P-Q3

IL-6, hsCRP, and the Development of Type 2 Diabetes Among HIV Positive Patients Taking ART

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BACKGROUND

- Untreated HIV is associated with higher levels of inflammatory markers, and initiation of continuous antiretroviral therapy (ART) results in a decline, but does not normalize these markers (1).
- Inflammation is hypothesized to play a role in development of type 2 diabetes (2); however, limited data addressing this issue among HIV positive patients have been reported (3).
- We investigated the association of interleukin 6 (IL-6) and high sensitivity C-reactive protein (hsCRP) with the development of type 2 diabetes in HIV positive individuals using data from the control arms of two large, international HIV trials (SMART and ESPRIT) (4)(5).

STUDY AIM

Estimate the incidence of diabetes and study the association of IL-6 and hsCRP (measured at baseline) with risk of diabetes in HIV positive individuals.

METHODS

- Participants from the control arms of SMART and ESPRIT were included if they did not have a history of diabetes at entry and had IL-6 and hsCRP measured at study entry using stored plasma.
- IL-6 was measured by Chemiluminescent Sandwich ELISA, R&D Sytems. hsCRP was measured by ELISA. For SMART participants, a NBTMII nephelometer, N Antiserum to Human CRP (Siemens Diagnostics) was used. For ESPRIT participants, an R&D Systems ELISA assay was used. The hsCRP assays, while different, compared very well on duplicate samples.
- Incident diabetes definition: participants were considered to have developed diabetes if they received a diagnosis of diabetes requiring drug treatment during follow-up.
- Hazard ratios (HR) with 95% confidence intervals (CI) per 2-fold higher level of baseline biomarker levels were estimated using Cox regression models.
- The association of incident diabetes with each biomarker was first studied in a model with no covariates and then in 3 regression models with the following baseline covariates: (I) age, race, sex, co-infection with hepatitis C or B, CD4+ count and HIV RNA level; (II) age, race, sex, co-infection with hepatitis C or B, CD4+ count, HIV RNA level, body mass index (BMI), use of lipid-lowering drugs, use of blood pressure–lowering drugs and use of ART; (III) all of the aforementioned covariates plus cigarette smoking status, LDL, HDL, and triglycerides (SMART only). The adjusted models yielded similar estimates and results from Model II are reported here.
- Interactions between each biomarker and the covariates were assessed.

RESULTS

- Among the 4,792 patients in the control arms of SMART and ESPRIT 240 (5%) reported taking drugs for diabetes at entry. Of those not taking drug treatment for diabetes at study entry, 3,965 had IL-6 and hsCRP measurements and attended at least one annual follow-up visit (1,719 in ESPRIT and 2,246 in SMART).
- These 3,965 patients with a median CD4+ count of 523 cells/mm3 were followed for an average of 4.6 years; 137 patients (3.5%, 8.18 per 1000 person years) developed diabetes during follow-up requiring drug treatment.
- Median levels of both IL-6 and hsCRP were significantly higher among those who developed diabetes: 3.45 versus 2.50 pg/ml for IL-6 and 4.91 versus 3.29 µg/ml for hsCRP (P<.001 for each marker) (Figure 1).
- Figures 2 and 3 give the cumulative percent developing diabetes by quartile of each biomarker. There is a clear separation in risk of diabetes for the upper 2 versus lower 2 quartiles.
- Higher baseline levels of IL-6, and hsCRP were significantly associated with the risk of developing diabetes. Unadjusted HRs associated with a doubling of IL-6 and hsCRP were 1.47 (95% CI: 1.26-1.70) and 1.32 (95% CI: 1.20-1.45), respectively.
- With adjustment for baseline covariates, the associations remained significant (Table 2).
- For comparison with a meta-analysis of studies in the general population, HRs as sociated with 1 natural log higher levels of IL-6 and hsCRP were estimated. With the Model II covariates, these HRs for IL-6 and hsCRP were 1.47 (95% CI: 1.13 to 1.89) and 1.37 (95% CI: 1.18 to 1.59), respectively. HRs from the meta-analysis for IL-6 and hsCRP were 1.31 (95% CI: 1.17-1.46) and 1.26 (95% CI: 1.16-1.37), respectively (Table 3).
- In the adjusted analysis, body mass index (P<0.001), age (P=0.01), and co-infection with hepatitis B or C (P=0.03) were associated with an increased risk of diabetes; smoking was associated with a decreased risk (P=0.03).
- There was evidence that the proportional hazards assumption may not hold for IL-6 (P-value=0.03). There was a stronger association between IL-6 and incident diabetes early in the follow-up period compared to later (HR of 1.5; 95% CI: 1.2-1.8; P<0.001 in the first 3 years of follow-up and HR of 1.0; 95% CI: 0.7-1.4; P=0.85 after 3 years). For hsCRP, the association with diabetes was similar over follow-up.

Table 1: Baseline characteristics for participants in the control arms of ESPRIT and SMART according to the development of diabetes during follow-up.

	Incident diabetes		
	No (n=3,828)	Yes (n=137)	P-value*
Study			0.002
ESPRIT	43.1	50.4	
SMART	56.9	49.6	
Age (years ± SD)	42.5 ± 9.4	47.5 ± 9.1	<0.001
Gender (% female)	23.0	20.4	0.79
Race (%)			<0.001
White	66.8	53.3	
Nonwhite	33.2	46.7	
Lipid lowering drug (%)	12.3	27.9	<0.001
Blood pressure lowering drug (%)	10.5	25.0	<0.001
Co-infected with hepatitis B or C (%)	16.3	18.2	0.60
Prior MI, stroke or CAD requiring surgery (%)	2.2	1.5	0.74
Mean BMI (kg/m^2 ± SD)	24.8 ± 4.7	28.6 ± 6.9	<0.001
Mean D-Dimer (µg/ml ± SD)	0.35 ± 0.52	0.35 ± 0.28	0.47
HIV RNA level/ART status			
On ART and HIV RNA ≤ 500 (%)	75.6	67.6	(ref.)
On ART and HIV RNA > 500 (%)	15.1	25.0	0.02
Off ART (%)	9.3	7.4	0.62
Baseline ART regimen			
PI-based regimen (%)	40.9	47.4	0.32
NNRTI-based regimen (no PI) (%)	38.7	31.4	0.10
Zidovudine (%)	44.1	31.9	0.05
Stavudine (%)	27.0	43.1	0.01
Mean CD4 count (per mm^3 ± SD)	589 ± 240	603 ± 276	0.02
Smoking (%)†	41.5	27.9	0.02
Mean HDL cholesterol (mg/dL ± SD)†	43.7 ± 14.9	41.3 ±15.5	0.19
Mean LDL cholesterol (mg/dL ± SD)†	114.7 ± 35.4	112.2 ± 35.9	0.87
Mean total cholesterol (mg/dL ± SD)†	195.3 ± 47.0	195.5 ± 49.7	0.94
Mean triglycerides (mg/dL ± SD)†	209.7 ± 190.4	282.8 ± 298.3	0.006

† Only available in the SMART study.
*P-value from univariate Cox proportional hazards models.

Table 2: Risk of diabetes associated with IL-6 and CRP levels at entry.

Type of adjustment	Quartile 1 HR (95% CI)	Quartile 2 HR (95% CI)	Quartile 3 HR (95% CI)	Quartile 4 HR (95% CI)	HR associated with doubling of biomarkers HR (95% CI)	P-value
IL-6						
IL6 range (pg/ml)	(0.15-1.15)	(1.16-1.80)	(1.81-2.82)	(2.83-88.63)	-	-
Number of patients with Incident diabetes (n) Crude Analysis (n=3,965; 3.45%)	14	27	44	52	-	-
Model II:	1.00 (Ref)	1.70 (0.89-3.25)	2.86 (1.56-5.24)	3.45 (1.91-6.23)	1.47 (1.26-1.70)	<0.001
hsCRP						
hs-CRP range (µg/ml)	(0.03-0.68)	(0.69-1.56)	(1.57-3.56)	(3.57-98.00)	-	-
Number of patients with Incident diabetes (n) Crude Analysis (n=3,965; 3.45%)	10	23	55	49	-	-
Model II:	1.00 (Ref)	2.32 (1.10-4.87)	5.52 (2.82-10.84)	5.13 (2.60-10.14)	1.32 (1.20-1.45)	<0.001
Model II:	1.00 (Ref)	2.25 (1.04-4.88)	4.44 (2.18-9.06)	3.81 (1.85-7.86)	1.24 (1.12-1.38)	<0.001

Model II: Adjusted for age, sex, race, CD4 count, co-infection with hepatitis B or C and viral load and ART status; stratified by study. 10 participants including 1 event are excluded due to missing baseline covariates, use of lipid lowering drugs, use of blood pressure lowering drugs and BMI; stratified by study. 32 participants including 2 events are excluded due to missing baseline covariates.

Table 3. HRs associated with 1 natural log higher levels of IL-6 and hsCRP (comparison with a meta-analysis of studies in the general population).

	HR associated with higher level of biomarkers	
	HR	95% CI
IL-6 (log pg/ml)		
Systematic review (meta-analysis)	1.31	(1.17-1.46)
SMART+ESPRIT*	1.47	(1.13 -1.89)
hsCRP (log µg/ml)		
Systematic review (meta-analysis)	1.26	(1.16-1.37)
SMART+ESPRIT*	1.37	(1.18-1.59)

*Using Model II

Figure 1: Boxplots of IL-6 and hsCRP by diabetes event status.

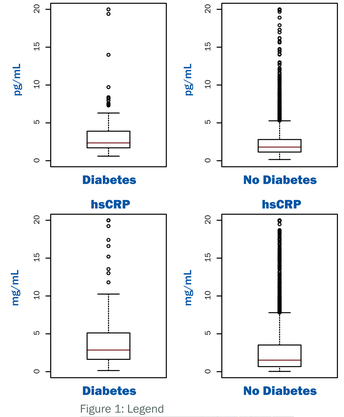


Figure 1: Legend

	Incident diabetes	
	No	Yes
IL-6 (log pg/ml)		
10th percentile	0.80	1.14
25th percentile	1.14	1.70
Median	1.80	2.35
75th percentile	2.80	3.90
90th percentile	4.46	6.30
hsCRP (log µg/ml)		
10th percentile	0.31	0.84
25th percentile	0.67	1.63
Median	1.52	2.86
75th percentile	3.52	5.12
90th percentile	7.35	11.80

Figure 2: Cumulative percent developing diabetes by quartile of IL-6.

The p-value corresponding to a logrank test with 3 degrees of freedom that compares the incidence of diabetes by quartile is <0.001.

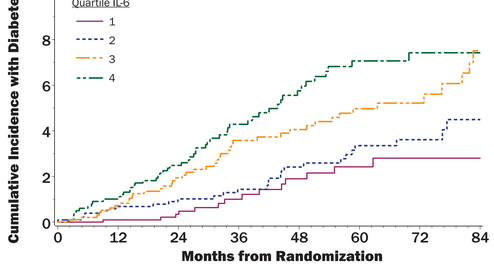
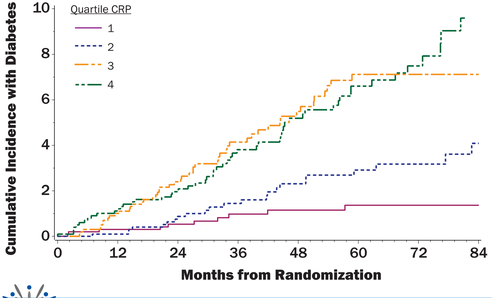


Figure 3: Cumulative percent developing diabetes by quartile of hsCRP.

The p-value corresponding to a logrank test with 3 degrees of freedom that compares the incidence of diabetes by quartile is <0.001.



CONCLUSIONS

- Elevated plasma levels of hsCRP and IL-6 are associated with type 2 diabetes among HIV infected patients taking ART. Our findings support the hypothesis that low-grade systemic inflammation is an underlying factor in the pathogenesis of type 2 diabetes.
- The association between hsCRP and IL-6 with type 2 diabetes was at least as strong as that observed in general population.
- These findings may have important implications for the prevention and treatment of type 2 diabetes among HIV patients.

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