Colonic microbiota exhibits disparate associations with HIV infection and sexual practices

21st International Workshop on Co-morbidities and Adverse Drug Reactions in HIV Nov 5-6 2019 Basel

Reported by Jules Levin

E Verheij1,2, O Sortino3,4, I Vujkovic-Cvijin5, J Sklar5, FW Wit1,6, NA Kootstra1,7, J Brenchley4, J Ananworanich2,8,9, Y Belkaid5, M Schim van der Loeff10, P Reiss1,2,6, I Sereti4, the AGEhIV Cohort Study Group
1Amsterdam University Medical Centers, University of Amsterdam, Department of Global Health and Division of Infectious Diseases, Amsterdam Infection and Immunity Institute and Amsterdam Public Health Research Institute, Amsterdam, the Netherlands;
2Amsterdam Institute for Global Health and Development, Amsterdam, the Netherlands;
3Clinical Monitoring Research Program Directorate, Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute;
4National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD, USA;
5MetaorganismImmunity Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA;
6HIV Monitoring Foundation, Amsterdam, the Netherlands;
7Amsterdam University Medical Centers, University of Amsterdam, Department of Experimental Immunology, Amsterdam Infection and Immunity Institute, Amsterdam, the Netherlands;
8The Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, USA;
9United States Military HIV Research Program; Walter Reed Army Institute of Research, Silver Spring, MD, USA;
10Public Health Service of Amsterdam, Department of Infectious Diseases, Amsterdam, the Netherlands

Conclusions

- Treated HIV infection exerts a robust effect on the gut bacterial community across human subject populations, in both men and women, that pervades sexual practice
- MSM microbiota signature is unique and does not overlap with that of HIV infection
- MSM represents a strong confounding variable in PLWH study population
- Receptive anal intercourse, regardless of condom use, is linked with specific gut bacterial communities, in both men and women, which may represent a driving factor for the consistently observed MSM-associated Prevotella-rich community
- HIV-associated microbiota signature correlates with CD4 nadir and prevalent non-communicable comorbidities

Objective: Effective antiretroviral therapy (ART) has prolonged survival and shifted the morbidity spectrum for people living with HIV (PLWH) from AIDS-associated opportunistic infections and malignancies towards age-associated non-communicable comorbidities (AANCCs), with these being more prevalent in PLWH
compared with in age-matched HIV-uninfected individuals. A key contributor to the current disease spectrum includes HIV-associated inflammation and immune activation, the aetiology of which in PLWH remains incompletely defined. Gut microbial dysbiosis is thought to be a potential important contributor, but data thus far are conflicting regarding the role that lifestyle factors, including sexual orientation and behaviour, and HIV-infection itself have on gut microbial dysbiosis.

**Methods:** Using 16S rRNA gene sequencing, we profiled the microbiota from fecal samples of PLWH with suppressed viraemia on ART and HIV-uninfected controls participating in the AGEhIV Cohort Study. PLWH were selected to include 40 men having sex with men (MSM), 20 men having sex with women (MSW) and 20 females (F) matched 1:1 by age, sex, sexual orientation, BMI, birth country and smoking status with HIV-uninfected controls.

**Results:** HIV-infection was associated with alterations in the gut microbiota including an enrichment in *Enterobacteriaceae* and *Desulfovibrionaceae* members and a depletion of short chain fatty acids-producing bacteria such as *Lachnospiraceae* and *Ruminococcaceae*. Furthermore, comparisons between MSM and non-MSM males revealed a unique MSM-associated micro biome signature characterized by an enrichment particularly in *Prevotellaceae* members (Figure 1), which was independent of HIV-infection. Finally, practicing receptive anal intercourse, regardless of condom use, was linked to a specific bacterial community variance independently of sex, which may explain the *Prevotella*-rich microbiome in MSM.

**Conclusions:** Our data provide unique evidence that colonic microbiota exhibit disparate associations with HIV-infection and sexual practices.
Studies show that comorbidities are associated with inflammation, and that inflammation is associated with the gut micro biome.
But what do we know about the microbiome of people living with HIV. In this figure, every column represents a different study, and each row shows which specie is either enriched - indicated by the red arrows - or depleted -as indicated by the blue arrows - in people living with HIV.

Pathogenesis

What are the mechanisms behind the association between the microbiome and HIV-associated inflammation?
First of all, translocation of Proteobacteria is known to activate systemic immunity. Depletion of Clostridiales is associated with less production of short chain fatty acids that maintain a healthy gut epithelium. Its also associated with increased disease severity in Crohn’s disease and leads to experimental colonic inflammation.
However, microbiota shifts in HIV infection, appears to parallel shifts in MSM versus non-MSM, possibly driven by the fact that the HIV positive populations consists mostly of MSM and the majority of the seronegative population is non-MSM.
Begs the question are the differences we’ve found due to HIV infection or due to MSM status?
And the second question I would like to answer is whether the gut microbiota is linked to clinical markers and comorbidity prevalence in PLWH.
In order to answer those questions, we actively selected from our AGEHIV study cohort PLWH who are chronically infected, with suppressed viremia on antiretroviral therapy and matched them 1 on 1 with HIV-uninfected controls. We created 3 subgroups, MSM, men who have sex with women (MSW) and females. They were all matched 1:1 on age, body mass index, and country of birth as we not from other studies that these variable have an impact on the microbiota.
Turning to the results: for each different group I’m going to discuss their microbiome. First we looked at the alpha diversity. Alpha diversity is a measure of how many different bugs are present in the gut. We see that in PLWH, the alpha diversity is decreased in compared to seronegative controls in all three subgroups. Secondly, we looked at beta diversity. The beta diversity is a measure of how different the microbial gut composition is, as compared to another. It reveals clustering of PLWH and HIV-negative controls in all three subgroups.
Importantly, our results mirror that of previous studies. Looking at this vulcanoplot, which shows on the right side of the figure the taxa enriched in PLWH and on the left site the taxa depleted in PLWH as compared to seronegative controls. You can see that Proteobacteria (in red) are enriched and Clostridiales (in blue) are depleted.
What do we see if we look at MSM?
Looking at MSM versus MSW and females, showed in increased alpha diversity in MSM. And the microbiome composition is different from MSM versus MSW

MSM vs MSW microbiota signature is distinct
Looking at this volcano-plot, on the right the species enriched in MSM and on the left species depleted in MSM shows that our results again mirror those of prior studies. Showing enrichment of Prev in MSM and depletion of Bact.

We were of course interested whether the same taxa observed to be enriched in PLWH are the same as those enriched in MSM or conversely if those depleted in PLWH were also depleted in MSM.

In order to do this, we selected the taxa that differed significantly between PLWH and seronegative controls and MSM versus MSW. When we plotted the taxa that overlapped, we surprisingly, saw that these taxa had opposing abundance trends. As you can see from the figure on the right you can see the taxa enriched in MSM and PLWH and the did not overlap. In fact, those enriched in MSM (indicated by the purple arrow) were depleted in HIV (mint green arrow). And vice versa.

**Microbiota shifts in MSM with recent receptive anal intercourse (RAI) vs. MSM without RAI resemble shifts of MSM vs MSW**
We tried to look if having recent anal intercourse had anything to do with the signature. Using the same trick as before, we looked at MSM who reported recent intercourse and we compared them with those without intercourse, and you can see that the shifts resemble that of MSM versus non-MSM.

*RAI = Receptive anal intercourse within 6 months prior to sampling*
Females practicing RAI, illustrated the same trend as MSM who report RAI. The figure on the left shows that the difference of the microbiome of females RAI is more similar to that of MSM/RAI+, females without RAI. Moreover, the taxa enriched in Females with RAI overlapped with taxa enriched in MSM with RAI and again vice versa.
To understand the clinical impact of the HIV-associated microbiome, we constructed a measure which is called the Dysbiosis Index, in which we collapsed into a single number, the shifts in bacterial taxa that were characteristic of PLWH versus seronegative controls. A higher number indicated higher dysbiosis.

So, DI is associated with lower CD4 nadir. Additionally, the index is associated with a higher number of prevalent comorbidities, in all three HIV-infected subgroups. And this effect is independent from CD4 nadir.
Acknowledgements (1)

NIH/NIAID

Ornella Sortino
Ivan Vujkovic-Cvijin
Irini Sereti
Yasmine Belkaid
Jack Sklar
Jason Brenchley

National Institutes of Health
Office of AIDS Research

National Institute of Allergy & Infectious Diseases

CANCER RESEARCH INSTITUTE

Dept. of Global Health,
Amsterdam University Medical Centers

Peter Reiss
Jintanat Ananworanich
Neeltje Koopstra
Ferdinand Wit
Maarten Schim van der Loeff
Anders Boyd