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Soluble Biomarkers and Morbidity and Mortality among People Infected with HIV: Summary of Published Reports from 1997-2010

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Abstract

Purpose of Review—Published articles from 1997 through May 2010 that reported findings on the relationship of soluble biomarkers with clinical outcomes among people infected with HIV were identified, and studies that examined the incremental value (over that of CD4+ count and HIV RNA level) that biomarkers had for predicting clinical outcomes were summarized.

Recent Findings—Over 1,500 articles were identified on MEDLINE databases that met selected MeSH terms. Thirty-eight met criteria for inclusion in the review. Fifteen of the articles were published since 2008. Most evaluated biomarkers reflecting inflammation and immune activation. For 25 studies, the relationship between the biomarker and all-cause mortality was evaluated. Stored samples were used for many studies, and those that did not usually focused on biomarkers that are measured as part of routine care. Eight of the reports utilized a case-control design and most of these were nested within a cohort study or a clinical trial.

Summary—Establishing the relationship between a biomarker and a clinical outcome is an important step in biomarker evaluation. To advance research on biomarkers relevant to people with HIV, large studies with long follow-up, carefully documented clinical events, and specimen repositories will be needed.

Keywords

Biomarkers; clinical outcomes; literature review

Introduction

Biomarkers have been extensively investigated as predictors of HIV clinical disease progression (AIDS or death) over the last two decades. This research has been aimed at informing disease pathogenesis among HIV-infected individuals, stratifying patients

according to risk of death and serious non-AIDS conditions (e.g., cardiovascular disease (CVD), non-AIDS-defining malignancies, renal disease, and liver disease), and potentially identifying drug targets for future intervention trials.

Biomarker studies are common in many areas of clinical research. In 2001, a National Institutes of Health working group developed standard definitions for a biomarker and surrogate outcome. The working group defined a biological marker (biomarker) as “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, or pharmacological responses to a therapeutic intervention”. This definition includes measures such as weight and blood pressure, blood tests, measurements based on imaging technology and genetic variations. The working group defined a surrogate marker as “a biomarker that is intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit (or harm or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence.”¹ A clinical endpoint is a characteristic or variable that reflects how a patient feels, functions or survives (e.g., death). Based on these definitions, surrogate markers can be considered a subset of biomarkers.

The working group classified biomarkers according to the following uses: 1) as a diagnostic tool; 2) as a tool for staging disease; 3) as an indicator of disease prognosis; and 4) for monitoring a response to an intervention. The two biomarkers studied most extensively in HIV research, CD4+ cell count and HIV RNA level, have been used for all four of the categories mentioned above, but primarily the last three. HIV RNA is considered a surrogate outcome for the clinical endpoint most often used in HIV treatment clinical trials, AIDS-defining conditions or death.

An Institute of Medicine (IOM) Committee adopted these same definitions in their recent report and described the following three steps for evaluating biomarkers: 1) assessment of the analytical performance of the assay; 2) evidence for association between the biomarker and different disease states, including the effects of interventions on both the biomarker and clinical outcomes; and 3) a contextual analysis of the proposed use of the biomarker that includes whether the analytical variation effects and evidence from the second step provides sufficient evidence for the proposed use.²

In this paper, we focus on one aspect of the second step described in the IOM report and review papers investigating the relationship of biomarkers with different disease states among people infected with HIV.

Methods

We searched for studies that related levels of soluble blood biomarkers (i.e., markers that could be measured in plasma or serum) with morbidity and mortality outcomes. We focused on soluble biomarkers because of their ease of collection, storage and cost. We excluded cellular blood components, including lymphocyte subsets, genetic markers and diagnostic markers (e.g., serology for hepatitis infection) from our review. We also excluded structural and functional markers that use imaging technologies (e.g., carotid IMT) and non-invasive measures of endothelial dysfunction. We aimed to identify studies that examined the incremental value (over that of CD4+ count and HIV RNA level) that novel biomarkers had for predicting clinical outcomes. We excluded cross-sectional studies because in these studies a temporal relationship could not be determined. A few studies evaluated markers at multiple time-points (e.g., at baseline and during follow-up). In those cases, we only focused on the baseline measurements.

We searched MEDLINE databases using the following MeSH terms: (“Biological Markers” [Mesh] OR “Cytokines/blood” [Mesh] OR “Lipids” [Mesh] OR “Lipoproteins” [Mesh]) AND (“HIV Infections” [Mesh] OR “Acquired Immunodeficiency Syndrome” [Mesh]) AND (“AIDS-Related Opportunistic Infections” [Mesh] OR “Mortality” [Mesh] OR “Disease Progression” [Mesh] OR “Acquired Immunodeficiency Syndrome/mortality” [Mesh] OR “HIV Infections/mortality” [Mesh] OR “Death” [Mesh] OR “Cardiovascular Diseases” [Mesh] OR “Myocardial Infarction” [Mesh] OR “Kidney Failure” [Mesh] OR “Liver Cirrhosis” [Mesh] OR “Neoplasms” [Mesh]). We also reviewed HIV journals for recently published articles. A total of 1,666 articles published in English after 1996 were identified with the MEDLINE search. We reviewed the abstract of each article and identified 38 that met our criteria.

3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40

A variety of methods were used to study biomarker-clinical outcome associations in the articles and individual patient data were not available for most articles. Thus, our summary, with few exceptions, is largely qualitative and meant to be a potential bibliographic resource for future investigations. Where possible, we cite literature from general population cohorts for comparison with the HIV studies we identified.

Results

Table 1 lists the articles identified by calendar date of publication. The first study meeting the selection criteria was published in 1998. More than one-half of the papers were published between 2006 and the first half of 2010; about 40% were published in the last two and one-half years. Research is increasing in this area which is reflected in recent conference abstracts not included in this review.^{41,42,43,44,45,46,47,48,49}

For 25 of 38 studies (66%), the relationship between the biomarker and all-cause mortality was evaluated. For 12 studies (32%), AIDS or the composite outcome AIDS or death was evaluated; 2 studies examined specific AIDS-defining events; and 4 studies examined CVD outcomes. All of the studies with a CVD outcome were published in 2008 or later.

Power for detecting associations between biomarkers and clinical outcomes depends on the number of participants who develop the clinical event of interest. Fourteen of the studies had fewer than 50 events (37%); 15 (39%) had more than 50 but fewer than 200 events; and 9 (24%) had more than 200 events. Two of the studies with more than 200 events were from the Multicenter AIDS Cohort Study (MACS) and involved follow-up of patients before combination ART was available.

Most of the studies used stored samples for the biomarker analyses, however six of the studies evaluated biomarkers collected as part of routine care (e.g., creatinine and lipids). Eight of the reports utilized a case-control design and most of these were nested within a cohort study or one or more clinical trials. The efficiency of nested case-control studies has been reviewed.⁵⁰ The other biomarker investigations were cohort studies.

In Table 2 we categorize the articles listed in Table 1 according to type of biomarker and clinical outcome. Findings are reviewed below.

Inflammatory/Immune Activation Markers

Much of the early biomarker work in HIV concerned markers of immune activation. Early studies in the MACS, prior to the availability of HIV RNA measurements, identified several cellular and serologic markers (e.g., β_2 -microglobulin, neopterin and CD38+ CD8+ cells) as independent predictors of disease progression and mortality.^{51,52,53} Later studies extended

the work on the cellular markers and considered the prognostic importance of cellular markers after adjustment for HIV RNA level and CD4+ cell count.^{54,55,56} Recent work on soluble markers that also considered the effects of HIV RNA level and CD4+ cell count is summarized in this section. In this category of inflammatory and immune activation markers, we include markers that reflect altered expression of cell surface antigens, products of cytokine activity, inflammatory markers, and other soluble markers that may represent immunologic changes. Twenty-four different published reports were identified that related these markers to all-cause mortality, AIDS, the composite outcome of AIDS or death, or CVD.

Associations between β_2 -microglobulin and clinical outcomes have been described in 7 reports. In three of the studies,^{7,13,19} a higher level of β_2 -microglobulin was associated with a significantly increased risk of death. One of these cohorts was described in a second paper that also considered adjustment for CD4+ CD28+ cells and the association remained significant with a similar hazard ratio.¹⁸ Three studies found that higher levels were associated with an increased risk of AIDS but with multivariate adjustment the association was not significant.^{3,4,10} One of these latter studies also reported a significant association with mortality that became non-significant after adjusting for HIV RNA level, CD4+ cell count and other factors.¹⁰

In all four studies evaluating neopterin, higher levels were associated with an increased risk of clinical outcomes.^{4,5,17,19} In one study, the association was not significant.¹⁹ Among the other three studies, one compared the upper tertile versus lower tertiles and the relative risk was 2.9⁴; another compared the upper versus lower quartile and the relative risk was 2.2¹⁷; and a third study reported a relative risk overall 5-6 fold higher for those with neopterin >20 nM compared to <12 nM, and 2-3 fold higher for those with neopterin 12-20 nM versus <12 nM. The relative risks associated with higher neopterin levels in this study were greater among patients with higher CD4+ cell count and with lower HIV RNA levels.⁵

The HIV-1 antigen, p24, was evaluated in two studies for its effect on progression to AIDS and mortality independent of CD4+ cell count and HIV RNA level.^{6,28} In both studies higher levels were associated with an increased risk of progression (relative risks were 2.9 for upper versus lower quartile and 1.6 for 10-fold higher levels).

Plasma levels of tumor necrosis factor alpha (TNF- α) were evaluated in five studies.^{3,8,16,23,30} In a study with advanced HIV, higher levels of TNF- α were associated with an increased risk of AIDS dementia.¹⁶ No association between TNF- α and the development of MAC bacteremia was reported in one small study.⁸ TNF- α was not related to all-cause mortality²³ and AIDS^{3,30} in three other studies, but each study had 50 or fewer events.

Soluble forms of receptors of TNF have also been investigated in studies that adjusted for HIV RNA level and CD4+ cell count.^{3,4,5,8,30,36} Baseline levels of TNF- α p75³⁰ and TNF-R55³ were not associated with progression to AIDS. One study found that higher levels of TNF-R75 were associated with AIDS⁴ and one study did not.³ Likewise results for TNF-RII were inconsistent. One study found an association with AIDS⁵ while two did not.^{8,36} One study reported a significant association between higher levels of TNF-RI and AIDS or death (hazard ratio = 3 per 615 pg/mL higher).³⁶

Several of the inflammatory markers studied in HIV cohorts have also been studied in general population cohorts. Two frequently studied markers were C-reactive protein (CRP) and interleukin-6 (IL-6). The relationship of CRP with all-cause mortality was evaluated in the Women's Interagency HIV Study (WIHS), a study of pregnant women in Tanzania, the Strategies for Management of Anti-Retroviral Therapy (SMART) study, and the Fat Redistribution and Metabolic Change in HIV Infection (FRAM) study.^{12,25,29,39} In all 4

studies, CRP was significantly related to all-cause mortality after adjustment for HIV RNA, CD4+ cell count and other factors. Relative risk/odds ratio estimates for the upper versus lowest quartile of CRP ranged from 1.7 in the Tanzanian study to 4.5 in WIHS. SMART was intermediate (relative risk = 3.1). In FRAM, the adjusted odds ratio estimate for the upper versus lowest quartile of CRP was 3.7.

In the Tanzanian study and in SMART, the association of CRP with AIDS was also evaluated. Significant associations were found in both studies with relative risks for the upper versus lowest quartile of CRP of 1.9 and 3.5.^{25,33} A report from the MACS preceded these studies and also found a significant association between CRP and AIDS. For those in the upper quartile of CRP (>2.3 mg/L) versus those ≤ 1.2 mg/L (lower 54% of the distribution), the relative risk was 2.0.²² In the Tanzanian study, a significant association between CRP and the composite outcome of AIDS or all-cause mortality was also reported with a relative risk (upper versus lowest quartile) equal to 2.2.

An overview of the association between CRP and all-cause mortality and between CRP and CVD outcomes in the general population has recently been published.⁵⁷ The authors concluded that associations of CRP with vascular and non-vascular mortality were similar. Graded relationships with CRP were evident for both outcomes.

Like CRP, IL-6 has been studied with different clinical outcomes. In SMART, the association between IL-6 and all-cause mortality was much greater than for CRP. The relative risk (upper versus lowest quartile) of IL-6 was 12.4.²⁹ Three studies reported significant associations between IL-6 and AIDS³³ or between IL-6 and AIDS or death.^{17,36} These associations, while significant, were not as strong as the association between IL-6 and all-cause mortality found in SMART but are similar to the association between CRP and AIDS. In one small study of disseminated MAC, there was no difference in IL-6 found among patients who developed MAC bacteremia and those who did not.⁸

IL-6 has been shown to be related to both CVD and all-cause mortality in the general population.^{58,59,60} For a number of years it has been recognized that IL-6 levels are elevated with HIV infection.⁶¹ Recently, the magnitude of this elevation for both IL-6 and CRP has been quantified among HIV-infected patients on combination ART and with suppressed HIV RNA levels.⁶²

In SMART, amyloid A and amyloid P, two other markers that have been evaluated in general population cohorts^{63,64} were studied in relation to all-cause mortality and AIDS. Higher levels of amyloid A and lower levels of amyloid P were seen for both outcomes, but the associations were not graded.^{29,33} To date, these markers have not been studied in other HIV cohorts.

Four studies evaluated soluble urokinase-type plasminogen activator receptor (suPAR) as a predictor of clinical outcomes.^{7,18,19,24} After adjusting for CD4+ cell count and HIV RNA level, higher levels of suPAR were associated with all-cause mortality in three of these reports.^{7,18,24} Two studies considered the increased risk associated with a 1 ng/mL increase in suPAR and hazard ratios were 1.6⁷ and 2.5¹⁸. A third study considered the risk associated with a one log increase in suPAR and the hazard ratio was 10.0.²⁴

Associations with interleukin-2 receptor (IL-2R) were reported in two studies. One study found IL-2R to be associated with progression to AIDS⁵ and the other found an association with all-cause mortality.¹⁴

A number of other markers related to immune activation have been evaluated in single studies. Immunoglobulin A (IgA) was associated with AIDS and all-cause mortality in

univariate analyses, however the associations were not significant after multivariate adjustment.¹⁰ Another study evaluated tumor necrosis factor β (TNF- β) and APO-1/Fas levels and found that patients who progressed to AIDS had higher TNF- β levels and lower APO-1/Fas levels compared to those who did not.³ A small study of disseminated MAC, evaluated transforming growth factor β (TGF- β) and Interleukin-10 (IL-10) and differences between those who developed MAC and those who did not were not significant.⁸ A study including patients with advanced HIV who were initiating ART evaluated E-selectin and soluble intercellular adhesion molecule-1 (ICAM-1) (IL-2R was also evaluated – see above).¹⁴ Higher levels of ICAM-1 were associated with mortality in univariate but not multivariate analyses. In a study that examined glutathione tripeptide (GSH) and GSH peroxidases (GSH-Px), these markers were not associated with progression to AIDS.³⁰ Finally, in a study that also evaluated other markers, soluble CD27 and CD40L were evaluated.³⁶ Higher levels of both CD27 and CD40L were associated with an increased risk of AIDS or death. The association was significant for CD40L, but not CD27, after adjustment.

Only one published study investigating an inflammatory marker and CVD was found. This case-control study investigated myeloperoxidase, and found no association with CVD.²⁷ Levels were actually lower in CVD cases (292 pmol/L) than matched controls (320 pmol/L).

Haemostatic Markers

In SMART, D-dimer and prothrombin fragment 1+2 were studied in relation to both all-cause mortality and AIDS.^{29,33} The association of D-dimer with all-cause mortality was striking (relative risk for 4th versus 1st quartile = 41; $p < 0.0001$), but was not significantly associated with AIDS.³³ Like IL-6 and CRP, D-dimer levels were elevated even among participants with suppressed HIV RNA level.⁶²

Recent abstracts have confirmed the association of D-dimer with mortality that was found in SMART.^{42,46,48} D-dimer has also been related to all-cause mortality and CVD in the general population,^{65,66} but the strength of the association was not as strong as seen in studies of HIV participants.

In FRAM, fibrinogen was evaluated.³⁹ Higher fibrinogen levels were associated with an increased risk of death (odds ratio for upper versus lowest tertile = 2.6). Fibrinogen is associated with vascular and non-vascular mortality in the general population.⁶⁷

Lipid Markers

Four studies reported associations between lipids/lipoproteins and clinical outcomes. Three of the four studies reported associations with CVD outcomes. In the single study that reported associations with all-cause mortality, the associations with non-HDL cholesterol and HDL cholesterol were weak ($p = 0.05$ and 0.06 , respectively) after adjustment for CD4+ cell count, HIV RNA level and CVD risk factors. Higher levels of both lipid fractions were associated with lower mortality.³⁷

The largest study to examine the association between lipids and CVD is the D:A:D study.³⁸ In DAD, total and HDL cholesterol were significantly associated (positive association for total cholesterol and inverse for HDL) with an increased risk of CVD, coronary heart disease and myocardial infarction (MI). A recent abstract from the DAD study, reported that triglycerides were associated with MI independent of both total and HDL cholesterol.⁴⁹

Two reports from SMART have been published. In the first report associations between usual lipid values with CVD were reported. In the continuous ART group, total cholesterol and the total/HDL cholesterol ratio were significantly associated with CVD.²⁶ The second report examined lipoprotein particle concentrations by nuclear magnetic resonance. Total,

large, and small HDL particle concentrations were significantly inversely associated with CVD, but VLDL and LDL particle concentrations were not.³⁴ The findings on usual lipids are in general agreement with a large body of evidence and an overview of studies carried out in the general population.⁶⁸ SMART is the only study that has evaluated the relationship of lipid particle concentrations and CVD in HIV-infected persons. The absence of an association of LDL particle concentrations with CVD in SMART, particularly small LDL particles, is in contrast to some studies in the general population.^{69,70}

Other Markers

The two most frequent markers studied in this category were albumin and creatinine and/or estimated glomerular filtration rate (eGFR). Biomarkers related to iron status were examined in two reports and the other markers were examined in single studies.

Associations between albumin levels and clinical outcomes were evaluated in four studies.^{10,15, 20,21} In each of these reports, reduced levels of albumin were associated with an increased risk of all-cause mortality. Two of these studies also reported the relationship between lower albumin levels and progression to AIDS.^{10,21} In the general population, lower albumin levels are associated with an increased risk of heart disease and all-cause mortality.⁷¹

Four studies evaluated creatinine or eGFR.^{9,11,35,40} One reported an increased risk of death associated with lower creatinine level ($<80 \mu\text{mol/L}$ for men and $<70 \mu\text{mol/L}$ for women).⁹ The other three studies found an increased risk of all-cause mortality with higher levels of creatinine (≥ 1.4 versus $<1.4 \text{ mg/dL}$)¹¹ and lower levels eGFR (<30 versus $\geq 30 \text{ mL/min/1.73m}^2$ or <60 versus $\geq 60 \text{ mL/min/1.73m}^2$)^{35,40} A recent meta-analysis of general population cohorts found that risk of death for eGFRs $<75 \text{ mL/min/1.73m}^2$ increased in a graded fashion.⁷²

Two studies evaluated the relationship between ferritin and all-cause mortality.^{31,32} In both of these reports, higher levels of ferritin were associated with an increased risk of death. One of these studies also examined the iron status biomarkers transferrin receptor (TfR), transferrin, transferrin index, the TfR/ferritin ratio (TfR:F) and iron.³² After adjustment for CD4+ count, HIV RNA level and other factors, lower levels of transferrin and TfR:F and higher levels of transferrin index were also associated with an increased risk of death.

A number of other markers were examined in individual studies. One report evaluated aspartate transaminase (AST), bilirubin and γ -glutamyl transpeptidase (GGT).¹⁰ These three markers were not associated with progression to AIDS or to all-cause mortality. GGT was univariately, but not multivariately, associated with AIDS and death. There was also a univariate association between AST and all-cause mortality, but this relationship did not remain significant after adjustment. One study reported an increased risk of death with higher fibrosis index (FIB) 4 levels.³⁵

Discussion

We identified 38 papers on the relationship of biomarkers with clinical outcomes. Most studies used stored specimens to measure biomarkers and for one-half of the studies, patients were enrolled in 1998 or before. The former statistic illustrates the potential importance of specimen repositories; the latter statistic and the small number of reports overall likely reflects the fact that since potent ART became available large cohorts with long follow-up are needed to accrue cases for biomarker studies as the risk of clinical events is low. The small number of studies investigating biomarkers with non-AIDS conditions (4 with CVD outcomes) may reflect both the low risk of these outcomes and the fact that

serious non-AIDS conditions have not been recognized as major causes of morbidity and mortality due possibly to both HIV infection and to complications of combination ART until the past few years. Many of the biomarkers have only been evaluated in one study, and many studies were small. Fourteen had 50 or fewer events.

The determinants of biomarker levels may differ for HIV-infected and non-infected participants. Thus, replication of studies in HIV cohorts may be important even when there is a substantial literature for the biomarker based on general population cohorts. A recent review discusses this for CVD outcomes.⁷³ The data reviewed herein support this. Elevated D-dimer levels appear to be associated with a greater risk of death among HIV participants than in general population cohorts. And, on the other hand, LDL cholesterol, particularly small LDL particle concentrations do not appear to be as strongly related to CVD outcomes among HIV participants as they are in the general population.

Large studies with long follow-up, carefully documented clinical events, and specimen repositories are needed to advance research on HIV biomarkers. Cohort studies and clinical trials are ideal for nesting case-control studies once a large number of clinical events have occurred.⁵⁰ The use of such studies enables good documentation of the target population from which both the cases and controls were chosen. With the clinical data and stored specimens for biomarker measurements, it should be possible to efficiently study novel biomarkers which may lead to improved systems for risk stratifying patients for AIDS and serious non-AIDS diseases. In HIV, this has already been done with HIV RNA and CD4+ cell count⁷⁴ and to some extent with other readily available laboratory tests.³⁵ Such risk stratification could be important for targeting treatments including the initiation of ART. Substantial biomarker research is ongoing in several other fields^{75,76,77}, and given the recent interest in risk of serious non-AIDS events⁷⁸, HIV research appears to be poised for an increase in such research. Thus, we would expect to see an increasing number of reports in the future concerning novel biomarkers to classify patients according to risk of specific non-AIDS conditions.

As noted in the IOM report, the evaluation of biomarkers requires multiple steps and several studies.² This review was aimed at identifying studies that examined associations between biomarkers and clinical outcomes. An important step which we did not address is the analytical performance of the different assays. Also critical to the evaluation of biomarkers and ultimately impacting morbidity and mortality, we did not review studies on interventions aimed at modifying biomarkers that are associated with increased risk of disease.

Multiple clinical trials will be needed that include both biomarker measurements and clinical outcomes to ensure that a biomarker can be safely used as a substitute for a clinical outcome in predicting the effect of an intervention. As noted in a classic paper on surrogate markers -- "a correlate does not a surrogate make".⁷⁹ Experience with research aimed at establishing HIV RNA level and CD4+ cell count as surrogate markers for progression of AIDS illustrates that point. These two biomarkers have been studied extensively for predicting AIDS events and mortality, for staging disease and as outcomes in clinical trials. The MACS established that single measurements of HIV RNA and CD4+ cell count were important determinants of AIDS or death.⁸⁰ Even though both HIV RNA level and CD4+ count are strongly related to risk of AIDS and all-cause mortality, and ART significantly impacts these markers, it took some time for HIV RNA to be accepted as a surrogate marker for clinical disease progression. A key step in the evaluation of surrogacy for HIV RNA level and CD4+ count was a meta-analysis of industry and government funded clinical trials that involved over 13,000 patients with HIV RNA and CD4+ measurements and clinical outcome assessments.⁸¹

Conclusion

In summary, initial research on biomarkers for predicting HIV progression focused on virologic and immunologic markers. Once HIV RNA levels and CD4+ cell count became widely used, HIV biomarker research slowed. There now seems to be a resurgence. Our review only considered soluble blood markers. Future research will likely also include functional markers, genetic biomarkers and a full array of technologies now available in “omics” toolboxes. While we can build on research findings in general population cohorts, it will be important to study biomarkers in HIV-infected cohorts and clinical trials. Future growth of this field of research will require large studies with carefully collected clinical data and specimen repositories.

Acknowledgments

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References

1. Biomarker Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Therap.* 2001; 69:89–95. [PubMed: 11240971]
2. IOM (Institute of Medicine). Evaluation of biomarkers and surrogate endpoints in chronic disease. Washington D.C.: The National Academies Press; 2010. **Provides recommendations for the evaluation of biomarkers and discusses how they are used including when they can be used as substitutes for clinical outcomes (surrogates) for assessing the risks and benefit of an intervention.
3. Medrano FJ, Leal F, Arienti D, et al. Tumor necrosis factor β and solubleAPO-1/Fas independently predict progression to AIDS in HIV-seropositive patients. *AIDS Res and Human Retroviruses.* 1998; 14:835–843. [PubMed: 9671212]
4. Zangerle R, Steinhuber S, Sarcletti N, et al. Serum HIV-RNA levels compared to soluble markers of immune activation to predict disease progression in HIV-1-infected individuals. *Int Arch Allergy Immunol.* 1998; 116:226–239.
5. Fahey JL, Taylor JMG, Manna B, et al. Prognostic significance of plasma markers of immune activation, HIV viral load and CD4 T-cell measurements. *AIDS.* 1998; 12:1581–1590. [PubMed: 9764776]
6. Ledergerber B, Flepp M, Böni J, et al. Human immunodeficiency virus type 1 p24 concentration measured by boosted ELISA of heat-denatured plasma correlates with decline in CD4 cells, progression to AIDS, and survival: comparisons with viral RNA measurement. *JID.* 2000; 181:1280–1287. [PubMed: 10751136]
7. Sidenius N, Sier CFM, Ullum H, et al. Serum level of soluble urokinase-type plasminogen activator receptor is a strong and independent predictor of survival in human immunodeficiency virus infection. *Blood.* 2000; 96:4091–4095. [PubMed: 11110678]
8. Havlir DV, Torriani FJ, Schrier RD, et al. Serum interleukin-g (IL-6), IL-10, tumor necrosis factor (TNF) alpha, soluble type II TNF receptor, and transforming growth factor beta levels in human immunodeficiency virus type 1-infected individuals with *Mycobacterium avium* complex disease. *J Clin Microbiol.* 2001; 39:298–303. [PubMed: 11136787]
9. Lewden C, Raffi F, Cuzin L, et al. Factors associated with mortality in human immunodeficiency virus type 1-infected adults initiating protease inhibitor-containing therapy: role of education level and early transaminase level elevation (APROCO-ANRS EP 11 Study). *JID.* 2002; 186:710–714. [PubMed: 12195361]
10. Sabin CA, Griffioen A, Yee TT, et al. Markers of HIV-1 disease progression in individuals with haemophilia coinfecting with hepatitis C virus: a longitudinal study. *Lancet.* 2002; 360:1546–1551. [PubMed: 12443592]
11. Gardner LI, Holmberg SD, Williamson JM, et al. Development of proteinuria or elevated serum creatinine and mortality in HIV-infected women. *J Acquir Immune Defic Syndr.* 2003; 32:203–209. [PubMed: 12571531]

12. Feldman JG, Goldwasser P, Holman S, DeHovitz J, Minkoff H. C-reactive protein is an independent predictor of mortality in women with HIV infection. *J Acquir Immune Defic Syndrome*. 2003; 32:210–214.
13. Ostrowski S, Gerstoft J, Pedersen BK, Ullum H. Impaired production of cytokines is an independent predictor of mortality in HIV-1 infected patients. *AIDS*. 2003; 17:521–530. [PubMed: 12598772]
14. Sipsas N, Sfrikakis PP, Touloumi G, et al. Elevated serum levels of soluble immune activation markers are associated with increased risk of death in HAART-naïve HIV-1 infected patients. *AIDS Patients Care and STDs*. 2003; 17:147–153.
15. Feldman JG, Gange SJ, Bacchetti P, et al. Serum albumin is a powerful predictor of survival among HIV-1 infected women. *J Acquir Immune Defic Syndrome*. 2003; 33:66–73.
16. Sevigny JJ, Albert SM, McDermott MP, et al. Evaluation of HIV RNA and markers of immune activation as predictors of HIV-associated dementia. *Neurology*. 2004; 63:2084–2090. [PubMed: 15596754]
17. Mildvan D, Sprizler J, Grossberg SE, et al. Serum neopterin, an immune activation marker, independently predicts disease progression in advanced HIV-1 infection. *CID*. 2005; 40:853–858.
18. Ostrowski SR, Piironen T, Høyer-Hansen G, et al. High plasma levels of intact and cleaved soluble urokinase receptor reflect immune activation and are independent predictors of mortality in HIV-1-infected patients. *J Acquir Immune Defic Syndr*. 2005; 39:23–31. [PubMed: 15851910]
19. Jaffar S, Van Der, Loeff MS, Eugen-Olsen J, et al. Immunological predictors of survival in HIV type 2-infected rural villagers in Guinea-Bissau. *AIDS Res and Human Retroviruses*. 2005; 21:56–564.
20. Brecht JR, Patrick PA, Visintainer P, Brand DA. Predictors of death within six months in patients with advanced AIDS. *Palliative and Supportive Care*. 2005; 3:265–272. [PubMed: 17039981]
21. Mehta SH, Astemborski J, Sterling TR, et al. Serum albumin as a prognostic indicator for HIV disease progression. *AIDS Res and Hum Retrovir*. 2006; 22:14–21. [PubMed: 16438640]
22. Lau B, Sharrett AR, Kingsley LA, et al. C-reactive protein is a marker of human immunodeficiency virus disease progression. *Arch Int Med*. 2006; 166:64–70. [PubMed: 16401812]
23. Sevigny JJ, Albert SM, McDermott MP, et al. An evaluation of neurocognitive status and markers of immune activation as predictors of time to death in advanced HIV infection. *Arch Neurol*. 2007; 64:97–102. [PubMed: 17210815]
24. Lawn SD, Myer L, Bangani N, et al. Plasma levels of soluble urokinase-type plasminogen activator receptor (suPAR) and early mortality risk among patients enrolling for antiretroviral treatment in South Africa. *BMC Infect Dis*. 2007; 7:41. [PubMed: 17509133]
25. Drain PK, Kupka R, Msamanga GI, et al. C-reactive protein independently predicts HIV-related outcomes among women and children in a resource-poor setting. *AIDS*. 2007; 1:2067–2075. [PubMed: 17885297]
26. Phillips AN, Carr A, Neuhaus J, et al. Interruption of antiretroviral therapy and risk of cardiovascular disease in persons with HIV-1 infection: exploratory analyses from the SMART trial. *Antiviral Therapy*. 2008; 13:177–187. [PubMed: 18505169]
27. El-Bejjani D, Hazen SL, Mackay W, et al. Higher plasma myeloperoxidase levels are not associated with an increased risk of cardiovascular events in HIV-infected adults. *HIV Clin Trials*. 2008; 9:207–211. [PubMed: 18547908]
28. Erikstrup C, Kallestrup P, Zinyama-Gutsire RBL, et al. p24 as a predictor of mortality in a cohort of HIV-1-infected adults in rural Africa. *J Acquir Immune Defic Syndr*. 2008; 48:345–349. [PubMed: 18545150]
29. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med*. 2008; 5(10):e203. [PubMed: 18942885] **A report that established that markers of inflammation and coagulation predict all-cause mortality even among HIV patients with high CD4+ counts and a suppressed HIV RNA level.
30. Morlat P, Pereira E, Clayette P, et al. Early evolution of plasma soluble TNF- α p75 receptor as a marker of progression in treated HIV-infected patients. *AIDS Res and Human Retroviruses*. 2008; 24:1383–1389. [PubMed: 18928394]

31. Rawat R, Humphrey JH, Ntozini R, et al. Elevated iron stores are associated with HIV disease severity and mortality among postpartum women in Zimbabwe. *Pub Health Nutr.* 2008; 12:1321–1329. [PubMed: 19000344]
32. McDermid JM, van der Loef MFS, Jaye A, et al. Mortality in HIV infection is independently predicted by host iron status and SLC11A1 and HP genotypes, with new evidence of a gene-nutrient interaction. *Am J Clin Nutr.* 2009; 90:225–233. [PubMed: 19474129]
33. Rodger AJ, Fox Z, Lundgren J, et al. Activation and coagulation biomarkers are independent predictors of the development of opportunistic disease in patients with HIV infection. *JID.* 2009; 200:973–983. [PubMed: 19678756]
34. Duprez DA, Kuller LH, Tracy R, et al. Lipoprotein particle subclasses, cardiovascular disease and HIV infection. *Atherosclerosis.* 2009; 207:524–529.
35. Justice AC, McGinnis KA, Skanderson M, et al. Towards a combined prognostic index for survival in HIV infection: the role of “non-HIV” biomarkers. *HIV Med.* 2010; 11:143–151. [PubMed: 19751364]
36. Kalayjian RC, Machekano RN, Nesrine R, et al. Pretreatment levels of soluble cellular receptors and interleukin-6 are associated with HIV disease progression in subjects treated with highly active antiretroviral therapy. *JID.* 2010; 201:1796–1805. [PubMed: 20446847]
37. Cockerham L, Scherzer R, Zolopa A, et al. Association of HIV infection, demographic and cardiovascular risk factors with all-cause mortality in the recent HAART era. *J Acquir Immune Defic Syndr.* 2010; 53:102–106. [PubMed: 19738484]
38. Friis-Møller N, Thiébaut R, Reiss P, et al. Predicting the risk of cardiovascular disease in HIV-infected patients: the Data Collection on Adverse Effects of Anti-HIV Drugs Study. *Euro J Cardiovasc Prev Rehabil.* 2010 epub ahead of print.
39. Tien PC, Choi AI, Zolopa Z, et al. Inflammation and mortality in HIV-infected adults: analysis of the FRAM Study Cohort. *J Acquir Immune Defic Syndr.* 2010 epub ahead of print.
40. Estrella MM, Parekh RS, Abraham A, et al. The impact of kidney function at highly active antiretroviral therapy initiation on mortality in HIV-infected women. *J Acquir Immune Defic Syndr.* 2010 epub ahead of print.
41. Duprez DA for the INSIGHT SMART Study Group. Inflammation, coagulation and cardiovascular disease in the Strategies for Management of Anti-Retroviral Therapy Study. Abstract P 1031-124. 58th Annual Scientific Session of the American College of Cardiology; Orlando, Florida. March 2009;
42. Ledwaba, L.; Maja, P. Pre-ART plasma levels of inflammation and coagulation makers are strong predictors of death after commencing ART in a South African cohort with advanced HIV disease. Abstract. 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention; Capetown. July 2009;
43. Sandler, N.; Wand, H.; Nixon, D., et al. Plasma levels of soluble CD14 predict mortality in HIV infection. Abstract 303. 17th Conference on Retroviruses and Opportunistic Infections; San Francisco, CA. February 2010;
44. Peters L for INSIGHT SMART Study Group. Biomarkers of inflammation and coagulation and risk of non-AIDS death (NAD) in HIV/hepatitis co-infected patients in the SMART study. Abstract 660. 17th Conference on Retroviruses and Opportunistic Infections; San Francisco, CA. February 2010;
45. Duprez DA for the INSIGHT SMART Study Group. N-terminal-proB-type natriuretic peptide (NT-proBNP) predicts cardiovascular disease events in HIV-infected patients. results of the SMART study. Abstract 712. 17th Conference on Retroviruses and Opportunistic Infections; San Francisco, CA. February 2010;
46. Boulware, D.; Huppler, Hullsiek K.; Puroon, C., et al. Higher levels of D-dimer, C-reactive protein, hyaluronic acid, and IL-6 before initiation of ART are associated with AIDS, IRIS and death among ART-naïve patients with a good virologic response to initial ART. Abstract 335. 17th Conference on Retroviruses and Opportunistic Infections; San Francisco, CA. February 2010;
47. Ambinder R for the INSIGHT SMART Study Group. Biomarkers of angiogenesis and γ Herpesviruses and the development of cancer in patients on episodic and continuous antiretroviral

- therapy in SMART. Abstract 760. 17th Conference on Retroviruses and Opportunistic Infections; San Francisco, CA. February 2010;
48. Ford, E.; Richterman, A.; Thompson, W., et al. Elevated D-dimer but not CRP levels in HIV+ patients prior to incident myocardial infarction or other cardiovascular disease event. Abstract 713. 17th Conference on Retroviruses and Opportunistic Infections; San Francisco, CA. February 2010;
 49. Worm, S.; Kamara, A.; El-Sadr, W., et al. Triglycerides and the risk of myocardial infarction in the D:A:D study. Abstract 127. 17th Conference on Retroviruses and Opportunistic Infections; San Francisco, CA. February 2010;
 50. Essebag V, Genest J, Suissa S, Pilote L. The nested case-control study in cardiology. *Amer Heart J*. 2003; 146:581–590. [PubMed: 14564310] *Nested case-control studies are efficient designs for evaluating the association of biomarkers with clinical outcomes. This is a useful discussion of the use of this design in cardiology.
 51. Fahey JL, Taylor JM, Detels R, et al. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. *N Engl J Med*. 1990; 322:166–172. [PubMed: 1967191] *An early report that established that investigated biomarkers as predictors of HIV progression and identified markers of immune activation as a potentially important contributor to HIV pathogenesis.
 52. Giorgi JV, Liu Z, Hultin LE, et al. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. *JAIDS*. 1993; 6:904–912.
 53. Liu Z, Cumberland WG, Hultin LE, et al. Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4+ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression. *JAIDS*. 1997; 16:83–92. [PubMed: 9358102]
 54. Giorgi JV, Lyles RH, Matud JL, et al. Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. *JAIDS*. 2002; 29:346–355. [PubMed: 11917238]
 55. Hazenberg MD, Otto SA, van Benthem BHB, et al. Persistent immune activation in HIV-1 infection associated with progression to AIDS. *AIDS*. 2003; 17:1–8. [PubMed: 12478064]
 56. Catalfamo M, Di Mascio M, Hu Z, et al. HIV infection-associated immune activation occurs by two distinct pathways that differentially affect CD4 and CD8 T cells. *PNAS*. 2008; 105:19851–19856. [PubMed: 19060209]
 57. The Emerging Risk Factors Collaboration. C-reactive protein concentration and risk of coronary heart disease, stroke and mortality: an individual patient meta-analysis. *Lancet*. 2010; 375:132–140. [PubMed: 20031199]
 58. Harris TB, Ferrucci L, Tracy RP, et al. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med*. 1999; 106:506–512. [PubMed: 10335721]
 59. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation*. 2000; 101:1767–1772. [PubMed: 10769275]
 60. Luc G, Bard JM, Juhan-Vague I, et al. C-reactive protein, interleukin-6, and fibrinogen as predictors of coronary heart disease: the PRIME Study. *Arterioscler Thromb Vasc Biol*. 2003; 23:1255–1262. [PubMed: 12775578]
 61. Breen EC, Rezai AR, Nakajima K, et al. Infection with HIV is associated with elevated IL-6 levels and production. *J Immunology*. 1990; 144:480–484. [PubMed: 2295799]
 62. Neuhaus J, Jacobs DR Jr, Baker JV, et al. Markers of Inflammation, coagulation and renal function are elevated in adults with HIV infection. *JID*. 2010; 201:1788–1795. [PubMed: 20446848] *A comparison of inflammatory and coagulation markers for HIV participants and those in the general population and established that levels are much in HIV participants.
 63. Johnson BD, Kip KE, Marroquin OC, et al. Serum amyloid A as a predictor of coronary artery disease and cardiovascular outcome in women: The National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). *Circulation*. 2004:726–732. [PubMed: 14970107]

64. Jenny NS, Arnold AM, Kuller LH, Tracy RP, Psaty BM. Serum amyloid P and cardiovascular disease in older men and women: results from the Cardiovascular Health Study. *Arterioscler Thromb Vasc Biol.* 2007; 27:352–358. [PubMed: 17138933]
65. Danesh H, Whincup P, Walker M, et al. Fibrin D-dimer and coronary heart disease: prospective study and meta-analysis. *Circulation.* 2001; 103:2323–2327. [PubMed: 11352877]
66. Cohen, HJ; Harris, T.; Piper, CF. Coagulation and activation of inflammatory pathways in the development of function decline and mortality in the elderly. *Am J Med.* 2003; 114:180–187. [PubMed: 12637131]
67. Danesh J, Lewington S, Thompson SG, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. *JAMA.* 2005; 294:1799–1809. [PubMed: 16219884]
68. Prospective Studies Collaboration. Blood cholesterol and vascular mortality by age, sex and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet.* 2007; 370:1829–1839. [PubMed: 18061058]
69. Blake GJ, Otvos J, Rifai N, et al. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. *Circulation.* 2002; 106:1930–1937. [PubMed: 12370215]
70. Rosenson RS, Otvos JD, Freedman DS. Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the pravastatin limitation of atherosclerosis in the coronary arteries (PLAC-1) trial. *Am J Cardiol.* 2002; 90:89–94. [PubMed: 12106834]
71. Danesh J, Collins R, Appleby P, Peto P. Fibrinogen, C-reactive protein, albumin and white cell count: meta-analysis of prospective studies of coronary heart disease. *JAMA.* 1998; 279:1477–1482. [PubMed: 9600484]
72. Chronic Kidney Disease Prognosis Consortium. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet.* 2010; 375:2073–2081. [PubMed: 20483451]
73. Schambelan M, Wilson PWF, Yarasheski KE, et al. Development of appropriate coronary heart disease risk prediction models in HIV-infected patients. *Circulation.* 2008; 118:e48–e-53. [PubMed: 18566316]
74. CASCADE Collaboration. Short-term risk of AIDS according to current CD4 cell count and viral load in antiretroviral drug-naïve individuals and those treated in the monotherapy era. *AIDS.* 2004; 18:51–58. [PubMed: 15090829]
75. Tice JA, Cummings SR, Smith-Bindman R, et al. Using clinical factors and mammographic breast density to estimate breast cancer risk: development and validation of a new predictive model. *Ann Intern Med.* 2008; 148:337–347. [PubMed: 18316752]
76. Cook NR, Ridker PM. Advances in measuring the effect of individual predictors of cardiovascular risk: the role of reclassification measures. *Ann Intern Med.* 2009; 150:795–802. [PubMed: 19487714]
77. Pepe MS, Feng Z, Janes H, Bossuyt PM, Potter JD. Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: standards for study design. *J Natl Cancer Inst.* 2008; 100:1432–1438. [PubMed: 18840817]
78. Phillips AN, Neaton J, Lundgren J. The role of HIV in serious diseases other than AIDS. *AIDS.* 2008; 22:2409–2418. [PubMed: 19005264]
79. Fleming TR, DeMets DL. Surrogate endpoints in clinical trials: are we being misled? *Ann Intern Med.* 1996; 125:605–613. [PubMed: 8815760]
80. Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med.* 1997; 126:946–954. [PubMed: 9182471] **A classic paper on the prognostic importance of the two most widely used biomarkers in HIV research.
81. HIV Surrogate Marker Collaborative Group. Human immunodeficiency virus type 1 RNA level and CD4 count as prognostic markers and surrogate end points: A meta-analysis. *AIDS Res Hum Retrovir.* 2000; 16:1123–1133. [PubMed: 10954887] *An illustration of the type of data and

methods needed for quantifying the concordance of risk reductions in clinical endpoints with changes in biomarkers (HIV RNA and CD4+ cell count) in order to assess surrogacy.

Summary of Studies Identified Ordered by Date of Publication

Table 1

Author (year)	Design	Target Population	Years of Enrollment	Outcome	Duration of Follow-up	Stored Samples Used	Soluble Blood Biomarkers	Sample Size/No. Events
Medrano (1998) ³	Matched case-control study	ART naïve patients in Spain; median CD4+ = 328 for cases and 335 cells/mm ³ for controls	1989-1995	AIDS	Mean = 143 weeks	Yes	β ₂ -microglobulin TNF-α TNF-β TNF-R55 TNF-R75 APO-1/Fas	21 cases and 21 matched controls
Zangerle (1998) ⁴	Cohort study	Untreated or treated with zidovudine monotherapy; median CD4+ count = 433 cells/mm ³	1991	AIDS, All-cause mortality	Median = 54 months	Yes	Neopterin β ₂ -microglobulin TNF-R75	31 patients for AIDS analysis, 11 developed AIDS; 47 patients for mortality analysis, 20 deaths
Fahey (1998) ⁵	Cohort study (MACS)	AIDS-free homosexual men in Los Angeles; median CD4+ = 514 cells/mm ³	1984-1985	AIDS	3 and 10 years	Yes	TNF-RII Neopterin IL-2R	659 patients, 141 with AIDS after 3 years and 441 with AIDS after 10 years
Ledergerber (2000) ⁶	Cohort study	Median CD4+ count = 255 for AIDS analysis and 141 cells/mm ³ for mortality analysis	1993-1994	AIDS, all-cause mortality	Median = 2.7 years	Yes	p24	108 patients for AIDS analysis, 35 developed AIDS; 169 patients for mortality analysis, 83 deaths
Sidenius (2000) ⁷	Cohort study	Median CD4+ = 204 cells/mm ³ ; not taking protease inhibitor treatment	1991-1992	All-cause mortality	Median = 38 months	Yes	suPAR β ₂ -microglobulin	314 patients, 167 deaths
Havlic (2001) ⁸	Case-control	CD4+ < 100, receiving MAC prophylaxis	1992-1994	Disseminated MAC		Yes	IL-6 IL-10 TNF-α TNF-RII TGF-β	15 cases and 15 controls
Lewden (2002) ⁹	Cohort study	Initiating PI-therapy	1997-1998	All-cause mortality	Median = 27 months	No	Creatinine	1155 patients, 48 deaths
Sabin (2002) ¹⁰	Cohort study	Men with hemophilia and hepatitis C co-infection in UK followed after sero-conversion; median CD4+ count = 600 cells/mm ³	1979-1985	AIDS, all-cause mortality	Median = 13.5 years	No	Albumin Bilirubin AST GGT IgA β ₂ -microglobulin	111 patients, 58 AIDS, 68 deaths
Gardner (2003) ¹¹	Cohort study (HERS)	Women free of AIDS; mean CD4+ count = 450 cells/mm ³	1993-1995	All-cause mortality	Average 4.9 years	No	Creatinine	885 patients, 239 deaths
Feldman (2003) ¹²	Cohort study (WHHS)	Women; median CD4+ count = 290 cells/mm ³	1994-1995	All-cause mortality	45 months	Yes	C-reactive protein	209 patients, 49 deaths

Author (year)	Design	Target Population	Years of Enrollment	Outcome	Duration of Follow-up	Stored Samples Used	Soluble Blood Biomarkers	Sample Size/No. Events
Ostrowski (2003) ¹³	Cohort study	Patients enrolled at a hospital in Denmark; 65% with prior AIDS or symptomatic disease	1994-1995	All-cause mortality	1-2 years	Unk	β_2 -microglobulin	88 patients, 17 deaths
Sipsas (2003) ¹⁴	Cohort study	Consecutive ART naïve patients without fever, neoplasm or concurrent opportunistic infection attending outpatient clinic; 38% with prior AIDS; median CD4+ 99 for those with AIDS and 482 cells/mm ³ without AIDS	1990-1993	All-cause mortality	Median = 46 months (until HAART initiation)	Yes	ICAM-1 IL-2R E-selectin	64, 34 deaths
Feldman (2003) ¹⁵	Cohort study (WHHS)	Women; median CD4+ count = 332 cells/mm ³	1994-1995	All-cause mortality	Median = 49 months	No	Albumin	1941 patients, 397 deaths
Sevigny (2004) ¹⁶	Cohort study	Patients with advanced HIV without dementia; median CD4+ count = 128 cells/mm ³	Beginning in 1998	AIDS dementia	Median = 20.7 months	No	TNF- α MCP-1 M-CSF MMP-2	203 patients, 74 AIDS dementia
Mildvan (2005) ¹⁷	Cohort study 1	Patients who had tolerated zidovudine for $\geq$ 16 weeks and had AIDS, AIDS-related complex with CD4+ $\leq$ 300 cells/ml, or asymptomatic with CD4+ $\leq$ 200 cells/ml; median CD4+ count = 75 cells/mm ³	1989	AIDS or death	Median = 344 days	Yes	Neopterin Endogenous interferon (primarily IFN- α) IL-6	152 patients, 52 AIDS
Ostrowski (2005) ¹⁸	Cohort study	Patients enrolled at a hospital in Denmark; median CD4+ count = 188 cells/mm ³	1994-1995	All-cause mortality	3 years	Yes	suPAR β_2 -microglobulin	99 patients, 14 deaths
Jaffar (2005) ¹⁹	Cohort study	HIV-2 infected patients in Guinea-Bissau; median CD4% = 28%	1989-1991	All-cause mortality	Median = 7 years	Unk	suPAR Neopterin β_2 -microglobulin	133 patients, 31 deaths
Brecht1 (2005) ²⁰	Cohort study	Mean CD4+ count = 104 cells/mm ³ ; 48% on HAART	1999	All-cause mortality	6 months	No	Albumin	152 patients, 61 deaths
Mehta (2006) ²¹	Cohort study	IDU, AIDS free; median CD4+ count = 290 cells/mm ³	1995	AIDS; AIDS-related mortality; all-cause mortality	Median = 4.6 years	No	Albumin	453 patients, 121 deaths, 135 AIDS
Lau (2006) ²²	Random sample from cohort study (MACS)	Homosexual men in U.S.; mean CD4+ count = 516 cells/mm ³	1984-1987	AIDS	Total follow-up = 2709 person-years	Yes	C-reactive protein	513 patients, 318 AIDS

Author (year)	Design	Target Population	Years of Enrollment	Outcome	Duration of Follow-up	Stored Samples Used	Soluble Blood Biomarkers	Sample Size/No. Events
Sevigny (2007) ²³	Cohort study	CD4+ < 200, or < 300 with cognitive impairment; mean CD4+ count = 139 cells/mm ³	1998-2002	All-cause mortality	Median = 25 months	No	TNF- α MCP-1 M-CSF MMP-2	329 patients, 50 deaths
Lawn (2007) ²⁴	Cohort study	ART-naïve patients beginning ART in South Africa; median CD4+ cell count = 47 cells/mm ³	2002-2005	All-cause mortality	Median = 5 months	Yes	suPAR	293 patients, 39 deaths
Drain (2007) ²⁵	Cohort study	ART-naïve pregnant women in Tanzania; 9% with CD4+ count < 200 cells/mm ³	1995-2003	AIDS, AIDS or death, all-cause mortality	Median=63 months	Yes	C-reactive protein	606 patients, 217 AIDS or death, 188 deaths
Phillips (2008) ²⁶	Cohort study	CD4+ > 350 in ART interruption trial	2002-2006	CVD	Mean = 16 months	No	Total cholesterol LDL cholesterol HDL cholesterol Total/HDL Triglycerides	5,472 patients, 79 CVD
El-Bejjani (2008) ²⁷	Case-control study	CVD cases between 1996 and 2001; controls with no CVD; site, age and gender matched; overall median CD4+ count = 304 cells/mm ³	1996-2006	CVD		Yes	Myeloperoxidase	62 cases and 62 matched controls
Erikstrup (2008) ²⁸	Cohort study	Untreated patients in Zimbabwe; median CD4+ count = 319 cells/mm ³	2001-2002	All-cause mortality	Range: 3-4.3 years	No	p24	198 patients, 58 deaths
Kuller (2008) ²⁹	Matched nested case control study (SMART)	Patients with CD4+ > 350 cells/mm ³ in ART interruption trial; median CD4+ count = 545 for deaths and 614 cells/mm ³ for controls	2002-2006	All-cause mortality	Median follow-up = 1.5 years	Yes	hsCRP Amyloid A Amyloid P IL-6 D-dimer F1.2	85 deaths (cases) and 170 controls
Morlat (2008) ³⁰	Nested case-control study	Patients starting a PL regimen in 1997-1998 in France; median CD4+ count = 120 cells/mm ³	1997-1998	AIDS	Cases in first year of treatment	Yes	TNF- α p75 TNF- α PAF-AH GSH GSH-Px	22 cases and 44 matched controls
Rawat (2008) ³¹	Cohort study	Women from Zimbabwe in a trial of Vitamin A following delivery; 80% with CD4+ <200	1997-2000	All-cause mortality	1-year post partum	Yes	Ferritin	643 patients, 26 deaths

Author (year)	Design	Target Population	Years of Enrollment	Outcome	Duration of Follow-up	Stored Samples Used	Soluble Blood Biomarkers	Sample Size/No. Events
McDermid (2009) ³²	Cohort study	Patients in The Gambia, West Africa; median CD4+ = 234 cells/mm ³ ; 67% HIV-1, 31% HIV-2, and 2% dual.	1991-2001	All-cause mortality	11.5 years (2714 person-years)	Yes	sTfR Iron Transferrin TfR:F Transferrin index Ferritin	1362 patients, 713 deaths
Rodger (2009) ³³	Matched nested case control study (SMART)	Patients with CD4+ > 350 cells/mm ³	2002-2006	AIDS	Median follow-up = 1.5 years	Yes	hsCRP Amyloid A Amyloid P IL-6 D-dimer F1,2	91 cases and 182 controls
Duprez (2009) ³⁴	Matched nested case control study (SMART)	Patients with CD4+ > 350 cells/mm ³ in ART interruption trial; median CD4+ count = 576 for CVD cases and 620 cells/mm ³ for controls	2002-2006	CVD	Average follow-up=2.8 years	Yes	Total HDLp Large HDLp Medium HDLp Small HDLp HDL size	248 cases and 480 controls
Justice (2010) ³⁵	Cohort study	US veterans initiating first ART regimen (at least 3 medications) between 1 Jan 1997 and 1 Aug 2002; median CD4+ count = 281 cells/mm ³	1997-2002	All-cause mortality	Median = 6.47 person-years	No	FIB 4 eGFR	9784 patients, 2566 deaths
Kalayjian (2010) ³⁶	Matched nested case control study	HAART-naïve patients who entered clinical trials of HAART regimens	1998-2001	AIDS or death	Median time to AIDS or death for cases = 12 weeks	Yes	TNF-RI TNF-RII CD27 CD40L IL-6	41 cases (AIDS or death) and 111 controls
Cockerham (2010) ³⁷	Cohort study (FRAM)	Median CD4+ count = 366 cells/mm ³	2004-2007	All-cause mortality	5 years	No	HDL cholesterol Non-HDL cholesterol	922 patients, 128 deaths
Friis-Møller (2010) ³⁸	Cohort study (DAD)	Median CD4+ count = 420 cells/mm ³	1999-2001	CVD, coronary heart disease (CHD), myocardial infarction (MI)	Average = 4.8 years	No	Total cholesterol HDL	22,625 patients, 663 CVD, 554 CHD, 375 MI
Tien (2010) ³⁹	Cohort study (FRAM)	Median CD4+ = 389 cells/mm ³ for those alive and 189 cells/mm ³ for death	2000-2002	All-cause mortality	5 years	Yes	CRP Fibrinogen	922 patients, 128 deaths
Estrella (2010) ⁴⁰	Cohort study (WHHS)	Women initiating combination ART; median CD4+ count < 300 cells/mm ³	1994-1995 and 2001-2002	All-cause mortality	5.7 years	No	eGFR	1425 patients, 335 deaths

Table 2
Summary of Markers Studied by Type of Marker and Outcome⁺

Outcome	Inflammatory/Immune Activation Markers	Haemostatic Markers	Lipid Markers	Other Markers
All-cause mortality	Amyloid A, Amyloid P, CRP, IL-6, β_2 -microglobulin, IgA, IL-10, sIL-2 α , M-CSF, MCP-1, MMP-2, neopterin, p24, suPAR, E-selectin, sICAM-1, TNF-R75, TNF- α , TNF- β 4,6,7,10,12,13,14,18,19,23,24,25,28,29,39	D-dimer, prothrombin fragment 1+2 (F1.2), fibrinogen 29,39	HDL, non-HDL cholesterol ³⁷	AST, albumin, bilirubin, creatinine, eGFR, FIB 4, ferritin, GGT, iron, sTfR, TfR:F, transferrin, transferrin Index 9,10,11,15,20,21,31,32,35,40
AIDS	Amyloid A, Amyloid P, CRP, IL-6, β_2 -microglobulin, IgA, IL-10, sIL-2 α , M-CSF, GSH, GSH-Px, MCP-1, MMP-2, neopterin, p24, APO-1/Fas, RTNF- α p75, TNF-RII, TNF-R55, TNF-R75, TGF- β , TNF- α TNF- β 3,4,5,6,8,10,16,22,25,30,33	D-dimer, F1.2, PAF-AH 30,33		AST, albumin, bilirubin, GGT, 10,21
AIDS or death	CRP, IL-6, Neopterin, sCD27, sCD40L, TNF-R1, TNF-RII, endogenous interferon (primarily IFN- α) 17,25,36			
CVD	Myeloperoxidase 27		Total cholesterol, HDL, LDL, triglycerides, Total/HDL, Total HDL-p, Large HDL-p, Medium HDL-p, Small HDL-p, LDL-p, VLDL-p, HDL size ^{26,34,37}	

⁺ References for the biomarkers in each category are given at the end (see Table 1 for description of study).