**NameofJournal:***World Journal of Gastroenterology*

**ManuscriptNO:**68607

**ManuscriptType:**ORIGINALARTICLE

***Basic Study***

**Effects of viremia and CD4 recovery on gut “microbiome-immunity” axis in treatment-naïve HIV-1-infected patients undergoing antiretroviral therapy**

Russo E *et al*. Gut“microbiome-immunity”axisin HIV-1 infectedpatients

EddaRusso,GiuliaNannini,GaetanaSterrantino,SebleTekleKiros,VincenzoDiPilato,MarcoCoppi,SimoneBaldi,ElenaNiccolai,FedericaRicci,MatteoRamazzotti,MarcoPallecchi,FilippoLagi,GianMariaRossolini,AlessandroBartoloni,GianlucaBartolucci,AmedeoAmedei

**EddaRusso,GiuliaNannini,GaetanaSterrantino,SebleTekleKiros,MarcoCoppi,SimoneBaldi,ElenaNiccolai,FedericaRicci,FilippoLagi,AlessandroBartoloni,AmedeoAmedei,** DepartmentofClinicalandExperimentalMedicine,UniversityofFlorence,Florence50134,Italy

**VincenzoDiPilato,**DepartmentofSurgicalSciencesandIntegratedDiagnostics,UniversityofGenoa,Genoa16126,Italy

**MatteoRamazzotti,MarcoPallecchi,**DepartmentofBiomedical,ExperimentalandClinical"MarioSerio",UniversityofFlorence,Florence50134,Italy

**GianMariaRossolini,**MicrobiologyandVirologyUnit,FlorenceCareggiUniversityHospital,UniversityofFlorence,Florence50134,Italy

**GianlucaBartolucci,**DepartmentofNeurosciences,Psychology,DrugResearchandChildHealth,UniversityofFlorence,Florence50019,Italy

**Authorcontributions:**RussoEandNanniniGcontributedequallytothework;SterrantinoG,RussoE,andAmedeiAdesignedthestudy;RussoEandSterrantinoGrevisedtheliteratureonthistopic;TekleKirosS,NiccolaiE,RicciF,RussoE,BaldiS, andNanniniGcollectedthedata;RussoE,CoppiM,DiPilatoV,NanniniG,BaldiS,RicciF, andLagiFanalysedthedata;RamazzottiM andDiPilatoVperformedmicrobiotaanalysis;RussoEwrotethemanuscript;RussoEeditedthemanuscript;AmedeiAsupervisedthemanuscript;AmedeiA,SterrantinoG,RossoliniGM,Bartoucci G, andBartoloniArevisedthemanuscript;RussoE,AmedeiA,SterratinoG,BartoloniA, andRossoliniGMprovidedfundingacquisition.

**Supportedby**UniversityofFlorence, No. XXXVPhdProgram.

**Correspondingauthor:AmedeoAmedei,BSc,PhD,Professor,Reader(AssociateProfessor),**DepartmentofClinicalandExperimentalMedicine,UniversityofFlorence,VialePieraccini,6,Florence50139,Italy.aamedei@unifi.it

**Received:**May28,2021

**Revised:**July 30, 2021

**Accepted:** January 13, 2022

**Publishedonline:**February 14, 2022

**Abstract**

BACKGROUND

Human immunodeficiency virus type 1 (HIV-1)infectionischaracterizedbypersistentsystemicinflammationandimmuneactivation,eveninpatientsreceivingeffectiveantiretroviraltherapy(ART).Convergingdatafrommanycross-sectionalstudiessuggestthat gutmicrobiota(GM)changescanoccurthroughoutincludinghumanimmunodeficiencyvirus(HIV)infection,treatedbyART;however,theresultsarecontrasting.Forthefirsttime,wecomparedthefecalmicrobialcomposition,serum andfecalmicrobialmetabolites,andserumcytokineprofileof treatment*-*naïvepatientsbeforestartingARTandafterreachingvirologicalsuppression,after24wkofARTtherapy.Inaddition,wecomparedthemicrobiotacomposition,microbialmetabolites,andcytokine profileofpatientswithCD4/CD8ratio< 1(immunologicalnon-responders [INRs])andCD4/CD8> 1(immunologicalresponders [IRs]),after24wkofARTtherapy.

AIM

Tocompareforthefirsttimethefecalmicrobialcomposition,serumandfecalmicrobialmetabolites,andserumcytokineprofileoftreatment*-*naïvepatientsbeforestartingARTandafterreachingvirologicalsuppression(HIVRNA<50copies/mL)after24wkofART.

METHODS

Weenrolled12treatment*-*naïve HIV-infectedpatientsreceivingART(mainlybasedonintegraseinhibitors).Fecalmicrobiotacompositionwasassessedthroughnextgenerationsequencing.Inaddition,acomprehensiveanalysisofabloodbroad-spectrumcytokine panelwasperformedthroughamultiplexapproach.Atthesametime,serumfreefattyacid(FFA)andfecalshortchainfattyacid levelswereobtainedthroughgas chromatography-mass spectrometry.

RESULTS

Wefirstcomparedmicrobiotasignatures,FFAlevels,andcytokineprofilebeforestartingARTandafterreachingvirologicalsuppression.Modestalterationswereobservedinmicrobiotacomposition,inparticularin theviralsuppressioncondition,wedetectedanincreaseof *Ruminococcus* and *Succinivibrio* andadecreaseof *Intestinibacter*.Moreover,inthesamecondition,wealsoobservedaugmentedlevelsofserumpropionicandbutyricacids.Contemporarily,areductionofserumIP-10andanincreaseofIL-8levelsweredetected in theviralsuppressioncondition.In addition,thesamecomponentswerecomparedbetweenIRsandINRs.Concerningthemicroflorapopulation,wedetectedareductionof *Faecalibacterium* andanincreaseof *Alistipes* inINRs.Simultaneously,fecalisobutyric,isovaleric,and2-methylbutyricacidswerealsoincreasedinINRs.

CONCLUSION

OurresultsprovidedanadditionalperspectiveabouttheimpactofHIVinfection,ART,andimmunerecoveryonthe“microbiome-immunityaxis”atthemetabolismlevel.Thesefactorscanactasindicatorsoftheactiveprocessesoccurringinthegastrointestinaltract.IndividualswithHIV-1infection,beforeARTandafterreachingvirologicalsuppressionwith24wkofART,displayedamicrobiotawithunchangedoverallbacterialdiversity;moreover,theirsystemicinflammatorystatusseems nottobe completelyrestored.Inaddition,weconfirmedtheroleoftheGMmetabolitesinimmunereconstitution.

**KeyWords:**HIV;Antiretroviraltherapy;Microbiome-immunityaxis;Microbiota;Cytokines;Shortchainfattyacid;Inflammation;Immunologicalresponders;Viremia

**©The** **Author(s) 2022.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Citation:** RussoE,NanniniG,SterrantinoG,KirosST,PilatoVD,CoppiM,BaldiS,NiccolaiE,RicciF,RamazzottiM,PallecchiM,LagiF,RossoliniGM,BartoloniA,BartolucciG,AmedeiA.Effects of viremia and CD4 recovery on gut “microbiome-immunity” axis in treatment-naïve HIV-1-infected patients undergoing antiretroviral therapy. *World J Gastroenterol* 2022;28(6): 635-652

**URL:** https://www.wjgnet.com/1007-9327/full/v28/i6/635.htm

**DOI:** https://dx.doi.org/10.3748/wjg.v28.i6.635

**CoreTip:**Eveninpatientsreceivingeffectiveantiretroviraltherapy(ART),human immunodeficiency virus type 1infectionischaracterizedbypersistentsystemicinflammationandimmuneactivation.ChangesinthegutmicrobiotacanoccurwithincludinghumanimmunodeficiencyvirusinfectionandtreatmentwithART;however,thedataarestillconflicting.Forthesereasons,wecomparedthefecalmicrobialcompositionandserumcytokine profileoftreatment*-*naïvepatientsbeforestartingARTandaftervirologicalsuppression.Finally,weevaluatedthemicrobiotacomposition,microbialmetabolites,andcytokine profileofpatientswithCD4/CD8ratio< 1andCD4/CD8> 1(immunologicalresponders).

**INTRODUCTION**

Themutualinteractionbetweenthehumanmicrobiotaandtheimmunesystemdefinestheso*-*called “microbiome-immuneaxis”.Thisaxishasalsobeenassociatedwithseveraldiseases,includinghumanimmunodeficiencyvirus(HIV)infection[1].Indeed,akeyplaceforHIVreplicationisthegastrointestinaltract. HIVreplicationinthegastrointestinaltractresultsinaseveredepletionofCD4+Tcellsthatleadstodecreasedfunctionoftheepithelialbarrier,allowingmicrobesandmicrobialproductstobetranslocated,whichcontributestothechronicinflammatoryresponse[2].HIVreplicationcanalsoresultinamicrobialdysbiosiscondition[3-5],whichhasbeencorrelatedwithincreasesinmarkersofdiseaseprogression,immuneactivation,andmicrobialtranslocation[3,5-7].Notably,HIV-infectedpeopleharbouradistinctgutmicrobiota (GM)[8,9]witha *Prevotella-*richcommunitycomposition,typicallyobservedinindividualsfromagrarianculturesorwithcarbohydrate-rich,protein-andfat-poordiets[10].Inaddition,thesignificantsubversionofthe *Bacteroidetes* and *Proteobacteria* phyla,withanimbalanced *Prevotella/Bacteroides* speciesratioandanabundancein *Enterobacteriaceae,* isoneofthemostpersistentchangesdocumentedinuntreatedHIVinfection[11-13].Moreover,theincreasednumberofgut-residentbacteriacapableofdirectlyproducinginflammationcanbeaprobablemechanisticlinkbetweenHIV-associateddysbiosisandhighsystemicimmuneactivation[14].However,convergingdatafromcross-sectionalstudiessuggestthattheGMcompositionanditsrelatedimmuneresponsecanchangeovertheprogressionofHIVinfection.Inparticular,correlatingthecompositionofthegastrointestinaltractmicrobiometoimmuneactivation,circulatingbacterialproductsandclinicalparameters,adecreaseofcommensalspecies,andagainofpathogenictaxawasobservedinHIV+subjectscomparedtocontrols[15].Additionally,analysingthefunctionalgenecontentof theGMinHIV+patientsandthemetabolicpathwaysofthebacterialcommunityassociatedwithimmunedysfunction,themetagenomesequencingrevealedanalteredfunctionalprofilewithsignificantinteractionsbetweenthebacterialcommunity,theiralteredmetabolicpathways,andsystemicmarkersofimmunedysfunction[16].Furthermore,analysingtheassociationsbetweenthe innatelymphoidcell (ILC)cytokinesandmeasuresofvirologic,immunologic,andmicrobiomeindices,itwasobservedthatinflammatoryILCscontributetogutmucosalinflammationandepithelialbarrierbreakdown,importantfeaturesofHIV-1mucosalpathogenesis[17].DespitegrowingevidencethattheGMhasaroleinHIVpathogenesis[11,18-20],theresults werecontrasting,withsomestudiessuggestinganinfluenceandothersnoHIVinfluenceonmicrobialdiversity[1,21]andcomposition[22,23].However,manystudiesontheGMinHIV-infectedpatientsareoftencarriedoutwith alackofadjustmentforconfoundingfactors,suchasdietanduseofdrugs[24,25].

Currently,antiretroviraltherapy(ART)hasincreasedthelifeexpectancyofHIV-infectedpatients,approximatingittothatofthegeneralpopulation[26].Interestingly,chronicinflammationandGMalterationspersistinpatientsvirologicallysuppressedbyART[27].Thesedataimplicatethatre-shapingthemicrobiotamaybeanadjuvanttherapyinpatientscommencingsuccessfulART[28].Ontheotherhand,suppressiveARTappearstohavealimitedeffectontherestorationof theGM[13,25,29,30].AlthoughthegutmicrobialcompositionofART-treatedpeoplediffersfromthatofuntreatedpeople,theformeralsohaveadifferentmicrobialcommunitystructure compared totheHIV-uninfectedpopulation[31,32].ThesefindingsraisethepossibilitythatpersistentgutdysbiosismayplayaroleinthedevelopmentofresidualclinicalillnessafterART.

Currently,theCD4/CD8ratioisconsidered oneofthebest-usedmarkersofimmunereconstitution.Notably,alowCD4/CD8ratioisassociatedwithanincreasedriskofnon-AIDS-relateddiseases[33].Furthermore,thedifferencesbetweentheelementsofthemicrobiome-immuneaxisbetweenpatients withnormalizedornon*-*normalized CD4/CD8ratioduringARThavenotbeenelucidatedsofar[34,35];however,thisquestionisrecognizedasacurrentresearchgap.

Moreover,withabetterunderstandingofthemicrobiota-immuneaxis,itisnowknownthatinadditiontotheintestinalfloraitself,itsmetabolitesarealsoinvolvedinregulatingvitalhostactivities,suchasenergymetabolism,cell-to-cellcommunication,andhostimmunity.Short-chainfattyacids(SCFAs)areimportantmetabolitesabletomodulatetheproductionofimmunemediators,suchaskeycytokinesfortherepairandmaintenanceofepitheliumintegrity[36].Inaddition,theSCFAsmodulatetheactivityofTcellsanddecreasetheoverexpressionofhistonedeacetylase,particularlybutyricandvalericacids[37].SCFAsareanimportantlinkbetweenmicrofloraandtheimmunesystem;theyinvolvedifferentmolecularmechanismsandcellulartargets,areessentialforthemaintenanceofintestinalhomeostasis,andfinallyplayaroleinHIVinfection[38].

Thepurposeofthisprospectiveobservationalstudywastocompareforthefirsttimethefecalmicrobialcomposition,serumandfecalmicrobialmetabolites,andserumcytokineprofileoftreatment*-*naïvepatientsbeforestartingARTandafterreachingvirologicalsuppression(HIVRNA<50copies/mL)after24wkofART.AnadditionalaimwastocorrelatetheGMcomposition,microbialmetabolites,andcytokineprofileofpatientswithCD4/CD8ratio<1andCD4/CD8>1afterantiretroviraltherapy.

**MATERIALSANDMETHODS**

***Patients***

Thestudypopulation,composedof12treatment*-*naïve HIV-infectedpatients receivingARTmainlybasedonintegraseinhibitors,wasenrolledbetweenApril2018andMay2019attheDepartmentofInfectiveandTropicalDiseaseatUniversityHospitalofCareggi,Florence,Italy(Table1).Thestudywasapprovedbylocalinstitutionalreviewboardsandwritteninformedconsentwasobtainedfrompatientsbeforeparticipation(RifCEAVC15035).

Weconductedaprospectiveobservationalcohortstudycomparingthechangesoccurringinthefecalmicrobiota,serumandfecalSCFA,serumfreefattyacids(FFAs),andserumcytokinesofpatientswithHIV-1infectionbeforeART(T0)andafter24wk(T1).Inaddition,patientsweredividedintotwogroups according towhethertheywereimmunologicalresponders(IRs, *n* =6)ornot(INRs, *n* =6)(INRsandIRs,basedonthenormalizationofCD4/CD8ratio:<1or≥1after24wkofART,respectively).Patientswhohadusedantibiotics,probiotics,orprebioticsorhadexperienceddiarrhoeaordigestivesymptomswithintheprevious1mo wereexcluded.

Personaldata,ARTregimen,HIV-RNAvalues,andnumberofCD4+andCD8+TcellspriortoARTstarting andatthetimeofvirologicsuppression wereincludedintheanalysis(Table1).Inthispilotexploratorystudy,noformalsamplesizecalculationwasperformed.AllpatientsfollowedaMediterraneandiet.

Plasma HIV-RNA wasmeasured usingTestv1.5RocheCOBASAmpliPrep,RocheTaqManHIV-1Testv2.0(RocheDiagnostics,Branchburg,NJ,UnitedStates)andSiemensVersantKPCR(SiemensHealthcareGmbH,Erlangen,Germany),withlowerlimitsofdetectionof50,20,and37copies/mL,respectively.

TheTcellcountsofpatientsweredeterminedusingaFACScantoflowcytometer(BDImmunocytometrySystems)[10].Immunophenotypingofperipheralbloodlymphocyteswasanalysedbythree-colorflowcytometry(EpicsXLFlowCytometry System;BeckmanCoulter,UnitedStates)aspreviouslydescribed[39].FreshlycollectedEDTAanticoagulatedwholebloodwasincubatedandtestedwithapanelofmonoclonalantibodiesdirectedagainstfluoresceinisothiocyanate/phycoerythrin/peridininchlorophyllproteincombinationsofCD3/CD4/CD8,CD3/CD16CD56/CD19,HLA-DR/CD8/CD38,andCD4/CD8/CD28andisotypecontrols(Immunotech,France).

Ateachtimepoint(0 and24wkafterstudyenrolment),wecollectedbloodandfecalsamples.Aftercollection,stoolsampleswereimmediatelyfrozenandstoredat−80 °CuntilDNAextraction.FecalsampleswereusedtoassessthemicrobiotacompositionandSCFAs, andwhilebloodsampleswereusedtomeasureSCFAsandFFAsandapanelof27selectedcytokines.

***Study follow-up***

Patientsunderwent medicalvisitsat0 and24 wk afterstudyenrolment.They alsounderwentacomprehensivephysicalexaminationandmedicalhistory inquiry,urinetoxicologypanel testing,clinicallaboratorytestsincludingplasmaHIVRNA,specimencollection,anddetailedbehaviouralquestionnaire survey.Demographicandclinicaldatawerecollectedinaspecificquestionnaireandreportedinanappropriatedatabase, includingthetimepointoffollow-upinmonths;theparticipant’sgender,age,weight,andheight;CD4+andCD8+Tcellcounts;theCD4/CD8ratio;HIV-1RNAlevels,art,andantibioticuse.Ifsubjectshadtostartantibiotics,theyprovidedalastfecalsampleandthestudyfollow-upwasimmediatelyterminated

***Fecal microbiota characterization***

TotalgenomicDNAwasextractedfromfrozen(-80 °C)stoolsamples,collectedatdifferenttimepoints(weeks0 and24; T0 and T24),usingtheDNeasyPowerLyzerPowerSoilKit(Qiagen,Hilden,Germany)accordingtothemanufacturer’sinstructions.ThequalityandquantityofpurifiedDNAwereassessedusingtheNanoDropND-1000(ThermoFisherScientific,WalthAP,US)andtheQubitFluorometer(ThermoFisherScientific),respectively.

ExtractedDNAsamplesweresenttoIGATechnologyServices(Udine,Italy)whereampliconsofthevariableV3–V4regionofthebacterial16SrRNAgeneweresequenced(2×300bppaired-end)ontheIlluminaMiSeqplatform,accordingtotheIllumina16SMetagenomicSequencingLibraryPreparationprotocol[40].

SequencingresultswereanalysedusingtheQIIME2suite(QuantitativeInsightsIntoMicrobialEcology)[41].Briefly,followingrawreadsdenoising(*i.e.*,estimationoferrorrates,removalofchimericandsingletonsequences, andjoinofdenoisedpaired-endreads)usingDADA2(DivisiveAmpliconDenoisingAlgorithm2)[42],denoisedreadsweredereplicatedandampliconsequencevariants(ASVs)wereinferred.TaxonomicclassificationofinferredASVswasperformedusingaNaiveBayesclassifiertrainedontheSILVA16Sreferencedatabase(release132)(https://www.arb-silva.de/documentation/release-132/).

***Evaluation of fecal short chain fatty acids and serum free fatty acids by gas chromatography-mass spectrometry***

Thefecal SCFAs,inparticularacetic,propionic,butyric,isobutyric,isovaleric,2-methylbutyric,valeric,andhexanoicacids,wereanalyzed using anAgilentGC-MSsystemcomposedwith a5971singlequadrupolemassspectrometer, a5890gas-chromatograph,and a7673autosampler.Thechemicals,GC-MSconditions,andcalibrationsparametersarereportedinsupportinginformation(Tables S1-S4)[43].Fecalsampleswerecollectedin15*-*mLFalcontubesandstoredat-80°C.Justbeforetheanalysis,eachsamplewasthawed,weighted(between0.5-1.0g),andaddedtosodiumbicarbonate10mmol/Lsolution(1:1w/v)ina1.5mLcentrifugetube.Theobtainedsuspensionwasbrieflystirredinavortexapparatus,extractedinanultrasonicbath(for5min),andthencentrifugedat5000rpm(for10min).Thesupernatantwascollectedandtransferredinto a1.5mLcentrifugetube(samplesolution).TheSCFAswerefinallyextractedasfollows:Analiquotof100µLofsamplesolutionwasaddedto50μLofinternal standardmixture,1mLoftert-butylmethylether,and50µLof1.0mol/LHClsolutionin a1.5mLcentrifugetube.Afterwards,eachtubewasshakeninavortexapparatusfor2min andcentrifugedat10000rpmfor5min,andfinallythesolventlayerwastransferredinto anautosamplervialandanalyzedby theGC-MSmethod.Eachsamplewaspreparedandprocessed,bythemethoddescribedabove,threetimes.Inaddition,serum FFAs,classifiedasSCFAs(acetic,propionic,butyric,isobutyricisovaleric,2-methylbutyri,andvalericacids),mediumchainfattyacids(MCFAs;hexanoic,heptanoic,octanoic,nonanoic,decanoic,anddodecanoicacids),andlongchainfattyacids(LCFAs;tetradecanoic,hexadecanoic,andoctadecanoicacids)wereanalyzedwithourpreviousdescribedGC-MSprotocol[44].Thechemicals,GC-MSconditions,GC-MSmethod,andcalibrationsparametersarereportedinsupportinginformation(TablesS5-S7).

Justbeforetheanalysis,eachsamplewasthawed.TheFFAswereextractedasfollows:Analiquotof300µLofplasmasamplewasaddedto10μLofinternal standard mixture,100μLoftert-butylmethylether,and20µLof6MHClplus0.5mol/LNaClsolutionin a0.5mLcentrifugetube.Afterwards,eachtubewasstirredinvortexfor2min andcentrifugedat10000rpmfor5min,andfinallythesolventlayerwastransferredinto avialwith amicrovolumeinsertandanalyzed.

***Molecular inflammatory response in serum***

Theinflammatoryresponse inserumsamplesofpatientsandhealthycontrolswas evaluated using aspecificallyassembledkitProCartaPlexMixMatchHuman27PanelforLuminexMAGPIXdetectionsystem(Affymetrix,eBioscience) followingthemanufacturers'instructions.

Indetail,thepanelincludedmacrophageinflammatoryprotein-1α(MIP-1α),interleukin(IL)-27,IL-1β,IL-2,IL-4,IL-5,interferongamma-inducedprotein10(IP-10),IL-6,IL-8,IL-10,IL-12p70,IL-13,IL-17A,interferon(IFN)-γ,IFN-α,tumornecrosisfactor-α(TNF-α),granulocyte-macrophagecolonystimulatingfactor(GM-CSF),monocytechemotacticprotein1(MCP-1),IL-9,P-selectin,IL-1α,IL-23,IL-18,IL-21,solubleintercellularadhesionmolecule-1(sICAM-1),IL-22,andE-selectin.

Allmeasurementswereperformedinablindedmannerbyalaboratorytechnicianwhowasexperiencedinexecutingthetechnique.Thelevelsofcytokineswereestimatedusinga5-parameterpolynomialcurve(ProcartaPlexAnalyst1.0)**.**Avalueunderthelowlimitofquantification(LLOQ)wasconsideredas0pg/mL.

***Statistical analysis***

StatisticalanalysesonASVsrepresentingthebacterialcommunitywereperformedinR(RCoreTeam,2014)withthehelpofthepackagesphyloseq1.26.1[45] andDESeq21.22.2[46],andotherpackagessatisfyingtheirdependencies,inparticularvegan2.5-5[47].RarefactionanalysisonASVswasperformedusingthefunctionrarecurve(step50reads), andfurtherprocessedtohighlightsaturatedsamples(arbitrarilydefinedassaturatedsampleswithafinalslopeintherarefactioncurvewithanincrementinASVnumberperreads<1e-5).Fortheclusteranalysis(completeclusteringoneuclideandistance)oftheentirecommunity,theOTUtablewasfirstnormalizedusingthetotalASVcountsofeachsampleandthenadjustedusingsquareroottransformation.ThecoveragewascalculatedbyGood'sestimatorusingtheformula (1- *n*/*N*)×100,where *n* isthenumberofsequencesfoundonceinasample(singletons),and *N* isthetotalnumberofsequencesinthatsample.

Richness,Shannon,Chao1,andevennessindiceswereusedtoestimatebacterialdiversityineachsampleusingthefunctionestimate\_richnessfromphyloseq[45].TheevennessindexwascalculatedusingtheformulaE=S/Log(R),whereSistheShannondiversityindexandRisthenumberofASVsinthesample.DifferencesinallindicesweretestedusingapairedWilcoxonsigned-ranktest.ThedifferentialanalysisofabundanceattheASVsaswellasatthedifferenttaxonomicranks(createdusingthetax\_glomfunctioninphyloseq)wasperformedwithDESeq2[46]usingatwogroupblockedbypatientdesigninordertoperformapairedtest[48].

Inaddition,thesoftwareGraphPadPrism(v.5)andStatgraphicsCenturionXVIsoftwarewereusedforimmunological dataanalysis.Numericaldataarepresentedas themean±SD.Theconcentrationsofseveralcytokinesinsomeofthesampleslaybelowthecurvefitofthestandards.Toavoidthebiasthatwouldhavebeenintroducedbyexcludingthesedata,theconcentrationsoftheimplicatedcytokineweresetathalfofthelowercutoffofthetestsystem,whichwasusuallyabout1pg/mL.Outliersattheotherendofthespectrum(higherthanthemean±SD)wereidentified *via* boxplotsandwereexcludedfromthestatisticalanalysis.ThecomparisonsbetweendependentgroupswereevaluatedbytheWilcoxonmatchedpairstest,whilethecomparisonsbetweentheindependentgroupswereassessedbytheMann-Whitneytest. A *P* valuelessthan0.05wereconsideredstatisticallysignificant.

***Data availability statement***

The16SrRNAsequencedatasethasbeendepositedintheNCBISequenceReadArchive(SRA)databaseandisavailableundertheBioProjectaccessionnumberPRJNA731648.

**RESULTS**

***Comparison of fecal microbiota and metabolic and inflammatory profiles after ART***

**ModestdifferencesinspecificfecalmicrobiotataxaassociatedwithHIVviremia:**Inthefirstpartofourstudy,wecomparedthefecalmicrobiota andmetabolicandinflammatoryprofilebeforeandafterARTstarting,inordertoexaminepotentialchangesresultingfromHIVinfectionand ARTtherapy.WefirstanalysedthelongitudinalvariationoffecalmicrobiotapopulationinthesamepatientsatT0(HIV+viremia- RNA>50copies/mL),definedas“highviremia”condition,andT24(HIV+suppression-RNA≤50copies/mL),definedas“viralsuppression”condition.ThealphadiversityofsamplesdidnotdisplaysignificantdifferencesforChao,Shannon, andevenness indices(Figure1).Theanalysisofthetaxonomiccompositionrevealedthatmorethan99%ofthesequencescollectedwereclassifiedintofourphyla: *Firmicutes* (65.46%), *Bacteroidetes* (21.54%), *Actinobacteria* (9.40%), and *Proteobacteria* (2.72%).Inordertoinvestigatesimilarityofpatients’microbiotaabundanceprofilesandtostudythepairednatureofsampling(*i.e.*,highviremiacondition *vs* viralsuppressioncondition),aclusteranalysisand PCoAonnormalizedASVcountswereperformed.

Thehierarchicalclusteringevidencedthatmicrobiotawasnotsufficientlyalteredaftertreatment(24wk)tobreakindividualcompositionsapart,resultinginaperfectmatchingofthetwotimepointsfromthesamepatient(Figure2A).ThisresultwasalsoconfirmedbythePCoA(Figure2B), whichshowedasubstantialproximityofeachpatientatT0andT24,indicatingthat,overall,theabundanceprofileofthesinglepatientwasnotaffectedbythe24*-*wktherapy.

Ontheotherhand,thepairedcomparisonoftheabundanceofsinglemicrobialranksrevealedsomesignificant(adj. *p* <0.05,abs(logFC)≥1)differencesbetweenthetwosamplesgroups.Inparticular,thegenera *Ruminococcus 2* and *Succinivibrio* werefoundtobesignificantlyincreasedinhigherviralsuppressioncondition.Onthecontrary,viralsuppressionwasrelatedwithadecreaseinthe *Intestinibacter* genus(medianabundance,~1%)(Figure3).

**AnalysisoffecalSCFAs displaysnodifferentlayoutbetween“highviremia”and“viralsuppression”conditions:**Aswenoticedminorchangesinfecalmicrobiomeprofile(justattheorderandgenuslevels),wewonderediftheGMmetabolicactivityhad beenalteredaswell,andwhetherthisactivitymightbemaskedbysimplyexaminingthemicrobiotacomposition.InordertoevaluatethepresenceofalterationsinGMmetabolicactivity,thelevelsofmicrobiallinearandbranchedSCFAsweremeasured infecalsamplesforeachpatient.However,theanalysisof linearSCFA(acetic,propionic,butyric, andvalericacids),andbranchedSCFA(isobutyric,isovaleric, and2*-*metilbutyricacids)abundancedidnotrevealanysignificantchangeafter24wkoftherapyforeachpatient.

**AnalysisofserumFFAs revealsasignificantlydifferentsubgroupofSCFAsbetween“highviremia”and“viralsuppression”conditions:**AswedidnotreportalterationsinthecompositionoffecalSCFAs,wewantedtoobserveiftherewereanyotheralterationsinmetabolicoutput,byanalyzingbothmicrobialandhostderivedFFAsinserum.Asknown,theimpairmentofgutintegrityduetodysbiosiscondition,leadstotranslocationofmicrobialelementsfromtheintestinalmucosatothebloodstream,whichisconsideredamajordrivingforceofchronicimmuneactivation[49]eveninpatientssuccessfullytreatedwithARTandachievingstablevirologicalsuppression[2].

TheanalysisofserumFFAlevelsshowedasignificantchangeoftwoSCFAsatT24comparedtothebaseline.Inparticular,propionicandbutyricacidswereincreasedinviralsuppressioncondition(Figure4).

**Inflammatoryprofilebetweenhighviremiaandviralsuppressionconditions:**Asknown,gutmicrobialdysbiosisislinkedtoaberrantimmuneresponses,asalterationsintheGMmayinducetheinterruptionofgutepithelialbarrierintegritywithsubsequentmicrobialtranslocation,increasedinflammation,andimmuneactivation,whichareoftenaccompaniedbyabnormaldifferentiationofimmunologicalcells[6,50].Sincewedetectedsignificantvariationsofmicrobialcommunitiesbetweenhighviremiaandviralsuppressionconditions,wedecidedtocharacterizealsotheserumimmunologicalprofile byevaluatingapanelof27cytokinesbetweenthetwomentionedconditions.Amongthe27cytokinesexamined,wedetectedasignificantreductionofIP-10(*p* =0.0244)andasignificantincrementofIL-8levels(*P* =0.0547)in thehighviremiasetting(Figure5).

***Association of GM composition and metabolic and inflammatory profiles with CD4+ T-cell counts***

**CorrelationbetweenfecalmicrobiotaandCD4/CD8ratio:**Inthesecondpartofourstudy,wedividedourcohortofpatientsintotwogroups:Immunologicalresponders(IRs)andimmunologicalnon-responders(INRs),basedon the CD4/CD8ratio>1or<1.Inthiscondition,theanalysisofmicrobiotarevealedthat,consideringonlytaxawithanoverallabundancehigherthan1%,membersofthe *Faecalibacteria* genusweresignificantlyreduced(adj. *p* <0.05,logFC=1.32)whilemembersofthe *Alistipes* genusweresignificantlyincreasedinresponders(adj. *p* <0.05,logFC=2.5) (Figure 6).

**DifferentbranchedSCFAprofiles inserumandfecalsamples betweenIRsandINRs:**AsweobservedsignificantvariationsinthecompositionofthefecalmicrobiotabetweenIRsandINRs,weassessediftherewereanyotheralterations inthefecalandserummicrobialmetabolitesaslinearandbranchedSCFAsderivedfrombacterialmetabolism.Wedocumentedsignificantchangesinisobutyric(*p =* 0.01),isovaleric(*p =* 0.04),and2-methylbutyric(*p =* 0.04)acids,whichwereincreasedinIRfecalsampleswhile wedidnotdetectsignificantdifferencesinserumsamples(Figure7).

**Inflammatoryprofileshowsnosignificantdifferences betweenIRsandINRs:**SincewedetectedsignificantvariationsofmicrobialcommunitiesbetweenIRsandINRs,wealsoevaluatedtheserumimmunologicalprofile.However,cytokinelevelsdidnotshowsignificantvariationsbetweenthe IRsandINRs.

**DISCUSSION**

Currently,themechanismsregulatingtheinterplaybetweenthehostimmunesystemandHIV-1,aswellastheexactchangesoccurringintheGMcompositionandfunctionality,remaintobedefined.Toclarifytheintricaterelationshipsbetweentheactorsofthe“microbiota-immunity”axis,weexaminedmicrobiotacompositionandfunctionality(SCFAs),seruminflammatoryresponse,andFFAcompositioninindividualsundergoing ARTindifferentHIVinfectionsettings.

Today,manystudiesonmicrobiotahavebeenperformedchieflycomparingHIV-infectedanduninfectedindividuals,revealingareducedGMdiversity(theso-calledHIV-associateddysbiosis)andanindependentassociationbetweenalpha-diversityofmicrobiotaandperipherallevelsofCD4+Tcellcountintreatment*-*naïveHIV*-*infectedpatients[28].However,cross-sectionalstudiesmaynotbesuitabletoprovideinformationaboutcause-and-effectrelationships,whereaslongitudinalonescouldbemorevalidforexaminingsuchrelationships.Besides,thereisalackofhumanlongitudinalobservationsofthe“microbiota-immunity”axisbeforeandafterfirstARTadministration.Onlyinfewlongitudinalstudies,whereHIV-1-infectedparticipantswerefollowedafterARTstarting,dataobtainedonbacterialflorashowedthatshiftsinthefecalmicrobiotapersistedinanumberofpatients[10,28].Ontheotherhand,arecentstudybyDillon *et al*[14]failedtofindasignificantchangeinasingletimepointstudyofthestoolofHIV-1-infectedpatients.

Inthisstudy,wefirstperformedalongitudinalinvestigationevaluatingtheGMbeforethetreatmentandafter“viralsuppression”(T24).AccordingtothelongitudinalstudyconductedbyDillon *et al*[14],ourresultsshowedmodestchangesintheGMcompositionafterART;indeed,wedidnotassesssignificantdifferencesinphylumcomposition.However,thepairedcomparisonoftheabundanceofsinglebacterialtaxarevealed asignificantalterationatthegenuslevelbetweenthetwosamplegroups(Figure3).Inparticular,thegeneraof *Ruminococcus,* and *Succinivibrio* weresignificantlyincreasedafterARTandtheviralsuppression.Conversely,thegenusof *Intestinibacter* was significantlydecreasedinthesamecondition.Wehypothesizethattheslightchangebetweenthetwogroupsmaybeduetopersistentinflammation(relatedtomicrobialtranslocationandreducedimmunoregulatoryfunction),HIVlatencythroughoutthegut,anddirecteffectsofantiretroviraldrugsonthebacterialpopulation.Moreover,ourresultsareinaccordancewithotherlongitudinalpreviousstudiesinnon-humanprimates,whichallowedtocontrolforconfoundersaffectinghumanstudies[51,52].Wealsoreportedanincreaseofthegenus *Succinivibrio* (*Proteobacteria* phylum)betweenthetwosamplesgroups.Inaddition,inagreementwithourdata,theproportionoftheraregenus *Succinivibrio*,wasalsofoundconsiderablyhighinthestoolofJapanesepatientstreatedwithART[53].Oneofthepossiblereasonsforthecontradictoryresultsreportedintheexamineddifferentstudiesmayincludethecross-sectionalnatureofthestudy,theusedsamplingmethod(stoolswab *vs* stool),andthemicrobialtaxonlevelapplied.

Basedonourfindings,the24wkofARTinhibitedHIV-1viralreplicationeffectively(indeed,allenrolledpatientsreachedviralsuppression),butdidnotheavilyaffecttheoverallbacterialcompositionofthegutmicroenvironment.ThemodestGMdiversitythatweobservedbetweenthetwosamplegroupsmightbeassociatedwiththeloweringofviremia.However,therewasevidencethatARTalsoinduceschangesinthegutmicrobiome,unrelatedtoHIVinfection.SomeauthorshaveimpliedthatARTmayenhancedysbiosis,whichisconsistentwiththehighfrequencyofgastrointestinalsideeffectsofthistreatment[28,54].

AstheGMinfluencestheimmunesystemthroughtheirbacterialmetabolites,likeSCFAs[55,56]*,* wemeasuredSCFAlevelsinbloodandstoolsamples,inordertohaveamoreaccurateassessmentofmicrobialmetabolismaftertheART.Asknown,themainSCFAsinclude,inorderofproportion,acetic,propionic,andbutyricacidsthatareproducedbyfibresfermentationbygutbacteria,particularlybymembersofthe *Firmicutes* phylum[57].Interestingly,forthefirsttime,weobservedasignificantchangeoftwoserumSCFAsaftertheART.Inparticular,propionicandbutyricacidswereincreasedin“viralsuppression”condition.ThisalteredSCFAprofilemayindicateapotentialrolefortheSCFA synthesispathwayintheregulationoftheHIV“microbiota-immunity”axisduringeffectiveART.Notably,wedidnotobserveanysignificantSCFAchangesinstoolsamples,probablybecauseinthecolon,about95%oftheproducedSCFAsarerapidlyabsorbedbylargeintestinalmucosalcellswhiletheremaining5%aresecretedinthefeces[58].Propionateisonlypresentat alowconcentrationintheperipherybecauseitismetabolizedintheliver[59].IthasbeenshownthatbutyratemayreducegutinflammationbyinducingtheregulatoryTcells(Tregs)andmodulatingactivationofantigen-presentingcells[17].Wemayspeculatethatbacterialflorarespondsreciprocallytoinflammationbyincreasingthebiosynthesisofanti-inflammatoryandpro-solvinglipidmediatorsthatcirculateinthebloodstream.Altogether,itisplausiblethatimmunesystem-bacteriasynergismmediatessolutionstoinflammation.Onthecontrary,aspreviouslyreported,somestudieshavefoundthatbutyrate-producingbacteriaareselectivelyreducedinstoolsamplesfromHIV-infectedcomparedtonon-infectedsubjects[17,54].Inparticular,Serrano-Villar *et al*[60]foundthatHIV-infectedindividualshadadistinctSCFAprofileinstoolcomparedtoHIV-negativecontrols,withincreasedpropionateandlowerlevelsofacetate.NodatafromtheliteratureareavailableregardingSCFAlevelsinHIV+serumsamples,exceptastudyofSegal *et al*[61]reportingthathighervaluesofserumSCFAs,inconsequenceofanincreasedabundanceofpulmonaryanaerobicbacteriainHIV+patientsonART,inhibitedtheimmuneresponseto *M. tuberculosis*,likelyenhancingtuberculosissusceptibility.Theyobservedthatbaselineserumbutyrateandpropionatewereassociatedwiththesubsequentincreasinghazardoftuberculosis.Moreover,wealsoevaluatedserumFFAcompositionbeforeandafterARTtreatment.Indeed,increasedlevelsofFFAandproinflammatorycytokineshavebeenreportedinsomeHIV-infectedpatientsunderART(reviewedinreference[62]).However,wedidnotappreciateanydifferenceattheexaminedtwotimepoints.

Regardingtheinflammationtone,thereisconsensusthatapro-inflammatorystatusremainsactiveevenafterARTinitiationinmostpatients[63,64].SincetheHIVlifecycleissuppressedthroughARTintreatedpatients,thechronicinflammatorystatusobservedinpatientsismaintainedbyfactorssecondarytoHIVreplication,includingmicrobialtranslocationandreducedimmunoregulatoryfunction.InordertoevaluatetheinflammatorystatusafterART,wemeasuredapanelofselectedmultifunctionaleffectormoleculesoftheimmuneresponseinserum.Amongthemeasuredcytokines,weobservedadecreaseofIP-10(*P* =0.0244)afterthetreatment,confirmingthedownregulationofthischemokineproductioninpatientswithHIV infection duringART[65-69].IP-10isinvolvedintraffickingimmunecellstoinflammatorysites, anditisconsideredanimportantpro-inflammatoryfactorintheHIVdiseaseprocess.Ithasbeenobservedthatitslevelscanbereduced,butnottonormallevels,byARTadministration.Interestingly,IP-10wasconsistentlyassociatedwithHIV[diseaseprogression](https://www.sciencedirect.com/topics/medicine-and-dentistry/disease-exacerbation)(basedonCD4+counts)duringtheperiod[70],suggestingitspotentialforuseasanindicatorofHIVinfectionand/oratherapeutictargetforHIVtreatment[71].Ontheotherhand,inagreementwithrecentdata,weobservedasignificantincreasedtrendofIL-8levels(*P* =0.0547)withsuppressedviralloadafter24wkofART.Indeed,increasedIL-8levelswereobservedinHIV-infectedindividualsonART[72].IthasbeenshownthatduringHIV-1infection,IL-8playsanimportantroleintherecruitmentofCD4+Tcellstothelymphnodes,thusgeneratingmoretargetsforviralreplication.OurresultsmaysuggestthatincreasedIL-8LevelsmayrepresentahallmarkofchronicinflammationinHIV+patientsonART.Inaccordancewithourfindings,Wada *et al*[73]observedsignificantlyhighercirculatingIL-8levelsinHIV+menonARTwithsuppressedviralloadincomparisontoHIV-uninfectedmen.

ItisnowestablishedthatthegutmicrobiomemayplayacrucialroleintheimmuneactivationinHIV-infectedpatientstreatedwithART[5,64,73-75].Recently,severalstudieshavereportedthatGMisassociatedwithCD4+TcellrecoveryinHIV-infectedpatients,playinganessentialroleinthereconstitutionofimmunefunction[76-78].Thepotentialmechanismincludestheformationofavirusshelter,resistancetoART,promotionofintestinalmucosalbarrierdamage,andfurtherentryofintestinalbacteria andtheirmetabolitesintothecirculatorysystem,resultinginlong-termimmuneactivation,inflammation,andmetabolicdisorderssuchascardiovasculardiseases,diabetesmellitus,liversteatosis,andlastly,cancer[8].AlthoughitremainsunclearwhetheranalteredimmunityafterHIVinfectiondrivesdysbiosisor *vice versa*,thegutdysbiosis,immunedysfunction,epithelialdamage,andmicrobialtranslocationarestillevidenteveninthesettingofART-mediatedviralsuppression,whichmightbethetreatmentdilemmaforHIVinfectionatpresent.DespitenumerousstudiesofthemicrobiotainHIV-infectedpatients,therearerelativelyfewreportsdiscussingthecompositionalGMchangesinpatientswithdifferentimmuneresponsestoART[79,80].

Toinvestigatethe role ofGM inimmunomodulationandimmunereconstitutionandwhichbacterialmetabolitesareimplicated,inthesecondpartofthestudy,wedividedthepatientsintotwogroups:PatientswithCD4/CD4ratio< 1withinsufficientreconstitutionofCD4+Tcellsdespiteachievingvirologicalsuppressionafter24wkofARTandthosewithCD4/CD8≥ 1whoreachedarobustreconstitutionofCD4+Tcells.Wefoundthatthe *Anaerostipes* genuswassignificantlyaugmentedinIRs;onthecontrary,the *Faecalibacterium* genuswassignificantlyincreasedinINRs.Notably,*Faecalibacterium* hasbeenreportedastheanti-inflammatorycommensalgenus[81].IthasbeenpositivelycorrelatedwiththeCD4/CD8ratioandanti-correlatedwithinflammationmarkersandLPSinarecentstudyinHIV-infectedpatients[82].

Regardingmicrobialmetabolites,wedetected asignificantincreaseinfecalisobutyric,isovaleric,and2-methylbutyricacidsintheIRs.However,wefoundthatthechangesassociatedwiththeIRgroupwerenotevidentintheblood.Basedonourresults,wehypothesizedthatchangesatthegenuslevelinthegutecosysteminHIV-infectedpatientsundergoingARTmightthusbebothaconsequenceandapotentialcauseoftherecoveryofsystemicimmunity.

Ourstudyhadsomelimitations.First,alownumberofpatientswereenrolledtoinvestigatetheelementsofthemicrobiota-immunityaxisanditcannotdeterminewhetherthealteredGMcontributedtoorwascausedbyimmunedysfunction.Second,onlytheeffectsof24*-*wkARTwereobservedinourstudy,andtoestablishamoremeaningfulconnectionbetweenGMandmicrobial/immuneparameters,futurestudiesshouldinvestigatetheGMalterationsandtherestorationofimmunefunctionafterlong-termeffectiveART.Finally,themicrobiotaoffeceswasaproxyforGMinthisstudy,whichwastheonlyrealisticsampleforanon-invasivestudy.However,fecalmicrobiotamayonlyrepresenttheGMcompositioninthelumenratherthanonthemucosalsurfaces,whichisanimportantdistinctionbecausethemucosa-associatedmicrobiotapotentiallyinteractswiththegut-associatedlymphoidtissueinHIV-1-infectedpatientsdirectly.

**CONCLUSION**

OurresultsprovidedanadditionalvisionabouttheimpactofHIVinfection,art,andimmunerecoveryinthemicrobiota-immunityaxisatthemetabolismlevel,whichareanindicatoroftheactiveprocessesoccurringinthegastrointestinaltract.Insummary,wedemonstratedthatpatientsinfectedbyHIV-1,afterreachingvirologicalsuppressionwithART,displayedafecalmicrobiotawithunchangedoverallbacterialdiversityexceptforfewgenera.Although24wkoftreatmentwithARTwaseffective,thesystemicinflammatorytonewasnotcompletelyrestoreddespitetheanti-inflammatoryserumbutyrateincrement.Inaddition,weconfirmedtheroleoftheGMinimmunereconstitution,withthepossibleimplicationofbacterialmetabolites;however,changesinthegutecosysteminHIV+patientsundergoing24wk ofARTmaythusbebothaconsequenceandapotentialcauseoftherecoveryofsystemicimmunity.

Futurelarger-scale,long-termARTandlongitudinalstudiesthatincludefunctionalmetagenomicandmetabolomicapproachestoidentifytherolesofthespecificdifferentialphylotypesarerequiredtobetterdefinetherelationshipbetweenmicrobiota-immunityaxisandHIV-1infectionandtoprovidenewinsightsintothetargetedtreatment,improvingtheimmunerecoveryanddampening inflammation.

**ARTICLEHIGHLIGHTS**

***Research background***

Human immunodeficiency virus type 1 (HIV-1)infectionischaracterizedbypersistentsystemicinflammationandimmuneactivation,eveninpatientsreceivingeffectiveantiretroviraltherapy(ART).Convergingdatasuggestthat gutmicrobiota(GM)changescanoccurthroughoutincludinghumanimmunodeficiencyvirus(HIV)infectiontreatedbyART.

***Research motivation***

ARThasincreasedthelifeexpectancyofHIV-infectedpatients;however,chronicinflammationandgutmicrobiotaalterationspersistinpatientsvirologicallysuppressedbyART.Thesedatasuggestthatre-shapingthemicrobiotamaybeanadjuvanttherapyinpatientscommencingsuccessfulART*.*

***Research objectives***

Thepurposeofthisprospectiveobservationalstudywastocompareforthefirsttimethefecalmicrobialcomposition,serumandfecalmicrobialmetabolites,andserumcytokineprofileoftreatment*-*naïvepatientsbeforestartingARTandafterreachingvirologicalsuppression(HIVRNA<50copies/mL)after24wkofART.

***Research methods***

The authorsenrolled12treatment*-*naïve HIV-infectedpatientsreceivingART.Fecalmicrobiotacompositionwasassessedthroughnextgenerationsequencing,andacomprehensiveanalysisofabroad spectrum ofcytokines in blood wasperformedthroughamultiplexapproach.Inaddition,serumfreefattyacid(FFA)andfecalshortchainfattyacid(SCFA)levelsweremeasuredthroughGC-MS.

***Research results***

The authorscomparedmicrobiotasignatures,FFAlevels,andcytokineprofilebeforestartingARTandafterreachingvirologicalsuppression.Modestalterationswere observedonmicrobiotacomposition;moreover,inthesamecondition,wealsoobservedaugmentedlevelsofserumpropionicandbutyricacids.AreductionofserumIP-10andanincreaseofIL-8levelweredetectedin theviralsuppressioncondition.Thereafter,thesamecomponentswerecomparedbetweenimmunologicalrespondersandnon-responders.Concerningthemicroflorapopulation,wedetectedareductionof *Faecalibacterium* andanincreaseof *Alistipes* inimmunologicalnon-responders.Simultaneously,fecalisobutyric,isovaleric,and2-methylbutyricacidswerealsoincreasedinimmunologicalnon-responders.

***Research conclusions***

TheresultsprovidanadditionalperspectiveabouttheimpactofHIVinfection,ART,andimmunerecoveryonthe“microbiome-immunityaxis”atthemetabolismlevel.Thesefactorscanactasindicatorsoftheactiveprocessesoccurringinthegastrointestinaltract.

***Research perspectives***

Futurelarger-scale,long-termARTandlongitudinalstudiesthatincludefunctionalmetagenomicandmetabolomicapproachestoidentifytherolesofthespecificdifferentialphylotypesarerequiredtobetterdefinetherelationshipbetweenmicrobiota-immunityaxisandHIV-1infectionandtoprovidenewinsightsintothetargetedtreatment,improvingtheimmunerecoveryanddampeninginflammation.

**REFERENCES**

1 **Vujkovic-Cvijin I**, Dunham RM, Iwai S, Maher MC, Albright RG, Broadhurst MJ, Hernandez RD, Lederman MM, Huang Y, Somsouk M, Deeks SG, Hunt PW, Lynch SV, McCune JM. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. *Sci Transl Med* 2013; **5**: 193ra91 [PMID: 23843452 DOI: 10.1126/scitranslmed.3006438]

2 **Brenchley JM**, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, Altmann D, Blazar BR, Rodriguez B, Teixeira-Johnson L, Landay A, Martin JN, Hecht FM, Picker LJ, Lederman MM, Deeks SG, Douek DC. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006; **12**: 1365-1371 [PMID: 17115046 DOI: 10.1038/nm1511]

3 **Dinh DM**, Volpe GE, Duffalo C, Bhalchandra S, Tai AK, Kane AV, Wanke CA, Ward HD. Intestinal microbiota, microbial translocation, and systemic inflammation in chronic HIV infection. *J Infect Dis* 2015; **211**: 19-27 [PMID: 25057045 DOI: 10.1093/infdis/jiu409]

4 **Guadalupe M**, Reay E, Sankaran S, Prindiville T, Flamm J, McNeil A, Dandekar S. Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. *J Virol* 2003; **77**: 11708-11717 [PMID: 14557656 DOI: 10.1128/jvi.77.21.11708-11717.2003]

5 **Klatt NR**, Funderburg NT, Brenchley JM. Microbial translocation, immune activation, and HIV disease. *Trends Microbiol* 2013; **21**: 6-13 [PMID: 23062765 DOI: 10.1016/j.tim.2012.09.001]

6 **Marchetti G**, Tincati C, Silvestri G. Microbial translocation in the pathogenesis of HIV infection and AIDS. *Clin Microbiol Rev* 2013; **26**: 2-18 [PMID: 23297256 DOI: 10.1128/CMR.00050-12]

7 **Mehandru S**, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C, Boden D, Racz P, Markowitz M. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. *J Exp Med* 2004; **200**: 761-770 [PMID: 15365095 DOI: 10.1084/jem.20041196]

8 **Zilberman-Schapira G**, Zmora N, Itav S, Bashiardes S, Elinav H, Elinav E. The gut microbiome in human immunodeficiency virus infection. *BMC Med* 2016; **14**: 83 [PMID: 27256449 DOI: 10.1186/s12916-016-0625-3]

9 **Rocafort M**, Noguera-Julian M, Rivera J, Pastor L, Guillén Y, Langhorst J, Parera M, Mandomando I, Carrillo J, Urrea V, Rodríguez C, Casadellà M, Calle ML, Clotet B, Blanco J, Naniche D, Paredes R. Evolution of the gut microbiome following acute HIV-1 infection. *Microbiome* 2019; **7**: 73 [PMID: 31078141 DOI: 10.1186/s40168-019-0687-5]

10 **Lozupone CA**, Li M, Campbell TB, Flores SC, Linderman D, Gebert MJ, Knight R, Fontenot AP, Palmer BE. Alterations in the gut microbiota associated with HIV-1 infection. *Cell Host Microbe* 2013; **14**: 329-339 [PMID: 24034618 DOI: 10.1016/j.chom.2013.08.006]

11 **Mudd JC**, Brenchley JM. Gut Mucosal Barrier Dysfunction, Microbial Dysbiosis, and Their Role in HIV-1 Disease Progression. *J Infect Dis* 2016; **214 Suppl 2**: S58-S66 [PMID: 27625432 DOI: 10.1093/infdis/jiw258]

12 **Klase Z**, Ortiz A, Deleage C, Mudd JC, Quiñones M, Schwartzman E, Klatt NR, Canary L, Estes JD, Brenchley JM. Dysbiotic bacteria translocate in progressive SIV infection. *Mucosal Immunol* 2015; **8**: 1009-1020 [PMID: 25586559 DOI: 10.1038/mi.2014.128]

13 **Pinto-Cardoso S**, Lozupone C, Briceño O, Alva-Hernández S, Téllez N, Adriana A, Murakami-Ogasawara A, Reyes-Terán G. Fecal Bacterial Communities in treated HIV infected individuals on two antiretroviral regimens. *Sci Rep* 2017; **7**: 43741 [PMID: 28262770 DOI: 10.1038/srep43741]

14 **Dillon SM**, Lee EJ, Kotter CV, Austin GL, Dong Z, Hecht DK, Gianella S, Siewe B, Smith DM, Landay AL, Robertson CE, Frank DN, Wilson CC. An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. *Mucosal Immunol* 2014; **7**: 983-994 [PMID: 24399150 DOI: 10.1038/mi.2013.116]

15 **Mutlu EA**, Keshavarzian A, Losurdo J, Swanson G, Siewe B, Forsyth C, French A, Demarais P, Sun Y, Koenig L, Cox S, Engen P, Chakradeo P, Abbasi R, Gorenz A, Burns C, Landay A. A compositional look at the human gastrointestinal microbiome and immune activation parameters in HIV infected subjects. *PLoS Pathog* 2014; **10**: e1003829 [PMID: 24586144 DOI: 10.1371/journal.ppat.1003829]

16 **Vázquez-Castellanos JF**, Serrano-Villar S, Latorre A, Artacho A, Ferrús ML, Madrid N, Vallejo A, Sainz T, Martínez-Botas J, Ferrando-Martínez S, Vera M, Dronda F, Leal M, Del Romero J, Moreno S, Estrada V, Gosalbes MJ, Moya A. Altered metabolism of gut microbiota contributes to chronic immune activation in HIV-infected individuals. *Mucosal Immunol* 2015; **8**: 760-772 [PMID: 25407519 DOI: 10.1038/mi.2014.107]

17 **Dillon SM**, Kibbie J, Lee EJ, Guo K, Santiago ML, Austin GL, Gianella S, Landay AL, Donovan AM, Frank DN, McCARTER MD, Wilson CC. Low abundance of colonic butyrate-producing bacteria in HIV infection is associated with microbial translocation and immune activation. *AIDS* 2017; **31**: 511-521 [PMID: 28002063 DOI: 10.1097/QAD.0000000000001366]

18 **Tincati C**, Douek DC, Marchetti G. Gut barrier structure, mucosal immunity and intestinal microbiota in the pathogenesis and treatment of HIV infection. *AIDS Res Ther* 2016; **13**: 19 [PMID: 27073405 DOI: 10.1186/s12981-016-0103-1]

19 **Parbie PK**, Mizutani T, Ishizaka A, Kawana-Tachikawa A, Runtuwene LR, Seki S, Abana CZ, Kushitor D, Bonney EY, Ofori SB, Uematsu S, Imoto S, Kimura Y, Kiyono H, Ishikawa K, Ampofo WK, Matano T. Dysbiotic Fecal Microbiome in HIV-1 Infected Individuals in Ghana. *Front Cell Infect Microbiol* 2021; **11**: 646467 [PMID: 34084754 DOI: 10.3389/fcimb.2021.646467]

20 **Luján JA**, Rugeles MT, Taborda NA. Contribution of the Microbiota to Intestinal Homeostasis and its Role in the Pathogenesis of HIV-1 Infection. *Curr HIV Res* 2019; **17**: 13-25 [PMID: 30854974 DOI: 10.2174/1570162X17666190311114808]

21 **Dillon SM**, Manuzak JA, Leone AK, Lee EJ, Rogers LM, McCarter MD, Wilson CC. HIV-1 infection of human intestinal lamina propria CD4+ T cells in vitro is enhanced by exposure to commensal Escherichia coli. *J Immunol* 2012; **189**: 885-896 [PMID: 22689879 DOI: 10.4049/jimmunol.1200681]

22 **Wu GD**, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011; **334**: 105-108 [PMID: 21885731 DOI: 10.1126/science.1208344]

23 **David LA**, Materna AC, Friedman J, Campos-Baptista MI, Blackburn MC, Perrotta A, Erdman SE, Alm EJ. Host lifestyle affects human microbiota on daily timescales. *Genome Biol* 2014; **15**: R89 [PMID: 25146375 DOI: 10.1186/gb-2014-15-7-r89]

24 **Li SX**, Armstrong A, Neff CP, Shaffer M, Lozupone CA, Palmer BE. Complexities of Gut Microbiome Dysbiosis in the Context of HIV Infection and Antiretroviral Therapy. *Clin Pharmacol Ther* 2016; **99**: 600-611 [PMID: 26940481 DOI: 10.1002/cpt.363]

25 **Pinto-Cardoso S**, Klatt NR, Reyes-Terán G. Impact of antiretroviral drugs on the microbiome: unknown answers to important questions. *Curr Opin HIV AIDS* 2018; **13**: 53-60 [PMID: 29028667 DOI: 10.1097/COH.0000000000000428]

26 **Marcus JL**, Leyden WA, Alexeeff SE, Anderson AN, Hechter RC, Hu H, Lam JO, Towner WJ, Yuan Q, Horberg MA, Silverberg MJ. Comparison of Overall and Comorbidity-Free Life Expectancy Between Insured Adults With and Without HIV Infection, 2000-2016. *JAMA Netw Open* 2020; **3**: e207954 [PMID: 32539152 DOI: 10.1001/jamanetworkopen.2020.7954]

27 **Ancona G**, Merlini E, Tincati C, Barassi A, Calcagno A, Augello M, Bono V, Bai F, Cannizzo ES, d'Arminio Monforte A, Marchetti G. Long-Term Suppressive cART Is Not Sufficient to Restore Intestinal Permeability and Gut Microbiota Compositional Changes. *Front Immunol* 2021; **12**: 639291 [PMID: 33717191 DOI: 10.3389/fimmu.2021.639291]

28 **Nowak P**, Troseid M, Avershina E, Barqasho B, Neogi U, Holm K, Hov JR, Noyan K, Vesterbacka J, Svärd J, Rudi K, Sönnerborg A. Gut microbiota diversity predicts immune status in HIV-1 infection. *AIDS* 2015; **29**: 2409-2418 [PMID: 26355675 DOI: 10.1097/QAD.0000000000000869]

29 **Villanueva-Millán MJ**, Pérez-Matute P, Recio-Fernández E, Lezana Rosales JM, Oteo JA. Differential effects of antiretrovirals on microbial translocation and gut microbiota composition of HIV-infected patients. *J Int AIDS Soc* 2017; **20**: 21526 [PMID: 28362071 DOI: 10.7448/IAS.20.1.21526]

30 **Deusch S**, Serrano-Villar S, Rojo D, Martínez-Martínez M, Bargiela R, Vázquez-Castellanos JF, Sainz T, Barbas C, Moya A, Moreno S, Gosalbes MJ, Estrada V, Seifert J, Ferrer M. Effects of HIV, antiretroviral therapy and prebiotics on the active fraction of the gut microbiota. *AIDS* 2018; **32**: 1229-1237 [PMID: 29683848 DOI: 10.1097/QAD.0000000000001831]

31 **McHardy IH**, Li X, Tong M, Ruegger P, Jacobs J, Borneman J, Anton P, Braun J. HIV Infection is associated with compositional and functional shifts in the rectal mucosal microbiota. *Microbiome* 2013; **1**: 26 [PMID: 24451087 DOI: 10.1186/2049-2618-1-26]

32 **Flygel TT**, Sovershaeva E, Claassen-Weitz S, Hjerde E, Mwaikono KS, Odland JØ, Ferrand RA, Mchugh G, Gutteberg TJ, Nicol MP, Cavanagh JP, Flægstad T; BREATHE Study Team. Composition of Gut Microbiota of Children and Adolescents With Perinatal Human Immunodeficiency Virus Infection Taking Antiretroviral Therapy in Zimbabwe. *J Infect Dis* 2020; **221**: 483-492 [PMID: 31549151 DOI: 10.1093/infdis/jiz473]

33 **Serrano-Villar S**, Sainz T, Lee SA, Hunt PW, Sinclair E, Shacklett BL, Ferre AL, Hayes TL, Somsouk M, Hsue PY, Van Natta ML, Meinert CL, Lederman MM, Hatano H, Jain V, Huang Y, Hecht FM, Martin JN, McCune JM, Moreno S, Deeks SG. HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8+ T cell activation, and increased risk of non-AIDS morbidity and mortality. *PLoS Pathog* 2014; **10**: e1004078 [PMID: 24831517 DOI: 10.1371/journal.ppat.1004078]

34 **McBride JA**, Striker R. Imbalance in the game of T cells: What can the CD4/CD8 T-cell ratio tell us about HIV and health? *PLoS Pathog* 2017; **13**: e1006624 [PMID: 29095912 DOI: 10.1371/journal.ppat.1006624]

35 **Serrano-Villar S**, Pérez-Elías MJ, Dronda F, Casado JL, Moreno A, Royuela A, Pérez-Molina JA, Sainz T, Navas E, Hermida JM, Quereda C, Moreno S. Increased risk of serious non-AIDS-related events in HIV-infected subjects on antiretroviral therapy associated with a low CD4/CD8 ratio. *PLoS One* 2014; **9**: e85798 [PMID: 24497929 DOI: 10.1371/journal.pone.0085798]

36 **Kelly CJ**, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ, Wilson KE, Glover LE, Kominsky DJ, Magnuson A, Weir TL, Ehrentraut SF, Pickel C, Kuhn KA, Lanis JM, Nguyen V, Taylor CT, Colgan SP. Crosstalk between Microbiota-Derived Short-Chain Fatty Acids and Intestinal Epithelial HIF Augments Tissue Barrier Function. *Cell Host Microbe* 2015; **17**: 662-671 [PMID: 25865369 DOI: 10.1016/j.chom.2015.03.005]

37 **Kim CH**, Park J, Kim M. Gut microbiota-derived short-chain Fatty acids, T cells, and inflammation. *Immune Netw* 2014; **14**: 277-288 [PMID: 25550694 DOI: 10.4110/in.2014.14.6.277]

38 **Corrêa-Oliveira R**, Fachi JL, Vieira A, Sato FT, Vinolo MA. Regulation of immune cell function by short-chain fatty acids. *Clin Transl Immunology* 2016; **5**: e73 [PMID: 27195116 DOI: 10.1038/cti.2016.17]

39 **Effros RB**, Cai Z, Linton PJ. CD8 T cells and aging. *Crit Rev Immunol* 2003; **23**: 45-64 [PMID: 12906259 DOI: 10.1615/critrevimmunol.v23.i12.30]

40 **Pagliai G**, Russo E, Niccolai E, Dinu M, Di Pilato V, Magrini A, Bartolucci G, Baldi S, Menicatti M, Giusti B, Marcucci R, Rossolini GM, Casini A, Sofi F, Amedei A. Influence of a 3-month low-calorie Mediterranean diet compared to the vegetarian diet on human gut microbiota and SCFA: the CARDIVEG Study. *Eur J Nutr* 2020; **59**: 2011-2024 [PMID: 31292752 DOI: 10.1007/s00394-019-02050-0]

41 **Bolyen E**, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS 2nd, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 2019; **37**: 852-857 [PMID: 31341288 DOI: 10.1038/s41587-019-0209-9]

42 **Callahan BJ**, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016; **13**: 581-583 [PMID: 27214047 DOI: 10.1038/nmeth.3869]

43 **Niccolai E**, Baldi S, Ricci F, Russo E, Nannini G, Menicatti M, Poli G, Taddei A, Bartolucci G, Calabrò AS, Stingo FC, Amedei A. Evaluation and comparison of short chain fatty acids composition in gut diseases. *World J Gastroenterol* 2019; **25**: 5543-5558 [PMID: 31576099 DOI: 10.3748/wjg.v25.i36.5543]

44 **Baldi S**, Menicatti M, Nannini G, Niccolai E, Russo E, Ricci F, Pallecchi M, Romano F, Pedone M, Poli G, Renzi D, Taddei A, Calabrò AS, Stingo FC, Bartolucci G, Amedei A. Free Fatty Acids Signature in Human Intestinal Disorders: Significant Association between Butyric Acid and Celiac Disease. *Nutrients* 2021; **13** [PMID: 33652681 DOI: 10.3390/nu13030742]

45 **McMurdie PJ**, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 2013; **8**: e61217 [PMID: 23630581 DOI: 10.1371/journal.pone.0061217]

46 **Love MI**, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014; **15**: 550 [PMID: 25516281 DOI: 10.1186/s13059-014-0550-8]

47 **Willis A**, Bunge J. Estimating diversity via frequency ratios. *Biometrics* 2015; **71**: 1042-1049 [PMID: 26038228 DOI: 10.1111/biom.12332]

48 **Russo E**, Giudici F, Ricci F, Scaringi S, Nannini G, Ficari F, Luceri C, Niccolai E, Baldi S, D'Ambrosio M, Ramazzotti M, Amedei A. Diving into Inflammation: A Pilot Study Exploring the Dynamics of the Immune-Microbiota Axis in Ileal Tissue Layers of Patients with Crohn's Disease. *J Crohns Colitis* 2021; **15**: 1500-1516 [PMID: 33611347 DOI: 10.1093/ecco-jcc/jjab034]

49 **Brenchley JM**, Douek DC. Microbial translocation across the GI tract. *Annu Rev Immunol* 2012; **30**: 149-173 [PMID: 22224779 DOI: 10.1146/annurev-immunol-020711-075001]

50 **Epple HJ**, Allers K, Tröger H, Kühl A, Erben U, Fromm M, Zeitz M, Loddenkemper C, Schulzke JD, Schneider T. Acute HIV infection induces mucosal infiltration with CD4+ and CD8+ T cells, epithelial apoptosis, and a mucosal barrier defect. *Gastroenterology* 2010; **139**: 1289-1300 [PMID: 20600014 DOI: 10.1053/j.gastro.2010.06.065]

51 **Raehtz KD**, Barrenäs F, Xu C, Busman-Sahay K, Valentine A, Law L, Ma D, Policicchio BB, Wijewardana V, Brocca-Cofano E, Trichel A, Gale M Jr, Keele BF, Estes JD, Apetrei C, Pandrea I. African green monkeys avoid SIV disease progression by preventing intestinal dysfunction and maintaining mucosal barrier integrity. *PLoS Pathog* 2020; **16**: e1008333 [PMID: 32119719 DOI: 10.1371/journal.ppat.1008333]

52 **Monaco CL**, Gootenberg DB, Zhao G, Handley SA, Ghebremichael MS, Lim ES, Lankowski A, Baldridge MT, Wilen CB, Flagg M, Norman JM, Keller BC, Luévano JM, Wang D, Boum Y, Martin JN, Hunt PW, Bangsberg DR, Siedner MJ, Kwon DS, Virgin HW. Altered Virome and Bacterial Microbiome in Human Immunodeficiency Virus-Associated Acquired Immunodeficiency Syndrome. *Cell Host Microbe* 2016; **19**: 311-322 [PMID: 26962942 DOI: 10.1016/j.chom.2016.02.011]

53 **Imahashi M**, Ode H, Kobayashi A, Nemoto M, Matsuda M, Hashiba C, Hamano A, Nakata Y, Mori M, Seko K, Nakahata M, Kogure A, Tanaka Y, Sugiura W, Yokomaku Y, Iwatani Y. Impact of long-term antiretroviral therapy on gut and oral microbiotas in HIV-1-infected patients. *Sci Rep* 2021; **11**: 960 [PMID: 33441754 DOI: 10.1038/s41598-020-80247-8]

54 **Noguera-Julian M**, Rocafort M, Guillén Y, Rivera J, Casadellà M, Nowak P, Hildebrand F, Zeller G, Parera M, Bellido R, Rodríguez C, Carrillo J, Mothe B, Coll J, Bravo I, Estany C, Herrero C, Saz J, Sirera G, Torrela A, Navarro J, Crespo M, Brander C, Negredo E, Blanco J, Guarner F, Calle ML, Bork P, Sönnerborg A, Clotet B, Paredes R. Gut Microbiota Linked to Sexual Preference and HIV Infection. *EBioMedicine* 2016; **5**: 135-146 [PMID: 27077120 DOI: 10.1016/j.ebiom.2016.01.032]

55 **Arpaia N**, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, Liu H, Cross JR, Pfeffer K, Coffer PJ, Rudensky AY. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 2013; **504**: 451-455 [PMID: 24226773 DOI: 10.1038/nature12726]

56 **Louis P**, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett* 2009; **294**: 1-8 [PMID: 19222573 DOI: 10.1111/j.1574-6968.2009.01514.x]

57 **Desai SN**, Landay AL. HIV and aging: role of the microbiome. *Curr Opin HIV AIDS* 2018; **13**: 22-27 [PMID: 29035948 DOI: 10.1097/COH.0000000000000433]

58 **Topping DL**, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev* 2001; **81**: 1031-1064 [PMID: 11427691 DOI: 10.1152/physrev.2001.81.3.1031]

59 **Cummings JH**, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* 1987; **28**: 1221-1227 [PMID: 3678950 DOI: 10.1136/gut.28.10.1221]

60 **Bandera A**, De Benedetto I, Bozzi G, Gori A. Altered gut microbiome composition in HIV infection: causes, effects and potential intervention. *Curr Opin HIV AIDS* 2018; **13**: 73-80 [PMID: 29045252 DOI: 10.1097/COH.0000000000000429]

61 **Philips JA**. HIV-associated anaerobes ferment TB risk. *Sci Transl Med* 2017; **9** [PMID: 28490669 DOI: 10.1126/scitranslmed.aan3781]

62 **Villarroya F**, Domingo P, Giralt M. Drug-induced lipotoxicity: lipodystrophy associated with HIV-1 infection and antiretroviral treatment. *Biochim Biophys Acta* 2010; **1801**: 392-399 [PMID: 19800025 DOI: 10.1016/j.bbalip.2009.09.018]

63 **Hunt PW**. HIV and inflammation: mechanisms and consequences. *Curr HIV/AIDS Rep* 2012; **9**: 139-147 [PMID: 22528766 DOI: 10.1007/s11904-012-0118-8]

64 **Deeks SG**, Tracy R, Douek DC. Systemic effects of inflammation on health during chronic HIV infection. *Immunity* 2013; **39**: 633-645 [PMID: 24138880 DOI: 10.1016/j.immuni.2013.10.001]

65 **Yao Y**, Luo Y, He Y, Zheng Y, Zhang Q, Zhou H, Zeng S, Chen Z, He B, He M. The effect of a year of highly active antiretroviral therapy on immune reconstruction and cytokines in HIV/AIDS patients. *AIDS Res Hum Retroviruses* 2013; **29**: 691-697 [PMID: 23151174 DOI: 10.1089/AID.2012.0275]

66 **Ramirez LA**, Arango TA, Thompson E, Naji M, Tebas P, Boyer JD. High IP-10 levels decrease T cell function in HIV-1-infected individuals on ART. *J Leukoc Biol* 2014; **96**: 1055-1063 [PMID: 25157027 DOI: 10.1189/jlb.3A0414-232RR]

67 **Cinque P**, Bestetti A, Marenzi R, Sala S, Gisslen M, Hagberg L, Price RW. Cerebrospinal fluid interferon-gamma-inducible protein 10 (IP-10, CXCL10) in HIV-1 infection. *J Neuroimmunol* 2005; **168**: 154-163 [PMID: 16091292 DOI: 10.1016/j.jneuroim.2005.07.002]

68 **Hattab S**, Guihot A, Guiguet M, Fourati S, Carcelain G, Caby F, Marcelin AG, Autran B, Costagliola D, Katlama C. Comparative impact of antiretroviral drugs on markers of inflammation and immune activation during the first two years of effective therapy for HIV-1 infection: an observational study. *BMC Infect Dis* 2014; **14**: 122 [PMID: 24589015 DOI: 10.1186/1471-2334-14-122]

69 **Relucio KI**, Beernink HT, Chen D, Israelski DM, Kim R, Holodniy M. Proteomic analysis of serum cytokine levels in response to highly active antiretroviral therapy (HAART). *J Proteome Res* 2005; **4**: 227-231 [PMID: 15822897 DOI: 10.1021/pr049930y]

70 **Jiao Y**, Zhang T, Wang R, Zhang H, Huang X, Yin J, Zhang L, Xu X, Wu H. Plasma IP-10 is associated with rapid disease progression in early HIV-1 infection. *Viral Immunol* 2012; **25**: 333-337 [PMID: 22788418 DOI: 10.1089/vim.2012.0011]

71 **Stylianou E**, Aukrust P, Bendtzen K, Müller F, Frøland SS. Interferons and interferon (IFN)-inducible protein 10 during highly active anti-retroviral therapy (HAART)-possible immunosuppressive role of IFN-alpha in HIV infection. *Clin Exp Immunol* 2000; **119**: 479-485 [PMID: 10691920 DOI: 10.1046/j.1365-2249.2000.01144.x]

72 **Ellwanger JH**, Valverde-Villegas JM, Kaminski VL, de Medeiros RM, Almeida SEM, Santos BR, de Melo MG, Hackenhaar FS, Chies JAB. Increased IL-8 levels in HIV-infected individuals who initiated ART with CD4+ T cell counts <350 cells/mm3 - A potential hallmark of chronic inflammation. *Microbes Infect* 2020; **22**: 474-480 [PMID: 32534178 DOI: 10.1016/j.micinf.2020.05.019]

73 **Nixon DE**, Landay AL. Biomarkers of immune dysfunction in HIV. *Curr Opin HIV AIDS* 2010; **5**: 498-503 [PMID: 20978393 DOI: 10.1097/COH.0b013e32833ed6f4]

74 **Klatt NR**, Chomont N, Douek DC, Deeks SG. Immune activation and HIV persistence: implications for curative approaches to HIV infection. *Immunol Rev* 2013; **254**: 326-342 [PMID: 23772629 DOI: 10.1111/imr.12065]

75 **Lederman MM**, Funderburg NT, Sekaly RP, Klatt NR, Hunt PW. Residual immune dysregulation syndrome in treated HIV infection. *Adv Immunol* 2013; **119**: 51-83 [PMID: 23886064 DOI: 10.1016/B978-0-12-407707-2.00002-3]

76 **Lu W**, Feng Y, Jing F, Han Y, Lyu N, Liu F, Li J, Song X, Xie J, Qiu Z, Zhu T, Routy B, Routy JP, Li T, Zhu B. Association Between Gut Microbiota and CD4 Recovery in HIV-1 Infected Patients. *Front Microbiol* 2018; **9**: 1451 [PMID: 30034377 DOI: 10.3389/fmicb.2018.01451]

77 **Ji Y**, Zhang F, Zhang R, Shen Y, Liu L, Wang J, Yang J, Tang Q, Xun J, Qi T, Wang Z, Song W, Tang Y, Chen J, Lu H. Changes in intestinal microbiota in HIV-1-infected subjects following cART initiation: influence of CD4+ T cell count. *Emerg Microbes Infect* 2018; **7**: 113 [PMID: 29934497 DOI: 10.1038/s41426-018-0117-y]

78 **Lee SC**, Chua LL, Yap SH, Khang TF, Leng CY, Raja Azwa RI, Lewin SR, Kamarulzaman A, Woo YL, Lim YAL, Loke P, Rajasuriar R. Enrichment of gut-derived Fusobacterium is associated with suboptimal immune recovery in HIV-infected individuals. *Sci Rep* 2018; **8**: 14277 [PMID: 30250162 DOI: 10.1038/s41598-018-32585-x]

79 **Siedner MJ**. START or SMART? Timing of Antiretroviral Therapy Initiation and Cardiovascular Risk for People With Human Immunodeficiency Virus Infection. *Open Forum Infect Dis* 2016; **3**: ofw032 [PMID: 26989755 DOI: 10.1093/ofid/ofw032]

80 **Havlir DV**, Currier JS. CROI 2015: Complications of HIV Infection and Antiretroviral Therapy. *Top Antivir Med* 2015; **23**: 56-65 [PMID: 25965312]

81 **Shaw KA**, Bertha M, Hofmekler T, Chopra P, Vatanen T, Srivatsa A, Prince J, Kumar A, Sauer C, Zwick ME, Satten GA, Kostic AD, Mulle JG, Xavier RJ, Kugathasan S. Dysbiosis, inflammation, and response to treatment: a longitudinal study of pediatric subjects with newly diagnosed inflammatory bowel disease. *Genome Med* 2016; **8**: 75 [PMID: 27412252 DOI: 10.1186/s13073-016-0331-y]

82 **Xie Y**, Sun J, Wei L, Jiang H, Hu C, Yang J, Huang Y, Ruan B, Zhu B. Altered gut microbiota correlate with different immune responses to HAART in HIV-infected individuals. *BMC Microbiol* 2021; **21**: 11 [PMID: 33407128 DOI: 10.1186/s12866-020-02074-1]

**Footnotes**

**Institutionalreviewboardstatement:**ThestudywasreviewedandapprovedbytheComitatoEticoAreaVastaCentro[(ApprovalNo. 15035)].

**Conflict-of-intereststatement:**Theauthorsdeclarethattheyhavenocompetinginterests to disclose.

**Datasharingstatement:**Noadditionaldataareavailable.

**Open-Access:**Thisarticleisanopen-accessarticlethatwasselectedbyanin-houseeditorandfullypeer-reviewedbyexternalreviewers.ItisdistributedinaccordancewiththeCreativeCommonsAttributionNon Commercial(CCBY-NC4.0)license,whichpermitsotherstodistribute,remix,adapt,builduponthisworknon-commercially,andlicensetheirderivativeworksondifferentterms,providedtheoriginalworkisproperlycitedandtheuseisnon-commercial.See:https://creativecommons.org/Licenses/by-nc/4.0/

**Provenance and peer review:** Invitedarticle; Externally peer reviewed.

**Peer-review model**: Single blind

**Peer-reviewstarted:**May28,2021

**Firstdecision:**June30,2021

**Articleinpress:**January 13, 2022

**Specialtytype:**Immunology

**Country/Territoryoforigin:**Italy

**Peer-reviewreport’sscientificqualityclassification**

GradeA(Excellent):0

GradeB(Verygood):0

GradeC(Good):C

GradeD(Fair):0

GradeE(Poor):0

**P-Reviewer:**HazafaA**S-Editor:**Ma YJ**L-Editor:** Wang TQ**P-Editor:** Yu HG

**FigureLegends**



**Figure1Box-plotsshowingalphadiversityindices(Chao1,Shannon, andevenness indices)insamples.**StatisticaldifferenceswereevaluatedusingpairedWilcoxonsigned-ranktestforChao,Shannon,andevennessindices. *P* valuelessthan0.05wereconsideredstatisticallysignificant.



A B

**Figure2Clusteranalysis(a)andprincipalcoordinateanalysisshowingthatsamplesdonotseparateintotwogroupsdependingontheircondition(0-24wk) (B).**



**Figure3Segmentplotsdepictingtaxawithsignificantlydifferencesbetweenhighviremia(time point0)andviralsuppression(time point24)conditions.**Linesconnectpairedsamplesandhighlightthedifferencesinnormalizedabundancefortheindicatedrank.Orangeorbluecolorshighlightdecreaseorincrease,respectively.Numbersinthetop-leftcornerrepresentcountsofincreased(orange)anddecreased(blue)measurementforpairedsamples.

*P* < 0.05

*P* < 0.05

**Figure4Boxplotsshowing statisticallydifferentlevelsofserumshort-chain fatty acids betweenhighviremiaandviralsuppressorpatients,assessedbytheWilcoxontest.** *p* value<0.05wasconsideredstatisticallysignificant.

*P* < 0.05

*P* < 0.05

**Figure5Boxplotsshowing statisticallydifferentlevelsofserumcytokinesbetweenhighviremiaandviralsuppressorpatients,assessedbytheWilcoxontest.**A *p* value<0.05wasconsideredstatisticallysignificant.



**Figure6Boxplotsshowingtheresultsoftaxa-leveldifferentialabundanceanalysisbetweenimmunological respondersandimmunological non-respondersat 24wk.**PlottitlesreporttheshrunkLog2foldchange(accordingtotheDESeq2functionlfcShrink).Allresultshavea *p* value<0.05.NR = INRs, R = IRs. IRs: immunological responders; INRs: immunological non-responders.



a

a

a

**Figure7Boxplotsshowing statisticallydifferentfecalshort-chain fatty acidabundancesbetweenimmunological responders andimmunological non-responders,assessedbytheMann-Whitneytest.**a*p* value < 0.05wasconsideredstatisticallysignificant.

**Table 1 Features of the enrolled patients**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Age** | **Sex** | **ART regimen** | **Comorbidities** | **Timepoints (wk)** | **Viral load (copies/mL)** | **CD4+ cells/mm3** | **CD8+ cells/mm3** | **CD4/CD8 ratio** |
| 1 | 37 | Male | 3TC/ABC/DTG | No | T0 | 597463 | 110 | 420 | 0.3 |
| T24 | < 20 | 520 | 832 | 0.6 |
| 2 | 38 | Male | FTC/TDF/EVG/C | No | T0 | 4489 | 630 | 670 | 0.9 |
| T24 | TND | 831 | 740 | 1.1 |
| 3 | 34 | Male | FTC/TDF/EVG/C | No | T0 | 165516 | 253 | 725 | 0.3 |
| T24 | TND | 504 | 363 | 1.4 |
| 4 | 39 | Male | FTC/TDF/EVG/c | No | T0 | 859883 | 360 | 974 | 0.4 |
| T24 | 33 | 781 | 986 | 0.8 |
| 5 | 38 | Male | 3TC/ABC/DTG | No | T0 | 4860 | 1341 | 928 | 1.4 |
| T24 | TND | 1881 | 988 | 1.9 |
| 6 | 41 | Male | FTC/TDF/RPV | Atrial fibrillation | T0 | 213 | 814 | 690 | 1.2 |
| T24 | TND | 845 | 519 | 1.6 |
| 7 | 25 | Male | 3TC/ABC/DTG | No | T0 | 23098 | 516 | 1149 | 0.4 |
| T24 | < 20 | 942 | 1019 | 0.9 |
| 8 | 22 | Male | FTC/TAF/EVG/c | No | T0 | 12188 | 654 | 1055 | 0.6 |
| T24 | TND | 668 | 733 | 0.9 |
| 9 | 48 | Male | 3TC/ABC/DTG | No | T0 | 175 | 833 | 1520 | 0.5 |
| T24 | TND | 941 | 1258 | 0.7 |
| 10 | 53 | Male | 3TC/ABC/DTG | Hypertension, HCV | T0 | 40545 | 863 | 1196 | 0.7 |
| T24 | TND | 612 | 515 | 1.2 |
| 11 | 40 | Male | 3TC/ABC/DTG | No | T0 | 859000 | 399 | 980 | 0.4 |
| T24 | 39 | 648 | 652 | 1 |
| 12 | 51 | Male | FTC/TDF DTG | Diabetes  | T0 | 4410 | 884 | 1066 | 0.8 |
| T24 | < 20  | 1130 | 1261 | 0.9 |

ART: Antiretroviral therapy; 3TC: Lamivudine; ABC: Abacavir; DTG: Dolutegravir; FTC: Emtricitabine; TDF: Tenovir; EVG/c: Elvitegravir/cobi; RPV: Rilpivirine.



Published by **Baishideng Publishing Group Inc**

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA

**Telephone:** +1-925-3991568

**E-mail:** bpgoffice@wjgnet.com

**Help Desk:** https://www.f6publishing.com/helpdesk

https://www.wjgnet.com



**© 2022 Baishideng Publishing Group Inc. All rights reserved.**