



"Non-HIV" Biomarkers Independently Predict Mortality and Are Associated with HIV Markers

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ABSTRACT

Background: We asked whether non-HIV biomarkers of anemia, liver injury, renal injury, and chronic viral hepatitis improve differentiation of mortality risk and whether these biomarkers are associated with HIV markers.

Methods: We identified veterans with HIV infection initiating combination antiretroviral treatment (CART) within the Veterans Health Administration (VA) between 1/1/97 and 8/1/02. Association between HIV and non-HIV biomarkers was tested using nonparametric tests. After splitting the data into development and validation sets, nested multivariable Poisson models were fitted to HIV markers (CD4 cell count, HIV-RNA, AIDS defining conditions) and to non-HIV biomarkers (hemoglobin, transaminases, platelets, creatinine, and Hepatitis B and C serology). All models were also adjusted for age and substance abuse or dependence. C statistics and quintiles of risk were compared. Sensitivity analyses employed inverse weighting for the propensity to be missing and included adjustment for year of CART initiation.

Results: Of 13,586 veterans initiating CART during this interval, 9789 (72%) had complete data and 2566 of these died. Subjects were predominantly Black (51%), male (98%), with a median age of 45 years. HIV biomarkers were associated with non-HIV biomarkers (p<0.0001). For example, the correlation with CD4 cell count was 0.39 for hemoglobin and -0.21 for an index of liver injury (FIB 4). In development and validation sets HIV biomarkers (C statistics in both: 0.69) and non-HIV biomarkers (C statistics: 0.72, 0.71) discriminated mortality. When models were combined, discrimination improved (C statistic in both 0.74, p<0.0001) resulting in better differentiation of risk. For example, among those at highest risk (5th quintile) mortality rates increased from 13.9, 95% CI 12.7-15.1 using the HIV biomarkers only to 17.1, 95% CI 15.6-18.7 deaths/100 PY when non-HIV markers were also included. Findings were robust after adjusting missing data and year of CART initiation.

Conclusions: Non-HIV biomarkers improve differentiation of mortality risk achieved by HIV markers, are strongly associated with HIV biomarkers, and therefore likely reflect HIV pathology. Combined with those of the SMART study, our findings underscore the overlapping nature of pathologic injury in HIV infection. After further validation, a combined index of HIV and non-HIV biomarkers may prove a superior management tool and surrogate endpoint for clinical trials.

INTRODUCTION

In a review of HIV cohort and SMART data Phillips and colleagues [1] found that: 1) Morbidity and mortality among those on CART was dominated by non-AIDS rather than AIDS events. 2) There was a strong positive association between non-AIDS death rates and both low CD4 counts and high HIV RNA. 3) The association with immunodeficiency was consistent across types of non-AIDS events including liver disease, renal disease, and non-AIDS malignancy.

The authors described a range of mechanisms by which HIV might raise the risk of non-AIDS events including complications of chronic immune activation and increased T cell turnover and concluded that, "We need to adapt our research priorities to better understand the full role of HIV in causing a wide range of clinical diseases. Clinicians caring for patients with HIV need to...become aware of the best means to try to prevent and to monitor for early signs of these [non-AIDS] outcomes..."

These goals would be facilitated by a prognostic index that combined HIV and HIV associated, "non-HIV", biomarkers. Excellent indices have been accomplished in HIV infection [2-3], but these have omitted biomarkers of anemia, liver disease, and renal disease despite established associations with immunodeficiency and survival. We develop and initially validate an index that combines HIV and "non-HIV" biomarkers.

METHODS

The Veterans Aging Cohort Study Virtual Cohort has been described in detail [4-5]. In brief, the Virtual Cohort consists of over 33,000 veterans with HIV infection treated within the national Veterans Affairs Healthcare System beginning in 1997 to the present. This sample identifies veterans at the point of initiating care for HIV infection and follows them using databases derived from the VA National Electronic Medical Record System. To ensure adequate follow up time, we identified subjects who initiated their first course of CART in the VA between Jan 1, 1997 through August 1, 2002. We used pharmacy data to identify individuals initiating a minimum of three antiretroviral medications and laboratory data to determine that they had received a minimal evaluation (CD4 count, HIV-RNA, and hemoglobin), within six months of initiating CART.

Available data included demographic factors (age, race/ethnicity, gender), administrative diagnostic codes (ICD-C Codes), routinely collected clinical laboratory data, pharmacy data, and long term mortality. All laboratory data were collected from the clinical sites through the Immunology Case Registry. Pharmacy data is drawn from the national VA Pharmacy Benefits Management Package. ICD-9 codes were used to determine diagnoses of drug abuse or dependence, alcohol abuse or dependence, and of AIDS-defining illnesses. Hepatitis C was defined as a positive antibody, qualitative or quantitative HIV RNA, or ICD-9 codes. Hepatitis B was defined as a positive surface antigen or ICD-9 codes.

ANALYSIS

Those who initiated after December 31, 1998 were in the development set and those initiating on or before this date were reserved for validation. We standardized the maximal observation interval for both samples to six years. We chose a nonrandom split based on calendar time to determine the temporal generalizability of our findings [6]. We present Poisson analyses as these results are the most directly interpretable. Proportions were compared using chi square test. Medians were compared using the rank-sum test. Discrimination was compared using C Statistics.

ART-CC is a carefully validated prognostic model based upon data from cohorts in Europe and North America [2] and includes: CD4 count (<50, 50-99, 100-199, 200-349, and 350 or more per mm³); HIV-1 RNA of five log or more; and presence of AIDS defining illness.

For "non-HIV" biomarkers we identified clinically available markers, employing previously validated specifications consistent with major organ system injury:

Liver injury FIB 4 [7]: [(years of age * AST)/(platelets in 10⁹/L * square root of ALT)]² Two thresholds of FIB 4 are recommended: > 3.25 consistent with high risk and <1.45 consistent with low risk for fibrosis/cirrhosis.

Renal injury, MDRD [8]: (eGFR: (186.3 * ((serum creatinine)-1.154) * (age-0.203) * (0.742 for women) * (1.21 if African American)))

Two thresholds for anemia: (hemoglobin 10-12 and <10 g/dL).

Hepatitis B (HBV) or C (HCV), 51% of those with chronic HBV also had HCV

The ART-CC model also adjusts for two demographic factors: Age of 50 or more years. Because our sample is older we adjusted both models for age 50-64 and 65 years and over. We also adjusted for history of intravenous drug use. We used ICD-9 diagnostic codes for abuse and dependence. We fit the same models in validation data and estimated C statistics. We then combined datasets.

RESULTS

Of 13,586 HIV infected veterans initiating CART between 1/1/1997 and 8/1/2002 with laboratory data, 9789 (72%) had complete data (analytic sample).

Table 1. Development and Validation Samples

	Full Cohort N = 9,789	Development N = 4,813	Validation N = 4,971
Age (years)			
<50 years (%)	6880 (70.3)	3121 (64.8)	3759 (75.6)
50-64 years (%)	2536 (25.9)	1487 (30.9)	1049 (21.1)
65+ years (%)	373 (3.8)	206 (4.3)	167 (3.4)
Male (%)	9578 (97.8)	4710 (97.9)	4863 (97.8)
Race/Ethnicity (%)			
White	4983 (50.9)	2388 (49.6)	2595 (52.2)
Black	3158 (32.3)	1476 (30.7)	1682 (33.8)
Hispanic/Other	1648 (16.8)	950 (19.7)	698 (14.0)
CD4 Cell Count (cells/mm³)			
<50 cells/mm ³ (%)	1225 (12.5)	806 (16.7)	419 (8.4)
50-99 cells/mm ³ (%)	723 (7.5)	440 (9.1)	293 (5.9)
100-199 cells/mm ³ (%)	1608 (16.4)	806 (16.7)	800 (16.1)
200-349 cells/mm ³ (%)	2354 (24.0)	1132 (23.5)	1222 (24.5)
>350 cells/mm ³ (%)	3871 (39.5)	1630 (33.9)	2241 (45.0)
HIV-1 RNA			
> 5 Log/ml (%)	1795 (18.3)	1219 (25.3)	576 (11.6)
Substance Addiction or Abuse (%)			
Drugs	2458 (25.1)	1188 (24.7)	1270 (25.5)
Alcohol	2258 (23.1)	1138 (23.6)	1120 (22.5)
Either one	3055 (31.2)	1512 (31.4)	1543 (31.0)
Hemoglobin			
10-12 g/dL (%)	1540 (15.7)	939 (19.5)	601 (12.1)
<10 g/dL (%)	558 (5.7)	400 (8.3)	158 (3.2)
Hepatic Measures			
Hepatitis B or C (%)	4676 (47.8)	2209 (45.9)	2467 (49.6)
FIB 4 >3.25 (%)	1187 (12.1)	606 (12.6)	581 (11.7)
FIB 4 < 1.45 (%)	5420 (55.4)	2625 (54.5)	2795 (56.2)
Renal Measures			
eGFR<30 (mL/min, %)	196 (2.0)	101 (2.1)	95 (1.9)
AIDS Diagnoses (%)			
PJP	527 (5.4)	311 (6.5)	216 (4.3)
MAITB	318 (3.2)	133 (2.8)	105 (2.1)
Bacterial Pneumonia	981 (10.0)	503 (10.4)	478 (9.6)

Table 2. Adjusted Poisson Models: HIV, "Non HIV", and Combined Biomarkers

DEVELOPMENT SET Initiated CART 1999-2002 n=4813	HIV Biomarkers (C stat=0.69)		"Non-HIV" Biomarkers (C stat=0.72)		Combined (C stat=0.74)	
	IRR	95% CI	IRR	95% CI	IRR	95% CI
log HIV RNA>5 copies/mm ³	1.28	1.12 1.45			1.16	1.02 1.33
CD4 50-99 cells/mm ³	0.79	0.65 0.96			0.76	0.62 0.93
CD4 100-199 cells/mm ³	0.70	0.59 0.84			0.71	0.59 0.85
CD4 200-349 cells/mm ³	0.57	0.48 0.68			0.64	0.53 0.77
CD4 350+ cells/mm ³	0.45	0.37 0.54			0.57	0.46 0.69
AIDS Defining Diagnosis	1.55	1.37 1.76			1.44	1.26 1.64
Hemoglobin<10 g/dL			2.34	1.76 2.77	1.70	1.41 2.05
Hemoglobin 10-12 g/dL			2.02	1.78 2.3	1.58	1.37 1.83
FIB 4 3.25+			1.67	1.44 1.93	1.66	1.42 1.93
FIB 4 <1.45			0.71	0.62 0.81	0.72	0.63 0.83
eGFR<30 mL/min			1.88	1.44 2.47	2.13	1.61 2.82
Viral Hepatitis			1.31	1.16 1.48	1.38	1.22 1.57
VALIDATION SET Initiated CART 1997-98 n=4971	HIV Biomarkers (C stat=0.69)		"Non-HIV" Biomarkers (C stat=0.71)		Combined (C stat=0.74)	
log HIV RNA>5 copies/mm ³	1.56	1.35 1.82			1.54	1.33 1.79
CD4 50-99 cells/mm ³	0.63	0.51 0.78			0.64	0.52 0.80
CD4 100-199 cells/mm ³	0.42	0.35 0.50			0.52	0.43 0.63
CD4 200-349 cells/mm ³	0.38	0.32 0.46			0.51	0.43 0.62
CD4 350+ cells/mm ³	0.24	0.20 0.28			0.36	0.30 0.44
AIDS Defining Diagnosis	1.47	1.30 1.66			1.38	1.22 1.56
Hemoglobin<10 g/dL			3.98	3.24 4.90	2.65	2.14 3.28
Hemoglobin 10-12 g/dL			2.13	1.86 2.44	1.66	1.44 1.90
FIB 4 3.25+			2.06	1.79 2.36	1.93	1.68 2.22
FIB 4 <1.45			0.56	0.49 0.63	0.65	0.57 0.75
eGFR<30 mL/min			1.85	1.42 2.43	1.98	1.50 2.58
Viral Hepatitis			1.19	1.05 1.34	1.25	1.11 1.42

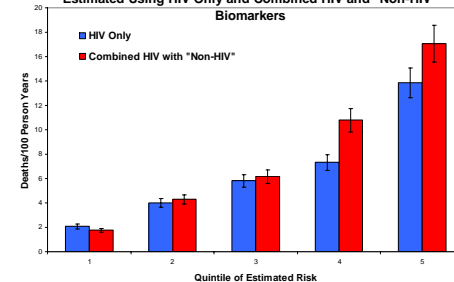
P for addition of Non-HIV Biomarkers to HIV Biomarkers<0.00001
 * All models also include two age variables (age 50-64 years and age 65 years and above) and a combined variable for Alcohol or Drug Abuse and Dependence. See methods section for rationale.
 FIB 4: [(years of age * AST)/(platelets in 10⁹/L * square root of ALT)]²
 eGFR: (186.3 * (serum creatinine)-1.154) * (age-0.203) * (0.742 for women) * (1.21 if African American))

Table 3. Biomarkers Characterized by Risk Quintile For Each Model*

	N	Deaths	95% CI	Age 65+ yrs.	Subst. Abuse/Dep.	HIV RNA (log)	HIV RNA (log)	HGB Cond.	HGB (g/dL)	FIB 4 >3.25	eGFR <30mL/min	Viral Hepatitis
HIV and "Non-HIV" Combined Quintiles												
1	4383	512	1.8 1.6 1.9	0.5%	20.1%	419	2.9	5.9%	14.5	0.0%	0.0%	29.0%
2	2009	514	4.3 3.9 4.7	3.2%	38.3%	261	3.7	21.5%	13.8	0.7%	0.3%	58.0%
3	1526	514	6.2 5.6 6.7	6.2%	35.2%	172	4.2	36.1%	12.9	18.2%	2.0%	61.1%
4	1049	514	10.8 9.9 11.8	8.4%	44.4%	123	4.6	43.6%	12.1	39.5%	3.7%	67.1%
5	823	513	17.1 15.6 18.7	12.5%	48.8%	48	5.1	61.0%	10.8	58.4%	14.2%	73.4%
HIV Quintiles												
1	3725	512	2.1 1.9 2.3	0.0%	19.5%	465	2.7	3.8%	14.5	5.9%	1.2%	43.0%
2	2162	514	4.0 3.6 4.4	0.0%	31.2%	269	3.6	11.5%	13.9	11.3%	1.7%	49.6%
3	1593	514	5.8 5.3 6.3	0.6%	42.0%	162	4	26.6%	13.4	17.0%	2.4%	54.1%
4	1380	514	7.3 6.7 8.0	10.7%	37.8%	114	4.5	52.3%	12.6	17.1%	2.9%	48.7%
5	929	513	13.9 12.7 15.1	12.9%	50.1%	26	5.3	73.4%	11.8	23.4%	3.9%	50.2%
"Non-HIV" Quintiles												
1	4090	512	2.0 1.8 2.1	16.8%	22.3%	363.5	3.2	13.8%	14.4	0.0%	0.0%	28.3%
2	2124	514	4.0 3.7 4.4	14.4%	21.8%	269	3.6	21.5%	14	0.0%	0.4%	44.6%
3	1681	514	5.5 5.0 6.0	6.9%	48.7%	240	3.9	31.1%	12.9	4.2%	0.8%	65.3%
4	1111	514	9.6 8.7 10.4	2.9%	42.1%	190	4.1	29.5%	12.5	51.7%	3.9%	78.4%
5	873	513	15.0 13.7 16.4	0.0%	48.1%	142	4.4	39.2%	10.7	62.2%	14.9%	72.1%

* Abuse/Dependence
 FIB 4: [(years of age * AST)/(platelets in 10⁹/L * square root of ALT)]²
 eGFR: (186.3 * (serum creatinine)-1.154) * (age-0.203) * (0.742 for women) * (1.21 if African American))

Figure 1. Observed Mortality Rate by Quintiles of Risk Estimated Using HIV Only and Combined HIV and "Non-HIV" Biomarkers



DISCUSSION

After accounting for conventional markers of HIV severity, routine clinical biomarkers of anemia, liver injury, renal injury, and chronic viral hepatitis, substantially improve discrimination of mortality among HIV infected veterans initiating CART. "Non-HIV" biomarkers both add independent information to risk estimation after adjustment for HIV biomarkers and are strongly associated with immunodeficiency (CD4 count and AIDS-defining conditions) and HIV RNA.

- Our study has unique advantages:
- Sufficient sample size and follow up to analyze mortality with uniform data sources and methods of data collection
 - Near complete mortality ascertainment
 - Older patient population.
 - Results validated in independent data

- Our study also has limitations:
- Only 75% of veterans are CART naïve at VA entry.
 - Few women.
 - HIV uninfected veterans in care experience higher rates of mortality, comorbid disease, and substance use than the general population; – less pronounced differences among veterans and nonveterans with HIV infection.

This study is an essential step toward development of a combined index. We have shown that "non-HIV" biomarkers of anemia, liver disease, renal disease, and viral hepatitis add important discrimination to HIV markers and are associated with immunodeficiency (CD4 count and AIDS-defining illnesses) and HIV RNA. Next steps include testing in nonveteran populations, especially among women, and longitudinal response to treatment effects. Once more completely tested, our index may offer a superior prognostic index and integrated surrogate endpoint for clinical research.

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Conflict of Interest: none.

Disclaimer: The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs

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