Reduced D-dimer levels and inflammation markers after probiotic supplement in HIV-infected on ART

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ABSTRACT

BACKGROUND:
Despite efficient antiretroviral treatment (ART) HIV-infected patients have increased morbidity and mortality compared to HIV seronegative individuals. Gut microbiota composition has potential impact on HIV pathogenesis and cardiovascular risk. Notably have markers of inflammation, coagulation and microbial translocation been reported to predict all-cause mortality. The aim of this study was to assess the efficacy of probiotic supplementation in HIV-infected individuals.

METHODOLOGY:
Thirty HIV-infected patients on ART treatment (median age 50.3y, 22 men, 22 Caucasians) with HIV-RNA < 50 copies/ml and CD4 count < 500 cells/ml were included. No antibiotics or probiotics were prescribed for the last 2 months, and no episodes of inflammatory bowel disease, immunomodulating therapy or infectious diarrhea for the last 6 months. Participants were randomized in a double blind fashion to the following study arms:
i) probiotics (n=14), ii) placebo (n=8), and iii) controls (n=8). The probiotic (Biola, Tine SA, Norway) consisted of 250 ml/day fermented skimmed milk supplemented with *Lactobacillus rhamnosus* GG (10^8 cfu/ml), *Bifidobacterium animalis* subsp. *lactis* B-12 (10^8 cfu/ml), and *Lactobacillus acidophilus* La-5 (10^7 cfu/ml). Heat-treated fermented skimmed milk served as placebo. The intervention period was 8 weeks. LPS was analyzed by Limulus Amebocyte Lysate colorimetric assay (LAL) (Lonza, USA), soluble (s)CD14 and IL-6 by ELISA (R&D Systems) and D-dimer was measured by clinically approved test. Non-parametric statistics were applied.

RESULTS:
Twenty-five completed the trial leaving 12 patients in the probiotic group, 7 placebo and 6 controls. No serious adverse events were recorded. In patients receiving probiotic, there was a 33% reduction from baseline to 8 weeks in the levels of the coagulation marker D-dimer, from a median of 320.3 ng/mL (239.8-474.7 IQR) to 214.2 ng/mL (142.0-392.6 IQR) (p=0.03, Wilcoxon). IL-6 levels decreased 13.8% from median 1.23pg/mL (0.94-2.46 IQR) to 1.06 pg/mL (0.93-1.39 IQR) in the probiotic group (p=0.06, Wilcoxon). No changes were found in the placebo group or controls. CD4 counts, plasma LPS or soluble CD14 did not change significantly in any of the study arms.

CONCLUSIONS:
Probiotic supplement significantly reduced D-dimer levels and possibly also levels of IL-6, both markers related to HIV-associated inflammation and cardiovascular disease. This reduction was apparently not related to changes in markers of microbial translocation. Additional studies to further elucidate mechanisms by which probiotics can influence upon gut microbiota, coagulation and inflammation are warranted.

BACKGROUND

- Gut microbiota composition has potential impact on HIV pathogenesis and cardiovascular risk and has recently been targeted as an intervention opportunity in HIV disease.
- Probiotics may decrease gut pathogens, improve gut barrier function and regulate inflammatory responses.[1]

OBJECTIVE

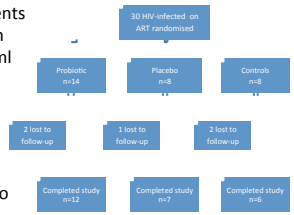
The objective was to assess safety and the efficacy of probiotic supplementation upon markers of inflammation, coagulation and microbial translocation in HIV-infected individuals on efficient ART.

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Reference: [1]Sanders, M. E.. Impact of probiotics on colonizing microbiota of the gut. J Clin Gastroenterol. 2011: 45; S115-9.

DESIGN

30 HIV infected patients on ART treatment with HIV-RNA < 50 copies/ml and CD4 count < 500 cells/ml were randomized in a 2:1:1 fashion to either a double blinded intervention or placebo arm, or to an open, untreated control arm. No antibiotics or probiotics were prescribed for the last 2 months, and no episodes of inflammatory bowel disease, immunomodulating therapy or infectious diarrhea for the last 6 months. 22 patients were recruited from Oslo University Hospital in Norway and 8 patients from Karolinska University Hospital in Sweden.



8 WEEKS OF INTERVENTION WITH:

1. Probiotic (Biola, Tine SA, Norway) 250 ml/day consisting of fermented skimmed milk supplemented with *Lactobacillus rhamnosus* GG (10^8 cfu/ml), *Bifidobacterium animalis* subsp. *lactis* B-12 (10^8 cfu/ml), and *Lactobacillus acidophilus* La-5 (10^7 cfu/ml).

   Or

2. Placebo consisting of heat-treated fermented skimmed milk.

FOLLOW UP:

Examination	Baseline	2 weeks	4 weeks	8 weeks
Clinical examination	X			X
Blood sample	X			X
Fecal sample	X			X
Adverse events		X	X	X

METHODS

CRP, CD4 and CD8 were analyzed from fresh serum/cells.
The following were analyzed from frozen samples stored at -80°C:

- LPS by Limulus Amebocyte Lysate colorimetric assay (LAL)
- Soluble (s)CD14, IL-6 and IP-10 by ELISA
- D-dimer by Asserachrom kit
- Thrombin generation by Calibrated Automated Thrombogram (CAT)

Non-parametric statistics with Wilcoxon Matched Pairs Test, Mann Whitney U Test or Fischers Exact Test were applied.

RESULTS

DESCRIPTION OF THE STUDY COHORT AT BASELINE

		Probiotic n=14	Placebo n=8	Control n=8
Age, (range)		50,3 (34,2-64)	50,1 (36-66,1)	52,5 (34,6-67,6)
Male gender, n (%)		10 (71,4)	4 (50)	8 (100)
Ethnicity, n (%)	Caucasian	10 (71,4)	6 (75)	6 (75)
	Non-caucasian	4 (28,6)	2 (25)	2 (25)
Risk group, n (%)	MSM	6 (42,9)	4 (50)	6 (75)
	IDU	1 (7,1)	1 (12,5)	0 (0)
	Other	7 (50)	3 (37,5)	2 (25)
Comorbidities, n (%)	Cardiovascular	3 (21,4)	2 (25)	4 (50)
	Diabetes	1 (7,1)	0 (0)	1 (12,5)
	Renal	3 (21,4)	0 (0)	0 (0)
Current smoker, n (%)		4 (28,6)	2 (25)	2 (25)
Years since HIV diagnosis, (range)		6,1 (1,5-26,4)	8 (1,8-26,5)	8,4 (1,3-19,8)
Years of effective ART, (range)		1,9 (0,7-5,9)	3 (0,7-19,6)	4,7 (0,9-14,1)
Year since nadir, (range)		3,4 (1,4-24,4)	3,1 (1,5-17,1)	4,6 (1,3-17,7)
CD4 nadir, (range)		145 (10-300)	130 (6-300)	109 (40-272)
CD4+ count, (range)		347 (188-741)	319 (190-449)	291 (196-470)
CD8+ count, (range)		704 (267-1658)	735 (276-1045)	645 (270-919)
CD4/CD8 ratio, (range)		0,4 (0,2-0,9)	0,4 (0,3-0,9)	0,5 (0,3-1,1)

Table 1: Data are given as median (range) or numbers (%). There were no significant differences between the groups. MSM, men who have sex with men, IDU, intravenous drug abuse.

IL-6 AND D-DIMER WERE INTERCORRELATED AND ASSOCIATED WITH sCD14 LEVELS

CORRELATIONS AT BASELINE

	IL-6	N	p-value
sCD14	0,40	29	0,022
LPS	0,47	30	0,008
D-dimer	0,40	29	0,031
	D-dimer	**n**	**p-value**
sCD14	0,38	28	0,05
IL-6	0,40	29	0,03

Table 2: Spearman Rank Order Correlations

Parameters of thrombin generation, IP-10 levels, CD4 counts, CD4/CD8 ratio, HIV RNA quantification, plasma LPS or soluble (s)CD14 did not change significantly in any of the study arms.

CHANGES IN D-DIMER, CRP AND IL-6 AFTER 8 WEEKS OF PROBIOTIC SUPPLEMENT

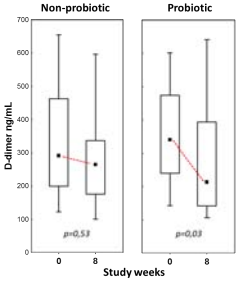


Fig. 1: Changes in D-dimer

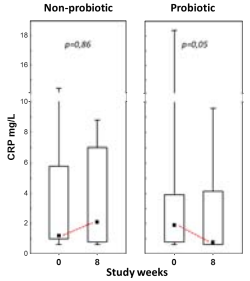


Fig. 2: Changes in CRP

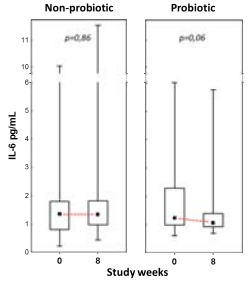


Fig. 3: Changes in IL-6

After 8 weeks of probiotic supplement d-dimer was significantly reduced (fig. 1) and there was a tendency towards decreased levels of CRP (fig. 2) and IL-6 (fig. 3). These changes were not seen in the placebo group or among the controls (combined in analyses, denoted "non-probiotic").

CONCLUSION

- **Probiotic supplement was well tolerated, no serious adverse events were recorded**
- **Probiotic supplement for 8 weeks significantly reduced D-dimer and possibly also levels of IL-6 and CRP**
- **This reduction was apparently not related to changes in markers of microbial translocation or coagulation, but is presumably a result of decreased inflammation due to other mechanisms. Microbiota and flowcytometry analyses are in progress**

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