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Randomized placebo-controlled trial of oral tannin supplementation on COVID-19 symptoms, gut dysbiosis and cytokine response

Silvia Molino [a], Andrea Pisarevsky [b], Shyam Badu [c,d], Qinglong Wu [c,d], Fabiana López Mingorance [e], Patricia Vega [b], Juan Pablo Stefanolo [f], Julieta Repetti [e], Guillermina Ludueña [b], Pablo Pepa [g], Juan Ignacio Olmos [g], Marcelo Rodriguez Fermepin [h], Tatiana Uehara [i], Elisa Viciani [j], Andrea Castagnetti [j], Tor Savidge [c,d,*], María Marta Piskorz [i,*]

[a] *Departamento de Nutrición y Bromatología, Instituto de Nutrición y Tecnología de los Alimentos, Centro de Investigación Biomédica, Universidad de Granada, Granada, Spain*
[b] *Universidad de Buenos Aires, Hospital de Clínicas José de San Martin, Departamento de Medicina Interna, Buenos Aires, Argentina*
[c] *Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, USA*
[d] *Texas Children's Microbiome Center, Department of Pathology, Texas Children's Hospital, Houston, TX, USA*
[e] *Universidad de Buenos Aires/ IBIMOL, Hospital de Clínicas José de San Martin, Programa de Estudios Pancreáticos, Buenos Aires, Argentina*
[f] *Hospital de Gastroenterología Dr Carlos Bonorino Udaondo, Buenos Aires, Argentina*
[g] *Universidad de Buenos Aires, Hospital de Clínicas José de San Martin, Servicio de Gastroenterología, Buenos Aires, Argentina*
[h] *Universidad de Buenos Aires, Hospital de Clínicas José de San Martin, Facultad de Farmacia y Bioquímica, Departamento de Bioquímica Clínica, Buenos Aires, Argentina*
[i] *Universidad de Buenos Aires, Hospital de Clínicas José de San Martin, Sector Neurogastroenterología del Servicio de Gastroenterología, Buenos Aires, Argentina*
[j] *Wellmicro Srl, Bologna, Italy*

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ABSTRACT

The clinical study aim was to investigate whether a tannin-based dietary supplementation could improve the efficacy of standard-of-care treatment of hospitalized COVID-19 patients by restoring gut microbiota function. Adverse events and immunomodulation post-tannin supplementation were also investigated. A total of 124 patients receiving standard-of-care treatment were randomized to oral tannin-based supplement or placebo for a total of 14 days. Longitudinal blood and stool samples were collected for cytokine and 16S rDNA microbiome profiling, and results were compared with 53 healthy controls. Although oral tannin supplementation did not result in clinical improvement or significant gut microbiome shifts after 14-days, a reduction in the inflammatory state was evident and significantly correlated with microbiota modulation. Among cytokines measured, MIP-1α was significantly decreased with tannin treatment ($p = 0.03$) where it correlated positively with IL-1β and TNF-α, and negatively with stool *Bifidobacterium* abundance.

1. Introduction

The major organs affected in individuals infected with SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) generally include the respiratory and cardiovascular systems. Less well appreciated in COVID-19 (Coronavirus disease 2019) patients is the dysfunction evident in other organs, including the gastrointestinal tract or onset of multiple organ failure (Chen et al., 2020; Gao et al., 2020). Disrupted crosstalk between the respiratory system and gut microbiota, termed the "gut-lung axis", is reported in COVID-19 patients who generally display depletion of symbionts (i.e., *Bifidobacterium* spp. and *Lactobacillus* spp.) with enrichment of opportunistic pathobionts that correlate with disease severity (Xu et al., 2020; Zuo et al., 2020). Moreover, the exacerbated immune response in COVID-19 is strongly linked to gastrointestinal dysfunction and dysbiosis, which in turn can lead to production of pro-inflammatory cytokines (Villapol, 2020). Thus, the gut microbiota

Abbreviations: AUC, area-under-curve; COVID-19, Coronavirus disease 2019; HC, Non COVID-19 healthy controls; LEFSe, Linear discriminant analysis effect size; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

* Corresponding authors at: Universidad de Buenos Aires, Hospital de Clínicas José de San Martin, Sector Neurogastroenterología del Servicio de Gastroenterología, Buenos Aires, Argentina (M.M. Piskorz).

E-mail addresses: tor.savidge@bcm.edu (T. Savidge), mpiskorz@hospitaldeclinicas.uba.ar (M.M. Piskorz).

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represents a potential target for therapy and diet could have a significant impact on the health status of the host.

Several reports have highlighted the influence of different diets on COVID-19 severity, which merits deeper investigation of therapeutic options based on nutritional support favoring restoration of gut microbiota function (Kalantar-Zadeh et al., 2020; Liang, 2020). Tannins are natural bioactive compounds that positively impact gut microbiota function, in part by promoting expansion of probiotic bacteria (Molino, Lerma-aguilera, et al., 2021; Molino et al., 2022). These polyphenol substrates are actively metabolized by gut microbes with end products, such as short chain fatty acids, urolithins and quercetin that can regulate local and systemic immune responses (Fanos et al., 2020; Molino et al., 2018). Despite the rollout of vaccination and booster campaigns against SARS-CoV-2, the threat of infection is still present, with a need for long-term clinical management of post-infectious symptoms becoming apparent. With the reported ability of oral tannin extract to modulate the gut microbiota composition and inflammatory response, this could potentially supplement COVID-19 standard therapies as a nutraceutical approach to mitigate SARS-CoV-2 infection.

The aim of this study was to investigate the efficacy and adverse events of a tannin-based dietary supplement added to standard-of-care treatment in patients with non-life-threatening COVID-19. The impact of oral tannin on dysbiosis and the systemic inflammatory response was also investigated. Furthermore, at baseline, the inflammation and gut microbiota of COVID-19 patients were compared with a cohort of non-COVID-19 controls.

2. Materials and methods

2.1. Study design

The study was approved by the ethics committee of the University Hospital of Universidad de Buenos Aires "Hospital de Clínicas José de San Martín", Argentina, and adhered to ethical principles outlined in the declaration of Helsinki convention. Registered on ClinicalTrials.gov (NCT04403646), all protocol details are shown in Supplementary Figure S1 and reported previously by us (Molino, Pisarevsky, et al., 2021). Eligibility of study participants was assessed within 24 h of randomization, with patients and clinicians blinded to treatment. Computer-generated randomization (1:1) assigned tannin supplement or placebo, with sample size estimated at 140 (70 patients per group) based on a calculated alpha error of 5 % and power of 80 %. All COVID-19 participants continued to receive standard-of- care treatment based on the Argentina Ministry of Health and hospital treatment guidelines (Ministerio de Salud Argentina, n.d.). Such treatments included antiviral medications, antibacterial medications, steroids, supplementary oxygen, and convalescent plasma. Participants in the treatment arms received 2 capsules per day of tannin (240 mg of quebracho and chestnut tannin extract blend + 0.72 μg B12 vitamin; Arbox Microbiota, Indunor S.A.) or identical capsules of placebo for 14 days (see Fig. 1).

Non COVID-19 healthy controls (HC) were age and sex-matched individuals with no medical history or use of antibiotic or corticosteroid within 3 months and tested negative for SARS-CoV-2. All authors had access to the study data and reviewed and approved the final manuscript.

2.2. Trial outcome endpoints

The primary outcome measure was time to hospital discharge within 28-days (T28) of receiving the first oral treatment. Secondary outcome measures included: 28-day all-cause mortality, invasive ventilation at T28, measurement of systemic inflammatory cytokine levels, and fecal microbiota composition between baseline T0 and 14 days of intervention (T14).

2.3. Sample collection

Blood and stool samples were collected at T0 for both COVID-19 patients and HC, while sample collections were repeated at T14 for COVID-19 patients only. Venous blood samples were drawn into Vacutainer tubes containing clot activator and serum obtained by centrifugation (3,000 g for 15 min, 4 °C). Stool samples were collected in μbeNAT preservation tubes (COPAN®, Italy) and stored at − 80 °C.

2.4. Serum cytokines

Cytokines were quantified using the commercial Bio-Plex Pro™ Human Cytokine 27-plex following kit instructions.

2.5. Microbiome sequencing and analysis

Microbial DNA was extracted from 100 mg of homogenized fecal samples using the MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit according to the manufacturer's instructions and was sequenced as described by Gaibani et al. (2021). DADA2 package (version 1.8) was used for processing de-multiplexed raw sequencing reads, with minor modifications (Klindworth et al., 2013). Specifically, raw sequencing reads were trimmed while maintaining the overlap regions for merging

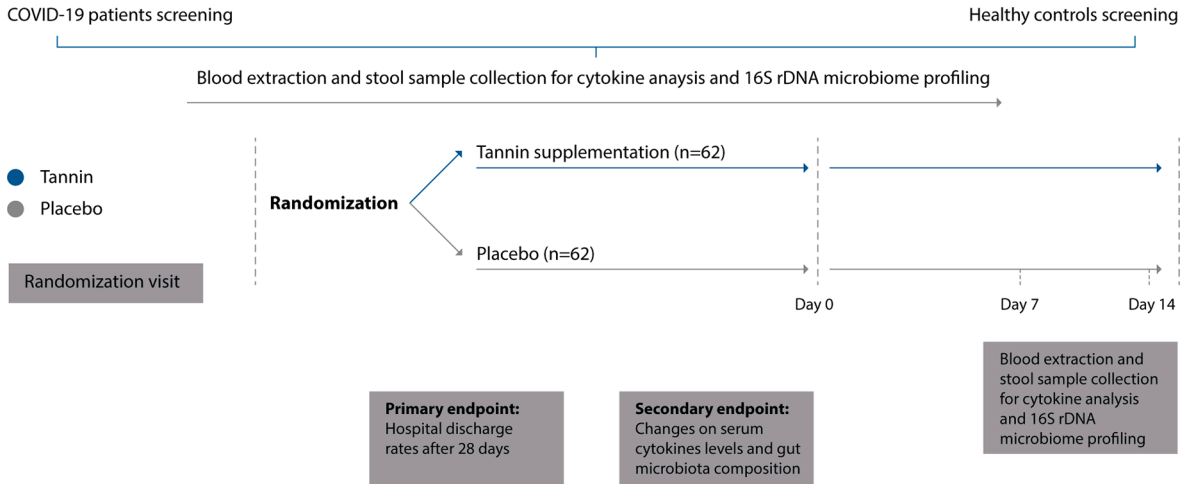

Fig. 1. Study design and treatment allocation. During screening, patients continued to receive standard-of- care treatment. At baseline, blood and stool samples were collected for COVID-19 patients and HC, for serum cytokine measurement and 16S rDNA microbiome profiling. 124 COVID-19 patients were randomized to receive tannin supplementation or placebo for 14 days. At day 14, blood and stool samples were collected only for COVID-19 patients.

paired-end reads; sequencing primers retained in forward and reverse reads were removed. IdTaxa function in DECIPHER package (version 2.6.0) (Callahan et al., 2016) and its pre-built training set (LearnTaxa function) of SILVA database (release 132) (Murali et al., 2018) was used for taxonomy assignment (threshold: 0.5) of amplicon sequence variants (ASVs) from DADA2 output. ASVs that were not classified as bacteria and chimeras were removed. Samples with less than 1,000 reads were excluded and total sum scaling method was applied to normalize feature data prior to downstream analysis.

2.6. Statistical analyses

Data were analyzed using the independent *t*-test, Mann–Whitney *U* test or chi2 test depending on their distribution. Comparison of cytokine levels between COVID-19 patients and controls (with and without adjustment for age, sex, and BMI by linear regression) was analyzed by linear regression with Stata software (Stata Corp., College Station, Texas, USA) version 17.0. Time-to-event data were analyzed using the Kaplan-Meier method. Hazard ratios were calculated using Cox proportional hazards models while treatment effects for secondary outcome measures applying odds ratios.

Random forest algorithm in the random Forest R package together with 5-fold cross-validation sampling was used to generate a supervised classification model using serum cytokine profiles to differentiate COVID-19 patients from HC. Alpha diversity analysis and Bray-Curtis dissimilarity distance were calculated at the ASV level using QIIME 1.9.1. Mann-Whitney test was used to compare alpha diversity metrics and ANOSIM non-parametric test was used to establish dissimilarity between groups. Relative abundances at each taxonomic level were compared by non-parametric Kruskal-Wallis test. Spearman correlation analysis between serum cytokines and stool microbiome features at ASV and genus levels was performed using the R based cor.test() function, Benjamini-Hochberg procedure was applied as multiple testing corrections.

3. Results

3.1. Study cohort

A total of 124 out of 143 screened COVID-19 patients were enrolled and randomized to receive oral tannin-based supplementation or placebo, added to standard-of-care treatment. Demographics of the study cohort are summarized in Supplementary Table S1. The mean age of the HC group was 52 ± 8 years (62 % women), while the COVID-19 patients were 54 ± 15 years (50 % women). Four COVID-19 patients in the interventional arm and one patient in the placebo group withdrew from the study, due to protocol violation and adverse event respectively. The median interval between the onset of symptoms and randomization was 5 days (IQR, 4–7 days) overall.

3.2. Primary outcome

Clinical characteristics did not present any significant differences between the interventional and placebo arms (Supplementary Table S2). Similarly, the primary (time to hospital discharge), and secondary outcomes (mortality, and invasive ventilation over a 28-day period from the start of the study) did not show significant differences between subjects supplemented with tannins or placebo (Supplementary Figure S2 and Table S3). No adverse events were recorded in the tannin group, whereas two patients reported diarrhea and abdominal pain in the placebo group.

3.3. Cytokine production in treatment-naïve COVID-19 patients versus post-treatment

At baseline, 17 cytokines were identified as differentially regulated in COVID-19 patients compared with HC (Supplementary Table S4). Hierarchical clustering and supervised learning accurately grouped COVID-19 patients from controls based on serum cytokine profiles, with an area-under-curve (AUC) value of 0.933 (Fig. 2A & B). After adjusting for age $\geq$ 60, sex and body mass index $\geq$ 30, significantly higher pro-inflammatory cytokines levels were found in COVID-19 patients at baseline (T0) compared to HC: IL-1ra ($p = 0.001$), IL-2 ($p < 0.001$), IL-6 ($p < 0.001$), IL-7 ($p < 0.001$), IL- 8 ($p = 0.05$), IL-13 ($p < 0.001$), IP-10 ($p < 0.001$), PDGF-bb ($p < 0.001$). In particular, COVID-19 patients with severe disease had significantly higher levels of IL-1ra ($p < 0.001$), IL-13 ($p < 0.001$) and IFN-γ ($p = 0.002$), when compared to mild and moderate disease.

A reduction in IL-1β, IL-1ra, IL-2, IL-8, IL-9, IL-13, MCP-1, MIP-1β, PDGF-β, TNF-α and RANTES was measured in the tannin-treated COVID-19 arm, but these differences did not meet statistical significance (Fig. 2C). MIP-1α was the only cytokine that was significantly decreased in patients post tannin treatment (Fig. 2D) (Supplementary Table S5). Strong positive correlations between MIP-1α and IL-1β, rho = 0.58 ($p < 0.001$) and rho = 0.31 ($p < 0.01$), and between MIP-1α and TNF-α, rho = 0.23($p < 0.01$) and rho = 0.41 ($p < 0.01$) were found in placebo and tannin arms, respectively.

3.4. Gut microbiome composition at baseline T0 is associated with disease severity

Differences in gut microbiota composition between HC and COVID-19 patients at baseline T0 was evaluated in 152 subjects (50 HC and 102 COVID-19 subjects). COVID-19 patients receiving antibiotics with standard of care treatments did not present a distinct gut microbiota composition (Data not shown).

COVID-19 patients possessed a lower α-diversity (Shannon diversity index, $p < 0.001$) (Fig. 3A) and richness (Chao1, $p < 0.001$) (Fig. 3B) compared to HC. The largest reduction in Shannon diversity index was evident in the most severe COVID-19 cases ($p = 0.032$). β-Diversity was calculated using Bray–Curtis dissimilarity, which measures differences in the relative abundance of ASVs across all T0 subjects. Although HC subjects clustered distinctly from COVID-19 patients ($p = 0.001$), no significant separation in β-diversity was evident with increasing disease severity (Fig. 3C) and may be reflective of large microbiome variation in this cohort (Bray-Curtis dissimilarity index, PCo1 and PCo2 contributed 5.23 and 4.84 % of the total variation).

Analysis of family-level abundances at baseline demonstrated a significant increase in Bacteroidaceae in COVID-19 patients compared with HC (Fig. 3D). LEFSe (Linear discriminant analysis Effect Size) analysis was used to illustrate specific microbiota changes associated with COVID-19. In particular, besides Bacteroidaceae (LDA score 4.723), also Clostridiales (LDA score 3.681), Pseudomonadiaceae (LDA score 2.859) and Atopobiaceae (LDA score 2.799) were significantly enriched in COVID-19 patients (Fig. 3E).

Spearman correlations of alpha-diversity and serum cytokine levels demonstrated that IL-15, MIP-1b, IFN-γ, IL-1rα, FGF-β, IL-9, IP-10, IL-6, IL-13, PDGF-ββ and IL-2 were statistically associated with Shannon diversity index after multiple testing correction. Out of these, six cytokines negatively and five positively correlated with alpha diversity (Fig. 3F).

3.5. Gut microbiome composition pre and post treatment

Shannon and Chao1 alpha-diversity indices were not altered in COVID-19 patients after tannin intervention (Fig. 4A & B). Similarly, no significant changes were evident in beta-diversity clustering based on Bray-Curtis dissimilarity-based analysis (Fig. 4C). Burkholderiaceae was significantly ($p = 0.0015$) elevated at T14 in patients after 14-days tannin treatment (Fig. 4D), as was *Enterococcus* (LDA score 3.095) and *Allisonella* (LDA score 2.733) whereas Lachnospiraceae (LDA score 2.676) demonstrated reduced abundance in the tannin-arm (Fig. 4E).

When assessing microbiome composition with cytokine levels, a

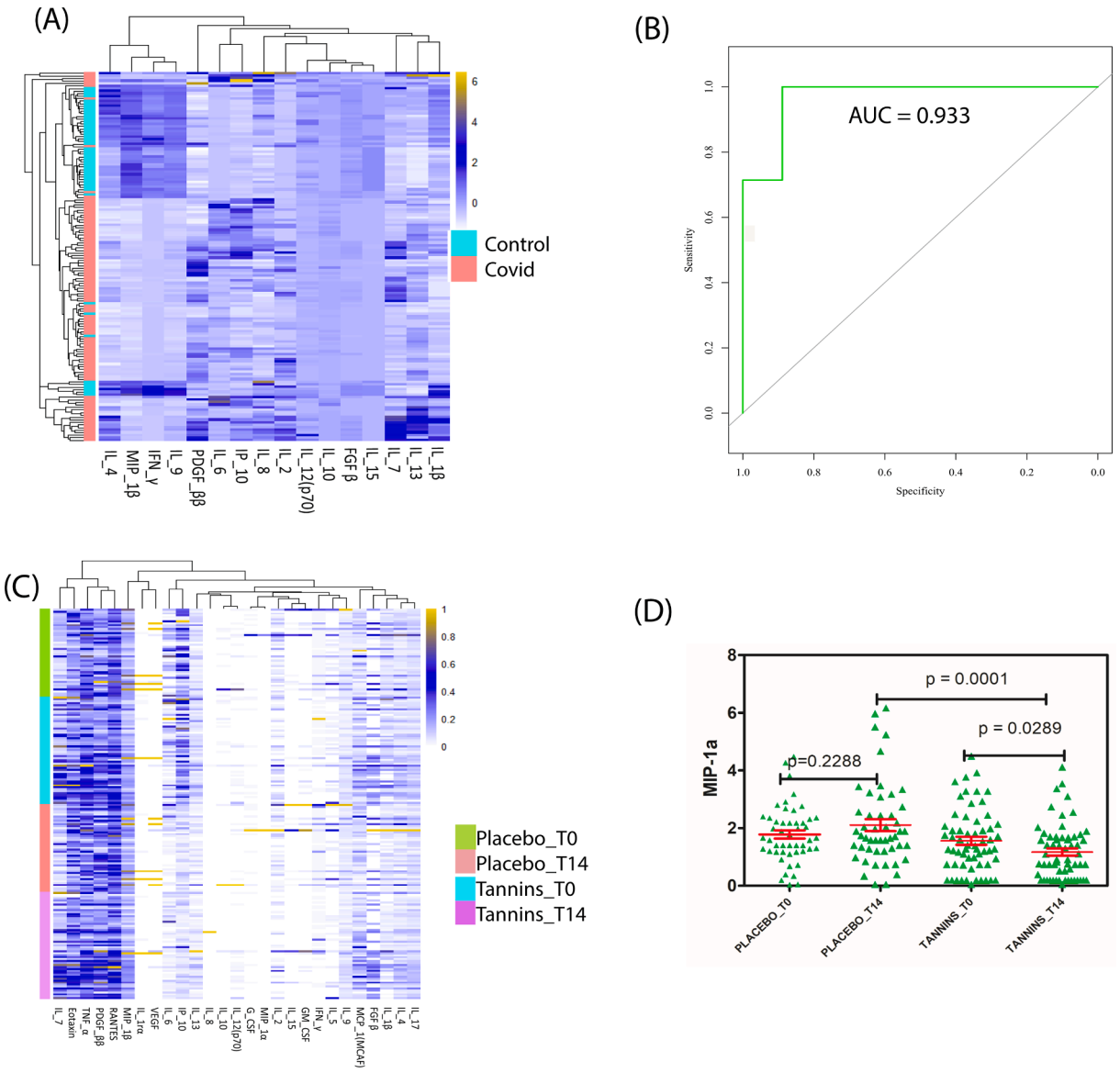

Fig. 2. (A) Heatmap representation of selected cytokines that are significant after group comparison between COVID (N = 101) and control (N = 50) subjects. (B) ROC plot corresponding to Random Forest Classification model. (C) Heatmap of cytokines stratified by subject with different treatment and time. (D) Abundance of MIP-1α cytokine stratified by treatment and time. (Mann Whitney test; significant p values for different groups are illustrated).

significant negative correlation between serum MIP-1α and stool *Bifidobacterium* was noted, especially with ASV103 which showed 100 % identity to *Bifidobacterium longum* (Fig. 4F).

4. Discussion

Continuously emerging SARS-CoV-2 variants contributing to COVID-19 being endemic in several countries, indicates that we must learn to coexist with this disease. Prevention and control measures other than vaccination are therefore needed. Dietary intervention represents one of the most realistic approaches to broadly manage the body's exacerbated inflammatory state to COVID-19 by maintaining a well-balanced gut microbiota. In the present study, we investigated this concept by testing the efficacy of oral tannin supplementation in COVID-19 patients with mild-to-severe disease severity.

Although our nutraceutical-intervention failed to improve clinical outcomes in acute COVID-19 patients receiving standard-of-care treatment, some notable findings were made that may direct treatment towards future management of acute and chronic illness linked to SARS-CoV-2 infection.

Before analyzing the effects of tannin supplementation on gut microbiota composition and inflammatory status of COVID-19 patients, we assessed how these features differed from a cohort of 53 healthy adult controls (HC). This comparison made it possible for us to confirm the patients' COVID-19 disease-associated features with other reports in geographically distinct locations.

Notably, the high level of systemic cytokines recorded by us in COVID-19 patients compared to HC reconfirms the findings reported by other authors (Chi et al., 2020). Indeed, eleven of the significantly increased proinflammatory cytokines (namely IL-1β, IL-1rα, IL-2, IL-6, IL-7, IL-8, IL-9, IL-10, IL-13, GM-CSF, IFN-γ) are in good agreement with Chi et al. (2020). This type of cytokine-mediated inflammatory response is shared by different infections (i.e. MERS-CoV, SARS, and more recently with SARS-CoV-2), which typically presents with pulmonary inflammation and acute lung injury (Huang et al., 2020; Mahallawi et al., 2018; Wong et al., 2004).

IL-1ra, IL-13, and IFN-γ were identified as cytokines that significantly discriminate severe from mild and moderate cases. These

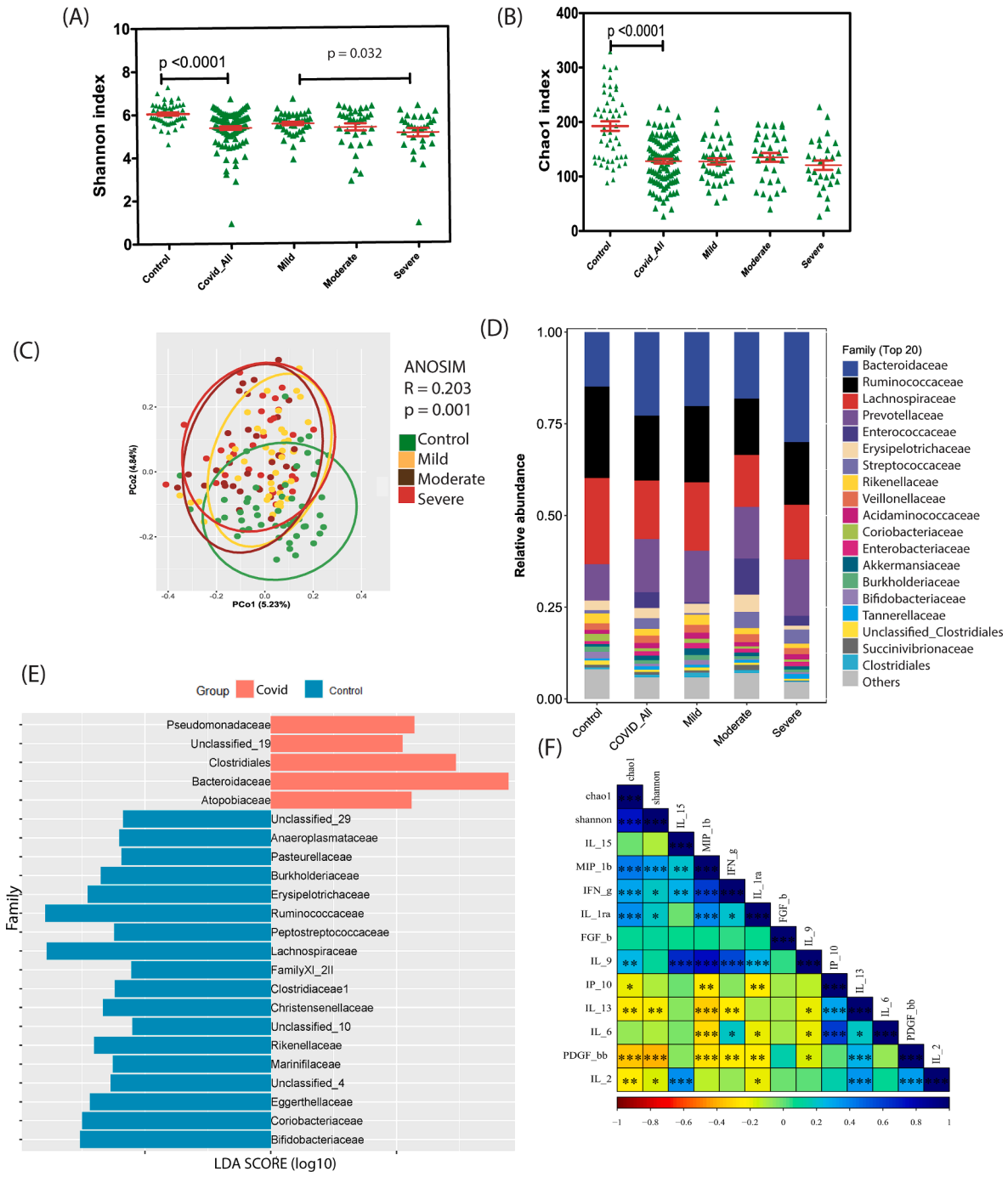

Fig. 3. Per sample alpha diversity at ASV level for HC vs COVID-19 subjects at various levels of severity of symptoms (A) Shannon index and (B) Chao1 richness index. (C) Bray Curtis dissimilarity metric for profiling samples stratified by severity of COVID-19 symptoms. Analysis of similarities non-parametric test results are R = 0.203 and p = 0.001 (D) Relative abundance of top 20 taxa at family level. (E) LEFSe analysis for group comparison of the features in COVID and HC subjects at family level. (F) Correlation heatmap for the Cytokines having spearman correlation significant with Shannon index.

cytokines markers were not included in previous studies and could serve as new predictors of morbidity (Chi et al., 2020; Huang et al., 2020; Qin et al., 2020).

When compared with HC, the gut microbiota composition was significantly altered in COVID-19 patients. As previously reported by other authors, in COVID-19 patients a similar dysbiosis was evident regardless of whether anti-microbials were prescribed and this is probably due to the severe impairment caused by the disease (Zuo et al., 2020).

An altered gut microbiome has already been associated respiratory viral infections, and this could lead to more severe clinical course due to secondary bacterial infections (Groves et al., 2018; Wang et al., 2014). The significant reduction in α-diversity (Shannon diversity) and richness (Chao1) in COVID-19 patients has been extensively described by other authors, who similarly to us have found an exacerbation of the situation in severe cases (Hilpert & Mikut, 2021). The alteration of gut microbiota balance was reflected also in a significant increase in the abundance of families containing opportunistic pathogens and the loss of beneficial symbionts. In particular, Zuo et al. (2020) reported an increase of taxa belonging to Bacteroidaceae (namely *Bacteroides nordii*) and Clostridiaceae family (namely *Clostridium hathewayi*), which were also found to be significantly enriched in our study (Zuo et al., 2020).

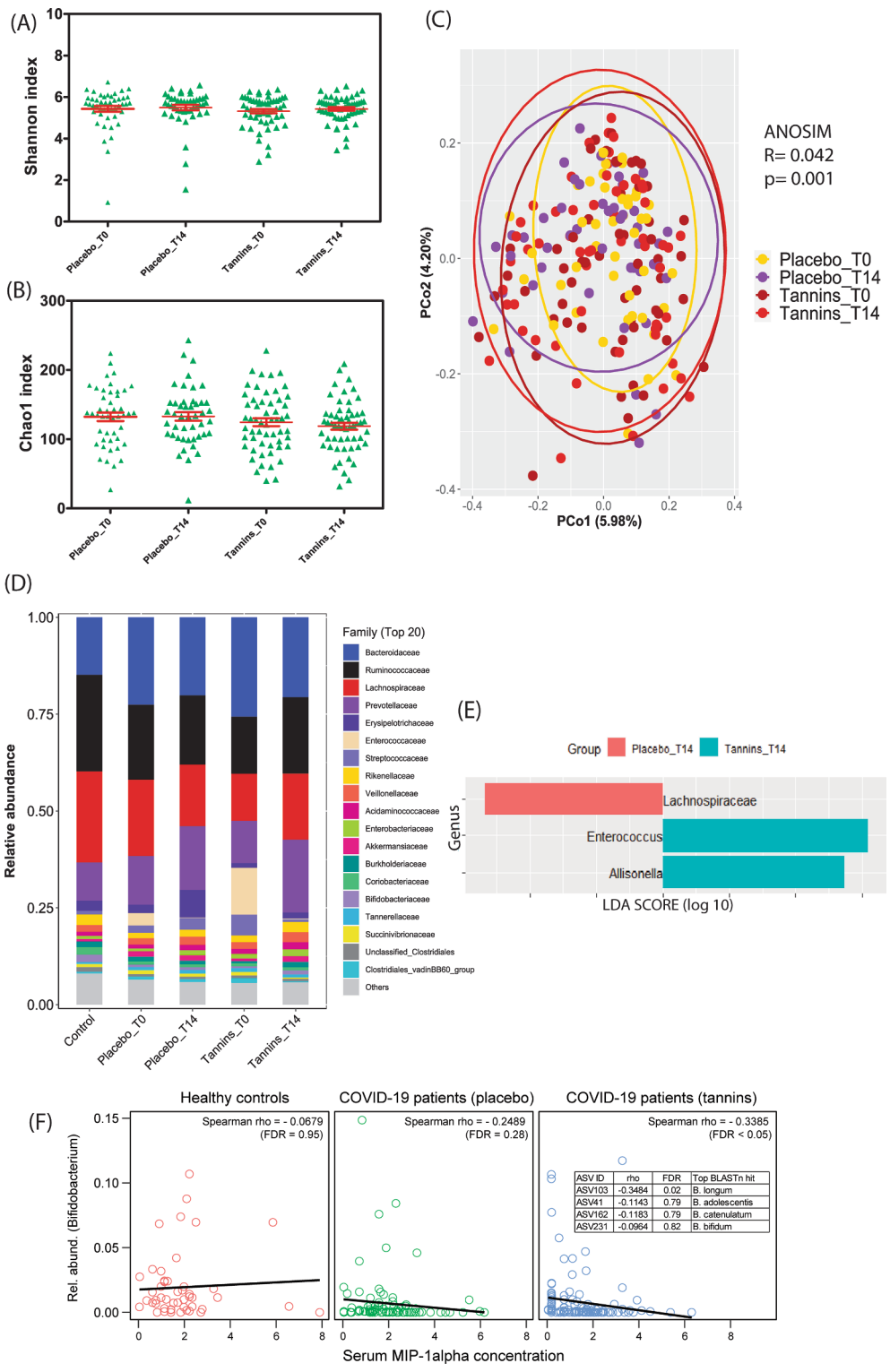

Fig. 4. Gut microbiota analysis of fecal samples at time T0 and T14 in COVID patients administered placebo (N = 46) or tannins (N = 56). (A) Shannon index (B) Chao1 index. (C) Bray Curtis beta diversity (D) Stacked bar plots to show the abundance of top 20 gut microbiota at family-rank to compare tannins vs placebo treatment arms at T14 (E) LEFSe analysis at genus-rank of placebo vs tannin treatment arms at T14. (F) Spearman correlation between serum MIP-1α levels and *Bifidobacterium* relative abundance for HC, placebo, and tannins arms.

Our study also found a correlation between the disruption of gut microbiota composition due to SARS-CoV-2 infection and the accentuated inflammatory state, suggesting that the intestinal microbial ecosystem could play a role in regulating the immune response in the host. In particular, the reduction of α-diversity in the COVID-19 cohort was linked to increased concentrations of IL-15, MIP-1b, IFN-γ, IL-1rα, FGF-β, IL-9, IP-10, IL-6, IL-13, PDGF-ββ and IL-2, consistent with immunological studies conducted by Yeoh et al., (2021).

As regards to the oral tannin supplementation, it led to a reduced inflammatory state, with the most significant inhibition being evident in the MIP-1α response. This chemokine plays a pivotal role in directing appropriate immune responses towards infection and inflammation. Moreover, several authors reported the impact of this mediator on lung defense (Driscoll, 1994; Jøntvedt Jørgensen et al., 2020) and its correlation with COVID-19 severity (Chi et al., 2020). MIP-1α acts by activating human granulocytes, with a consequent release of other pro-inflammatory cytokines (i.e. IL-6 and TNF-α) (Ren et al., 2010).

The relatively short tannin intervention could represent one

explanation why we did not observe significant shifts in α-diversity nor β-diversity, or in tannin-induced compositional shifts which we have previously been reported for Bacteroidaceae, Lachnospiraceae and Ruminococcaceae families (Molino et al., 2022; Molino, Lerma-aguilera, et al., 2021). Members of these families are recognized for being producers of short chain fatty acids (SCFAs), interesting metabolites with a powerful effect in counteracting the inflammatory state (Akhtar et al., 2022).

The correlation study highlighted an inverse correlation between the MIP-1α serum levels and the abundance of *Bifidobacterium* species in the stool samples of tannin-supplemented COVID-19 patients. This finding suggests that specific gut bacteria, such as *Bifidobacterium* could regulate aspects of the gut-lung axis, through modulating systemic levels of cytokines.

Reinforcing these results, previous studies on oral tannins showed similar effects in less dysbiotic individuals, where the anti-inflammatory effect could be related to the gut microbiota-derived metabolites known to be effective in inflammatory events (Molino et al., 2018, 2022). In particular, the authors highlighted that the inmunomodulatory effect, through the microbiota modulation could be due to both the production of SCFAs and the release of metabolites such as quercetin and urolithins (A and B) (Molino et al., 2018). These compounds are powerful antioxidants, able to decrease the inflammatory state and, especially, to inhibit the release of MIP-1α (Alexander Haslberger et al., 2020; Noh et al., 2014).

A major limitation of our study is related to the short tannin intervention time (14 days) restricted to the hospitalization of COVID-19 patients. In a previous study we showed that supplementation with a tannin blend led to an increase in SCFA release after the two weeks, whereas significant changes in microbiota composition emerged only after 4 weeks of treatment (Molino et al., 2022). This might explain why in our study we could begin to observe a reduction in cytokines after 14 days of tannin supplementation, but shifts in microbiota composition were not readily evident. Thus, the duration of the oral tannin intervention may have been suboptimal.

In addition, it should be considered that the composition of the microbiota of COVID-19 patients at T0 was highly affected by the virus infection, reporting a decreased alpha diversity and an altered composition compared to the HC. Thus, probably the gut microbiota community structure was too compromised to observe significant beneficial tannin modulation.

Taking all these results together, they indicate that tannin supplementation may be more amenable to chronic illness such as long-COVID where longer treatment duration is merited, and patients are less likely to marked dysbiosis.

5. Conclusions

In this study, a tannin supplementation was administered to 124 COVID-19 patients receiving standard-of-care treatment. Comparison of the patients with 53 healthy volunteers showed that the COVID-19 patients were characterized by a pronounced intestinal dysbiosis and high inflammatory status.

Although the short course of oral tannin supplementation (14 days) failed to provide significant clinical improvement or favorable hospital discharge rate after 28 days, oral tannins were associated with a decrease in systemic inflammation correlated with fecal *Bifidobacterium* abundance. Our study sets the stage for future clinical investigation of prophylactic nutraceutical management of COVID-19 symptoms, through precision restoration of gut microbiota members that modulate adverse host immune responses to infection.

Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Hospital de Clínicas Jose de San Martin, Buenos Aires University, Argentina and approved on 12/06/2020. The study protocol was also registered with ClinicalTrials.gov under the number NCT04403646, on May 27th, 2020. Informed consent was obtained from all subjects involved in the study.

The study was approved by the ethics committee of the University Hospital of Universidad de Buenos Aires "Hospital de Clínicas José de San Martín", Argentina, and adhered to ethical principles outlined in the declaration of Helsinki convention. All participants gave written informed consent, and they were able to withdraw from the survey at any time without giving a reason.

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Transparency Statement

The data sets, including the redacted study protocol, redacted statistical analysis plan, and individual participant data supporting the results reported in this article, will be made available within 3 months from initial request to researchers who provide a methodologically sound proposal. The data will be provided after its de-identification, in compliance with applicable privacy laws, data protection, and requirements for consent and anonymization. Sequencing data were deposited in the European Nucleotide Archive (ENA) at EMBL-EBI (Project: PRJNA856489).

CRediT authorship contribution statement

Silvia Molino: Conceptualization, Investigation, Formal analysis, Writing – review & editing. **Andrea Pisarevsky:** Conceptualization, Investigation, Formal analysis, Supervision, Visualization, Writing – original draft, Writing – review & editing, Resources. **Shyam Badu:** Investigation, Formal analysis, Data curation, Data curation, Software, Supervision, Writing – original draft, Writing – review & editing. **Qinglong Wu:** Investigation, Formal analysis, Data curation, Software, Writing – original draft, Writing – review & editing. **Fabiana López Mingorance:** Investigation, Formal analysis, Data curation, Writing – review & editing. **Patricia Vega:** Investigation, Formal analysis, Writing – review & editing. **Juan Pablo Stefanolo:** Investigation, Formal analysis, Visualization, Writing – review & editing. **Julieta Repetti:** Investigation, Formal analysis, Writing – review & editing. **Guillermina Ludueña:** Investigation, Formal analysis, Writing – review & editing. **Pablo Pepa:** Investigation, Formal analysis, Writing – review & editing. **Juan Ignacio Olmos:** Investigation, Formal analysis, Writing – review & editing. **Marcelo Rodriguez Fermepin:** Investigation, Writing – review & editing, Resources. **Tatiana Uehara:** Investigation, Writing – review & editing. **Elisa Viciani:** Investigation, Software, Writing – original draft, Writing – review & editing. **Andrea Castagnetti:** Investigation, Software, Writing – review & editing. **Tor Savidge:** Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Resources, Funding acquisition. **María Marta Piskorz:** Conceptualization, Formal analysis, Data curation, Supervision, Visualization, Writing – original draft, Writing – review & editing, Funding acquisition.

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

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2022.105356.

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