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Assessment of a novel functional food modulating the microbiota-inflammation-brain axis in patients with heart failure and/or /atrial fibrillation patients (the AMBROSIA study): Protocol for a randomized controlled trial

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ABSTRACT

Background and aims: Atrial fibrillation (AF), heart failure (HF), and undernutrition represent a complex triad with major clinical and socioeconomic consequences in older adults, often predisposing to frailty. Undernutrition often remains underdiagnosed due to a reliance on weight-based measures and limited awareness of inflammation-related cachexia. The AMBROSIA study aims to fill these gaps by exploring the response of the microbiota–inflammation–brain axis to a targeted, fortified food product-based intervention, with comprehensive outcome assessments, alongside mechanistic/exploratory -omics analyses and gut microbiota (GM) functional profiling.

Methods and results: This single-center, prospective, parallel-group randomized controlled trial aims to enroll 120 older adults with confirmed AF and/or HF. Participants will be randomized 1:1 into an intervention group (n = 60) or control group (n = 60). All participants receive individualized dietary counseling; the intervention group additionally consumes one AMBROSIA nutritional bar daily for six months. The bar contains hydrolyzed proteins, inulin, CoQ_{10}, and probiotics (*L. rhamnosus* IMC 501® and L. *paracasei* IMC 502®) in a flavonoid-rich chocolate matrix. Clinical, cognitive, and nutritional data, along with blood, saliva, urine, and stool samples, will be collected at baseline, 3, and 6 months. The primary endpoint is the change in skeletal muscle mass, physical function and frailty, while secondary endpoints include changes in nutritional status, inflammation, GM, metabolomics, and quality of life.

Conclusion: By integrating cutting-edge omics tools and a multidimensional nutritional strategy, AMBROSIA aims to uncover mechanisms driving undernutrition and identify biomarkers to support personalized interventions for older patients with AF and HF.

1. Introduction

Atrial Fibrillation (AF) and Heart Failure (HF) are highly prevalent cardiovascular conditions in older adults that substantially increase the risks of stroke, hospitalization, dementia, and lastly, mortality [1,2]. AF is a supraventricular arrhythmia characterized by completely disordered

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atrial electrical activity and is the most common rhythm disorder in the elderly, affecting approximately 6 million people across Europe and posing relevant risks such as stroke, HF exacerbation and cognitive decline [3]. HF, in turn, is a clinical syndrome resulting from structural or functional cardiac abnormalities and manifests as dyspnea and fatigue due to inadequate organ perfusion [4]. It is the leading cause of hospitalization in people over 65, resulting in a dismal prognosis and in a heavy financial burden on healthcare systems. HF currently affects approximately 15 million individuals across Europe, with prevalence continuing to rise [5].

Notably, AF and HF often coexist and synergistically promote frailty and undernutrition in older adults, further worsening morbidity and mortality [6]. According to the WHO, frailty is a clinical state characterized by reduced ability to cope with stressors due to a decline in physiological homeostasis. It is a multifactorial and dynamic condition involving multiple organ systems and is closely associated with, often bidirectionally, cardiovascular diseases [7]. Remarkably, frailty should not be interpreted as a condition for withholding beneficial therapies; rather, it should prompt the implementation of targeted strategies aimed at enhancing resilience and preserving functional capacity in affected individuals [8].

Undernutrition in HF may arise from decreased appetite, malabsorption, or elevated metabolic demands, and can progress to cachexia, a chronic, inflammation-driven wasting syndrome affecting fat, skeletal muscle and bone [9]. Although overt cachexia affects approximately 15 % of HF patients, in part because there are no standardized diagnostic criteria and clinicians often rely solely on observed weight loss, broader malnutrition definitions identify up to 50 % of this population as at-risk. This risk may result from difficulties in obtaining, eating, or absorbing food, as well as markedly increased energy requirements [10]. Notably, recent evidence highlights the significance of sarcopenic obesity, a condition marked by the simultaneous presence of muscle loss and excess body fat. Sarcopenic obesity is often observed in frail and disabled individuals and is associated with an increased risk of cancers, cardiovascular conditions, bone fractures, kidney diseases, and higher mortality rates [11].

Intriguingly, recent evidence links chronic inflammation and malnutrition to gut microbiota (GM) changes and decreased production of short-chain fatty acids (SCFAs), key microbial-derived metabolites that appear to be key regulators of gut-barrier integrity and systemic immune homeostasis [12]. In detail, the bidirectional communication between the gut and brain, called “gut-brain axis”, plays a fundamental role in the regulation of appetite, energy storage, and expenditure. Given the relevance of early nutritional support in undernourished older patients with AF and HF, therapeutic strategies targeting the gut-inflammation-brain axis may open new avenues for intervention.

In HF patients, micronutrient supplementation has been associated with improvements in left ventricular function and quality of life [13]. Probiotic interventions have documented consistent promise in undernutrition management in other population groups. For instance, *Bifidobacterium breve* has supported weight gain in severely malnourished children, and strains such as *Lactobacillus fermentum*, *Lactobacillus ingluviei*, and *Lactobacillus acidophilus* have been linked to increased weight in adult populations [14]. Overall, dietary strategies that positively influence dietary habit in parallel with microbially-mediated improvements to systemic health represent cost-effective and realistic approaches to improving host metabolism and nutritional status in this vulnerable population.

Recent advances in high-throughput “omics” technologies (*e.g.* metagenomics, metabolomics, lipidomics) have allowed the generation of large-scale datasets that may uncover novel biomarkers of undernutrition [15], or elucidate mechanisms underlying responses to interventions. In parallel, machine-learning approaches could integrate these multidimensional data, handling moderate sample sizes, class imbalance, and potential confounders, to identify predictive molecular and clinical features for improved risk stratification and intervention monitoring [16].

Hence, AMBROSIA study aims to define if a novel functional food targeting the microbiota–inflammation–brain axis, combined with dietary counseling, can improve undernutrition and benefit skeletal muscle mass, frailty, cognitive function and other clinical outcomes in older patients with AF and HF. A secondary aim is to analyze biological samples (faeces, saliva, plasma and urine) throughout the intervention to evaluate mechanistic responses to treatment at the level of the GM and wider systemic effects.

2. Methods/design

2.1. Study design and setting

This is a single-center, prospective, parallel-group randomized controlled trial (RCT) registered at the US National Institutes of Health (ClinicalTrials.gov ID: NCT05912309) and will be conducted at the Research Unit of Medicine of Ageing of the Department of Experimental and Clinical Medicine of the University of Florence (Italy). Eligible participants, patients attending the day hospital or recently discharged from the Geriatric Intensive Care Unit will be screened and enrolled to establish a clinically homogeneous and relevant cohort. Following finalization of the study protocol, staff training, and regulatory approvals (anticipated within a 5-months’ preparatory period), participant screening and enrollment will take place over the subsequent 15 months. Enrolled patients will then begin a 6-months’ intervention phase. Participants randomized to the intervention group (IG) will receive individualized dietary counseling in combination with the “AMBROSIA” chocolate-based nutritional bar, to be consumed approximately fifteen minutes before breakfast each day. The control group (CG) will receive only individualized dietary counseling, in line with standard care practices. The intervention phase will span a total of 22 months, beginning with the first patient enrollment and concluding with the final follow-up visit. The final six months of the AMBROSIA study will be dedicated to data cleaning and statistical analyses.

The AMBROSIA project will be conducted in strict compliance with data protection regulations and in agreement with full ethics committee approval (19882_spe).

2.2. Eligibility criteria

Eligible, Caucasian, participants must be at least 70 years old at the time of screening. All patients must have a confirmed diagnosis of AF, HF, or both. For AF, the diagnosis must have been established within the 12 months preceding the screening using a standard 12-lead electrocardiogram, Holter monitoring, or an implantable loop recorder; diagnoses based solely on pacemaker interrogation are not considered enough. In patients with AF alone, the arrhythmia must be classified as persistent (lasting more than seven days), long-standing persistent, or first-diagnosed. In cases where AF is associated with HF, any form of AF, including paroxysmal or permanent, is tolerable.

Eligibility for HF requires the presence of both signs and symptoms consistent with clinical diagnosis, coupled with objective instrumental findings. For HF patients with mid-range or reduced ejection fraction, eligibility requires a left ventricular ejection fraction below 50 %, elevated levels of natriuretic peptides (such as B-type natriuretic peptide or N-terminal pro-brain natriuretic peptide), and evidence of structural heart disease or diastolic dysfunction. For HF patients with preserved ejection fraction, inclusion criteria include an ejection fraction of 50 % or higher, elevated natriuretic peptides, and supporting evidence of structural cardiac abnormalities or diastolic dysfunction. In addition to cardiovascular criteria, patients must demonstrate either normal nutritional status or be at risk of malnutrition. This will be assessed using both the Mini Nutritional Assessment (MNA) [17] and the Malnutrition Universal Screening Tool (MUST) [18]. Finally, all patients must exhibit adherence to a Mediterranean dietary pattern, as determined by the

MEDILITE (Mediterranean Diet Adherence Score) questionnaire [19] (Table 1).

2.3. Exclusion criteria

Subjects with a Body Mass Index (BMI) >30 kg/m^2 or < 18.5 kg/m^2 will not be eligible. Additionally, patients will be excluded if they received antibiotic therapy within the six months prior to screening or are currently using systemic corticosteroids or immunosuppressive medications. A history of severe allergic reactions or the presence of any active infectious disease at the time of screening will also lead to exclusion, such as a history of significant chronic or recurrent infections possibly affecting study outcomes. The recent use of non-steroidal anti-inflammatory drugs (NSAIDs; within two weeks prior to screening), or to undergo any minor medical intervention (*e.g.* a dental extraction) within the same period, will preclude participation. Participants treated with SSRIs, SNRIs, and other antidepressant agents will be eligible; however, individuals who initiate or discontinue such treatments during the study will be excluded from the analysis.

Similarly, patients with a history of major surgical procedures within the past three months will not be included. Participants with a confirmed immunodeficiency, or who have traveled to exotic areas within the 12 months prior to screening, will also be excluded due to potential immunological and microbial variability.

Additional exclusion criteria include significant comorbidities such as uncontrolled diabetes mellitus (glycated hemoglobin >9 %), active cancer, advanced liver disease (including decompensated cirrhosis), or severe chronic kidney disease (KDIGO stages 4–5; glomerular filtration rate consistently <30 mL/min/1.73 m^2). Patients undergoing chemotherapy or radiotherapy at the time of enrollment will also be excluded.

Neurocognitive and functional impairments will also serve as exclusion criteria. Specifically, patients diagnosed with dementia or severe psychiatric disorders that may impair compliance or the ability to provide informed consent will be excluded, as will those who are unable to self-administer medications and require a caregiver for the daily medication management (Table 1).

Table 1
Inclusion and exclusion criteria for patients with AF and HF.

Condition	Inclusion Criteria	Exclusion criteria
AF	• ≥70 years at screening • Caucasian ethincity • AF confirmed within 12 months by 12-lead ECG, Holter, or loop recorder (pacemaker interrogation alone insufficient) • AF alone: persistent (>7 days), long-standing persistent, or first-diagnosed • AF + HF: any AF subtype allowed • Normal nutritional status or at risk of malnutrition (MNA and MUST) • Mediterranean diet adherence (MEDILITE score)	• BMI <18.5 or > 30 kg/m^2 • Antibiotic use within 6 months • Use of systemic corticosteroids or immunosuppressive medications • History of severe allergic reactions or chronic disease • Presence of any active infectious disease • Use of NSAIDs within 14 days prior to screening • Minor medical/dental procedures within 14 days days prior to screening • Start or discontinuation of SSRIs, SNRIs, or antidepressants during the study
HF	• ≥70 years at screening • Caucasian ethincity • HF patients with mid-range or reduced ejection fraction: left ventricular ejection fraction<50 % with elevated natriuretic peptides and structural heart disease or diastolic dysfunction • HF patients with preserved ejection fraction: left ventricular ejection fraction>50 % with elevated natriuretic peptides and structural heart disease or diastolic dysfunction. • Normal nutritional status or at risk of malnutrition (MNA and MUST); • Mediterranean diet adherence (MEDILITE score)	• Major surgery within the past 3 months • Confirmed primary or secondary immunodeficiency • Travel to exotic/tropical regions within the past 12 months • Decompensated cirrhosis or advanced chronic liver diseas • Uncontrolled diabetes mellitus (glycated hemoglobin >9.0 %) • Severe chronic kidney disease (eGFR <30 mL/min/1.73 m^2; KDIGO stage 4–5) • Dementia or cognitive/physical deficits impairing consent/ compliance • Undergoing chemotherapy or radiotherapy at the moment of enrollment

2.4. AMBROSIA study protocol

2.4.1. Fortified food product design

The AMBROSIA bar (AB) was developed through a rigorous selection process involving commercially available protein hydrolysates, chosen for their high protein content, balanced essential amino acid profiles, and demonstrated *in vitro* cardioprotective properties. The final formulation incorporates hydrolyzed proteins (Carbery Group, Ireland), dietary fibers (including inulin, ACEF, Italy) Coenzyme Q_{10} (INDENA, Italy), and a proprietary SYNBIO® probiotic blend (Synbiotec Srl, Italy). This blend consists of two human-derived strains, *Lacticaseibacillus rhamnosus* IMC 501® and *Lacticaseibacillus paracasei* IMC 502®, both patented for their robust mucosal adhesion, resistance to gastric acidity, and ability to inhibit pathogenic microorganisms [20,21]. Delivered in a chocolate-based matrix rich in flavonoids [22], the AB is gluten-free, free from artificial additives, and specifically optimized for palatability, affordability, and digestibility in older adults. Developed in collaboration with patients' associations included in the project, the AB is a convenient and functional tool designed to support nutritional status, GM balance, and overall psycho-physical well-being in older patients living with AF and HF. The detailed AB composition is provided in Table 2**,** and the product appearance is shown in Fig. 1.

The taste acceptance and gastrointestinal AB tolerance, both characteristics were evaluated using a structured questionnaire adapted from De Luis et al. [23]. This tool assessed both sensory perception and potential digestive symptoms in a sample of 150 participants (87 women, 63 men), divided into three age groups: <40 years, 40–70 years, and > 70 years, to capture a broad age distribution.

Each participant received a single portion of the AB after having been instructed to consume it in a single time. The evaluation process included two phases:

1. Acceptance (Immediate Sensory Evaluation). Immediately after consumption, participants completed the section, rating sensory characteristics such as taste, sweetness, bitterness, and aftertaste on a 0–10 scale, where 0 indicated "Not at all" and 10 indicated "Very much."
2. Tolerance (End-of-Day Assessment). At the end of the day, participants completed the section, documenting any symptom experienced, including nausea, bloating, acid reflux, and changes in stool consistency, reporting any adverse effect or gastrointestinal symptom using the same 0–10 scale.

The questionnaire was administered in paper format, and participants were instructed to return it on the same day to ensure timely data collection and maximize accuracy and compliance. Analysis of the

Table 2
Detailed composition of the AMBROSIA bar.

Ingredients	g/dose
Chocolate	7
Synbio® Probiotics lyophilized powder	0.12
Hydrolysed proteins	1
Inulin	1
CoQ10	0.32
TOTAL	9.44

Fig. 1. Appearance of the AMBROSIA bar.

responses revealed that 75 % of participants found the AB acceptable, with positive evaluations distributed consistently across all three age-groups. Furthermore, over 95 % of participants reported no gastrointestinal symptoms, such as nausea, vomiting, acid reflux, abdominal pain, bloating, flatulence, and stomach discomfort.

2.4.2. *Intervention protocol*

Following enrollment, patients will be randomly assigned in a 1:1 ratio to either the IG or the CG. To ensure balanced allocation across groups, block randomization will be used, with block sizes predefined and randomly varied to minimize allocation predictability.

All enrolled patients will receive comprehensive, individualized dietary counseling. Participants in the IG will additionally receive a daily supply of the AB; in contrast, the CG will receive dietary counseling alone, representing the standard of usual care (Fig. 2).

The study protocol will begin with an early screening phase during which potential participants will be rigorously assessed according to the predefined inclusion and exclusion criteria to ensure their suitability for the study. Following successful screening, the study officially starts at the baseline visit (T0). During this visit, all enrolled patients will receive detailed information regarding the study objectives, procedures, and sample collection protocols. Written informed consent will be obtained from each participant prior to beginning any study-related activities. At T0, in addition to the collection of serum, saliva, urine, and fecal samples, a comprehensive set of baseline assessments will be conducted, encompassing clinical evaluations, nutritional assessments, and a Geriatric Multidimensional Assessment. These include:

- Clinical and Sociodemographic Data: Family history, sociodemographic factors, lifestyle habits, medical history, physical examination and blood pressure measurement;
- Cardiac Evaluation: Standard Electrocardiogram (ECG) and Echocardiography;
- Anthropometric and Body Composition Measurements: weight, height, BMI and Bioelectrical Impedance Analysis (BIA);
- Frailty and physical function: Phenotype frailty index including handgrip measure of arm strength [24], Clinical Frailty Scale [25] and the Short Physical Performance Battery (SPPB) test [26];
- Cognitive and Emotional Status: Mini-Mental State Examination (MMSE) [27] and the 15-item Geriatric Depression Scale (GDS-15) [28];
- Nutritional Assessment: MNA, MUST and MEDILITE;
- Quality of Life and Bowel Function: Gastrointestinal Quality of Life Index (GIQLI).

At the baseline visit, patients will also receive individualized dietary counseling, aimed at promoting healthier food choices in accordance with national food-based dietary guidelines [29], with focus on clinical recommendations for HF and AF patients. To further assess dietary habits, each participant will be asked to complete a 4-day unweighed food diary just prior to the T0 visit. Following the completion of all baseline assessments, patients randomized to the IG will receive the ABs (an adequate amount to cover three months), along with detailed instructions regarding their right storage and daily consumption.

After three months, participants will attend a second study visit (T1). Patients will be asked to complete an additional 4-day unweighed food diary for the T1 visit. This diary will serve as a valuable tool for assessing dietary adherence in both groups. This intermediate evaluation will include nutritional assessment, consisting of anthropometric measurements and body composition analysis. Nutritional status will be evaluated using again the MNA, the MUST, and the MEDILITE. At this visit, IG participants will also receive a new supply of ABs, enough to cover the remaining three months of the intervention period.

The six-month follow-up visit (T2) will serve as the final clinical evaluation for the study's primary endpoints. In preparation for this visit, patients will again be asked to complete a 4-day unweighed food diary. At T2, in addition to the collection of serum, saliva, urine, and fecal samples, all assessments conducted at T0 will be thoroughly repeated. A complete clinical re-evaluation, including cardiologic and geriatric assessment, will also be conducted.

Throughout the entire study length, a rigorous system will be in place to carefully monitor and document all adverse events, ensuring participant safety and the integrity of the research findings.

2.5. *Outcomes*

PrimaryEndpoint.

The primary endpoint of this study is the evaluation of the effects of the ABon skeletal muscle mass, assessed using BIA.

This measure is a robust and clinically relevant indicator, closely associated with functional capacity and known to be modifiable through nutritional interventions [30]. The effects on physical function and frailty will also be assessed.

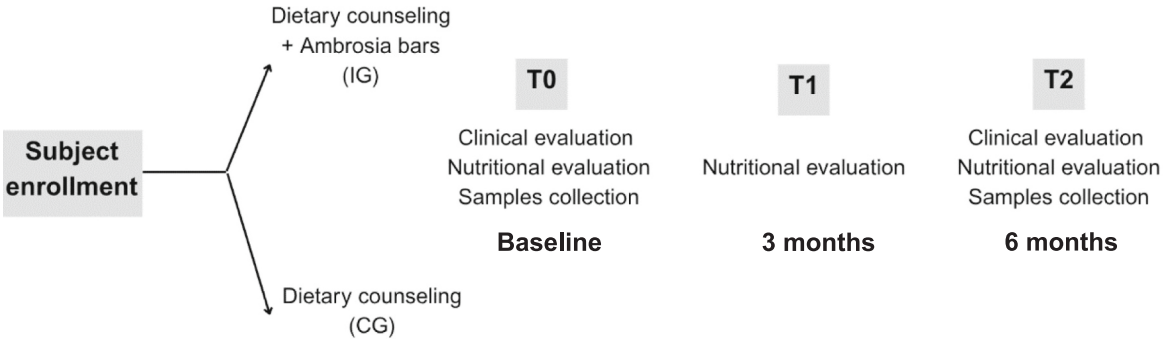

Fig. 2. Schematic representation of the AMBROSIA study protocol.

Secondary Endpoints.

Secondary endpoints, designed to provide a broader understanding of the potential mechanistic effects of the AB, include:

- Changes in body composition and anthropometric measures;
- Impacts on participants' quality of life, depressive symptoms and overall physical performance;
- Evaluation of changes in the GM profile through 16S rRNA sequencing;
- Changes in circulating inflammatory markers, mainly plasma cytokine profiles;
- Modifications in the blood lipid profile, including cholesterol and triglyceride levels;
- Changes in blood markers indicative of undernutrition;
- Alterations in metabolomic profiles in plasma, saliva, and stool samples to identify signatures associated with undernutrition and response to intervention.

2.6. Sample size

The sample size has been calculated to ensure adequate statistical power, seeing the innovative nature of the proposed nutritional intervention and its expected impact on body composition. Considering that a previous multiple nutrient supplementation trial reported an effect size exceeding 0.8 in appendicular muscle mass improvement [31], to adopt a conservative approach (accounting for increased variability in the target population) we have based our calculation on a smaller effect size of 0.3. This yields a required total sample of 82 participants (41 per arm) to detect a statistically significant difference between groups, with 80 % power and a two-sided alpha level of 0.05. To compensate for potential attrition due to loss to follow-up, non-compliance, or protocol violations, an estimated drop-out rate of 15 % has been considered. Accordingly, the target recruitment has been set at 120 participants, ensuring enough power for primary outcome analysis despite anticipated exclusions. Basing on previous literature studies [32,33], we could hypothesize to enroll 64/120 patients with AF and 56/120 patients with HF. The sample size calculation was conducted using G*Power software (Heinrich Heine Universität, Düsseldorf, Germany.

2.7. Data collection

The AMBROSIA's success in monitoring, evaluation, and research is grounded in the systematic collection and rigorous analysis of high-quality quantitative and qualitative data, which are essential for evidence-based decision-making. All patients' data will be collected and analyzed primarily according to the intention-to-treat (ITT) principle, ensuring that all randomized patients are included in the analysis regardless of adherence to the intervention protocol. Additionally, a per-protocol analysis will be conducted as a secondary approach to further assess the efficacy of the intervention among participants with high adherence. Comprehensive clinical, nutritional, functional, cognitive, anthropometric, and biological data, including serum, saliva, stool, urine samples, will be collected at T0 and T2. Information on dietary habits and nutritional status will be systematically obtained at T0, T1 and T2. These assessments will include the validated MEDILITE questionnaire to evaluate adherence to the Mediterranean diet, 4-day unweighted food diaries to capture dietary intake, and the MNA and MUST to determine nutritional status.

2.8. Data management

A comprehensive Data Management Plan will be developed to ensure responsible handling of all data generated throughout AMBROSIA. This plan will outline the nature of the data collected, strategies for its exploitation, procedures to ensure accessibility for verification and reuse, and detailed approaches for long-term curation and preservation. All data will be securely stored in a dedicated digital database, with robust safeguards to protect participants confidentiality. Personally, identifiable information will be encrypted and stored separately from clinical and experimental datasets. To maintain anonymity, all sensitive data will be pseudonymized and a unique code will be assigned to patients. Pseudonymized datasets essential for machine learning (ML) applications and statistical analyses will be transferred *via* secure, encrypted connections to a password-protected AMBROSIA semantic knowledgebase. This platform will support structured data integration, cross-domain analysis, and intelligent querying, enabling efficient data reuse and interpretation. Data handling procedures will strictly adhere to the principles of the General Data Protection Regulation and the former Directive 95/46/EC, ensuring legal and ethical compliance. All procedures started only after full approval by the relevant ethics committee (19882_spe). In agreement with the FAIR principles (Findable, Accessible, Interoperable, Reusable), data will be catalogued with appropriate metadata using electronic lab notebooks and standardized formats. The AMBROSIA semantic knowledgebase will adhere to the FAIR principles to simplify interoperability and long-term preservation. Where appropriate, anonymized datasets will be deposited in well-curated public repositories, contributing to open science initiatives and enabling participation in open data pilots.

2.9. Statistical analysis

Statistical analyses will primarily adhere to the ITT principle, with efforts to minimize follow-up losses. For the primary outcome, changes in skeletal muscle mass, frailty indices and physical performance from baseline to six months will be compared between groups using an unpaired *t*-test. If necessary, linear mixed-effects models for repeated measures will be used for adjustment to potential confounders.

One important source of confounding may be participants' pharmacotherapy profiles. Indeed, several drugs used for AF and HF can exert anti-inflammatory or metabolic effects, and antidepressant agents may affect inflammatory and neuropsychiatric outcomes. Baseline differences in medication prescriptions will therefore be assessed, and drug-related subgroups analysis will be conducted if the sample size will be sufficient to evaluate specific effects on clinical and biological outcomes. Nonetheless, we anticipate that randomization will help mitigate the potential impact of therapeutic differences between groups.

Secondary outcomes will be analyzed similarly, and correction for multiple testing will be applied to control for Type I error. Beyond classical methods, the study will integrate advanced statistical and ML methods to identify predictive features from the "Microbiota-Inflammation-Brain axis," aiming to discover biomarkers for undernutrition and treatment response. This includes specific analyses of high-throughput sequencing data (*e.g.*, using Shannon indexes and Wilcoxon rank-sum tests) and the development of ML models (*e.g.* unsupervised dimensionality reduction and supervised regression and classification methods) to identify subtle effects, cluster patients, and correlate outcomes with experimental data.

2.10. Ethics approval and considerations

AMBROSIA will be conducted in full accordance with the Declaration of Helsinki, the Oviedo Convention on Human Rights and Biomedicine, and applicable European regulations and directives, including EU Regulation No. 536/2014 on clinical trials and Directive 2004/23/EC on standards for the donation and handling of human samples. Prior to the initiation of any study procedures, full ethical approval will be obtained from the appropriate national or local ethics committee (19882_spe). All patients will receive detailed written and verbal information about the study's objectives, procedures, potential risks, and benefits, and will be required to provide written informed consent in accordance with ethical and legal standards. Additionally, the study will operate under a Data and Safety Monitoring Plan. The

Principal Investigator, in collaboration with the designated study team, will be responsible for the continuous monitoring of safety data and the prompt reporting of any adverse events or serious adverse events to the ethics committee and relevant regulatory bodies, in line with standard clinical research practice.

3. Discussion

AF, HF, undernutrition, loss of physical function and frailty represent a complex, interrelated network with substantial clinical and socioeconomic implications, especially among older individuals [34]; thus, addressing these coexisting conditions demands innovative, multidisciplinary approaches. Recent advances highlight the GM as a central modulator of nutritional status, systemic inflammation, and energy homeostasis. The microbiota–gut–brain axis, in detail, is now recognized as a critical regulator of appetite, metabolic signaling, and immune function in patients with AF and HF [35]. State-of-the-art strategies increasingly focus on targeted nutritional interventions, such as micronutrient supplementation, specialized diet counseling, and probiotic therapy, as effective means to counteract undernutrition and improve clinical and functional outcomes [36]. Concurrently, the integration of high-throughput 'omics' technologies (including metagenomics, metabolomics, lipidomics) with ML techniques represents a transformative advance in the identification of novel biomarkers and the personalization of nutritional interventions [37]. Despite this progress, undernutrition often remains underdiagnosed, largely due to the missing of standardized definitions and limited clinical awareness of inflammation-driven cachexia. A paradigm shift is urgently needed, moving beyond weight-centric metrics toward a multidimensional understanding that includes inflammatory pathways, GM dynamics, and physical and cognitive function.

In this scenario, by combining detailed anthropometric, functional, cognitive, and nutritional evaluations with advanced "omics" analyses and GM profiling, AMBROSIA will investigate the complex interplay across the microbiota–inflammation–brain axis in parallel with primary outcomes related to nutritional status and clinical outcomes. This holistic approach not only enhances mechanistic understanding but also paves the way for evidence-based, personalized strategies to prevent undernutrition and improve long-term outcomes in older patients with cardiovascular comorbidities.

CRediT authorship contribution statement

Simone Baldi: Writing – review & editing, Conceptualization. **Francesca Cuffaro:** Writing – review & editing, Conceptualization. **Edda Russo:** Writing – review & editing, Conceptualization. **Kate Porter:** Writing – review & editing, Conceptualization. **William Cheung:** Writing – review & editing, Conceptualization. **Maria Magdalena Coman:** Writing – review & editing. **Marco Garcia Vaquero:** Writing – review & editing, Conceptualization. **Thomas Lingner:** Writing – review & editing. **Maria Cristina Verdenelli:** Writing – review & editing, Conceptualization. **Gwendolyn Barceló-Coblijn:** Writing – review & editing, Conceptualization. **Iain Brownlee:** Writing – review & editing, Conceptualization. **Stefano Fumagalli:** Writing – review & editing, Conceptualization. **Amedeo Amedei:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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