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Highlights

- The gut microbiota is an overlooked factor for malnutrition in clinical settings.
- Malnourished hospitalized older adults had an altered gut microbiota profile.
- Gut microbiota profile declined during hospitalization and with malnutrition severity.
- Gut microbiota might be a missing piece in the high prevalence of hospital malnutrition.

Gut microbiota disturbances in hospitalized older adults with malnutrition and clinical outcomes.

Shirley S. Muñoz-Fernandez[1,*], Flavia B. Garcez[2,3], Julio C. G. Alencar[4], Amália A. Bastos[1], John E. Morley[5], Tommy Cederholm[6,7], Ivan Aprahamian[8], Heraldo P. de Souza[4], Thiago J. Avelino-Silva[2], Laure B. Bindels[9], Sandra M. L. Ribeiro[1,10]

[1] Nutrition Department, School of Public Health, University of São Paulo. São Paulo, SP, Brazil.

[2] Laboratorio de Investigacao Medica em Envelhecimento (LIM 66), Servico de Geriatria, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo. São Paulo, SP, Brazil.

[3] Hospital Universitario, Departamento de Medicina, Universidade Federal de Sergipe. Aracaju, SE, Brazil

[4] Disciplina de Emergencias Clínicas, Departamento de Clínica Medica, Faculty of Medicine, University of São Paulo. São Paulo, SP, Brazil.

[5] Division of Geriatric Medicine, School of Medicine, Saint Louis University. St. Louis, MO, USA.

[6] Clinical Nutrition and Metabolism, Department of Public Health and Caring Sciences, Uppsala University. Uppsala, Sweden.

[7] Karolinska University Hospital. Stockholm, Sweden.

[8] Group of Investigation on Multimorbidity and Mental Health in Aging (GIMMA), Division of Geriatrics, Department of Internal Medicine, Jundiaí Medical School. Jundiaí, SP, Brazil.

[9] Metabolism and Nutrition Research Group, Louvain Drug Research Institute, Université catholique de Louvain. Brussels, Belgium.

[10] School of Arts, Science, and Humanity, University of São Paulo. São Paulo, SP, Brazil.

*Correspondence: Shirley S. Muñoz Fernandez, Av. Dr. Arnaldo 715, São Paulo 01255-000, ssmufer@gmail.com

Declaration of competing interest

The authors have no conflict of interest to declare.

Author contributions

SSMF: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – Original Draft, Review & Editing. FBG: Data curation, Funding acquisition, Investigation, Methodology, Resources, Writing – Manuscript review. JCGA: Data curation, Investigation, Methodology, Resources, Writing –Manuscript review. AAB: Investigation, Writing – Manuscript review. TC, IA, and JEM: Writing – Review & Editing. HPS and TJAS: Funding acquisition, Methodology, Project administration, Resources, Writing – Manuscript review. LBB: Methodology, Software, Supervision, Writing – Review & Editing. SMLR: Conceptualization, Methodology, Supervision, Project administration, Writing – Review & Editing.

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Abstract

Objective: Malnutrition is one of the most threatening conditions in geriatric populations. The gut microbiota has an important role in the host's metabolic and muscular health, however, its interplay with disease-related malnutrition is less well understood. We aimed to identify the association of malnutrition with the gut microbiota and predict clinical outcomes in hospitalized acutely ill older adults.

Methods: We performed a secondary longitudinal analysis in 108 geriatric patients from a prospective cohort evaluated at admission and 72 hours of hospitalization. We collected clinical, demographic, nutritional, and 16S rRNA gene-sequenced gut microbiota data. Microbiota diversity, overall composition, and differential abundance were calculated and compared between patients with and without malnutrition. Microbiota features associated with malnutrition were used to predict clinical outcomes.

Results: Patients with malnutrition (51%) had a different microbiota composition compared to well-nourished during hospitalization (ANOSIM R=0.079, P=0.003). Patients with severe malnutrition showed poorer α-diversity at admission (Shannon P=0.012, Simpson P=0.018) and follow-up (Shannon P=0.023, Chao1 P=0.008). Differential abundance of *Lachnospiraceae NK4A136 group*, *Subdoligranulum*, and *Faecalibacterium prausnitzii* were significantly lower and inversely associated with malnutrition, while *Corynebacterium*, *Ruminococcaceae Incertae Sedis*, and *Fusobacterium* were significantly increased and positively associated with malnutrition. *Corynebacterium*, *Ruminococcaceae Incertae Sedis*, and the overall composition were important predictors of critical care in patients with malnutrition during hospitalization.

Conclusion: Older adults with malnutrition, especially in a severe stage, may be subject to substantial gut microbial disturbances during hospitalization. The gut microbiota profile of patients with malnutrition might be able to predict worse clinical outcomes.

Keywords: acute disease, critical care, gut microbiota, GLIM criteria, malnutrition, older adults.

INTRODUCTION

Malnutrition is one of the most prevalent and threatening syndromes in hospitalized patients, particularly older adults [1]. It can result from starvation, diseases, the aging process, or a combination of these factors. Malnutrition is manifested as altered body composition and diminished biological function, leading to physical decline and reduced recovery [2]. Moreover, it decreases the ability to tolerate and respond to medical interventions, predisposing affected people to complications and poorer prognosis [3]. Recent studies are increasingly supporting a strong link between the gut microbiome and skeletal muscle, the so-called "gut-muscle axis". This axis influences many physiological processes, including digestion, metabolism, inflammation, and immunity; suggesting a potential role in the development, progress, and/or consequences of malnutrition [4]. Evidence on other related muscle wasting disorders such as cachexia, sarcopenia, and frailty, confirming these observations may support this premise [4,5].

Age-related changes can induce gut microbiota imbalances, in addition to factors such as diet, nutritional status, physical activity, chronic diseases, medications, geographic location, and housing [6,7]. Studies have reported that older adults living in nursing homes showed poorer microbiota compared with community dwellers. They have identified higher interindividual variability and lower diversity during aging, especially an increased abundance of pathobionts and reduced commensals [6,8]. Members of the latter produce short-chain fatty acids (SCFA), which can have various effects on health, for example, modulate immune cells and enterocytes and stimulate the expression of tight junction proteins by activating specific receptors [9]. The reduction of SCFA affects the integrity of the gut epithelium, increasing its permeability and favoring the translocation of intestinal content from the lumen to the peripheral circulation boosting the systemic inflammatory activity [9,10]. These alterations entail metabolic and homeostatic dysfunctions resulting in anorexia,

anabolic resistance, increased muscle catabolism, and energy expenditure [4,5], contributing to the exacerbation of malnutrition, chronic diseases, and other geriatric syndromes [3,6], which increase the usage of hospital services [11].

This rationale supports that nutritional status influences the modulation of the structure, function, and interaction of the intestinal microbiota with health status and vice versa [5]. However, this relationship during acute conditions in a hospital setting has been underinvestigated. Therefore, we investigated the association between disease-related malnutrition and the composition and metabolic potential of the gut microbiota in acutely ill hospitalized older adults and its influence on clinical outcomes.

MATERIALS AND METHODS

Participants and design

A secondary longitudinal analysis was performed on 108 participants of a prospective cohort of acutely ill older adults ≥ 65 years sequentially admitted to the hospital through the Emergency Department due to acute conditions (Fig S1) [12]. Recruitment, follow-up, and data collection were conducted from September 2019 to March 2020 at the *Hospital das Clínicas* in São Paulo, Brazil. Patients with acute gastrointestinal disease or structural central nervous system injuries (as required for the primary cohort), using antibiotics for more than 24 hours before inclusion, receiving nutritional support, using prebiotics, probiotics, or symbiotics in the last month, admitted for palliative care, or expected hospital stay shorter than 48 hours were excluded. Participants were evaluated within the first 24 hours after admission, and at 72 hours of hospitalization.

Demographic, clinical, and hospital data, such as medical conditions for admission, comorbidities, blood parameters, length of hospital stay, and date of discharge or death, were collected from participants and electronic medical records. The Hospital's Ethics Committee

for Research approved the project (CAPPesq, n° 006688019.0.0000.0068, 7 February 2019), following the Declaration of Helsinki. We obtained informed consent from all participants before enrolment.

Nutritional assessment

A comprehensive description of the nutritional assessment is reported elsewhere [13]. Briefly, we screened participants using the Mini Nutritional Assessment (MNA) short form. Patients at risk were investigated for malnutrition using the GLIM framework [2] (Supplementary Methods, Table S1). Anthropometric measurements were evaluated at the bedside and on the left side whenever possible, namely, mid-upper arm circumference and triceps skinfold thickness to calculate the arm muscle area, subscapular skinfold, calf circumference, adductor pollicis muscle thickness, and handgrip strength (HGS). Self-reported height, and usual and current weight by participants or their caregivers were used to calculate weight changes and the body mass index, when participants were unaware of these measurements (14%), they were estimated from predictive equations [14].

Gut microbiota

Sample collection.

Microbiota samples were collected by trained registered nurses immediately after inclusion in the study and 72 hours later, through a deep rectal swab (Copan Liquid Amies Swab, ESwab, COPAN Italia) inserted ±2 cm beyond the anal canal, rotated for material collection, and extracted avoiding contact with perianal skin. Samples were transported in a swab screw cap tube with a preservation medium and temporarily preserved at -20ºC before long-term storage in the laboratory at -80 °C for further analysis.

Sequencing.

Bacterial DNA was extracted from the frozen samples. The primers of the V4 region of the 16S rRNA gene were used for DNA amplification by PCR. Sequencing was performed using the Ion Torrent Personal Genome Machine (PGM) platform.

Bioinformatics.

Sequence libraries were processed using *DADA2* to identify amplicon sequence variants (ASV) and *PICRUSt2* to infer metabolic functional potential. We determined the microbial diversity using Chao1, observed ASV, Heip, Shannon, and Simpson indices; the differential relative abundance of taxa and metabolic pathways, its compositional contribution to the explanation of microbiome variation, and analysis of similarities (ANOSIM). To identify possible covariates of microbiota explanation, we tested and classified patients' metadata on demographics, clinical, health, medication, biomarkers, and nutritional. More details are described in the Supplementary Methods.

Statistical analysis

Statistical analyses were performed by groups, without malnutrition (WN) vs. with malnutrition (MN) and WN vs. moderate malnutrition (MMN) vs. severe malnutrition (SMN), at admission and after 72 hours of hospitalization. Patients discharged by the 72-hour were not included in the corresponding analysis. Statistical significance was set at a two-tailed p-value < 0.05 and correction for false discovery rate (FDR) using the Benjamini-Hochberg method < 0.1. All statistical, graphical, and bioinformatic analyses were performed in R v 4.1.3.

Values are presented as median and interquartile range or frequency and percentage and compared by Fisher's Exact or Pearson's Chi-squared test unless otherwise noted. Comparisons in the α-diversity metrics and differential abundance between nutritional groups were tested by the Wilcoxon rank-sum or Kruskal-Wallis tests when appropriate. Post-hoc

exploratory subgroup analyses were conducted to determine possible interactions with the use of antibiotics and the presence of pre-specified diseases.

We performed cross-validated generalized linear models via penalized maximum likelihood after CLR transformation (CLR-LASSO) for the selection of microbial taxa potentially associated with nutritional status. To evaluate the performance and accuracy of the models, we calculated the proportion of explained deviance (R^2), misclassification error (ME), and the area under the curve (AUC) for the selected features, the latest only for binomial analyses [15]. The association of selected microbial features with the nutritional diagnosis was confirmed by fitting individual generalized additive models (GAMs) with smoothing splines and adjusting by significant covariates. To decipher whether gut microbiota composition and malnutrition would influence clinical outcomes, we used the significantly associated taxa, α-diversity metrics, and compositional variation to predict the need for critical care and in-hospital death by building decision classification and survival trees, respectively, and subtrees according to the severity of malnutrition. Further details are supplied in the Supplementary Methods.

RESULTS

Characterization of participants

Participants had a median age of 72 years, most of them were males, self-identified as White, admitted due to cardiovascular diseases. Of the 84.3% screened at risk, 50.9% had a confirmed diagnosis of malnutrition, of these, 32.4% were in a severe stage. Participants with malnutrition had significantly higher thrombocytosis and lower hemoglobin levels, higher inflammatory activity, longer hospital stays, and a higher rate of transference to the ICU for critical care and in-hospital death (Table 1).

Microbial diversity and composition in malnutrition

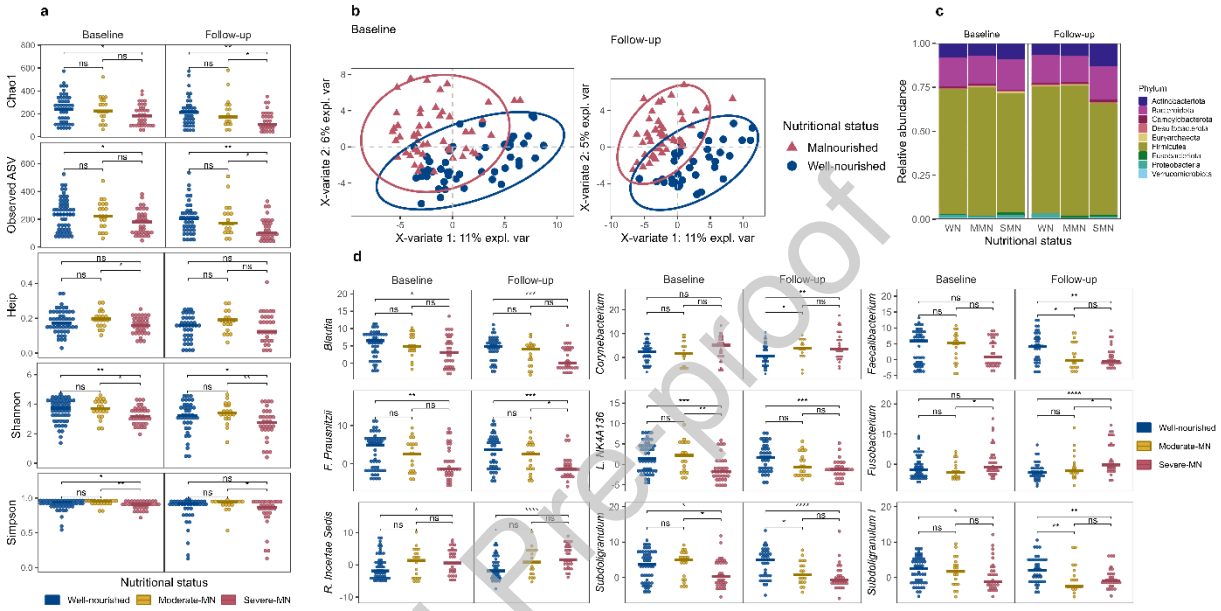
At baseline, the SMN group had a significantly lower diversity compared to MMN and WN (Shannon P=0.012; Simpson P=0.018). At 72-hour hospitalization, the richness was significantly lower in MN *(*Chao1 P=0.024, Observed ASV P=0.02), and extremely lower in SMN (Chao1 P=0.008, Observed ASV P=0.006, Shannon P=0.023) (Fig 1a, Table S2). Subgroup analysis showed a lower microbial richness at admission in WN with type 2 diabetes, in SMN with hypertension, and at follow-up in MN receiving antibiotics compared to MN without antibiotics (Fig S2).

ANOSIM revealed a significantly dissimilar overall gut microbiota composition between WN and MN, with a modest effect, at admission (R=0.018, P=0.045) and follow-up (R=0.079, P=0.003), but not according to the severity of malnutrition (admission R=0.032, P=0.184; follow-up R=0.029, P=0.164). A marked differentiation was observed by the components selected based on malnutrition classification (Fig 1b). At baseline, *Faecalibacterium prausnitzii* was the main contributor in WN, *Oscillospiraceae NK4A214 group* in MN and SMN, and *Lachnospiraceae NK4A136 group* in MMN. At follow-up, unclassified *Subdoligranulum* was enriched in WN, *Ruminococcaceae Incertae Sedis* in MN, *Corynebacterium* in MMN, and *Fusobacterium* in SMN (Fig S3).

Of the nine phyla detected, *Firmicutes, Bacteroidota*, and *Actinobacteriota* were the most abundant. At follow-up, we identified a significant increase in the differential relative abundance of *Actinobacteriota* and *Fusobacteriota* and a decrease in *Proteobacteria* in MN contrariwise to WN, but not at admission (Fig 1c). At the species level, there were no significant differences after FDR between MN and WN at admission. At follow-up, species of *Blautia*, *Faecalibacterium*, *L. NK4A136,* and *Subdoligranulum* had significantly lower abundance, while *Corynebacterium* and *R. Incertae Sedis* were significantly higher in MN (Table S3). Significant differences in the abundance of several taxa were observed in SMN compared to WN and MMN (Fig 1d). Subgroup analysis showed a lower abundance of

Blautia obeum and *Faecalibacterium* in WN and MN with diabetes. At follow-up, *B. obeum* was lower in MMN with gastrointestinal/hepatobiliary diseases and *L. NK4A136* when other diseases were present, and *F. prausnitzii* was increased in SMN using antibiotics. (Fig S4**).**

Fig 1. Gut microbiota diversity and compositional abundance according to nutritional status.



(a) α-diversity indices. (**b)** PLS-DA sample plot comparing clusters of patients. (**c)** Relative abundance at the phylum level. (**d)** Significant taxa between groups identified by ALDEx on the expected value of effect sizes. *P<0.05, **P<0.01, ns: non-significant.

The core microbiome showed that *Finegoldia magna* was present in all participants regardless of their nutritional status. The core differences for MN were detected at 75%. Unlike WN, unclassified *Corynebacterium* and *Anaerococcus vaginalis* were observed in the MN group (Table S4).

Microbial functional potential

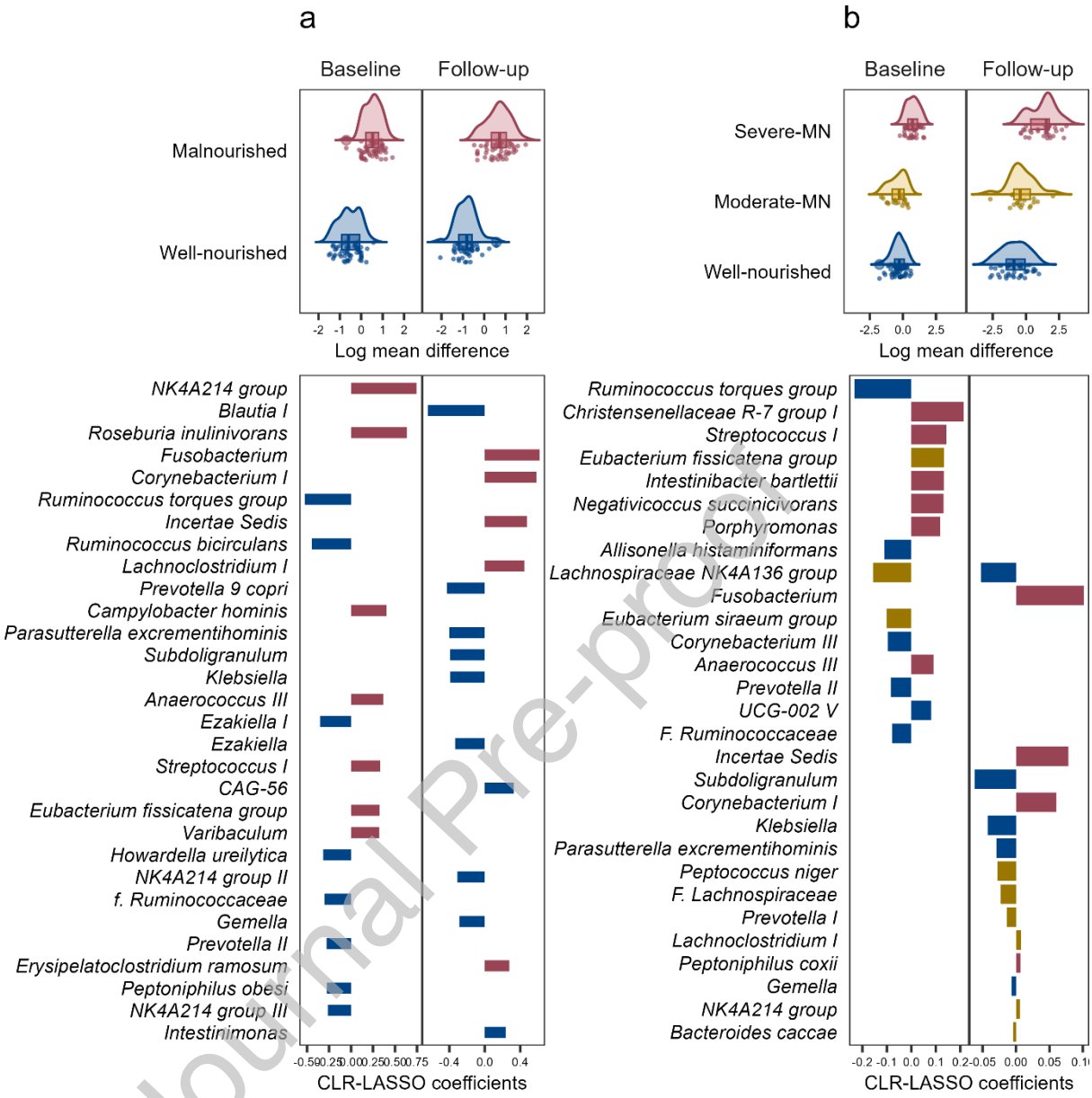
The overall composition of inferred metabolic pathways between WN and MN, and according to severity, was not statistically significant at admission or follow-up. None of the significantly abundant pathways remained significant after correcting for FDR (Table S3).

Association of malnutrition with microbial features

α-diversity. At baseline, Chao1, Observed ASV, and Shannon displayed significant negative associations with SMN; and at follow-up, Chao1 and Observed ASV with MN and SMN. Only Shannon in SMN (β=-0.31, P=0.049) and Heip in MMN (β=0.03, P=0.029) at admission remained significant in the final adjusted models (Table S5).

Taxa. CLR-LASSO selected 66 taxa to potentially discriminate malnutrition (R^2=0.863, ME=0.046, AUC=0.996) and 71 taxa its severity (R^2=0.789, ME=0.046) at admission. *Ruminococcus torques group, L. NK4A136 group,* and *Christensenellaceae R-7 group* were the greatest contributors in WN, MN, and SMN, respectively (Table S6, Fig 2). GAMs confirmed 10 taxa significantly associated with malnutrition and 17 with its severity. In the final adjusted models, *Ezakiella, L. NK4A136 group, Oscillospiraceae UCG-002,* and *R. torques group* had significant negative associations with MN and SMN*;* and *O. NK4A214 group* and *Peptoniphilus* a positive association with MMN (Table S7). At follow-up, 50 taxa predicted malnutrition (R^2=0.908, ME=0.296, AUC=0.74) and 27 taxa its severity (R^2=0.373, ME=0.18). *Blautia* and *Subdoligranulum* were the greatest contributors in WN, *Fusobacterium* in MN, unclassified taxa of the family *Lachnospiraceae* in MMN, and *R. Incertae Sedis* in SMN (Table S6, Fig 2). GAMs confirmed a significant association of 13 and 19 of the selected taxa, respectively. In the final models, *L. NK4A136, Prevotella copri,* unclassified *Subdoligranulum, Klebsiella, Gemella,* and *F. prausnitzii* had significant negative associations and *Corynebacterium, Fusobacterium,* and *R. Incertae Sedis* positive associations with MN/SMN; and unclassified species of *Prevotella* and the family *Lachnospiraceae* with MMN (Table S7).

Fig 2. Selected taxa to discriminate nutritional status.



Top taxa selected by CLR-LASSO based on their contribution to discriminate (**a)** malnutrition and (**b)** its severity. Top panel: Raincloud plots characterize the distribution of the log-mean difference scores for each group. Bottom panel: bar plots show the coefficients of the selected taxa. The color assigned to each taxon indicates the group with the larger median contribution.

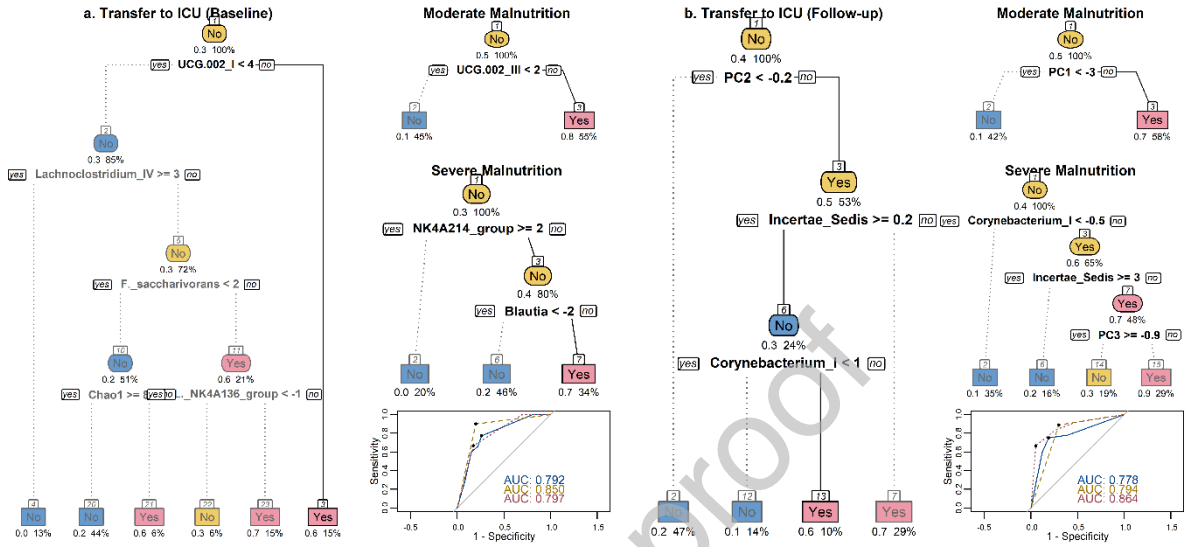
Inferred metabolic potential. CLR-LASSO selected 27 metabolic pathways for possibly predicting malnutrition (R^2=0.42, ME=0.17, AUC=0.933) and 40 for its severity (R^2=0.387,

ME=0.213) at baseline. Of these, 9 and 15 pathways had significant associations in individual GAMs. Peptidoglycan biosynthesis IV, GDP-mannose biosynthesis, and superpathway of guanosine nucleotides de novo biosynthesis I were the respective main contributors in WN, MN/MMN, and SMN. The first two had negative and positive associations with MN, respectively. At follow-up, 51 pathways were selected for malnutrition, although with suspected performance (R^2=0.94, ME=0, AUC=1), and 32 for its severity (R^2=0.38, ME=0.29). GAMs confirmed significant associations of 15 and 13 pathways, mostly negative. Calvin-Benson-Bassham cycle and chorismate biosynthesis from 3-dehydroquinate had the highest contribution in WN, the second negatively associated with SMN; arginine, ornithine, and proline interconversion in MN, and TCA cycle VI in MMN (Table S8).

Prediction of clinical outcomes from the malnourished microbiota profile

The gut microbiota profile in malnutrition predicted the need for critical care with good accuracy. At admission, *O. UCG-002* was the most important predictor in all participants (AUC=0.79, SP=0.77, SV=0.74) and MMN (AUC=0.85, SV=0.9, SP=0.8); and *Blautia* and *O. NK4A214 group* in SMN (AUC=0.79, SV=0.67, SP=0.83) (Fig 3a, Table S9). During hospitalization, the overall composition (represented by component 2), *R. Incertae Sedis,* and *Corynebacterium* predicted the need for critical care with good accuracy in all participants (AUC=0.78, SV=0.75, SP=0.81) and SMN (AUC=0.78, SV=0.75, SP=0.81), and component 1 in MMN (AUC=0.79, SV=0.89, SP=0.7) (Fig 3b, Table S9). At admission, the survival tree showed good accuracy in predicting in-hospital mortality using component 1, *Ezakiella, Lachnoclostridium,* and *Howardella ureilytica* (AUC=0.89, SV=0.8, SP=0.93). The overall composition, α-diversity metrics, and *Corynebacterium* were the main predictors in the baseline subtrees and follow-up models; however, possible imprecise performance was suspected (Table S10).

Fig 3. Decision trees for critical care in acutely ill geriatric patients based on the gut microbiota features of malnutrition.



(**a)** Baseline. (**b)** Follow-up. Each node shows the transfer to ICU (yes or no), predicted probability, and percentage of observations. Dark solid lines represent the pruned tree based on the optimal complexity parameter. AUC plots exhibit the accuracy of prediction, solid lines in all participants, dashed lines in MMN, and dotted lines in SMN.

DISCUSSION

Based on our findings, malnutrition seemed to be associated with the reduction of microbiota α-diversity; however, it may not prevail when considering other factors during hospitalization. Experiments have shown that mice transplanted with fecal microbiota from malnourished patients had a worse nutritional status [16]. Investigations into the gut microbiota's influence on muscle wasting syndromes have revealed divergent results. Non-acute older adults with low muscle mass [17] and malnourished patients with cancer [16] displayed lower gut bacteria richness and evenness. In contrast, geriatric patients with

undernutrition showed a higher α-diversity [18], while patients with cachexia, frailty, and sarcopenia have reported no difference [19–22]. Still, all of them had a significantly different overall composition compared to their counterparts without the muscle wasting-related condition [16–19,22], as we observed in our sample.

Microbiota diversity and overall composition were among the most important predictors of hospital outcomes. Prior studies in critically ill patients demonstrating a dramatic loss of microbial diversity and potentially beneficial bacteria, and overgrowth of pathogens support this finding [23]. Likewise, the gut microbiota of malnourished patients with colorectal cancer predicted poorer outcomes [16]. Moreover, patients with cachexia transplanted with fecal microbiota from healthy obese donors had higher median survival than those who received the autologous transplantation [24].

After three days of hospitalization, significant shifts were detected, especially in severe malnutrition, suggesting that these patients may be more prone to gut microbiota imbalances. In the *Actinobacteriota* phylum, increased in MN, have been identified several opportunistic and pathogenic species, bearers of highly virulent and multidrug resistance genes [25,26]. This increase in the MN was explained by the enrichment of *Corynebacterium*, which has been previously associated with low BMI in critically ill older adults [23], *Varibaculum*, an underinvestigated genus isolated from clinical specimens [27], and *Eggerthella lenta*, also associated with sarcopenia and frailty in older adults [21].

In the *Firmicutes* phylum, the *Oscillospiraceae NK4A214 group* and *Ruminococcaceae Incertae Sedis* were also highly enriched and positively associated with malnutrition. The former was reduced in obesity after lactic acid bacteria supplementation [28] and in animal models treated with a ghrelin antagonist [29]. The latter was increased in adults with overweight/obesity after intermittent fasting [30] and healthy older adults supplemented with soluble corn fiber alone or combined with *Lactobacillus rhamnosus* GG,

suggesting a fermentative role [31]. More research is needed regarding the interactions of these bacterial genera with host health and metabolism. *R. Incertae Sedis*, alongside *Corynebacterium*, were accurate predictors of critical care in malnourished patients during hospitalization. Although different taxa have been identified as biomarkers in critical care, few have been investigated in the context of malnutrition [23,32].

Fusobacteriota, representing the genus *Fusobacterium* in our sample and significantly increased and positively associated with malnutrition, has been reported as an opportunistic oncobacterium associated with a variety of necrotizing infections and septicemia [33], including critical patients [32]. The family *Fusobacteriaceae* was significantly increased in older adults with low muscle mass and positively correlated with fat mass [17]. *Fusobacterium nucleatum* was distinguished as one of the most predominant taxa in moderately malnourished patients with colorectal cancer [16]. Also, a culturomics study confirmed the enrichment of *Fusobacterium* in children with severe malnutrition [34].

Conversely, the genera *Blautia*, *Lachnospiraceae*, *Faecalibacterium*, *Fusicatenibacter*, and *Subdoligranulum*, decreased and negatively associated with malnutrition, are mainly producers of SCFA and other secondary metabolites, associated with contrasting inflammatory and metabolic effects in different health conditions [28,35–39]. Consistent with our results, *Blautia* has shown a lower abundance associated with undernutrition in older adults [18] and a significant positive association with appendicular skeletal mass in younger adults [40]. *Subdoligranulum* and *F. prausnitzii*, have been increased in people with normal muscle mass, without sarcopenia or cachexia [17,19,41]. Among other taxa inversely associated with malnutrition, studies showed a decreased abundance of *P. copri* in people with cachexia, which has been linked with the biosynthesis and blood levels of branched-chain amino acids [19], and increased abundance of *Klebsiella* and *Oscillospiraceae UCG-002* in older adults with poor appetite [18]. Based on this reduced abundance of SCFA

producers, older adults with undernutrition and low muscle mass have exhibited reduced levels of fecal acetate and butyrate, respectively, which correlated with skeletal muscle mass index and the abundance of SCFA producers [17,18].

Similar to Fluitman et al [18], we did not identify significantly enriched metabolic pathways between groups. However, we observed significant associations of metabolic pathways with malnutrition, mainly of the biosynthesis class at admission and degradation and biosynthesis class at follow-up. Pathways positively associated with malnutrition were related to carbohydrate and amino acid metabolism for the biosynthesis of GDP-mannose and L-alanine, key compounds for protein glycosylation and peptidoglycan, respectively [42]. Studies have reported dysfunction of protein glycosylation in muscle diseases [43] and increased levels of carbohydrate-deficient transferrin as a biomarker of glycosylation activity in malnourished children [44]. Pathways negatively associated with malnutrition were also related to sucrose degradation [42], which might explain the reduced sucrose absorption observed in critically ill patients [45], who are at higher risk of malnutrition which increases with age [23]. Whether the microbiota plays a mechanistic role in these alterations is unclear and should be further explored.

The restricted availability of substrates for the gut microbiota, secondary to the reduced food intake/assimilation, may affect its composition and function. This might disturb, along with the disease-related inflammatory activity, metabolic pathways, and mediating molecules involved in energy metabolism, muscle health, and feeding behavior [4,5,46]. Since malnutrition is a multifactorial syndrome and the microbiome a complex interactive bionetwork, individual taxa may not be able to influence the nutritional status strongly enough to be considered a biomarker for malnutrition. Furthermore, analyzing the microbiota for this purpose in a hospital setting would be unlikely. It seems plausible to deem the gut microbiota as a whole and its interaction with the host as a potential bidirectional mechanism of action,

considering other related factors, and therefore a possible emerging target for ancillary personalized therapeutic/preventive interventions, which could optimize the efficacy of traditional therapies [47,48].

Based on the literature, this is a pioneer work to explore and associate the gut microbiota composition and metabolic potential of hospitalized geriatric patients with malnutrition diagnosed by the GLIM criteria and clinical outcomes. Our findings may provide new perspectives for understanding the physiopathology of malnutrition [4,6]. However, this study was performed in a nonrepresentative small sample size, which might underpower the statistical analyses. Limitations regarding nutritional aspects were reported elsewhere [13]. The sequencing technology used limited the information to attain lower taxa levels and more accurate functional annotation. Moreover, the compositional nature and transformation of these data may limit their interpretation and possibly induce bias. Therefore, these aspects should be considered when generalizing our findings.

Conclusion

Acutely ill geriatric patients with malnutrition had different gut microbiota composition and poorer diversity, lower abundance of potentially beneficial bacteria, and increased pathogenic/opportunistic bacteria during hospitalization, mainly in severe malnutrition. A "malnourished gut microbiota" may be able to predict adverse hospital outcomes. We opened new standpoints for larger clinical investigations and mechanistic preclinical exploration in disease-related malnutrition.

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Table 1. Baseline demographic and clinical characteristics of participants.

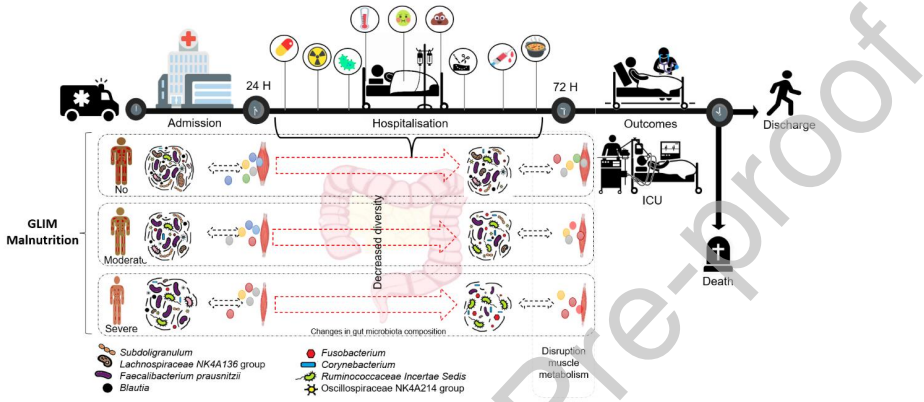
Characteristics	**All participants** n=108 (100%)	**Without malnutrition** n= 53 (49.1%)	**With malnutrition** n= 55 (50.9%)	**P**	**Moderate malnutrition** n= 20 (18.5%)	**Severe malnutrition** n= 35 (32.4%)	**P**
Age, y	72±11	73±10	70±11.5	0.516	69.5±8.3	73±14.5	0.281
Sex, n (%)							
Males	63 (59)	31 (29)	32 (30)	1	14 (13)	18 (17)	0.405
Females	45 (42)	22 (20)	23 (21)		6 (6)	17 (16)	
Ethnicity, n (%)							
White	58 (54)	29 (27)	29 (27)		10 (9)	19 (18)	
Mixed	34 (32)	16 (15)	18 (17)	0.99	7 (6)	11 (10)	0.990
Black	14 (12)	7 (6)	7 (6)		2 (3)	4 (4)	
Asian	2 (2)	1 (1)	1 (1)		0 (0)	1 (1)	
Length of stay, n days	8 (11.3)	7 (10)	9 (17)	0.069	14 (24)	8 (9.5)	0.067
Main diagnosis for admission, n (%)				0.432			0.521

Respiratory disease	13 (12.0)	4 (3.7)	9 (8.3)		3 (2.8)	6 (5.6)	
Traumatic injury	3 (2.8)	3 (2.8)	0 (0)		0 (0)	0 (0)	
Cardiovascular disease	49 (45.4)	26 (24.1)	23 (21.3)		12 (11.1)	11 (10.2)	
Infectious diseases	5 (4.7)	2 (1.9)	3 (2.8)		1 (0.9)	2 (1.9)	
Cancer	7 (6.5)	2 (1.9)	5 (4.6)		2 (1.9)	3 (2.8)	
Urinary/kidney disease	13 (12.1)	6 (5.6)	7 (6.5)		1 (2.8)	6 (5.6)	
Gastrointestinal/hepatobiliary diseases	6 (5.6)	3 (2.8)	3 (2.8)		0 (0)	3 (2.8)	
Other diseases	12 (11.1)	7 (6.5)	5 (4.6)		1 (0.9)	4 (3.7)	
Critical care, n (%)	35 (32)	13 (12)	22 (20)	0.102	10 (9)	12 (11)	0.112
In-hospital death, n (%)	10 (10)	3 (3)	7 (7)	0.321	3 (3)	4 (4)	0.407
CRP, g/L (n= 95)	28.9±74	16.3±63.9	40.2±101.6	0.081	37.3±65.9	55.9±106.7	0.077
PLR (n= 107)	145.7±139.5	121.3±131.8	167.5±123.3	**0.014**	151.5±126.8	184.9±196.7	0.005
NLR (n= 107)	3.6±5.2	3.2±3.9	4.1±6.3	0.216	2.4±3.02	6.2±10.3	0.014
Hb, g/dL (n= 107)	11.8±3.3	12.7±3.5	11±2.9	**<0.001**	11.1±2.2	10.7±3.3	**<0.001**
BMI, kg/m^2 (n=106)	24.8±6.2	27.2±5.2	22.8±5.2	**<0.001**	23.1±6.2	22.5±3.9	**<0.001**
APM, mm (n=106)	17±6.4	19±6.0	14.2±5.5	**<0.001**	18±5.3	13±3.9	**<0.001**
HGS, kg (n= 73)	16.2±12.7	18±12.5	13±9.1	0.07	17.7±15.5	12.5±9.7	**0.045**
MNA n (%)				**<0.001**			**<0.001**

Normal	17 (16)	17 (16)	0 (0)		0 (0)	0 (0)	
At risk	48 (44)	29 (27)	19 (18)		9 (8)	10 (9)	
Malnourished	43 (39)	7 (6)	36 (33)		11 (10)	25 (23)	
GLIM malnutrition, n (%)							
Phenotypic criteria							
Nonvolitional weight loss (n=83)	39 (47)	4 (5)	35 (42)	**<0.001**	10 (12)	25 (30)	**<0.001**
Low BMI (n=106)	23 (26)	4 (5)	19 (22)	**0.002**	5 (4)	14 (17)	**<0.001**
Low muscle mass (n=107)	40 (37)	3 (3)	37 (35)	**<0.001**	9 (8)	28 (26)	**<0.001**
Etiologic criteria							
Reduced food intake/Assimilation	88 (82)	38 (35)	50 (46)	**0.013**	19 (18)	31 (29)	**0.031**
Disease burden/inflammation	83 (77)	39 (36)	44 (41)	0.497	14 (13)	30 (28)	0.303
Medications, n (%)							
Antibiotics	12 (11)	3 (3)	9 (8)	0.124	3 (3)	6 (5)	0.203
Proton-pump inhibitor	24 (22)	11 (10)	13 (12)	0.818	6 (6)	7 (6)	0.649
Polypharmacy	41 (39)	19 (18)	22 (21)	0.842	8 (8)	14 (13)	0.932

Categorical variables are expressed as frequency (%) and compared using Fisher's exact or Pearson's Chi-square tests. Numerical variables are presented in median±IQR and tested using Wilcoxon rank-sum or Kruskal-Wallis tests. APM: adductor pollicis muscle thickness, BMI: body mass index, CRP: C-reactive protein, Hb: Hemoglobin, HGS: handgrip strength, MNA: Mini Nutritional Assessment, NLR: neutrophils-to-lymphocytes ratio, PLR: platelets-to-lymphocytes ratio.

Graphical abstract



Declaration of Interests

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: