Hospital del Mar
Barcelona

PROTOCOL OF CLINICAL RESEARCH

Randomized Study to Assess the Impact of Mediterranean Diet Optimization on Metabolic Profile, Immune Activation, and Cardiovascular Risk in People with HIV on ART

Version of the protocol and date	Version: 1.2 date: 06/15/2024
Code of the protocol	
Qualification abbreviated either acronym	*VIHMET*
Promoter	
CRO (when proceed)	*If applicable*
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SUMMARY

Qualification	Randomized Study to Assess the Impact of Mediterranean Diet Optimization on Metabolic Profile, Immune Activation, and Cardiovascular Risk in People with HIV on ART
Goals	- Assess changes in he profile metabolic and lipid in the week 24 - immunoactivation profile at week 24 - To assess changes in arterial stiffness levels measured by pulse wave velocity at week 24 - Proportion of participants with >20% decrease in TMAO in week 24 compared to baseline. - Proportion of participants with an increase of >2 points in the MEDAS scale of adherence to the Mediterranean diet at week 24 - Proportion of participants improving on the EQ-5D quality of life questionnaire at week 24 - Assess persistence of all changes from Objective 1 to 6 at Week 48 - To evaluate the presence and composition of coronary atheromatous plaque by means of coronary CT angiography before and after the intervention.
Design	Rehearsal clinical randomized open
Inclusion-exclusion criteria	<u>Criteria of inclusion:</u> - Participant ≥ 18 years. - Infection by HIV confirmed by serology. - Treatment antiretroviral asset (TAR). - Burden viral undetectable during to the less 12 months (HE allows a blip below 200 copies). - LDL >140 mg/ dL . - Mediterranean Diet Adherence Screener (MEDAS) less than 9 points (adherence low) for ease the detection of changes after the intervention. <u>Criteria of exclusion:</u> - Participants in treatment lipid-lowering. - Treatment with anti-inflammatory chronic. - Hepatitis B, C or Others conditions inflammatory chronicles active. - Hypercholesterolemia familiar No treated. - Hypothyroidism No controlled. - Impossibility of give he consent.
Treatments or interventions	Advice dietary, analysis blood, measurement of the vibe arterial pulse
Variables	Cholesterol total, cLDL,cHDL , triglycerides <u>Number, size and composition of particles of lipoproteins</u> <u>Anthropometric profile</u> CD 3,CD45,CD4,CD8,CCR7,CXCR3,CCR6,CD103,FOXP3,CD25,CD45RA and CD45RO IL-6, TNF-alpha, IFN-gamma, sCD163, sCD14, MADCAM-1, sCD14 and LPS. Rigidity arterial extent by the speed of vibe of pulse Trimethylamine -N-Oxide Scale MEDAS of adherence to the diet Mediterranean

	Atheroma plaque (number of plaques) Plaque composition (% fibrosis, % calcium, % fat) Coronary calcium index

	Questionnaire of quality of life EQ- 5D
Population and No. subjects	100 patients of the unit of HIV
Participating centers	Hospital of the Sea. Barcelona
Study Description	Study comparative of the intervention dietetics conventional with a personalized dietary intervention
Overall duration from the study	January 2025- December 2026
Duration of the study for the patient	48 weeks

Board 1. Calendar of the study / procedures for a subject

	Screening (max 28 days before baseline visit)	**visit (randomization)**	**W4 (+/- 7d)**	**W12 (+/-7d)**	**W24 (+/-7d)**	**W48 (+14-7d)**
Type of visit **Intervention group** **Cluster control**	In person	In person	Virtual No visit	Virtual No visit	In person	In person
Informed consent	**x**					
Criteria of inclusion and exclusion	**x**					
Demographic data	**x**					
History medical	**x**					
Related story with HIV	**x**					
Exam physical		**x**			**x**	**x**
Anthropometric data		**x**			**x**	**x**
Questionnaires (MEDAS, FFQ, SMAQ, EQ-5D and IPAQ)	**X** (only MEDAS)	**x**			**x**	**x**
Concomitant medication	**x**	**x**		**x**	**x**	**x**
Adverse effects		**x**		**x**	**x**	**x**
Test of pregnancy		**x**			**x**	**x**

if applicable						
Analytical studies	**Valid available last 28 days**	**x**			**x**	**x**
Sample storage		**x**			**x**	**x**
Pulse wave velocity		**x**			**X**	
Interview with nutritionist		**x**	**x***	**x***	**x**	
Noninvasive coronary artery angiography using CT (CCTA)		**x**				x

*Only cluster intervention

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1. LIST OF ABBREVIATIONS

HIV	Virus of the immunodeficiency human
PLHIV	Person that lives with he HIV
TAR	Treatment antiretroviral
AIDS	Syndrome of immunodeficiency acquired human
LDL	Lipoproteins of low density
MRI	Resonance magnetic nuclear
TMAO	N-oxide of trimethylamine

2. HISTORY OF VERSIONS

Version	Date	Description of the change	Brief justification
1.2	June 15, 2024	Introduction to the amendment Noninvasive coronary artery angiography using CT (CCTA)	Given the possibility of having CCTA experience for coronary plaque detection at our center, this amendment is included to evaluate subclinical coronary disease and possible changes.

3. FRAME THEORIST

3.1 BACKGROUND AND JUSTIFICATION OF THE STUDY

Since the implementation of highly active antiretroviral therapy (ART), HIV-related mortality has been dramatically reduced. Morbidity and mortality are no longer caused by acquired immunodeficiency syndrome. Liver disease, cardiovascular disease, and non-AIDS-defining malignancies are the main causes of death. Compared with the general population, people living with HIV (PLHIV) have some degree of chronic immune activation leading to accelerated immune senescence. This process may drive the development of more non-AIDS-related comorbidities compared with uninfected individuals. By it so much, the PLHIV develop further events cardiovascular (MACE). Finding new targets to reduce cardiovascular risk is critical to decreasing MACE and improving the quality of life of PLHIV. Multiple mechanisms may drive these non-AIDS-related events, such as chronic immune activation, inflammation, and treatment side effects. ART use has been associated with metabolic complications such as dyslipidemia, endurance to the insulin and distribution altered of the fat. Besides, These metabolic complications have been linked to an increased risk of myocardial infarction and cardiovascular disease. Different types of ART appear to have a different impact on the metabolic/lipid profile.

Diet also has a role in metabolic control independently of ART. The impact of dietary intake of saturated fat and cholesterol on lipids The relationship between serum cholesterol and subsequent development of cardiovascular disease has been consistently demonstrated. On the contrary, the degree of dietary fat and cholesterol intake, the composition of that fat and the interaction of ART in PLHIV have an impact a stranger in the levels of lipids serum. Several studies previous to

Small-scale studies in HIV-infected individuals demonstrated no relationship between saturated fat intake and serum lipid levels.

Profile lipid

HIV infection is known to be associated with a highly atherogenic pattern characterized by increased levels and reduced size of low-density lipoproteins (LDL) and decreased levels of high-density lipoproteins. The key factor in the relationship between he HIV and the dyslipidemia go on without stay up late. The infection by HIV and ART are probably involved. In any case, the consequence of this persistent dyslipidemia could increase cardiovascular risk and the progression of atherosclerosis, which in addition to persistent inflammation could increase MACE. The standardization of the determination of LDL cholesterol (cLDL) is technically complex. This fact, together with the variability of some lipid components between individuals, has generated difficulties in the interpretation of the results of the lipid profile and its association with cardiovascular risk. The conventional methods of lipoprotein quantification used in clinical laboratories only allow the quantification of concentrations. Therefore, it is necessary to find new techniques capable of estimating cardiovascular risk with greater precision. This requires the ability to distinguish between different classes and subclasses of lipoproteins and to quantify particle size using nuclear magnetic resonance (NMR) spectroscopy. NMR has been shown to be a more accurate technique in different types of population. To date, few studies have been conducted using NMR in people living with HIV. With NMR we can estimate with precision he risk cardiovascular to the identify and quantify different types of lipoproteins according to their physicochemical characteristics, which allows determining with greater precision and accuracy the degree of penetration of these particles into the endothelium vascular. This technique can allow us have a better comprehension of quantitative and qualitative changes in lipoprotein profiles. Some studies explored the relationship between lipoprotein subclass profiles assessed by MRI and subclinical atherosclerosis by noninvasive means, such as intima-media thickness or aortic pulse wave velocity.

Speed of vibe of pulse

Arterial stiffness may lead to the development of vascular complications and premature vascular aging . It is a reliable biomarker of cardiovascular damage. Aortic pulse wave velocity is an indicator of aortic stiffness and is used as a predictor of adverse cardiovascular events. In addition, this technique is able to detect changes before intima-media thickness. Data on arterial pulse wave velocity in PLHIV are scarce. Since it is a simple, noninvasive technique, it could be useful to assess cardiovascular risk in this population.

Biomarkers metabolic of risk cardiovascular

Trimethylamine N-oxide (TMAO) is a gut microbiota-dependent metabolite generated from the oxidation of trimethylamine . TMAO is an independent cardiovascular factor related to inflammation, oxidative stress, cardiac fibrosis, endothelial injury, hypertension, and platelet activation. PLWH are potentially more susceptible to TMAO due to increased intestinal permeability. Increased intestinal permeability would increase the translocation of this bacterial product and, consequently, increase immune activation and inflammatory cytokine expression. The Diet has an impact on TMAO. Vegetarians have reduced TMAO levels

while people who did not follow the Mediterranean diet had increased levels according to two studies. Dietary changes in TMAO measurements have not been investigated before in HIV infection. Acting on the intestinal microbiota and the diet could be a via potential for reduce he risk cardiovascular in PLWH. Any impact on diet and lifestyle could change the TMAO profile and could be considered as an indirect marker of cardiovascular disease. in a via different to the metabolism lipid. His measurement could provide additional information on lipids and be an outcome of interest to evaluate the result of a dietary intervention.

Risk cardiovascular and activation immunological

Previous studies in HIV have separately associated subclinical atherosclerosis with markers serum of inflammation, activation widespread of cells T, activation of cytomegalovirus-specific T cells and soluble CD163 (a marker of monocyte activation). The role of T cells and the adaptive immune response in atherosclerosis has long been known. Although murine models suggested that CD4+ cells may be more atherogenic than CD8+ cells within plaques, circulating CD8+ cells can also promote atherosclerosis in response to activation by intracellular infections such as cytomegalovirus or *Chlamydia pneumoniae* . Kaplan et al have shown that in HIV, CD8+ activation may be more predictive of carotid plaque whereas CD4+ activation is more closely associated with carotid stiffness, another measure of disease vascular subclinical. All the data clinical published until he moment suggest that exists a narrow relationship between the activation immunological derivative of HIV infection and the extent of cardiovascular damage.

Diet Mediterranean in PLHIV

The Mediterranean diet, supplemented with virgin olive oil and nuts, has shown, in people at high cardiovascular risk, a reduction in MACE in primary and secondary prevention. The Mediterranean diet has also been associated with a reduction in age-related inflammation. Stradling et al. conducted a feasibility study in PLWH who adopted a Mediterranean portfolio diet supplemented with with walnuts reducers of fat. The results They suggested improvements in blood pressure, LDL cholesterol, and diet quality after 6 months. Another study in PLWH showed improvement in metabolic parameters, immune activation, regulatory T cell subsets, and microbiota .

Noninvasive coronary artery angiography using CT (CCTA)

Noninvasive coronary angiography using computed tomography allows a detailed and noninvasive assessment of the coronary arteries, their anatomy, the presence of atherosclerotic plaques, their location and level of severity , and the presence of qualitative characteristics in the plaques that increase their risk of rupture. Currently, CCTA can be performed with very low doses of radiation (around 3 mSv), and the total time of the test is just a few minutes. The test requires the use of intravenous contrast for a better definition of the coronary lumen, although relevant complications associated with the use of contrast are very rare. Due to its advantages, CCTA has become a key test in the assessment of patients with stable chest pain, it is recommended as a class I test by the guidelines of the European Society of Cardiology (), and its clinical use is increasing rapidly in our environment.

In conclusion, the Mediterranean diet in the general population with cardiovascular risk factors reduced the incidence of major adverse cardiovascular events. In PLWH, the diet Mediterranean ha shown some improvements immune and metabolic. Reduce he risk

cardiovascular associated to the HIV is at the moment one of the Main objectives of the investigation in HIV. HE they have identified several techniques for estimate the risk, but we do not yet have an adequate tool to reduce it.

3.HYPOTHESIS

This study HE base in the following hypothesis:

1. HIV infection and ART negatively impact the lipid profile of people living with HIV.
2. HIV infection is associated with an increase in cardiovascular disease through different pathways (classical risk factors, persistent inflammation, antiretroviral treatment, etc.).

3. The infection HIV HE associates to a profile lipid abnormal so much of the cholesterol total, HDL, LDL and lipoprotein subclasses measured by proton magnetic resonance imaging.
4. HIV infection drives early atherosclerosis mediated, in part, by an adverse lipid profile and persistent immune activation.
5. Modifying the diet towards a Mediterranean diet will produce changes in the lipid profile, in immunoactivation markers , in inflammatory parameters, in clinical markers of subclinical atherosclerotic disease such as the pulse wave and in markers of metabolites associated with cardiovascular disease such as serum Trimethylamine -N-Oxide (TMAO).

4. Objectives

Based in the previous hypothesis We propose the following Objectives:
Objective 1. Evaluate changes in the metabolic and lipid profile:

- Objective 1a. To evaluate changes in the lipid profile (total cholesterol, LDL-C , HDL-C , triglycerides) at week 24 compared to baseline.
- Objective 1b To evaluate the number, size and composition of lipoprotein particles by proton magnetic resonance spectroscopy at week 24 compared to baseline.
- Objective 1c. Percentage of people with increased high-density lipoprotein (HDL) levels >5%.
- Objective 1d. To assess changes in the anthropometric profile (weight, body mass index, abdominal circumference) at week 24 compared to baseline.

Aim 2 . Assess changes in he profile inflammatory and of immunoactivation :

- Aim 2a. Assess the changes in the activation immune of the T cell compartment at week 24 compared to baseline.
- Objective 2b. Percentage of patients with reduced inflammatory and bacterial translocation markers at week 24 compared to baseline.

Objective 3. To evaluate changes in arterial stiffness levels measured by pulse wave velocity at week 24 compared to baseline.
Aim 4 . Proportion of Participants with decline of >20% of TMAO in the week 24 compared to baseline.
Aim 5 .Proportion of Participants with increase of >2 points in the scale MEDAS adherence to the Mediterranean diet at week 24 compared to baseline
Objective 6. Proportion of participants who improve in the quality of life questionnaire. EQ-5D life at week 24.
Objective 7. Evaluate the persistence of all changes included in objectives 1 to 6 at week 48 (24 weeks after the end of the intervention)
Objective 8. To assess the burden of subclinical atherosclerotic coronary disease and changes 1 year after intervention.

5. METHODOLOGY

5.1. Design of study

Study randomized prospective of intervention with optimization dietetics in people with HIV in treatment antiretroviral with dyslipidemia (Cholesterol total >200mg/dl either LDL cholesterol >160mg/dl) and without treatment lipid-lowering.

5.2. Study population, inclusion/exclusion criteria and subject withdrawal criteria.

Criteria of inclusion:

- Participant ≥ 18 years.
- Infection by HIV confirmed by serology.
- Treatment antiretroviral asset (TAR).

- Burden viral undetectable during to the less 12 months (HE allows a blip below 200 copies).
- LDL >140 mg/ dL .
- Mediterranean Diet Adherence Screener (MEDAS) lower to 9 points (low adhesion) to facilitate the detection of changes after the intervention.

Criteria of exclusion:
- Participants in treatment lipid-lowering.
- Treatment with anti-inflammatory chronic.
- Hepatitis B, C or Others conditions inflammatory chronicles active.
- Hypercholesterolemia familiar No treated.
- Hypothyroidism No controlled.
- Impossibility of give he consent.

Criteria of withdrawal:
Subjects may withdraw from the study at any time without obligation to provide a reason. He IP can give of low to a subject of the study by reasons of safety or efficacy.

The reasons of abandonment of the study will be the following:
- Failed of screening: All patient that, a time informed is spoiled in participating in the study, does not meet the inclusion and/or exclusion criteria.
- Withdrawal of the consent: All patient that, by any reason, remove your consent
- Consumption of anti-inflammatory either lipid-lowering drugs during further of 7 days.
- Diagnosis of hypothyroidism either diabetes mellitus.
- Any event adverse that prevent continue any diet indicated.
- Loss of follow-up.

5.3. Definition of the variables of study

Main Variables
- Total cholesterol, cLDL , cHDL , triglycerides in mg/ dL . It will be measured at baseline, week 24 and 48.

Secondary Variables
- Number, size and composition of lipoprotein particles by proton magnetic resonance spectroscopy at baseline, week 24 and 48.
- Anthropometric profile (weight, body mass index, abdominal circumference) at baseline, week 24 and 48.
- CD 3,CD 45,CD4,CD8,CCR7,CXCR3,CCR6,CD103,FOXP3,CD25,CD45RA and CD45RO. Quantification in blood in visits basal, 24 weeks and 48 weeks of cells with markers.
- IL-6, TNF-alpha, IFN-gamma, sCD163, sCD14, MADCAM-1, sCD14 and LPS. Quantification in serum in visit basal, 24 weeks and 48 weeks of
- Rigidity arterial extent by the speed of vibe of pulse in the visit basal, week 24 and 48.
- Levels of Trimethylamine -N-Oxide serum (TMAO) in the in the visit basal, week 24 and 48.

- Scale MEDAS of adherence to the diet Mediterranean measures in the Baseline visit, week 24 and 48
- Questionnaire of quality of life EQ-5D in the visit basal, week 24 and 48.
- Presence of atheromatous plaque at the coronary level at the baseline visit and at week 48
- Presence of calcium, fibrous and fatty fraction in the coronary plaque at the baseline visit and at week 48
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6. PROCEDURES OF THE STUDY

- Pre-screening visit (Recruitment): carried out by the regular doctor in the outpatient clinics of the HIV unit at Hospital del Mar. Potential candidates who meet the inclusion criteria will be identified. They will be given an appointment for: Initial visit: informed consent will be signed, blood tests will be performed with blood samples (to achieve objectives 1 to 6). A comprehensive history of clinical history, HIV infection, and drug history will be taken. Randomization will be carried out following randomization tables according to age, gender, and ART. They will be referred to an intervention consultation.
- Visit 1 (baseline) : anthropometric measurements will be taken, scales predetermined in the study (annexes) will be passed and the measurement of the pulse wave velocity (according to methods described later). A dietary approach will then be carried out with a different visit schedule:
  ***Cluster Intervention (67 participants):**
  The intervention group will receive at the beginning a motivational interview with a personalized portfolio provided by a nutritionist on the Mediterranean diet and adherence to the diet: with information on the food components; desirable frequency of intake; recipes, use of olive oil, walnuts, hazelnuts and almonds. Individual guidelines will be planned individually to improve the lipid profile and improve food choices considering dietary habits, socioeconomic status and nutritional needs (and the score obtained on the MEDAS scale).
  At weeks 4 and 12 (virtual), the patient will have a new interview with the nutritionist to review the recommendations, discuss difficulties with diet adherence, design weekly dietary plans and shopping lists. At week 24 and 48, an in-person visit will be held to perform all the procedures of the baseline visit again (including blood draws).
  ***Cluster treatment standard (33 participants).**
  Baseline visit (in person), In addition to the initial assessment identical to that of the intervention group, general dietary information will be provided in writing, as is currently done in the HIV unit.
  New visits will be made at week 24 (in person) and week 48 (in person). The same measurements, dietary assessments and blood draws will be made as at the baseline visit.

  Detailing of tasks to carry out for achievement of the goals:
- Task 1. Adherence to the Mediterranean diet, anthropometric and quality of life measurements (objectives 5 and objective 6). All carried out by a trained researcher.
  - Task 1a. 118-item semiquantitative food frequency questionnaire (FFQ), MEDAS questionnaire, and a 24-hour dietary recall .
  - Task 1b. Anthropometric measurements: height and weight will be measured using a mechanical anthropometric scale. BMI (BMI = weight (kg) / [height (cm)]2). Waist circumference halfway between the lower rib margin

and the iliac crest. Hip circumference will be measured as the widest measurement over the buttocks.

- Task 1c. Measuring quality of life using the EQ-5D questionnaire. Specific questions on alcohol consumption patterns and the IPAQ Physical Activity Questionnaire, Simplified Medication Adherence Questionnaire (SMAQ).

- Task 2. Measurement of the profile lipid (for achievement of goals 1 (1a,1b,1c)
  - Task 2a. Blood collection after fasting for more than 8 hours, between 8am and 10am. Serum will be obtained for the usual clinical measurement by means of of the practice clinic usual and in he laboratory of Reference from Catalonia for the measurement of total cholesterol, HDL and LDL (according to standard practice). Serum, plasma and cells will be preserved (task 4)
  - Task 2b. With the samples of serum obtained and preserved to -80ºC and when recruitment and monitoring have been completed, the analysis of the lipoprotein subclass profile will be performed by means of Proton Nuclear Magnetic Resonance (NMR) in Metabolomics Platform IISPV, CIBERDEM. Rovira i Virgili University , Bisofer Teslab . Lipoprotein analysis of serum samples will be performed using Liposcale ® (Biosfer Teslab , Reus, Spain), a method based on 2D diffusion-ordered 1H-NMR spectroscopy. This protocol evaluates lipid concentrations, particle size and number of three different classes of lipoproteins (vLDL), LDL and HDL), as well as the number of particles of nine subclasses (large, medium and small vLDL , LDL and HDL). This technique records 2D 1HRMN spectra on a BrukerAvance III 600 spectrometer, operating at a proton frequency of 600 MHz to 310 KHz (Bruker (BioSpin , Rheinstetten , Germany). The signal obtained makes it possible to determine the nine lipoprotein subclasses. (J. Clin. Med . 1875).) The study of lipoprotein subclasses makes it possible to obtain a profile that, when compared with a population database, makes it possible to estimate cardiovascular risk based on the lipid composition of the lipoprotein subclasses in a more specific way than by means of the classic scales for estimating cardiovascular risk.

- Task 3. Measurement of arterial stiffness by pulse wave velocity (to achieve objective 3). Arterial pulse wave velocity will be determined by sequential applanation tonometry (Millar Tonometer, SPC-301; Millar Instruments, Houston, TX) in the carotid and femoral arteries synchronized with a three-lead electrocardiogram using the SphygmoCor device (SphygmoCor ; AtCor , Sydney , Australia). Wave arrival latency time will be calculated using a foot-wave method. The surface distance from the suprasternal notch to each recording point will be measured. Total transit distance will be calculated by subtracting the distance between the sternal notch and the carotid from the distance between the sternal notch and the femoral artery. Arterial pulse wave velocity will be calculated using total transit distance divided by time. of latency. HE will reject the measurements of the speed of the vibe pulse that do not meet the automatic quality controls specified by SphygmoCor software . Two pulse wave velocity measurements will be averaged for each subject for all calculations.
- Task 4. Study of immunoactivation markers in PBMCs (peripheral blood mononuclear cells) (objective 2a). PBMCs will be obtained from heparinized blood by density gradient centrifugation (Ficoll - Paque ; Amersham Biosciences).

- Task 4a. Flow cytometry. A panel of antibodies will be used to characterize adaptive immune cells, including CD3, CD 45, CD 4, CD8, CCR7, CXCR3, CCR6, CD103, FOXP3, CD25, CD45RA a nd
  CD45RO. HE will identify the cells T of memory (CD45RA-/CD45RO+) and naïve and effector T cells (CD45RO-/CD45RA+). Within memory cells (CD45RA-), central memory cells (CCR7+) and effector memory cells (CCR7-); and inside of the cells T that No are of memory (CD45RO-
  /CD45RA+), naïve cells (CCR7+) and effector cells (CCR7-). Using lineage marker (CD3+) and cytotoxic lymphocytes (CD8+), helper lymphocytes (CD4+) are separated into subtypes by expression or not of the chemokine receptors type 3 (CXCR3) and 6 (CCR6) such as: Th1 (CXCR3+CCR6-),Th 2 (CXCR3-CCR6-),Th17 (CXCR3-CCR6+) and Th1- Th17
  (CXCR3+CCR6+). The percentage of activated cells will be differentiated using the activation markers HLA-DR and CD38. Regulatory T cells will be identified as FOXP3+CD25+. Appropriate positive and negative controls will be performed. Cell populations will be acquired from LSR- Fortessa (UPF central facilities) and analyzed using Flowjo software (Treestar Inc. CA).
- Task 5. Study of inflammatory markers and bacterial translocation (to achieve objective 2b). A multiplex ELISA for different biomarkers: at least the multiplex must include: IL-6, TNF-alpha, IFN-gamma, sCD163, sCD14, MADCAM-1, sCD14 and LPS. Plasma samples will be stored at -80 °C to perform the analysis at the same time in order to reduce variability.
- Task 6. Determination of the levels plasmatic of TMAO (for achievement of aim 4). HE will preserve to -80C all the samples and HE will perform a Once the study is completed, the determination of TMAO levels in fasting plasma will be quantified by stable isotope dilution liquid chromatography with on-line tandem mass spectrometry (LC/MS/MS) on an APi 500 triple quadruple mass spectrometer (AB SCIEX, Framingham, Massachusetts) available at the Bioanalytics department of Middlesex University , London, with whom the PI has a stable collaboration and will allow the measurement to be carried out reliably and consistent.
- Task 7. Upon approval of the amendment (version 1.2 of the protocol), all enrolled patients will undergo a CCTA. CCTAs in the study will be performed in an identical manner to those performed daily in our center as part of routine clinical practice, using identical protocols for both image capture and interpretation/reporting . The radiation dose used will follow the ALARA principle (“as low as reasonably possible”), as low as possible while maintaining image quality. The same principle will apply to the intravenous contrast dose. Patients will sign an updated informed consent to participate in the VIHMET study (insert the name of your study), which will include a specific description of the CCTA, its objectives and associated risks. Once in the study, participants will receive the usual pre-CCTA preparation by specialized nursing staff and directed by cardiologists with experience in cardiac imaging. This includes general recommendations on hydration and fasting, as well as the potential use of a low-dose beta-blocker (or ivabradine) to control heart rate if it is above 70 bpm .
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7. PERIOD OF THE STUDY

For can carry to cape all he project it we have divided in different stages:

1. Preparation and validation of the protocol. Patient preselection circuits and diagnostic tests.
   January 2025 to February 2025.
   This phase will be carried out by the researchers: Design of the patient selection circuit within the HIV unit and promotion of the study among the professionals of the unit focused on the need to address the optimization of the lipid profile of this group of people. We will review the processing of the samples (labeling of the samples, contact with the BioBank service , presentation of the study to the doctors for their knowledge). An anonymous registry of candidate patients will be created.
2. Recruitment of patients
   From March 2025 to March 2026. It is estimated that it will take at least one year to recruit the 100 patients.

Recruitment will be carried out at the Infectious Diseases Service of Hospital del Mar (HM). The referent will be Dr. Roberto Güerri Fernández (IP The researchers who will coordinate the recruitment process will be Dr. Alicia González. Patients who meet the inclusion criteria will be referred to a specific consultation created ad hoc to carry out the selection visit. This phase will be carried out interchangeably by Dr. Esperanza Cañas, Dr. Alicia González, Dr. Itziar Arrieta, Dr. Mariano Pascual or Dr. Roberto Güerri. During this visit, informed consent will be signed, the first sample will be extracted for the complete analysis and general data will be collected: background, history of HIV infection, treatment history and the questionnaires and the participant will be randomized.

3. Follow-up of the patient during 48 weeks . From
   March 2025 to October 2026.
   At this stage, the specific intervention itself will be carried out. Nutritionist researcher Jordi Argimon together with Dr. Silvia Ballesta and Dr. Juan José Chillarón from the Endocrinology service will carry out a complete nutritional assessment. Anthropometric measurements and a measurement of the speed of movement will be carried out. of the vibe of pulse. HE will perform follow-up according to it notarized.
4. study visits (March 2025 to October 2026). The final study visit with follow-up at 48 weeks will be carried out in all participants. The tests contemplated in the protocol will be carried out. This stage will be carried out by he equipment of the Dr Squealer (Jordi Argimon together with the Dr. Silvia Crossbow and Dr. Juan Jose Chillaron) .
5. Sample collection and processing : blood collection and processing to obtain PBMCs , plasma and serum. The processing and conservation of the samples will be carried out in the IMIM laboratory by Dr. Güerri -Fernández and Dr. García Giralt.
6. Sample preparation for FACS . Once initial recruitment is complete, preserved samples will be thawed, stained and prepared for FACS (Dr. Güerri-Fernández, Dra García-Giralt). Data reading and interpretation will be performed by Dr. Güerri-Fernández and an external collaborator (Prof David M. Asmuth (University of California).
7. Sample preparation and shipment to Metabolomics Platform IISPV, CIBERDEM . Rovira i Virgili University , Bisofer Teslab . When the follow-up is completed, samples will be sent (baseline samples , 24 weeks and 48 weeks). weeks) to the Rovira i Virgili University for further processing and interpretation of the results of the lipoprotein subclasses.
8. Preparation and shipment of plasma samples to Middlesex University , London (Dr. laboratory Sha). Once follow-up is complete, samples (baseline, 24-week and 48-week samples) will be sent to the Middlesex Hospital. university for the determination of TMAO according to the described protocol.
9. Analysis and publication of results (from January 2026 (preliminary) to December 2026) will be carried out by the entire research team.

8. ETHICS

The study will be submitted to the rules ethical of the Investigation Biomedical in Humans. The study will be conducted in accordance with the ethical principles derived from the Declaration of Helsinki (Fortaleza, Brazil, October 2013). In addition, the study will be conducted in accordance with the protocol, good clinical practice (GCP) in accordance with the guidelines of the International Conference on Harmonization (ICH) and the regulatory requirements for participating institutions. The study will be conducted according to a protocol reviewed by a Committee. The benefits of the study are considered to be in proportion to the risks; and the rights and well-being of the subjects

will be respected.

Patients will be informed and given an approved informed consent form in compliance with GCP. Likewise, all participants will sign the PsMar Biobank consent . Aspects related to confidentiality will follow established regulations. Before including subjects in the study, the researcher will review the information sheet with the potential participant, and if the participant agrees, agrees to participate, the informed consent document will be signed and dated. The researcher will provide a copy of the signed informed consent form to each subject and will keep a copy in the study file. The subject will be informed, as provided for in Organic Law 15/99 on the Protection of Personal Data, that these may be subject to automated processing and of the rights that the study participants have to consult, modify or delete their personal data from the file. The subject will be informed that the database will be handled by the IP using an access code and the name will not appear in the registry. The responsibility of the file global corresponds to the Institute Hospital of the Sea of

Research Institute (IMIM) of Barcelona. This study will comply with the Code of Good Scientific Practices (http://www.imim.es/imim/cas/c-CBPC.htm).

No financial compensation is expected for participants or reimbursement of expenses arising from their participation

9. MANAGEMENT OF SAMPLES

As previously described, during the study, blood will be drawn and processed to obtain PBMCs , plasma and serum. The processing and conservation of the samples will be carried out in the IMIM laboratory by Dr. Güerri - Fernández and Dr. García Giralt. When the initial recruitment is completed, the preserved samples will be thawed, stained and prepared to undergo FACS. When the follow-up is completed, samples will be sent (baseline samples , 24 weeks and 48 weeks) to Rovira i Virgili University for further processing and interpretation of lipoprotein subclass results. Likewise, samples (baseline, 24-week and 48-week samples) will be sent to Middlesex University for TMAO determination.
The samples will be pseudo-anonymized and when the study is completed, the remaining samples will be collected in the PSmar biobank .

10. CONFIDENTIALITY OF THE DATA

This study will be carried out in accordance with the General Data Protection Regulation 2016/679 and Organic Law 3/2018, of December 5, on the Protection of Personal Data and the Guarantee of Digital Rights.
Patient recruitment will be carried out in the outpatient clinics of the HIV unit at Hospital del Mar. Once the participant signs the informed consent, he or she will be assigned a study number that will correspond to his or her medical record number. This will be saved in an encrypted file with a password to which the IP will have access.
The database that will be created ad hoc for the study will not include any data that would allow the patient to be identified: no date of birth, no phone number, no medical record number. Only the study ID. This database will be in T h e RedCap environment will only be accessible to the study's researchers.

Study data must be verifiable with the source data, which requires access to all original records, laboratory reports, and subject records. Confidentiality of data and patient identity will be maintained during and after the study is completed . Only the principal investigator and authorized study personnel will have access to these confidential records.

None fact used in he analysis and later divulgation of the results of the study will contain some identifiable reference to patient names.

Once the study is completed, the results will be communicated to the relevant authorities, as appropriate, in accordance with local legislation. The data generated will also be published in conferences and scientific journals.

11. LITERATURE

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12. ANNEXES:

- Archives additional (exhibit MEDAS (1), EQ-5D-5L (2), SMAQ (3))