19-21 GIUGNO ROMA
UNIVERSITA' CATTOLICA DEL SACRO CUORE CENTRO CONGRESSI EUROPA
RESEARCH and CARE: FROM BENCH, TO BEDSIDE, TO COMMUNITY
Promosso da SIMIT
16° CONGRESSO NAZIONALE ICAR
Italian Conference on AIDS and Antiviral Research
P 90
POSTER 4 YOU ICAR 2024

Plasma markers of gastrointestinal mucosal barrier dysfunction in PLWH receiving cART

V. Artusa[1], R. Zamarato[1], A. De Nicolò[2], A. D'Avolio[2], L. Benedetti[3], M. Compagno[3], V. Malagnino[3], M. Iannetta[3], L. Duca[4], F. Ceccherini Silberstein[4], M. Biasin[1], D. Trabattoni[1], M.S. Clerici[5,6]

[1]University of Milan, Department of Biomedical and Clinical Sciences, Milan, Italy [2]University of Turin, Department of Medical Sciences, Turin, Italy [3]Tor Vergata University, Department of System Medicine, Rome, Italy [4]Tor Vergata University, Department of Experimental Medicine, Rome, Italy [5]University of Milan, Department of Pathophysiology and Transplantation, Milan, Italy [6]IRCCS Don Carlo Gnocchi Foundation, Milan, Italy

Introduction

Despite receiving effective combined antiretroviral therapy (cART), people living with HIV (PLWH) still encounter elevated rates of morbidity and mortality compared to age-matched uninfected individuals.[1]

HIV-induced mucosal CD4 T cell depletion is accompanied by epithelial barrier dysfunction and increased levels of microbial translocation.[2]

Gut dysfunction is a common feature of HIV infection and can occur at any stage of the disease, it is particularly prevalent in advanced stages of HIV infection. The combination of the structural impairment of the epithelial barrier and mucosal immunodeficiency are critical in driving HIV disease progression.[3]

The invasiveness of anorectal biopsies emphasizes the need for validated non-invasive plasma biomarkers to evaluate gastrointestinal damage in PLWH.

This study aimed to unveil the link between the altered expression of structural and immunological genes at the GI level and the presence of plasma biomarkers indicating gut damage and microbial translocation in PLWH receiving cART.

Study Design

40 PLWH were enrolled in the **MARISA project** (PRIN, Prot. 202074AA2B), and stratified according to their clinical stage (A, B, C) or the cART regimen (BIC- or DTG-based) they were receiving.

Sex (M/MtF)	39 M /1 MtF
Age (years)	44.22 ± 1.45
Current CD4⁺ count (cells/mm³)	807.8 ± 41.13
Current CD8⁺ count (cells/mm³)	851.5 ± 62.17
CD4⁺ nadir (cells/mm³)	385.46 ± 32.79
HIV RNA zenit (copies/mL)	7700 - 10000000<
Current HIV RNA (copies/mL)	TND - 20<
Clinical stage (CS)	A1 22.5%; A2 35%; A3 2.5%; B1 2.5%; B2 17.5%; B3 7.5%; C2 5%; C3 7.5%
cART INSTI/NRTI	BIC/TAF/FTC 52.5%; DTG/TAF/FTC or DTG/ABC/3TC 45%; RAL/TAF/FTC 2.5%
Years of cART (years)	5.22 ± 0.97

Values expressed as mean +/- standard error or range.

Table 1. Clinical characteristics of patients enrolled in the study.

Anorectal biopsy and plasma have been analyzed. Gene expression of 14 structure- and 14 immune-related genes have been assessed via RT-PCR analysis. Plasma biomarkers of intestinal damage, microbial translocation and systemic inflammation have been measured via ELISA assay. Correlation analyses and multiple linear regression models were applied to evaluate potential correlations between patterns of gene expression in the mucosa and the presence of plasma markers.

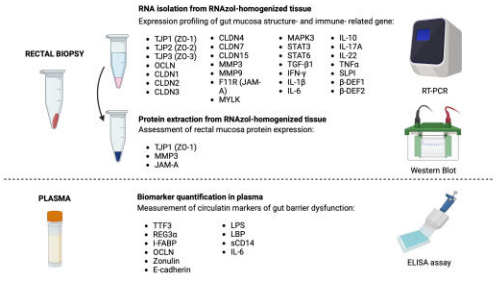

Figure 1. Workflow of experimental analyses. Techniques used for sample analysis: RNA and protein were obtained from the biopsies to study their expression by RT-PCR and Western blot, respectively. Plasmas were analyzed using ELISA tests to check for plasma markers.

Results

Gene Expression Profiling of Rectal Biopsies

Differential mucosal gene expression was observed in PLWH at various clinical stages (**Fig. 2A**). We observed decreased expression of *CLDN3* (N fold A: 929 (612 – 1808) vs. C: 100 (12 - 405), *p*=0.009), *CLDN7* (N fold A: 266 (218 - 403) vs. C: 98 (33 - 107), *p*=0.025) and *CLDN15* (N fold A: 134 (73 - 264) vs. C: 61 (7 - 84), *p*=0.020), *TJP3* gene expression (N fold A: 503.0 (192.5 – 742.0) vs. C: 100.0 (0.000 – 221.5), *p*=0.0263) and increased expression of *IL-22* (N fold A: 0.000 (0.000 – 63.50) vs. C: 246,0 (50.00 – 994.0), *p*=0.0488).

Comparing groups receiving different therapy regimens (**Fig. 2B**), we observed decreased expression of *CLDN3* (N fold BIC: 1157 (678 -1488) vs. DTG: 529 (181 - 1285), *p*=0.046). Additionally, we observed a decrease in *MMP3* transcription between the BIC-based group and the DTG-based group (N fold BIC: 360 (43 - 1245) vs. DTG: 0 (0 - 303), *p*=0.0054). Also, *SLPI* expression differs between the two therapy regimens, being higher in the BIC-based group compared to the DTG-based group (N fold BIC: 302.0 (149.0 – 449.0) vs. DTG: 75.50 (41.50 – 177.5), *p*=0.,0039).

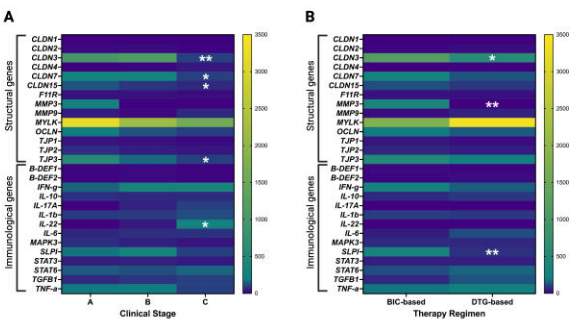

Figure 2. Gene expression profiling of structure- and immune-related genes and correlation analysis between mucosal gene expression and plasma biomarkers. Mucosal gene expression analysis of different groups of subjects stratified using clinical stage (**A**) or cART regimen (**B**) as criteria. *p<0.05 **p<0.01, A vs C; BIC vs DTG.

Measurements of Enhanced Intestinal Permeability and Systemic Inflammation Biomarkers in Plasma

No differences where observed comparing plasma concentration of intestinal permeability and systemic inflammation markers comparing PLWH with different clinical stages (data not shown).

On the contrary, we identified significant differences in plasma soluble CD14 (sCD14) levels between individuals receiving BIC-based therapy and those receiving DTG-based therapy (**Fig. 3**).. Specifically, individuals in the DTG- based therapy group exhibited higher plasma sCD14 levels compared to those in the BIC-based therapy group (BIC: 5.629 (5.014 – 6.909) ng/mL vs. DTG: 6.970 (6.301 – 9.295) ng/mL, *p*=0.0052).

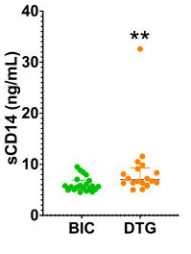

Figure 3. Plasmatic levels of sCD14 in BIC- or DTG-treated PLWH. Scatter plot of the concentration of sCD14 detected by ELISA test, in PLWH stratified according to the therapeutic regimen (BIC: green, DTG: orange).

Results

Correlation Analysis

Spearman's correlation analysis (**Flg. 4A**) showed a number of statistically significant correlations (**Fig. 4B**) amoung plasma markers of gut dysfunction and systemic inflammation and mucosal gene expression.

This data also confirmed the role of clinical stage and therapy regimen in altering mucosal gene expression. Interestingly, we observed a positive correlation between the severe clinical stage and the DTG-based therapy.

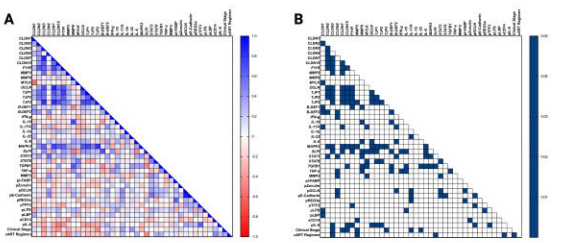

Figure 4. Correlation analysis between mucosal gene expression, plasma markers concentration, clinical stage and therapy regimen. Spearman correlation *r* values (A) and *p* values (B). (A) Blue indicates a positive correlation, conversely, red indicates a negative correlation. The intensity of the color reflects the strength of the relationship between the variables. (B) Dark blue squares identify correlations with *p* values lower than 0.05.

The following graphical representation (**Fig. 5**) show the relationships among plasma biomarkers, mucosal gene expression, clinical stage and therapy regimen.

Nodes with the highest degree of connections are considered the most relevant.

In our analysis, IL-6, OCLN, and E-cadherin exhibit a high degree of connectivity with other nodes, suggesting their central role in influencing various aspects of gut health, systemic inflammation, and disease progression in HIV infection.

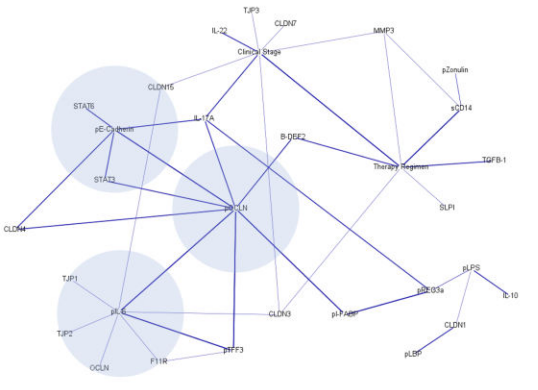

Figure 5. Network graph portraying the relationships between plasma markers, gene expression, clinical stage and therapy regimen. Darker lines denote a positive correlation, while lighter lines refer to a negative relationship. degree of connectivity with other nodes.

Conclusions

Measuring **OCLN**, **E-cadherin** and **IL-6** in plasma offers an indirect evaluation of gut barrier function. These results could help in developing non-invasive diagnostic strategies to assess GI integrity in PLWH and might be useful to identify risk biomarkers for non-AIDS comorbidities.

References

1. Zicari S, Sessa L, Cotugno N, Ruggiero A, Morrocchi E, Concato C, Rocca S, Zangari P, Manno EC, Palma P. Immune Activation, Inflammation, and Non-AIDS Co-Morbidities in HIV-Infected Patients under Long-Term ART. Viruses. 2019 Feb 27;11(3):200. doi: 10.3390/v11030200. PMID: 30818749; PMCID: PMC6466530.
2. Zevin AS, McKinnon L, Burgener A, Klatt NR. Microbial translocation and microbiome dysbiosis in HIV-associated immune activation. Curr Opin HIV AIDS. 2016 Mar;11(2):182-90. doi: 10.1097/COH.0000000000000234. PMID: 26679414; PMCID: PMC4752849.
3. Dillon SM, Frank DN, Wilson CC. The gut microbiome and HIV-1 pathogenesis: a two-way street. AIDS. 2016 Nov 28;30(18):2737-2751. doi: 10.1097/QAD.0000000000001289. PMID: 27755100; PMCID: PMC5101180.