Long-term Elevated IL-6 and D-dimer after Delayed ART Initiation in the START Trial

0240

Jason V. Baker[1], Birgit Grund[2], Shweta Sharma[2], Keith Henry[1], Jennifer Hoy[3], Stefan Esser[4], Silvia Nozza[5], Kiat Ruxrungtham[6], Adam Rupert[7], H. Clifford Lane[8], Jens D. Lundgren[9], and James Neaton[2] for the INSIGHT START study group

[1]Hennepin Healthcare Research Institute, Minneapolis, Minnesota; [2]University of Minnesota, Minneapolis, Minnesota; [3]Monash University, Melbourne, Victoria, Australia; [4]University Hospital Essen, Essen, Germany; [5]San Raffaele Scientific Institute, Milan, Italy; [6]Chulalongkorn University, Bangkok, Thailand; [7]Leidos Biomedical Research, Inc., Frederick, Maryland; [8]National Institutes of Health, Bethesda, Maryland; [9]University of Copenhagen, Copenhagen, Denmark

START

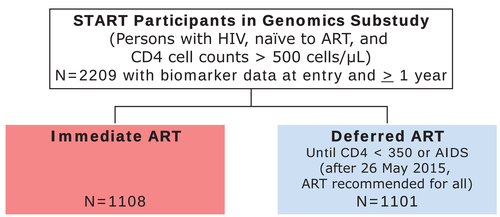
insight

BACKGROUND

- Inflammation and coagulation are associated with disease risk among persons living with HIV.
- Antiretroviral treatment (ART) reduces inflammation, but it is not known whether delays in ART initiation (e.g., due to delayed diagnosis) result in long-term increased levels of inflammation.
- We studied the interleukin-6 (IL-6) and D-dimer trajectories in the immediate versus deferred groups of the START trial to:
  1) Quantify the duration of excess inflammation over time as a consequence of delaying ART.
  2) Determine if a longer duration of untreated HIV prior to ART influences the degree of inflammation during viral suppression.

METHODS

Figure 1. Study design



Participants were enrolled between 15 April 2009 and 23 December 2013. All participants were recommended ART after 26 May 2015. Annual biomarker data were obtained through 2017.

Outcome Measures

- IL-6 and D-dimer levels were measured at baseline, month 8, and annually up to 7 years.
- Laboratory assays were high-sensitivity ELISA for IL-6 (R&D Systems) and VIDAS system (BioMerieux) for D-dimer.
- Baseline and month-8 samples were analyzed in 2016; month-12 and later samples were analyzed in 2018 (by Leidos Biomedical Research, MD, USA). In order to adjust for possible systematic differences between 2016 and 2018 assays, we re-tested 160 IL-6 and 140 D-dimer baseline and month-8 samples in 2018. Linear conversion equations for $\log_2$ biomarker levels were derived and used to convert 2018 measures to the 2016 scale.
- Testing was blinded to treatment groups. Run order for year 1+ samples was by study visit, but randomized across treatment groups.

Statistical Methods

- Mean change in $\log_2$-transformed IL-6 and D-dimer from entry were compared between the deferred versus immediate groups by intent-to-treat using longitudinal mixed models. Subgroups were defined by baseline factors.
- To assess the effect of delaying ART on biomarker levels after/during sustained viral suppression, we compared biomarker changes from ART initiation, between the deferred and immediate groups. These are not randomized comparisons.
- All analyses were performed on $\log_2$-transformed biomarker levels. Results were presented as percent change from baseline. Models were adjusted for age, sex, geographic region (high versus medium/low income), baseline biomarker levels, and visit.

REFERENCES

1. INSIGHT START Study Group. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. N Engl J Med. 2015;373:795-807.
2. Baker JV, Sharma S, Grund B, et al. Systemic Inflammation, Coagulation, and Clinical Risk in the START Trial. Open Forum Infectious Diseases. 2017:ofx262-ofx262.

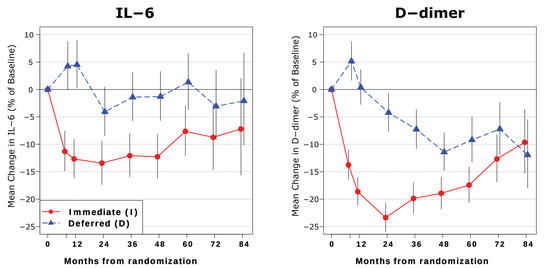
ACKNOWLEDGMENTS

We thank the participants in the START study, as well as investigators and staff.

Funding: NIH Grants UM1-AI068641, UM1-AI120197 and 1U01-AI36780. ClinicalTrials.gov ID: NCT00867048.

Compared to immediate ART in the START trial, delayed ART resulted in higher levels of IL-6 and D-dimer over at least 5 years, and potentially higher levels over at least 2 years after viral suppression was achieved.

Figure 2. Percent change in IL-6 and D-dimer levels over time in START. Comparison by intent-to-treat. Percent changes are calculated relative to the study entry levels. Vertical lines show 95% CIs.



No. of participants

I:	1108	1066	1049	996	973	788	521	281
D:	1101	1042	1001	967	947	768	515	270

Deferred-Immediate

Est. Diff:	9.1	8.9	3.4
P−value:	<0.001	<0.001	0.31

Overall Est. Diff: 10.3 (95% CI: 7.6 to 12.9), p-value:<0.001

No. of participants

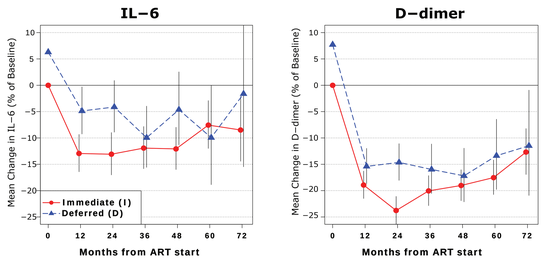
I:	1103	1061	1044	991	968	787	520	280
D:	1088	1031	989	954	934	758	511	266

Deferred-Immediate

Est. Diff:	19.4	8.2	6.9
P−value:	<0.001	<0.001	0.02

Overall Est. Diff: 14.0 (95% CI: 11.4 to 16.5), p-value:<0.001

Figure 4. IL-6 and D-dimer levels after ART initiation. Percent changes from study entry, with t=0 plotted as date of ART initiation. Comparisons are not randomized. Vertical lines show 95% CIs.



No. of participants

I:	1090	1053	1034	983	961	782	514
D:	963	913	806	480	356	196	83

Deferred-Immediate

Est. Diff:	7.1	11.3	8.7
P−value:	0.003	<0.001	0.17

Overall Est. Diff: 6.7 (95% CI: 3.6 to 9.9), p-value:<0.001

No. of participants

I:	1085	1048	1029	978	956	781	513
D:	952	902	797	474	352	193	83

Deferred-Immediate

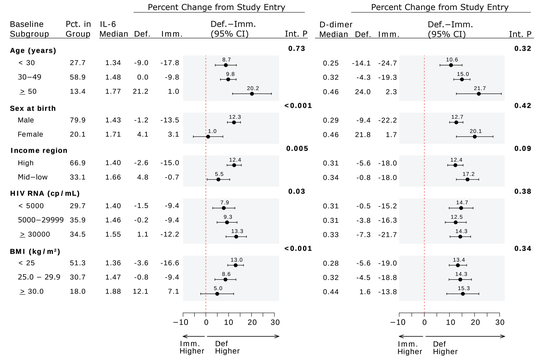
Est. Diff:	9.6	3.7	2.9
P−value:	<0.001	0.14	0.59

Overall Est. Diff: 7.3 (95% CI: 4.5 to 10.1), p-value:<0.001

Deferred ART group: t=0 at ART initiation. Because the ART start often did not coincide with an annual visit, the t=0 biomarker levels were obtained from the most recent available biomarker levels, at a median 125 days (IQR 36 to 220) before ART initiation.

Immediate ART group: t=0 at study entry; participants who did not start ART in the first year were excluded (n=18). Baseline biomarker levels were obtained at a median 16 days (IQR 7 to 29) before ART initiation.

Figure 3. Subgroup analyses for IL-6 and D-dimer. Differences between the immediate versus deferred ART groups comparing percent change from study entry through follow-up within and across subgroups. Comparisons within subgroups are by intent-to-treat, and protected by randomization.



Baseline Subgroup	Pct. in Group	IL-6 Median	IL-6 Def.	IL-6 Imm.	IL-6 Def.−Imm. (95% CI)	IL-6 Int. P	D-dimer Median	D-dimer Def.	D-dimer Imm.	D-dimer Def.−Imm. (95% CI)	D-dimer Int. P
Age (years)						0.73					0.32
< 30	27.7	1.34	-9.0	-17.8	8.7		0.25	-14.1	-24.7	10.6	
30−49	58.9	1.48	0.0	-9.8	9.8		0.32	-4.3	-19.3	15.0	
≥ 50	13.4	1.77	21.2	1.0	20.2		0.46	24.0	2.3	21.7	
Sex at birth						<0.001					0.42
Male	79.9	1.43	-1.2	-13.5	12.3		0.29	-9.4	-22.2	12.7	
Female	20.1	1.71	4.1	3.1	1.0		0.46	21.8	1.7	20.1	
Income region						0.005					0.09
High	66.9	1.40	-2.6	-15.0	12.4		0.31	-5.6	-18.0	12.4	
Mid−low	33.1	1.66	4.8	-0.7	5.5		0.34	-0.8	-18.0	17.2	
HIV RNA (cp/mL)						0.03					0.38
< 5000	29.7	1.40	-1.5	-9.4	7.9		0.31	-0.5	-15.2	14.7	
5000−29999	35.9	1.46	-0.2	-9.4	9.3		0.31	-3.8	-16.3	12.5	
≥ 30000	34.5	1.55	1.1	-12.2	13.3		0.33	-7.3	-21.7	14.3	
BMI (kg/m²)						<0.001					0.34
< 25	51.3	1.36	-3.6	-16.6	13.0		0.28	-5.6	-19.0	13.4	
25.0 − 29.9	30.7	1.47	-0.8	-9.4	8.6		0.32	-4.5	-18.8	14.3	
≥ 30.0	18.0	1.88	12.1	7.1	5.0		0.44	1.6	-13.8	15.3	

There was no evidence for differences in the treatment effect across subgroups formed by CD4 cell counts, CD8 cell counts, CD4:CD8 ratio, IL-6 and D-dimer levels at baseline, estimated time since HIV infection (<6 months, 6 months - 2 years, >2 years), and smoking status. Median levels for IL-6 are presented in pg/mL, for D-dimer in μg/mL.

Table 1. Participant characteristics at study entry

	All Participants (N=2209) Median [IQR] or n(%)
Demographics	
Age (years)	36 [29, 45]
Female sex	443 (20.1)
Race	
Black	494 (22.4)
White	1227 (55.5)
Other (Latino, Asian, Other)	488 (22.1)
Geographic Location	
High income (US, Europe, Australia)	1477 (66.9)
Mid-Low income (Africa, Latin America)	732 (33.1)
Clinical Characteristics	
Time known to be HIV-positive (years)	1.1 [0.4, 2.9]
CD4 (cells/μL)	649 [584, 761]
Nadir CD4 (cells/μL)	545 [473, 642]
CD8 (cells/μL)	1074 [798, 1433]
CD4:CD8 Ratio	0.6 [0.5, 0.8]
HIV RNA (copies/mL)	14884 [3460, 45100]
HIV RNA ≤ 200 copies/mL	95 (4.3)
Hepatitis B/C	47 (2.2)
Prior CVD (stroke, MI, revascularization)	21 (1.0)
Body Mass Index (kg/m^2)	24.9 [22.5, 28.3]
Current Smoking	867 (39.2)
Biomarkers	
IL-6 (pg/mL)	1.47 [1.02, 2.24]
D-Dimer (μg/mL)	0.31 [0.22, 0.47]

RESULTS

Participant Characteristics, ART Use, and Viral Suppression (<200 cp/mL)

- Table 1 summarizes participant characteristics at study entry. Median levels of IL-6 and D-dimer were 1.47 pg/mL and 0.31 μg/mL, respectively.
- In the Immediate group, 94-97% had viral suppression at all annual visits.
- In the Deferred group, median time to ART initiation was 2.5 years (IQR 1.4 to 3.6). Viral suppression rates increased over time, as more participants initiated ART: 18%, 61%, 89%, and 95% at years 1, 3, 5, 7, respectively.
- In the Deferred group, of the 963 participants who started ART, 603 (63%) started before and 360 (37%) after 26 May 2015, i.e., when all participants were recommended to start ART.

IL-6 and D-dimer Through Follow-up

Randomized comparison by intent-to-treat

- IL-6 and D-dimer levels remained significantly higher in the deferred versus the immediate group through 5 years (Figure 2). Through follow-up, the treatment difference in IL-6 was 10.3% (95% CI: 7.6 to 12.9, p<0.001), and in D-dimer 14.0% (95% CI: 11.4 to 16.5, p<0.001).
- Subgroup analyses are shown in Figure 3.

Comparison from time of ART initiation

- In the deferred group, biomarkers increased between study entry and ART initiation (Figure 4, t=0): IL-6 levels had increased by 7% (from 1.47 to 1.59 pg/mL), and D-dimer levels by 9% (from 0.31 to 0.35 μg/mL) by the time of ART start; the annual increases were 2.3% per year for IL-6 and 2.8% for D-dimer.
- When comparing treatment groups from the time of ART start, biomarker levels remained higher in the deferred compared to the immediate group over at least 2 years of ART (Figure 4). These comparisons are not protected by randomization.
  - At 2 years on ART, viral suppression was >96% in both groups. The estimated difference in percent change from baseline (Def-Imm) was 7.1% (95% CI: 2.5 to 1.8; p=0.003) for IL-6 and 9.6% (95% CI: 6.0 to 13.2, p<0.001) for D-dimer.
  - The higher biomarker levels in the deferred group compared with immediate ART at 2+ years after ART initiation appeared primarily due to the biomarker increase during the period of untreated ART and the resulting higher levels

LIMITATIONS

- Comparing biomarker levels between the immediate and deferred groups with t=0 at ART start is not protected by randomization (Figure 4).
- In the deferred group, time to ART initiation was variable, and the start dates usually did not coincide with annual visits when biomarker levels were measured.
- Numbers of participants in follow-up decreased after year 4 in both treatment groups (Figure 2), due to staggered enrollment. In addition, for analyses setting t=0 at ART start, follow-up on ART is shorter in the deferred group and the sample size decreases substantially after year 2 on ART (Figure 4).

CONCLUSIONS

- Compared to immediate ART, delayed ART was associated with higher levels of IL-6 and D-dimer over at least 5 years, with differences diminishing over time.
- IL-6 and D-dimer levels increase during untreated HIV, leading to higher absolute levels of inflammation at the time of ART initiation when treatment is delayed.
- The excess inflammation associated with delayed HIV treatment does not appear to be overcome during the first 2 years of ART despite viral suppression.
- After viral suppression on ART and the initial decrease in biomarker levels, IL-6 levels remained stable over time but D-dimer levels increased after 2 years; reasons for this and the implications for disease risk are unclear.
- Follow-up continues in START to determine the clinical consequences of excess inflammation from delayed HIV diagnosis and treatment.