-based screen, an increase in the acute phase protein serum amyloid A (A-SAA) in plasma was the first sign of an immune response [12^{*}]. A-SAA was demonstrated to have antiviral properties and was detected 5 days prior to the first measurable viral RNA, thus posing as a possible biomarker of hyperacute infection. A-SAA displayed a biphasic response, the first during viral eclipse phase and the second during the viral expansion phase that overlapped with the cytokine storm associated with acute HIV-1 infection [13] (Fig. 1).

During the cytokine storm in acute infection, the immune system produces a milieu of cytokines

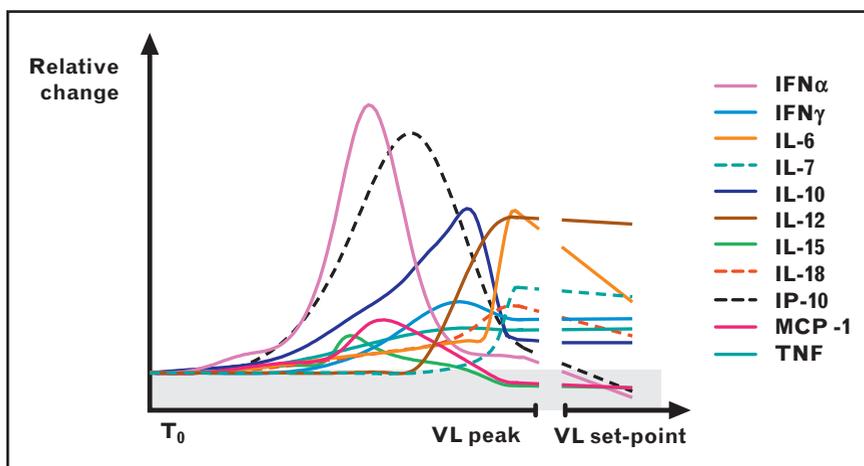


FIGURE 1. The storm of cytokines in plasma during acute HIV-1 infection harbours potential biomarkers. IFN, interferon; IL, interleukin; TNF, tumour necrosis factor. Data from [13] and [14].

that may work to help or hinder virus growth and reservoir establishment. Two studies explored a range of cytokines in a cohort from preinfection to 3 weeks postinfection [13], and in follow-up out to 16–24 weeks postinfection [14] (Fig. 1). These soluble factors can be regarded as candidate biomarkers of the subsequent clinical development. Building on these results, Jiao *et al.* [15] found that higher levels of IFN γ -induced protein 10 (IP-10) during Fiebig stages III, IV and V [16] was strongly associated with low CD4 cell counts 2 years after the initial infection, suggesting that IP-10 might be a useful acute stage biomarker of disease progression. Roberts *et al.* [17^{*}] confirmed the elevation of tumour necrosis factor (TNF), IP-10 and interleukin (IL)-10 six weeks after initial infection, and also proposed a model for predicting viral load set-point 12 months after infection from five cytokines measured during acute infection. Plasma concentrations of IL-12p40/70, IFN γ , IL-7 and IL-15 together predicted 66% of viral set-point variation [17^{*}]. Interrogation of these plasma cytokine networks for prediction of future viral load set-point and disease progression may have great potential [18].

SOLUBLE BIOMARKERS OF CHRONIC HIV-1 DISEASE

Chronic untreated HIV-1 infection is characterized by a state of persistent immune activation, and this broad activation is a strong contributor to disease progression (reviewed in [19]). As discussed above, components of the acute stage cytokine storm may be biomarkers to predict the viral load set-point [17^{*}]. Deeks *et al.* [20] in 2004 found that the immune activation set-point, defined by the levels

of T-cell activation, predicts disease progression independently of viral load. The link between the acute cytokine storm and the T-cell activation set-point and chronic stage activation levels remains to be defined. Studies such as the large Early Capture HIV Cohort Study (ECHO), or RV217, by the US Military HIV Research Program, will be important to ascertain such relationships and define broadly applicable soluble biomarkers.

MICROBIAL TRANSLOCATION AND INNATE IMMUNE ACTIVATION

HIV-1 infection is associated with severe loss of CD4 T cells and disruption of immune homeostasis at mucosal sites. This in turn leads to impaired mucosal barrier function and enhanced microbial translocation into the tissues and eventually into circulation. This topic has recently been reviewed elsewhere [21,22].

Monocyte and macrophage activation and their dysregulation in HIV disease are partly caused by the high sensitivity of these cells to elevated microbial products in the systemic circulation, and partly due to direct effects of HIV-1 replication. Activated monocytes and macrophages release several measurable biomarkers in the plasma and cerebrospinal fluid, including soluble (s)CD14, sCD163 and IL-6. Perhaps the most widely used biomarkers of microbial translocation are lipopolysaccharide (LPS) and sCD14 [23]. LPS is a direct biomarker of bacterial biomaterial in plasma and probably reflects translocation of Gram-negative bacterial material [24]. Marchetti *et al.* [25] observed that LPS levels are predictive of disease progression, suggesting that LPS is a functionally relevant biomarker of microbial translocation. Redd *et al.* [26], however, did not

observe such a relationship in a Ugandan cohort. It is possible that environmental and microbiome differences might affect the utility of LPS as a biomarker in different parts of the world. Sandler *et al.* [27[■]] also did not observe a predictive association between plasma LPS and clinical outcome in the Strategies for Management of Anti-Retroviral Therapy (SMART) study. However, this study instead found levels of sCD14 to be significantly associated with all-cause mortality [27[■]]. A similar relationship was observed in an HIV-uninfected cohort of patients undergoing haemodialysis, suggesting that sCD14 might reflect health status unrelated to HIV infection [28[■]]. Eller *et al.* [29] observed no significant association between sCD14 levels and HIV-1 disease progression in a Ugandan cohort, whereas a recent study by Thiebaut *et al.* [30] did observe a significant association between sCD14 and HIV-2 disease progression.

Microbial translocation products from the gut would normally arrive in the liver via the portal circulation. Sandler *et al.* [31[■]] observed that sCD14 is a biomarker of liver cirrhosis in hepatitis C virus (HCV) infected patients. Furthermore, sCD14 was associated with interferon and ribavirin treatment outcome in HCV/HIV-1 coinfecting patients [32,33[■]]. Thus, systemic sCD14 levels are likely influenced not only by the rate of microbial translocation but perhaps also by the status of the liver (Fig. 2). Furthermore, we have observed that sCD14 levels increased during interferon treatment in the setting of HCV/HIV-1 coinfection, suggesting that the type I interferon response also influences the levels of sCD14 in circulation [33[■]]. In addition to LPS and sCD14, recent studies have highlighted the role of IL-6 [34], intestinal fatty acid binding protein (I-FABP) [27[■]] and bacterial 16S DNA [35], as possible biomarkers of microbial translocation [21,22].

SOLUBLE BIOMARKERS OF COMORBIDITIES

Activation and turnover of cells of the macrophage lineage are implicated in HIV-associated comorbidities, including the development of atherosclerosis, cardiovascular disease (CVD) and HIV-associated neurocognitive disorders (HANDs) [36,37]. HIV-1 disease is also associated with persistent inflammation, which is thought to disrupt the homeostatic balance of the coagulation pathway, shifting it to a net procoagulant state [38,39].

COAGULOPATHY AND INFLAMMATION

Coagulopathy has been linked to an increased incidence of comorbidities from CVD and all-cause mortality observed among HIV-infected people. Findings from the SMART study show that levels of D-dimer, a fibrin degradation product and a biomarker of coagulation, as well as IL-6 and high-sensitivity (hs)CRP, biomarkers of inflammation, are associated with an increased risk of all-cause mortality in the combination antiretroviral therapy (cART)-untreated group [40]. More recent findings from the SMART cohort show that HIV-infected people with higher levels of IL-6, High-sensitivity C-reactive protein (hsCRP) and D-dimer also have an increased risk of CVD even after adjustment for other traditional risk factors, with similar associations in the untreated and cART-treated groups [41[■]]. Higher pre-ART levels of IL-6, hsCRP and D-dimer are linked with an increased risk of AIDS and death among the Flexible Initial Retrovirus Suppression Therapy (FIRST) cohort [42[■]], as well as those of hyaluronic acid, a biomarker of tissue fibrosis, although caution should be exercised as hyaluronic acid was more likely to be detectable in hepatitis virus coinfection than in HIV mono-infection alone [42[■]]. Indeed, hyaluronic acid levels

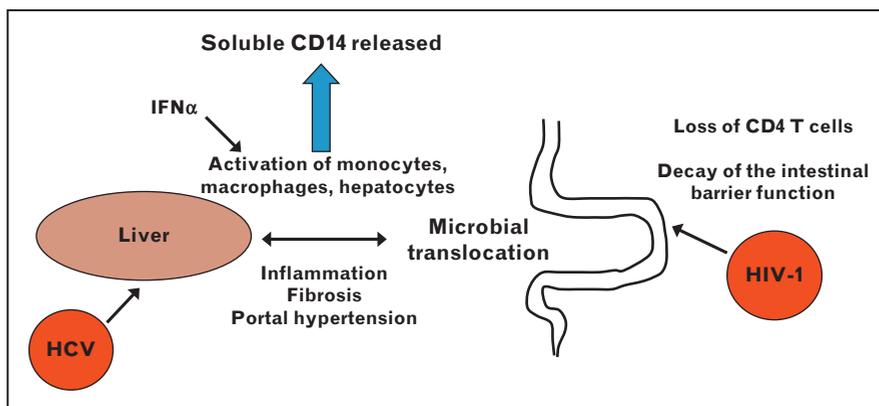


FIGURE 2. Soluble CD14 as an integrated soluble biomarker of microbial translocation, liver status and type I interferon stimulation. HCV, hepatitis C virus; IFN α , interferon alpha.

Table 1. Soluble biomarkers of HIV immunopathogenesis

Soluble biomarker	Role in HIV-1 disease	References
Markers of immune activation		
IFN γ	Antiviral cytokine that increases in acute infection and peaks along with VL. Stays at a level higher than baseline during infection. Also associated with lower levels of transmission via breast milk.	[4,13]
RANTES	(CCL5) chemokine for recruiting leukocytes, seen to be increased in breast milk of HIV-positive mothers and is associated with a transmission risk to infants.	[3]
MIP-1 β	(CCL4) chemokine used to recruit NK cells and monocytes. Elevated in breast milk from HIV-positive mothers with a possible association with transmission.	[3,4]
H β D-2	(SAP-1) An antibacterial, with a possible antiviral activity, which is increased in CVL of seroconverters, probably a biomarker of environmental stimulus predisposing a higher risk of HIV-1 acquisition.	[6 [■] ,7]
A-SAA	An antiviral and first measurable immune response to infection, before measurable viral RNA. Possible biomarker of hyperacute infection	[12 [■]]
IP-10	(CXCL10) Chemokine secreted in response to IFN γ . Higher levels in acute infection are associated with low CD4 cell counts in chronic phase. May be a useful biomarker for disease progression.	[16]
IL-12p40/p70, IFN γ , IL-7, IL-15	Measures in acute infection have been used in an algorithm to predict 66% of viral set-point variation. Likewise for CVL IL-12p70 and IL-15.	[9 [■] ,17 [■]]
Markers of coagulation, tissue fibrosis and inflammation		
D-dimer	Elevated in HIV disease. Association with endothelial dysfunction even in virologically suppressed patients; increased risk of CVD, IRIS, AIDS events or all-cause mortality, including during treatment interruption. Appears to correlate with viral loads.	[27 [■] ,40,41 [■] ,42 [■] ,44,47]
Fibrinogen	Elevated in HIV disease. Independent association with all-cause mortality, regardless of CD4 cell count in the FRAM cohort. Appears to correlate with higher plasma viral loads.	[45,46]
hsCRP	Elevated in HIV disease. Increased risk of CVD, AIDS events, IRIS or all-cause mortality, including during treatment interruption. Association with viral loads in the SMART cohort, but little association in the FRAM cohort.	[40,41 [■] ,42 [■] ,45,46,47]
Hyaluronic acid	Pre-cART levels are associated with an AIDS-defining illness or death. HBV or HCV coinfecting patients are more likely to have detectable HA levels than HIV mono-infected patients. HA levels predict increased risk of non-AIDS mortality in coinfecting patients in the SMART cohort.	[42 [■] ,43]
IL-6	A classical marker of inflammation that is strongly associated with CVD, IRIS, AIDS events or all-cause mortality in several, large cohort studies. Appears to correlate with higher plasma viral loads, particularly in the setting of low CD4 cell counts.	[27 [■] ,29,40,41 [■] ,42 [■] ,45,47]
Markers of enterocyte damage, microbial translocation and monocyte activation		
EndoCAB	Endogenous endotoxin (LPS) core antibody is a marker of endotoxin exposure. Plasma levels are decreased in both untreated and treated HIV-infected patients in the SMART study, but there was no association with comorbidities or death. More studies are needed.	[27 [■]]

Table 1 (Continued)

Soluble biomarker	Role in HIV-1 disease	References
I-FABP	Intestinal fatty acid binding protein is a marker of enterocyte damage. Plasma levels are increased in both untreated and treated HIV-infected patients in the SMART study. No association with comorbidities or death, but patients who died had significantly higher I-FABP levels. More studies are needed.	[27 [■]]
LPS	A classical marker for microbial translocation that is increased in HIV infection. Contradicting reports on association with disease progression exist. The LPS detection assay is relatively challenging and results may vary among different operators. More studies are needed, as well as more sensitive and consistent microbial translocation measurements.	[25,26,27 [■]]
sCD14	Levels are associated with subclinical atherosclerosis progression and neurocognitive impairments, and in the SMART cohort associated with all-cause mortality. No association with disease progression is found in a Ugandan cohort. Levels appear to correlate with levels of IL-6, D-dimer and hsCRP.	[27 [■] ,28 [■] ,29,48–50]
sCD163	A novel marker of monocyte activation that is increased in HIV infection. ART initiation reduced levels to baseline in early, but not chronic disease. Independent association with noncalcified coronary plaques; correlation with aortic inflammation.	[51,52,53 [■]]

ART, antiretroviral therapy; A-SAA, acute phase protein serum amyloid A; cART, combination antiretroviral therapy; CCL5, chemokine (C-C motif) ligand 5; CVL, cervicovaginal lavage; FRAM, Study of Fat and Redistribution and Metabolic Change in HIV infection; HA, hyaluronic acid; HBV, hepatitis B virus; HCV, hepatitis C virus; hsCRP, high-sensitivity C-reactive protein; IFN γ , interferon-gamma; IL, interleukin; IRIS, immune reconstitution inflammatory syndrome; LPS, lipopolysaccharide; MIP-1 β , macrophage inflammatory protein-1 β ; NK, natural killer; SMART, Strategies for Management of Anti-Retroviral Therapy; VL, viral load.

predict time to a major liver-related event and non-AIDS mortality in the SMART study [43]. Elevated levels of D-dimer have been associated with endothelial dysfunction as measured by brachial artery flow-mediated dilation, despite effective virological suppression [44]. Furthermore, detectable viraemia has been linked with increased levels of IL-6 [27[■],45], and D-dimer in the SMART cohort [27[■]], and fibrinogen [27[■],45], a biomarker of coagulation that is associated with excess mortality among HIV-infected people from the Study of Fat and Redistribution and Metabolic Change in HIV infection (FRAM) cohort [46]. Higher plasma levels of IL-6 are also associated with faster progression to AIDS in a Ugandan cohort [29]. Higher levels of hsCRP, D-dimer and IL-6 before and during cART are furthermore associated with the development of immune reconstitution inflammatory syndrome in the FIRST and another Ugandan cohort [42[■],47]. These findings together indicate that IL-6, hsCRP, D-dimer, fibrin and hyaluronic acid can be clinically useful in predicting comorbidities in HIV-1-infected patients both on and off cART.

MONOCYTE AND MACROPHAGE ACTIVATION IN COMORBIDITIES

Plasma levels of sCD14 are independently associated with all-cause mortality and directly correlate with IL-6, hsCRP and D-dimer levels in the SMART cohort [27[■]]. Elevated plasma concentrations of sCD14 are furthermore associated with increased rates of carotid artery intima-media thickening, a surrogate marker for progression of subclinical atherosclerosis [48]. Interestingly, levels of sCD14 in plasma and CSF are also linked with neurocognitive impairment in advanced HIV-1 infection despite suppressive ART [49,50]. Levels of the soluble scavenger receptor for haemoglobin, CD163, are elevated in both early and chronic infection, and parallel to viral load and associated with monocyte expansion [51]. Interestingly, the same investigators discovered that levels of sCD163 are independently associated with noncalcified coronary plaque in HIV-infected people, but not in uninfected controls with similar CVD risk factors [52]. Indeed, another study [53[■]] has linked sCD163 levels with arterial inflammation in patients receiving cART as determined by PET.

In nonhuman primates, pathogenic simian immunodeficiency virus (SIV) infection of pig-tailed and rhesus macaques results in elevated levels of D-dimer, sCD14 and sCD163, and evidence of cardiovascular lesions at the histological level, but not in the nonpathogenic SIV infection of African green monkeys and sooty mangabeys [54[¶]]. Administration of LPS to replicate microbial translocation in the nonpathogenic, chronically SIV-infected African green monkeys led to elevated sCD14 and D-dimer levels [54[¶]]. Collectively, these studies strengthen the notion that microbial translocation and the concomitant monocyte and macrophage activation in HIV-1 disease may play a direct role in coagulopathy and associated comorbidities. Furthermore, they identify a set of soluble biomarkers that may prove useful in predicting such comorbidities.

POSSIBLE NEW SOLUBLE BIOMARKERS, AND COMBINATION OF BIOMARKERS, IN HIV DISEASE

Although many soluble biomarkers that reflect different pathological processes in HIV-1 infection have been identified, it is essential to continue the search for novel useful biomarkers. Asymmetric dimethylarginine, an endogenous inhibitor of the nitric oxide synthase pathway that reflects endothelial dysfunction, may be one such new marker [55]. Furthermore, in the SMART cohort, levels of this factor decrease following therapy [56].

It is possible that assessment of combinations of soluble biomarkers may prove more powerful than the single factors alone in predicting disease. As discussed above, a combination of five cytokines may be predictive of viral set-point variation [17[¶]]. Another recent example in this direction is the observation that soluble factors from T cells inhibiting C-X-C chemokine receptor type 4-using HIV strains are composed of a mixture of three β -chemokines and two RNases [57].

CONCLUSION

A vast array of soluble biomarkers has been shown to be associated with various aspects of HIV-1 disease throughout the duration of infection (Table 1). Correlates between disease stages or manifestations and soluble markers are being very instructive in forming our understanding of this complex disease. New soluble factors that can function as biomarkers in HIV-1 infection will probably be discovered. Looking further into the future, it is tempting to speculate that integration of many biomarkers derived from a single plasma sample might become

a powerful tool in clinical practice to optimize and individualize treatment and care.

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Conflicts of interest

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