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Association of HIV clinical disease progression with profiles of early immune activation: results from a cluster analysis approach

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Abstract

Objective—CD4 and CD8 T-cell activation are independent predictors of AIDS. The complete activation profile of both T-cell subtypes and their predictive value for AIDS risk is largely unknown.

Design—A total of 564 AIDS-free women in the Women's Interagency HIV Study were followed over 6.1 years (median) after T-cell activation assessment. A cluster analysis approach was used to evaluate the concurrent activation patterns of CD4 and CD8 T cells at the beginning of follow-up in relation to AIDS progression.

Methods—Percentages of CD4 and CD8 T cells with HLA-DR± and CD38± were assessed by flowcytometry. Eight immunologic variables (four on each $CD4^+$ and $CD8^+$: DR± and CD38±) were assessed to yield a 4-cluster solution on samples obtained before clinical endpoints. Proportional hazards survival regression estimated relative risks for AIDS progression by cluster membership.

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Conflicts of interest
The authors have no conflict of interest.

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Results—Compared with the other three clusters, outstanding activation features of each distinct cluster of women were: Cluster 1: higher $CD8^{+}CD38^{-}$ DR^{-} (average = 41% of total CD8 T-cell pool), $CD4^{+}CD38^{-}$ DR^{-} (average = 53% of total CD4 T-cell pool), and $CD8^{+}CD38^{-}$ DR^{+} (28%); Cluster 2: higher $CD8^{+}CD38^{+}DR^{-}$ (44%) and $CD4^{+}CD38^{+}DR^{-}$ (58%); Cluster 3: higher $CD8^{+}CD38^{+}DR^{+}$ (49%) and $CD4^{+}$ $CD38^{+}DR^{-}$ (48%); Cluster 4: higher $CD8^{+}CD38^{+}DR^{+}$ (49%), $CD4^{+}CD38^{+}DR^{+}$ (36%) and $CD4^{+}CD38^{-}$ DR^{+} (19%). Compared with cluster 1, women in cluster 4 had two-fold increased risk of AIDS progression (Hazard ratio = 2.13; 95% confidence interval = 1.30–3.50) adjusted for CD4 cell count, HIV RNA, and other confounders.

Conclusion—A profile including CD4 and CD8 T-cell activation provided insight into HIV pathogenesis indicating concurrent hyperactivation of CD4 and CD8 T cells is associated with AIDS progression.

Keywords

AIDS; cluster analysis; immune activation

Introduction

T-lymphocyte activation is a hallmark of HIV infection that can lead to accelerated T-cell apoptosis [1,2]. Immune activation contributes more to CD4 decline than does the direct effect of HIV [1,3,4]. Immune activation may also reach a set point or threshold early in HIV infection, which can predict CD4 decline independent of HIV viral load [5]. In addition to well established documentation that low CD4 and CD8 T-cell counts predict AIDS among HIV-infected individuals, a growing body of evidence suggests that CD4 and CD8 activation also predicts HIV disease progression [3,6,7]. We have shown that HIV-infected women coinfected with hepatitis C virus (HCV) have higher levels of immune activation and greater risk of disease progression compared with women infected with HIV only [8,9]. A better understanding of the markers of immune activation is needed to maximize the utilization of T-cell activation as a predictor of HIV disease progression.

CD38 expression is a well documented marker of early T-cell activation and is also associated with cytokine production and rapid T-cell differentiation [10–12]. In the Multicenter AIDS Cohort Study, CD38 expression was a better predictor of HIV disease progression than other markers of immune activation [7]. Although most studies assessed immune activation by measuring percentages of T cells expressing CD38, the prognostic value of T-cell activation increased when immune activation was assessed by co-expression of CD38 with HLA-DR [13] or CD45R0 [14], suggesting expression of multiple markers on T cells could be indicative of a hyperactivation status. T cells dually activated with CD38 and HLA-DR significantly predicted progression to AIDS among HIV-infected men, and the same cohort of women in the Women's Interagency HIV Study (WIHS) as the current study [9]. Although numerous studies have evaluated the impact of CD4 or CD8 T-cell activation on risk of progression to AIDS, none evaluated the concurrent activation status of CD4 and CD8 T cells. It is possible that a global activation, characterized by hyperactivation of both CD4 and CD8 T cells, is indicative of a more general immune dysregulation leading to high T-cell turnover, severe immunodeficiency, and progression to AIDS. We undertook this study to identify clusters of women with a similar pattern of CD4 and CD8 T-cell activation based on CD38 and/or HLA-DR expression, which will reflect the concomitant CD4 and CD8 T-cell activation profile in HIV-infected individuals. We further examined the risk of AIDS for women in each cluster to identify whether there is a high-risk immune activation profile. We hypothesized that different immune profiles would demonstrate differential associations with progression to AIDS.

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Methods

Study population

WIHS enrolled HIV-infected (n = 2059) and uninfected (n = 569) women from October 1994 to November 1995 at six sites in the United States [15]. An additional 739 HIV-infected and 406 uninfected women were enrolled between 2001 and 2002. Participants are assessed at baseline and every 6 months with an extensive battery of questionnaires and laboratory tests. The current study includes 564 HIV-infected women who were AIDS-free at baseline and had immune activation markers assessed before a diagnosis of AIDS. Written informed consent was obtained from all study participants of the WIHS. The study was approved by the institutional review boards and ethics committees of all six participating sites.

Laboratory evaluations

CD4 and CD8 T-cell counts were measured by flow cytometry in laboratories participating in the National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS Flow Cytometry quality assurance program [15]. The fluorochrome-conjugated antibodies for three-color cytometry were anti-CD3, CD4, CD8, HLA-DR, and CD38 (Becton Dickinson, San Jose, California and Pharmigen, San Diego, California, USA) [8,16,17]. HIV-1 RNA quantification was performed every 6 months using real-time isothermal nucleic acid sequence-based amplification (Organon Teknika Corp., Durham, North Carolina, USA) [18]. HCV antibody testing was done at baseline using Abbott EIA 2.0 or 3.0 assays. HCV RNA was measured by polymerase chain reaction using COBAS Amplicor Monitor 2.0 assay (Roche Diagnostics, Branchburg, New Jersey, USA) [19].

Demographic, lifestyle, and clinical variables

Structured interviews every 6 months included questions on sociodemographics, medical and health history, obstetric, gynecologic, and contraceptive history, tobacco, alcohol, and drug use, sexual behavior, healthcare access utilization, and psychosocial measures [15]. Using the 1993 Centers for Disease Control classification system, the self-reported occurrence of an AIDS-defining clinical condition (ADC) in the previous 6 months was also recorded. This event was confirmed by review of medical records and matching to AIDS registries. HCV status was determined at baseline and categorized as: antibody negative (HCV^{-}), antibody positive nonviremic ($HCV^{+}RNA^{-}$), or viremic, which was further categorized using the median HCV RNA cutoff as HCV RNA less than 2 400 000 IU/ml. Self-reported ethnicity/race was: white, African–American, Hispanic, or other. Age was categorized based on tertiles of distribution (<35, 35–40, 41 years). We followed our prior publication [9] for categorizing the following variables: IDU (yes, no), smoking (never, former, current), alcohol consumption (0, 1–3, 4–10, 11 drinks/week), HIV RNA (4000; 4001–20 000; 20 001–55 000; 55 001–100 000; >100 000 copies/ml), CD4 cell count (200, 201–350, 351–500, >500 cells/μl), and antiretroviral therapy (ART) (none, mono, combination, HAART).

Statistical methods

Pearson correlations were used to evaluate the correlation among the eight immunologic variables. Principal components analysis on these variables was performed to determine the number of clusters. Modeling the percentages of CD4 and CD8 cells that were DR± and CD38± as continuous variables, a four-factor solution accounted for at least 90% of the variance in these immune activation markers. To evaluate the sensitivity of our results to the initial number of clusters, we performed the cluster and survival analyses with one less and one more cluster than was determined by the principal components method. The four

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clusters identified by the analysis were then numbered 1 to 4 based on their immunologic (CD4 cell count) and virologic (HIV viral load) status and the associated AIDS risk (1 = lowest; 4 = highest risk group) for convenience of interpretation of the prediction model results.

To determine the clusters, we used a nonhierarchical k-means clustering algorithm that allows women to move from one cluster to another. The centroids from average linkage clustering were used as the seeds for clustering. The algorithm used each woman's first visit with immune activation data, which ranged from the first to 14th study visit. For 54% of women, these data were available for the first visit.

The clusters were compared on baseline demographic factors, substance use behavior and clinical factors using analysis of variance (ANOVA) or χ^2 tests. The mean value of each immune activation variable was compared among the clusters using ANOVA.

Incident AIDS was defined as the first self-reported ADC. Time to AIDS was defined as the time from the visit with immune marker assessment to the midpoint between the last AIDS-free and first ADC visits. Women not reporting an ADC were censored at their last follow-up visit and Cox regression analytic approaches were used to test differences in AIDS risk among the clusters. In Cox regression models, cluster 1 was the reference group as women in this cluster had the best clinical profile with the highest CD4 cell count and lower HIV viral load. Variables, other than the immunologic factors, differing across clusters were included as covariates: HCV status (baseline), ART (time-dependent), and HIV viral load (time-dependent). Age and ethnicity/race (both at baseline), and IDU, current smoking, and alcohol drinking (all time-dependent) were included as covariates based on their known association with AIDS progression. As CD4 cell count correlated with most of the immune activation markers (correlation coefficients, 0.2–0.5), multivariate models with and without CD4 cell count were evaluated.

Results

Baseline demographic and clinical characteristics: the median (range) age at baseline (time of immune activation assessment) was 38 years (18–68); 57% of the women were African–American, 25% were Hispanic, and 17% were white; 14% reported IDU; 59% were current smokers; half reported no alcohol use (Table 1).

Immune activation profile of the clusters: complete activation profiles including all eight immunologic variables for the four clusters are displayed in Table 2. Compared with the other clusters, women in cluster 1 (*n* = 117) had the highest percentages of $CD8^+CD38^-DR^-$ and $CD4^+CD38^-DR^-$ T cells, whereas women in cluster 4 (*n* = 60) had the highest percentages $CD4^+CD38^+DR^+$ and relatively higher percentages of $CD8^+CD38^+DR^+$ T cells. Cluster 2 (*n* = 165) included women with the highest percentages of $CD8^+CD38^+DR^-$ and $CD4^+CD38^+DR^-$ T cells. Women in cluster 3 (*n* = 222) had the highest percentage of $CD8^+CD38^+DR^+$ and relatively higher percentage of $CD4^+CD38^+DR^-$ T cells. Women in cluster 1 had the highest percentages of $CD8^+CD38^-DR^+$, whereas women in cluster 4 had the highest percentages $CD4^+CD38^-DR^+$ T cells.

Correlation among the eight components of T-cell activation: the proportion of $CD4^+CD38^-DR^-$ T cells was highly positively correlated with proportions of $CD8^+CD38^-DR^-$ ($r = 0.62$) and $CD8^+CD38^-DR^+$ + T cells ($r = 0.41$), which is the outstanding feature of cluster 1 (Tables 2 and 3). The proportion of $CD4^+CD38^+DR^+$ T cells was highly positively correlated with $CD8^+CD38^+DR^+$ T cells ($r = 0.49$), which is the outstanding feature of cluster 4. A high positive correlation was also observed between

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$CD8^{+}CD38^{+}DR^{-}$ and $CD4^{+}CD38^{+}DR^{-}$ T cells (r = 0.55), which is consistent with the cluster 2 profile.

Baseline demographic and clinical factors by cluster: age, ethnicity, IDU, smoking, and alcohol use did not differ across the clusters (Table 1), but CD4 cell count and HIV RNA level did differ significantly. Compared with cluster 1, women in clusters 3 and 4 had significantly lower median CD4 cell counts and higher HIV RNA levels. In addition, compared with cluster 1, women in cluster 3 and 4 were less likely to be ART naive, and more likely to have very high HCV RNA (2.4 million IU/ml) if coinfected with HCV. Women in clusters 1 and 2 were similar with respect to CD4 cell count, HIV RNA level, and ART.

Relation of immune activation clusters to AIDS development: among the 564 women, 228 developed AIDS over a median of 6.1 years (range 0.2–12.3 years) after activation marker assessment. Figure 1 shows the cumulative AIDS-free survival of the four clusters. Clusters 1 and 2 had relatively lower and nearly identical risk of AIDS. Cluster 3 had an intermediate risk, whereas cluster 4 had the highest risk (Log rank $P < 0.0001$).

Three multivariate proportional hazard models were tested to determine whether clusters based on immune activation profile were significant predictors of AIDS risk (Table 4). The first model included age, race, smoking, alcohol use, IDU, ART, and HCV status as covariates. The second included CD4 cell count in addition to model 1 covariates. The third included HIV RNA in addition to model 1 covariates. In the first model, the adjusted risk of AIDS was significantly higher in clusters 3 and 4 than cluster 1 [hazard ratio (HR) (95% CI) = 1.58 (1.05–2.37), 2.70 (1.65–4.40), respectively]. When CD4 cell count was added, the relative risk for AIDS was attenuated but remained significantly elevated for cluster 4 [HR (95% CI) = 1.76 (1.05–2.96)]. When adjusted for HIV RNA, the relative risk of AIDS also remained significantly elevated only for women in cluster 4 [HR (95% CI) = 2.13 (1.30–3.50)]. When both CD4 cell count and HIV RNA were included in the model, the relative risk of AIDS remained elevated for women in cluster 4 with a borderline significance [HR (95% CI) = 1.68 (0.99–2.85)]. An additional multivariate model including CD4/CD8 ratio also showed a significant increased risk of AIDS progression associated with cluster 4 (HR = 1.73; 95% CI = 1.05–2.91). As cluster 4 included relatively less white women (8%) compared with the other clusters (range: 14– 22%), we analyzed models excluding the white women to rule out any possible bias. The results were almost identical to the ones reported in Table 4 adjusted for the confounders and HIV RNA levels.

The sensitivity analyses for all models using 3 or 5 cluster solutions revealed similar results.

Discussion

Our results show that immune activation patterns among HIV-infected women, assessed by CD38 and HLA-DR expression, correlate well between CD8 and CD4 T cells. Using cluster analysis, four groups of women were identified, who had distinct immune activation profiles. We show that women with high percentages of $CD8^{+}CD38^{+}DR^{+}$ and $CD4^{+}CD38^{+}DR^{+}$ T cells are at significant risk of AIDS compared with women with high levels of $CD8^{+}CD38^{-}DR^{-}$ and $CD4^{+}CD38^{-}DR^{-}$ cells. Women in cluster 4 demonstrated a state of hyper-activation of both CD4 and CD8 T cells and also had lower CD4 cell counts compared with the other clusters. This is the first study to identify an extended immune activation profile incorporating activation patterns of both CD4 and CD8 T cells and to examine the value of such profiles in predicting the risk of AIDS progression, shedding insight to the immunopathogenesis of HIV disease.

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Binding of HIV to CCR5 or CXCR4 coreceptors on CD4 T cells induces *env*-mediated signals and activates CD4 cells by inducing expression of immune markers such as CD25, CD38, CD57, CD69, CD70, and HLA-DR. Activated CD4 cells release soluble factors including cytokines which in turn activate CD8 cells [20]. HIV-induced T-cell activation is essential for viral replication, and activated T cells that are not productively infected eventually die [21]. A decline in CD4 cell count, which is the most important phenotypic characteristic of HIV disease progression, is due to a combination of factors including apoptosis of infected CD4 cells, bystander activation of uninfected T cells by HIV virions in tissue reservoirs, and cytokines that may cause global activation of the immune system [4,20,22]. Activation of T cells is followed by expansion of the T-cell pool and death of a large number of T cells through apoptosis. Notably, the dynamics of activation, expansion, and apoptosis differ between CD8 and CD4 cells [23–25]. The expanded CD8 cell pool survives longer upon activation and forms a stable resting pool of memory cells, whereas activated CD4 cells die fairly quickly [25–27]. This difference could explain the differential predictive value of CD4 and CD8 activation for HIV disease progression. CD8 activation has been associated with HIV seroconversion [28] as well as disease severity [29]. CD8 activation predicted CD4 decline [14] and progression to AIDS in several reports [1,3,6,9,30]. CD8 activation is also a marker of residual viral replication in treated HIV-infected patients [13] and has prognostic value even in patients with undetectable plasma viral load [31]. Activation of CD8 cells occurs at a lower viral threshold because of their cytotoxic potential, but hyperactivation leads to CD8 cell exhaustion and anergy.

In contrast, only a few studies documented the role of CD4 activation in HIV disease and progression to AIDS. Giorgi *et al*. [3] reported that CD4 activation was associated with shorter survival among advanced HIV-infected patients but did not correlate with HIV viral load. Another report showed that preconversion levels of elevated CD4 activation were associated with increased risk of AIDS after seroconversion [6]. We evaluated the predictive value of both CD8 and CD4 activation status for progression to AIDS. Our findings suggest global activation of T cells may explain the more general immunologic dysregulation noted with HIV infection leading to high T-cell turnover, immune senescence, anergy, and finally cell death [32–35].

Most studies have assessed immune activation by measuring percentages of T cells expressing CD38, the most well characterized of the activation markers identified. However, the prognostic value of both CD8 and CD4 activation increased when immune activation is assessed by coexpression of CD38 with HLA-DR [13] or CD45R0 [14]. T cells dually activated with CD38 and HLA-DR significantly predicted progression to AIDS among HIV-infected men, and in the same cohort of women in the WIHS as the current study [9]. We took the analysis one step further in the current study to identify AIDS risk based on simultaneous activation status of both CD4 and CD8 T cells. We hypothesized that patterns of CD4 activation are associated with those of CD8 activation. Despite the difference in activation, expansion, and apoptosis dynamics between CD8 and CD4 T cells, the percentages of nonactivated ($CD38^-$ and DR^-), partially activated ($CD38^+$ or DR^+), or dually activated ($CD38^+$and DR^+) CD8 T cells correlated strongly with corresponding activation patterns of CD4 cells (correlation coefficients, 0.41–0.62; $P < 0.0001$), most likely due to global activation of the immune system by expanded cytokine production [36].

Proportions of the eight immune activation components within the profiles of each cluster reflected the highly correlated components of CD8 and CD4 activation. Although the proportions of nonactivated and dually activated T cells are expected to be strongly inversely correlated, as we found in clusters 1 and 4, the presence of relatively higher proportions of CD8 cells expressing HLA-DR only in cluster 1 and CD4 cells expressing HLA-DR only in cluster 4 is intriguing. Multiple reports have identified the presence of

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HLA-DR on CD8 cells without CD38 expression as a marker of long-term nonprogression, stable CD4 cell count, and survival among HIV-infected patients [37–41]. We reported this in the setting of HIV/HCV co-infection [9]. In this report, we show that the combination of high proportions of nonactivated CD8 and CD4 T cells and HLA-DR-expressing CD8 cells confers the best clinical conditions and lowest AIDS risk in HIV-infected women. This suggests that CD8 T cells that express HLA-DR only may have enhanced cytotoxic capacity and ability to expand rapidly to control HIV infection and destroy HIV-infected cells in tissue reservoirs. Conversely, CD4 T cells expressing HLA-DR only were present in association with high proportions of dually activated T cells among women who had a detrimental clinical profile and significant AIDS risk. The underlying mechanism for the differential implications of HLA-DR expression on CD8 vs. CD4 T cells demands further attention.

The significance of CD38 upregulation on T cells in HIV disease is not well understood. Some proportion of $CD38^+DR^-$ cells can essentially indicate naive T cells, that are not activated [12]. It is possible that cluster 2 is describing a group of individuals with a high proportion of such cells, which partially explains the apparent good prognosis of this group of women. In our analysis, women in cluster 3 also had high levels of dually activated CD8 T cells, yet their AIDS risk was not significantly elevated after adjustment for CD4 cell count or HIV RNA level. A possible explanation is inadequate elevation of other immune activation components compared with cluster 4 such as $CD4^+CD38^+DR^+$ (18 vs. 36%) and $CD4^+CD38^-DR^+$ (8 vs. 19%). The increased AIDS risk was partially explained by CD4 cell count or HIV viral load. Of note, women in cluster 3 had high HIV viral load, low CD4 cell count, and high prevalence of HCV coinfection. In contrast to cluster 3, the increased risk of AIDS in cluster 4 women was independent of HIV viral load and CD4 cell count, indicating some underlying mechanisms, perhaps global activation of the immune system that were not explained by these factors. More research is warranted to explore the mechanisms of accelerated progression to AIDS among women with aggressive immune activation defined as expression of CD38 and HLA-DR on CD4 and CD8 T cells. Further, an increased understanding of the mechanism of T-cell activation, including the sequence of upregulation of markers on T cells or subsets of T cells that may be predominant during different phases of HIV infection, would be important.

Our results have important clinical implications. Although CD4 cell count is the most widely used predictor of AIDS in clinical practice, a growing body of evidence suggests a significant predictive value of immune activation markers. We have shown that, at a given time, expression of CD38 and HLA-DR on CD4 and CD8 T cells results in distinct activation profiles, which provided insight into HIV pathogenesis. Our results indicate that concurrent hyperactivation of both CD4 and CD8 T cells is associated with AIDS progression. It is important to note that almost all (93%) of our study population was HAART naive when activation markers were measured. Levels of T-cell activation decrease after treatment with HAART but usually do not completely reverse to the levels of HIV-uninfected persons, whereas immune activation remains abnormally elevated in some individuals despite HAARTuse [42–45]. Therefore, correlations between the activation markers need to be evaluated among HAART-experienced patients, and the predictive value of post-HAART T-cell activation profiles for AIDS risk needs to be investigated in future studies. Such studies will be more useful given most HIV-infected patients initiate HAART at an early stage of the disease. It will also be clinically useful to evaluate the predictive value of similar immunologic profiles before and after initiating HAART on immune recovery, cytokines, markers of inflammation, and HIV-associated non-AIDS outcomes.

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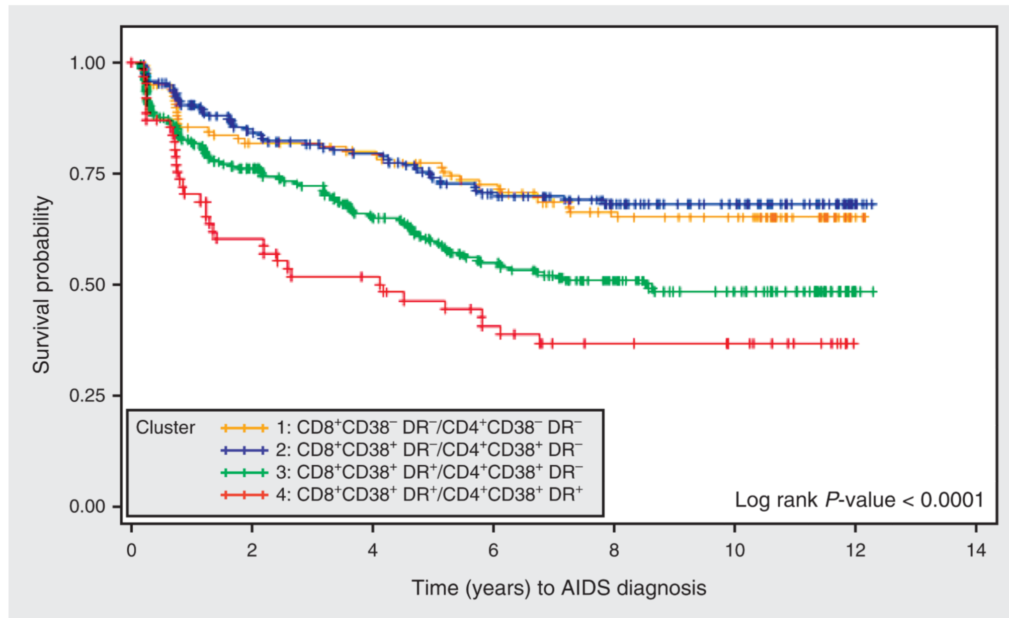

Fig. 1.

Cumulative probability of remaining AIDS-free by the immune activation marker clusters.

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Table 1

Baseline demographic and clinical characteristics across the four clusters of 564 HIV-infected women.

	Cluster 1 (n = 117)	Cluster 2 (n = 165)	Cluster 3 (n = 222)	Cluster 4 (n = 60)	P
Age, years	38 (18–57)	38 (21–68)	38 (24–60)	39 (29–67)	0.69
Ethnicity					0.42
White	17 (14%)	36 (22%)	36 (16%)	5 (8%)	
African–American	63 (54%)	92 (56%)	129 (58%)	39 (65%)	
Hispanic	36 (31%)	36 (22%)	55 (25%)	15 (25%)	
Other	1 (1%)	2 (1%)	2 (1%)	1 (2%)	
IDU (current)					0.56
Yes	15 (13%)	18 (11%)	35 (16%)	8 (16%)	
No	102 (87%)	147 (89%)	185 (84%)	52 (86%)	
Missing	0	0	2	1	
Smoking status					0.38
Never smoker	27 (23%)	43 (26%)	48 (22%)	14 (27%)	
Former smoker	20 (17%)	36 (22%)	38 (17%)	6 (10%)	
Current smoker	70 (60%)	85 (52%)	134 (61%)	39 (66%)	
Missing	0	1	2	1	
Alcohol use (current)					0.37
0 drinks/week	48 (41%)	91 (56%)	101 (47%)	33 (56%)	
1–3 drinks/week	33 (28%)	43 (26%)	63 (29%)	13 (22%)	
4–10 drinks/week	12 (10%)	12 (7%)	17 (8%)	5 (8%)	
11^{+} drinks/week	23 (20%)	17 (10%)	34 (16%)	8 (14%)	
Missing	1	2	7	1	
CD4 cell count (cells/μl)	529 (6–1972)	471 (9–1331)	316 (2–1096)	206 (5–1322)	<0.0001
Pairwise comparison with cluster 1	ref	P = .06	P < 0.0001	P < 0.0001	
HIV RNA (copies/ml)	4000 (80–1 400 000)	5600 (80–1 300 000)	20 000 (80–1 700 000)	14 500 (80–670 000)	<0.0001
Pairwise comparison with cluster 1	ref	P = 0.15	P < 0.0001	P = 0.003	
Antiretroviral therapy					0.003
None	55 (47%)	69 (42%)	77 (35%)	15 (25%)	
Mono	20 (17%)	23 (14%)	66 (30%)	15 (25%)	
Combo	38 (33%)	61 (36%)	62 (28%)	25 (42%)	
HAART	4 (3%)	13 (8%)	16 (7%)	5 (8%)	
Missing	0	0	1	0	
Pairwise comparison with cluster 1	ref	P = 0.38	P = 0.02	P = 0.03	
HCV status at WIHS baseline visit					<0.0001
HCV^{-}	51 (45%)	69 (42%)	71 (33%)	27 (46%)	
HCV^{+}, Nonviremic	26 (23%)	11 (7%)	20 (9%)	6 (10%)	
HCV^{+}, RNA<2 400 000 IU/ml	27 (24%)	49 (30%)	52 (24%)	12 (20%)	
HCV^{+}, RNA 2 400 000 IU/ml	9 (8%)	35 (21%)	74 (34%)	15 (25%)	

	Cluster 1 (n = 117)	Cluster 2 (n = 165)	Cluster 3 (n = 222)	Cluster 4 (n = 60)	*P*
Missing	4	1	5	0	
Pairwise comparison with cluster 1	ref	$P < 0.0001$	$P < 0.0001$	0.007	

Median (range) reported for continuous variables, *P*-values from Kruskal-Wallis test; *n* (%) reported for categorical variables, *P*-values from χ^2 test.

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Table 2

Mean (SD) percentages of immune activation markers by the four clusters of 564 HIV-infected women.

Activation markers on T-cell subtypes	Cluster 1 (*n* = 117)	Cluster 2 (*n* = 165)	Cluster 3 (*n* = 222)	Cluster 4 (*n* = 60)
$CD8^{+}CD38^{-}DR^{-}$	40.8 (18.9)	23.9 (11.4)	13.2 (7.3)	15.0 (11.7)
$CD8^{+}CD38^{+}DR^{-}$	12.7 (10.8)	44.0 (11.7)	29.2 (9.7)	19.9 (11.8)
$CD8^{+}CD38^{-}DR^{+}$	27.8 (18.6)	5.9 (4.0)	8.4 (6.1)	16.9 (15.1)
$CD8^{+}CD38^{+}DR^{+}$	18.8 (12.5)	26.1 (9.5)	49.1 (11.5)	48.8 (15.7)
$CD4^{+}CD38^{-}DR^{-}$	53.3 (11.1)	28.4 (12.2)	26.1 (11.4)	22.4 (11.4)
$CD4^{+}CD38^{+}DR^{-}$	29.4 (9.2)	58.4 (13.2)	48.2 (12.2)	22.9 (8.4)
$CD4^{+}CD38^{-}DR^{+}$	9.5 (5.0)	4.4 (2.4)	7.7 (3.4)	18.7 (7.4)
$CD4^{+}CD38^{+}DR^{+}$	7.8 (4.9)	8.8 (5.3)	18.0 (8.6)	35.9 (16.6)

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Table 3

Correlation between activation markers on CD8 and CD4 T cells.

Activation markers subtypes	$CD4^+CD38^-DR^-$	$CD4^+CD38^+DR^-$	$CD4^+CD38^-DR^+$	$CD4^+CD38^+DR^+$
$CD8^+CD38^-DR^-$	0.62 (<0.0001)[a]	–0.27 (<0.0001)	–0.08 (0.05)	–0.39 (<0.0001)
$CD8^+CD38^+DR^-$	–0.43 (<0.0001)	0.55 (<0.0001)	–0.44 (<0.0001)	–0.005 (0.90)
$CD8^+CD38^-DR^+$	0.41 (<0.0001)	–0.41 (<0.0001)	0.41 (<0.0001)	–0.16 (0.0001)
$CD8^+CD38^+DR^+$	–0.50 (<0.0001)	0.07 (0.10)	0.15 (0.004)	0.49 (<0.0001)

[a]correlation coefficient r (*P*-value).

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Table 4

Risk of progression to AIDS for immune activation marker clusters.

	Univariate model			Multivariate models								
				No CD4 cell count/HIV RNA			Model with CD4 cell count			Model with HIV RNA		
Variables in the model	**HR[1]**	**95% CI**	***P***	**HR[1]**	**95% CI**	***P***	**HR[1]**	**95% CI**	***P***	**HR[1]**	**95% CI**	***P***
Clusters												
[a]Cluster 1 (CD8$^+$CD38$^-$DR$^-$, CD4$^+$CD38$^-$DR$^-$)	1.00			1.00			1.00			1.00		
Cluster 2 (CD8$^+$CD38$^+$DR$^-$, CD4$^+$CD38$^+$DR$^-$)	0.98	0.64–1.52	0.93	1.03	0.66–1.62	0.88	1.03	0.65–1.61	0.91	0.95	0.61–1.50	0.83
Cluster 3 (CD8$^+$CD38$^+$DR$^+$,CD4$^+$CD38$^+$DR$^-$)	1.71	1.16–2.51	0.006	1.58	1.05–2.37	0.03	1.18	0.77–1.81	0.46	1.70	0.77–1.78	0.47
Cluster 4 (CD8$^+$CD38$^+$DR$^+$, CD4$^+$CD38$^+$DR$^+$)	2.46	1.53–3.94	0.0002	2.70	1.65–4.40	<0.0001	1.76	1.05–2.96	0.03	2.13	1.30–3.50	0.003
HCV Status at WIHS Baseline Visit												
HCV Ab$^-$				1.00			1.00			1.00		
HCV Ab$^+$ RNA$^-$				0.88	0.52–1.48	0.62	0.93	0.55–1.58	0.80	1.02	0.61–1.74	0.92
RNA <2 400 000 IU/ml				1.01	0.67–1.54	0.96	0.96	0.63–1.46	0.85	1.07	0.71–1.63	0.74
RNA 2 400 000 IU/ml				1.38	0.91–2.10	0.13	1.34	0.88–2.04	0.18	1.32	0.87–2.01	0.20
IDU												
No				1.00			1.00			1.00		
Yes				1.62	1.06–2.48	0.03	1.80	1.17–2.76	0.007	1.59	1.03–2.45	0.04
Antiretroviral therapy												
None				1.00			1.00			1.00		
Mono				1.15	0.73–1.78	0.55	1.03	0.65–1.64	0.89	1.13	0.72–1.78	0.60
Combo				0.80	0.54–1.17	0.47	0.77	0.51–1.16	0.21	0.99	0.66–1.50	0.97
HAART				0.80	0.54–1.17	0.24	0.79	0.54–1.15	0.21	1.18	0.76–1.65	0.58
CD4 cell count (cells/μl)												
>500							1.00					
351–500							1.03	0.64–1.56	0.99			
201–350							1.31	0.86–2.00	0.21			
0–200							3.45	2.34–5.09	<0.0001			
HIV RNA (copies/ml)												
0–4000										1.00		
4001–20 000										3.00	1.55–3.41	<0.0001

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Variables in the model	Univariate model			Multivariate models								
				No CD4 cell count/HIV RNA			Model with CD4 cell count			Model with HIV RNA		
	HR[1]	95% CI	*P*	HR[1]	95% CI	*P*	HR[1]	95% CI	*P*	HR[1]	95% CI	*P*
20 001–55 000										2.00	1.24–3.24	0.005
55 001–100 000										3.06	1.84–5.07	<0.0001
>100 000										5.34	3.55–8.05	<0.0001

All models adjusted for age, race, smoking and alcohol use. All covariates except for age, race, and HCV status were time dependent.

[a]Cluster 1 was considered baseline as women in this cluster had the highest CD4 cell counts and lowest HIV RNA levels.