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Biomarkers of Inflammation and Coagulation and Risk of Non-AIDS death in HIV/Hepatitis Coinfected Patients in the SMART Study

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INTRODUCTION

In the Strategies for Management of Anti-Retroviral Therapy (SMART) study, hepatitis B, and C co-infected participants randomized to the drug conservation (DC; interrupt antiretroviral therapy [ART] until CD4+ <250/µL) group were at increased risk of non-AIDS death if their baseline plasma level of the liver fibrosis marker hyaluronic acid (HA) was elevated (>75 ng/mL), while the risk of non-AIDS death in the viral suppression (VS; continued use of ART) group was considerably lower and did not depend on baseline HA level (fig 1). The excess mortality seen in co-infected participants was not due to any particular causes, suggesting that interruption of ART affects multiple, and not a single, pathological process. Another biomarker sub-study within SMART showed that higher levels of the coagulation and inflammation markers D-dimer, interleukin-6 (IL-6) and high sensitivity C-reactive protein (hsCRP) at study entry were significantly associated with an increased risk of all-cause mortality (Kuller et al; PLoS Med 2008).

The findings of these different studies raise the possibility that impaired liver function is associated with activation of both inflammatory and coagulation processes, which could be further exacerbated by ART interruptions and increase the risk of death.

OBJECTIVES

1. To assess IL-6, D-dimer and hsCRP at baseline and at month 6 among all hepatitis co-infected SMART participants with known baseline HA level.
2. To examine predictors for non-AIDS death during follow-up.

HYPOTHESES

1. Hepatitis co-infected participants with existing liver impairment (as determined by elevated HA) would have higher levels of the coagulation and inflammation markers D-dimer, IL-6 and hsCRP relative to participants with normal liver function (HA within the normal range).
2. The ability of biomarkers to predict non-AIDS death would differ by baseline HA level.

METHODS

Participants

All participants positive at baseline for HCV-RNA (>615 IU/mL; denoted HCV+) and/or HBsAg (denoted HBV+), with baseline HA level and with stored plasma samples were included. The study includes data through the end of follow-up.

Biomarkers

D-dimer, IL-6 and hsCRP were measured in stored plasma samples at baseline and at month 6 during follow up by the Laboratory for Clinical Biochemistry Research at the University of Vermont. IL-6 was measured with Chemiluminescent Sandwich ELISA (R&D Systems); hsCRP with a NB™II nephelometer, N Antiserum to Human CRP (Siemens Diagnostics); and D-dimer levels with immunoturbidometric methods on the Sta-R analyzer, Liatest D-DI (Diagnostica Stago).

Statistical methods

- Baseline biomarker levels were compared using Wilcoxon rank sum test
- Percent change in biomarker level from baseline to month 6 was calculated after $\log_e$ transformation and adjustment for baseline level
- Risk of non-AIDS death was estimated using Cox regression analysis adjusting for treatment group, age, gender, race, prior AIDS, baseline CD_4 and RNA, nadir CD_4, baseline ART status, alcohol abuse and Hepatitis B status

Figure 1

Risk of non-AIDS death in co-infected participants according to baseline HA and treatment arm

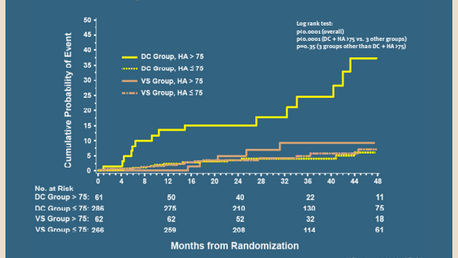

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Table 1

Baseline characteristics

	DC (n=337)	VS (n=318)	Total (n=655)
HBsAg+ (%)	17.5	15.1	16.3
HCV-RNA+ (%)	81.3	82.7	82.0
HBsAg+ and HCV-RNA+ (%)	1.2	2.2	1.7
Female sex (%)	27.0	23.6	25.3
History of alcohol abuse (%)	26.4	24.8	25.6
HIV-RNA ≤400 cps/mL (%)	67.6	60.9	64.3
Age, years*	45 (40, 51)	46 (41, 50)	46 (40, 50)
Baseline CD_4+, cells/µL*	600 (463, 759)	566 (459, 701)	582 (461, 737)
Hyaluronic acid*	29.4 (16.9, 54.7)	30.9 (18.3, 59.6)	30.0 (17.5, 57.0)
hsCRP, µg/mL *	1.10 (0.44, 2.81)	1.33 (0.45, 3.05)	1.16 (0.44, 2.90)
IL-6, pg/mL*	2.60 (1.65, 4.23)	2.62 (1.75, 4.25)	2.61 (1.69, 4.25)
D-dimer, µg/mL *	0.27 (0.17, 0.48)	0.28 (0.17, 0.50)	0.27 (0.17, 0.49)

*Median (IQR)

Table 2

Biomarker levels at baseline according to plasma hyaluronic acid level

		Baseline HA > 75 ng/mL		Baseline HA ≤ 75 ng/mL	
Baseline Marker	N	Median (IQR)	N	Median (IQR)	P-value*
hsCRP (µg/mL)	122	1.44 (0.55 – 2.83)	533	1.11 (0.41 – 2.93)	0.18
IL-6 (pg/mL)	121	4.41 (2.68 – 6.42)	523	2.33 (1.59 – 3.69)	<0.001
D-dimer (µg/mL)	122	0.31 (0.17 – 0.76)	531	0.27 (0.17 – 0.44)	0.01

*Wilcoxon rank sum comparing baseline hyaluronic acid groups

RESULTS

Baseline characteristics

Out of 5,472 participants enrolled in the SMART study from January 2002 - January 2006, 655 (12.0%) were HBV+ or HCV+, had HA measured at baseline and had baseline plasma samples available for biomarker analysis. **Table 1** shows the baseline characteristics of the participants according to randomization group. 18.6 % had a HA level above the upper normal range (75 ng/mL). There were no significant differences in baseline variables between the two randomization groups.

Follow-up

At month 6 the median (IQR) CD_4+ cell counts in the DC and VS groups were 441 cells/µL (328 – 572) and 596 cells/µL (447 – 758), respectively, while 28.1% and 72.1% had HIV-RNA ≤400 copies/mL.

50 participants died from non-AIDS causes (30 in DC and 20 in VS). Breakdown of the different causes of death (N;%) was: infection (7; 14%), non-AIDS cancer (6; 12%), substance abuse (6; 12%), hepatic (4; 8%), cardiovascular (4; 8%), renal (4; 8%), accident/violent/suicide (4; 8%), chronic obstructive pulmonary disease (1; 2%), CNS disease (1; 2%), unknown cause (13; 26%). The median (IQR) interval between the measurement of baseline biomarkers and non-AIDS death was 17 (9 - 34) months.

Biomarker levels at baseline and during follow-up

The baseline levels of the three biomarkers are shown in **table 1**. The levels of all three biomarkers were higher in participants with elevated baseline HA (>75 ng/mL) compared with participants with HA in the normal range (≤ 75 ng/mL), but the difference was only statistical significant for IL-6 and D-dimer, **table 2**. Interruption of ART led to a significantly higher percent change in D-dimer, but not hsCRP and IL-6 levels, from baseline to month 6 (**fig. 2**).

Participants randomized to the DC group with an elevated HA level had a 47.3% increase in IL-6 from baseline to month 6 compared with a 0.7% decrease in DC group participants with HA within the normal range. The increase in IL-6 was not associated with an increase in hsCRP, which decreased slightly in all groups (**fig. 3**).

Predictors for non-AIDS death

All participants were stratified into four equal sized groups based on baseline median HA and median biomarker level. Overall the subgroups with both HA and either hsCRP, IL-6 or D-dimer above the median at baseline accounted for 52%, 62% and 48% of all non-AIDS deaths, respectively. On the contrary, only 8% of all non-AIDS deaths were seen in the subgroups having both HA and either biomarker below the median baseline level (**fig. 4**). The interaction p-values for dichotomous HA x biomarker for non-AIDS death were 0.88, 0.89 and 0.63 for hsCRP, IL-6 and D-dimer, respectively. Adjusted hazard ratios (95% confidence interval) for non-AIDS death comparing those with both HA and biomarker above the median to those with both HA and biomarker below the median were 6.1 (2.1-17.7), p=0.001; 5.9 (2.0-17.3), p=0.001 and 4.4 (1.5-13.3), p=0.008 for hsCRP, IL-6 and D-dimer, respectively. The results of the univariate analysis were similar.

CONCLUSIONS

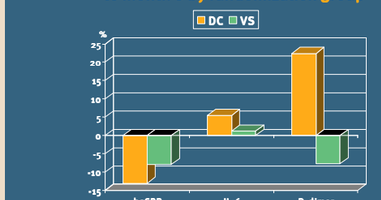
- Baseline levels of IL-6 and D-dimer were significantly higher in HIV/hepatitis co-infected participants with elevated levels of the liver fibrosis marker HA.
- Interruption of ART led to increased levels of IL-6 only in participants who also had elevated HA, but unexpectedly corresponding increases in hsCRP were not observed.
- During follow-up the risk of non-AIDS death in participants with elevated baseline levels of either hsCRP, IL-6 and D-dimer was highest if HA was also elevated.

Perspective

Our data suggest that among HIV-viral hepatitis co-infected persons, those with impaired liver function are particularly in a pro-inflammatory state associated with excess risk of death from causes other than AIDS - and interruption of ART further exacerbates this pro-inflammatory state.

Figure 2

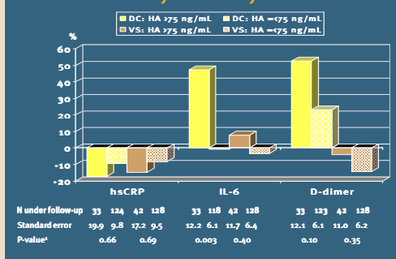
Percent change in biomarker level from baseline to month 6 by randomization group



¹Difference between DC and VS at month 6 using $\log_e$ transformed levels after adjustment for baseline level

Figure 3

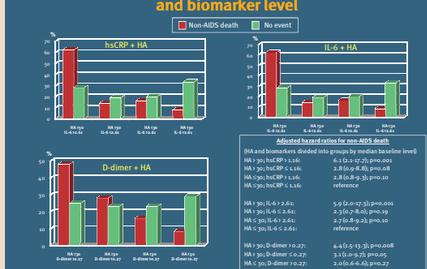
Percent change in biomarker level from baseline to month 6 by baseline hyaluronic acid level



²Difference between HA groups at month 6 using $\log_e$ transformed levels after adjustment for baseline level

Figure 4

Distribution of individuals with and without non-AIDS death according to baseline hyaluronic acid and biomarker level



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