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Máster en Microbiota, Probióticos y Prebióticos

IMPROVEMENT OF THE GUT MICROBIOTA BALANCE IN PATIENTS WITH AXIAL SPONDYLOARTHRITIS AFTER ONE YEAR OF BIOLOGICAL THERAPY

MEJORA DEL EQUILIBRIO DE LA MICROBIOTA INTESTINAL EN PACIENTES CON ESPONDILOARTROPATIA AXIAL DESPUES DE UN AÑO DE TRATAMIENTO BIOLOGICO

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"Mejora Del Equilibrio De La Microbiota Intestinal En Pacientes Con Espondiloartropatía Axial Después De Un Año De Tratamiento Biológico"

Introducción

La disbiosis de la microbiota intestinal podría estar asociada al desarrollo y progresión de la Espondiloartropatía axial (axSpA). Los medicamentos biológicos modificadores de la enfermedad (bDMARDs) han demostrado tratar eficazmente la axSpA, pero la falta de respuesta al tratamiento sigue siendo un reto que podría estar relacionado con la microbiota intestinal.

Objetivo

Explorar los cambios en la microbiota intestinal en pacientes con axSpA después de un año de tratamiento con bDMARDs.

Métodos

En una extensión de la Cohorte de Inicio Prospectiva de Espondiloartritis Alemán (GESPIC) se incluyeron pacientes con axSpA radiográfica y alta actividad de la enfermedad a pesar del tratamiento con antiinflammatorios no esteroideos (AINEs) antes de empezar la terapia con bDMARDs. Se recogieron las evaluaciones de los parámetros de actividad de la enfermedad y muestras fecales al inicio y después de un año de iniciar el tratamiento. Se compararon con individuos con dolor crónico de espalda en los que se descartaron enfermedades inflamatorias. La composición de la microbiota se analizó mediante secuenciación del 16S rRNA y se clasificó taxonómicamente con SILVA138 database. La respuesta al tratamiento con bDMARDs se definió como una mejora en el índice de ASDAS de al menos 1.1 puntos.

Resultados

Se incluyeron 99 pacientes con axSpA y 63 controles, según la disponibilidad de datos clínicos y de microbiota. Los pacientes con axSpA tenían una edad media de 36.4 ± 10.4

años y el 64.4% (64) eran hombres. La prevalencia de HLA-B27 fue mayor en pacientes con axSpA que en controles (89.9% vs 7.9%, respectivamente).
La diversidad alfa y beta se acercaron al grupo control después de un año de tratamiento, aunque no de manera significativa. Al inicio, los pacientes con axSpA presentaron menos abundancia relativa en taxones de la familia *Lachnospiraceae* como *Blautia, Roseburia* y *Fusicatenibacter*, y mayor en *Collinsella* en comparación con los controles. Al cabo de un año, la mayoría de los taxones aumentaron en abundancia, especialmente *Blautia*. Los niveles de *Collinsella* permanecieron estables, mientras que cambios en *Prevotella* y *Bacteroides* se correlacionaron con cambios en ASDAS.

Conclusiones

La composición de la microbiota intestinal en pacientes con axSpA cambió para asemejarse más a los controles después de un año de tratamiento con bDMARDs. El enriquecimiento resistente a tiempo y tratamiento de *Collinsella* sugiere un posible papel como biomarcador de la enfermedad.

Palabras clave

Espondiloartropatia, microbiota, bDMARDs, 16S rRNA, biomarcador

2 Title and Abstract (English version)

"Improvement Of The Gut Microbiota Balance in Patients With Axial Spondyloarthritis After One Year Of Biological Therapy"

Background

Dysbiosis of the gut microbiota might be associated with the development and progression of axial spondyloarthritis (axSpA). Biological disease-modifying antirheumatic drugs (bDMARDs) effectively treat axSpA, but non-response to therapy remains a challenge that might be related to the gut microbiota.

Objectives

To explore the gut microbiota changes in patients with axSpA following one year of bDMARD treatment.

Methods

In an extension of the prospective German Spondyloarthritis Inception Cohort (GESPIC), patients with radiographic axSpA and high disease activity despite NSAID treatment were recruited prior to bDMARD therapy initiation. Assessments of disease activity and fecal samples were collected before treatment and one year after. The results were compared with individuals with chronic back pain where inflammatory disease was ruled out. Microbiota composition was analyzed using 16S rRNA gene sequencing and SILVA138 database profiling. Treatment response to bDMARDs was defined as improvement in ASDAS by at least 1.1 points.

Results

The analysis included 99 patients with axSpA and 63 controls, based on availability of clinical and microbiome data. Patients with axSpA had a mean age of 36.4 ± 10.4 years and 64.4% (64) were males. HLA-B27 prevalence was higher in patients with axSpA than in controls (89.9% vs 7.9%, respectively).

Alpha diversity and beta diversity moved toward resembling the control group post-treatment, though not significantly. At baseline, patients with axSpA were depleted in Lachnospiraceae taxa such as Blautia, Roseburia and Fusicatenibacter, and enriched in Collinsella compared to controls. After treatment, most taxa increased in abundance, particularly Blautia. Collinsella levels remained stable, while shifts in Prevotella and Bacteroides correlated with ASDAS change.

Conclusions

The gut microbiota composition of patients with axSpA changed and resembled closer controls after one year of treatment with bDMARDs. The persistent enrichment of Collinsella despite time and treatment suggests it potential role as a disease biomarker.

Keywords

Spondyloarthritis, microbiota, bDMARDs, 16S rRNA, biomarker

3 Introduction

3.1 Axial spondyloarthritis

Axial Spondyloarthritis (axSpA) represents an auto-immune mediated disease predominantly affecting the axial skeleton, including the spine and sacroiliac (SI) joints. The disease presents as chronic back pain and morning stiffness, usually low back pain and buttocks, but can be located in any part of the spine. The back pain is typically inflammatory, characterized by morning stiffness, improvement with movement, worsening with stillness and awaking on the second half of the night. Over time, this inflammation can eventually lead to fusion of the vertebrae, a condition known as ankylosis bringing irreversible stiffness to patients (Figure 1). Other manifestations are peripheral musculoskeletal symptoms such as arthritis, enthesitis and dactylitis(1). The onset of the disease is in the early adulthood by the second or third decade of life, with a prevalence of 0.3%-1.4% worldwide(1).

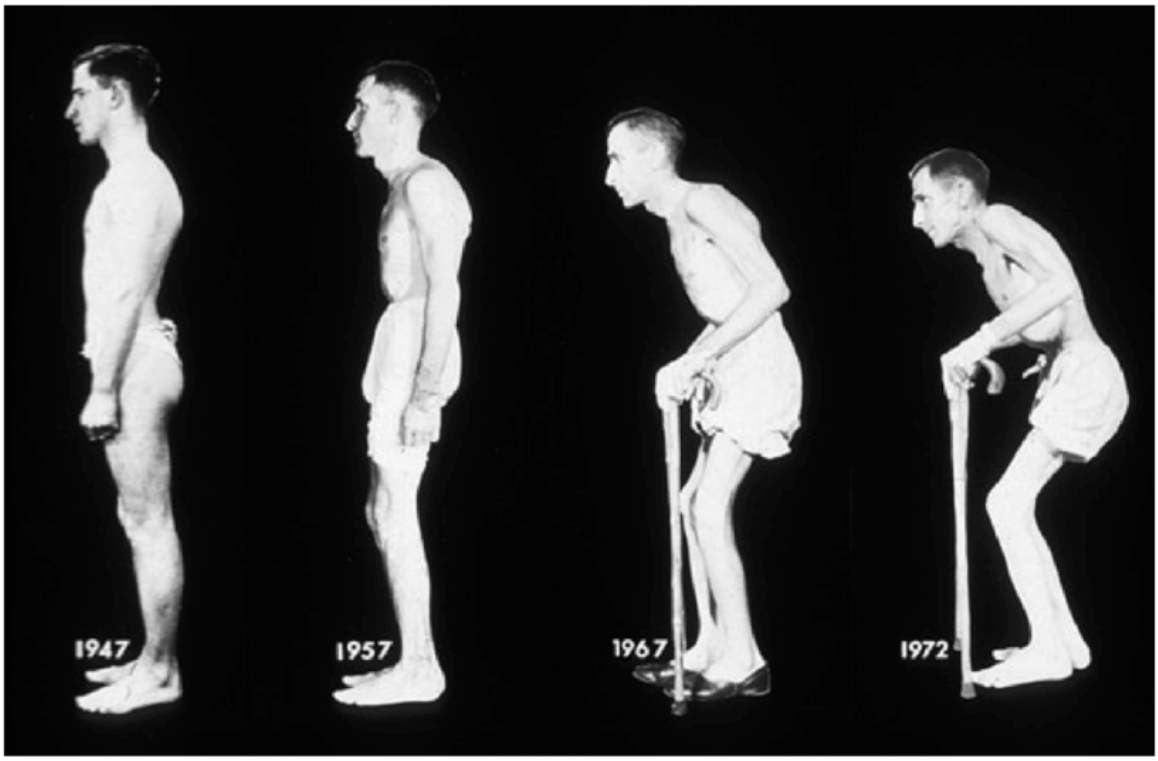


Figure 1. Axial Spondyloarthritis 35 years of natural evolution of the disease.
Little H. et al. Am J Med. 1976

The disease has the strongest disease-HLA association until now described, with a heritability estimated around 90%(2). The presence of the HLA-B27 is a key genetic biomarker in the diagnosis of SpA, although remains still unclear the exact role that its expression interplays in the pathogenesis of the disease.

AxSpA can be broadly classified into radiographic axSpA (r-axSpA), also known as Ankylosing Spondylitis (AS), and non-radiographic axSpA (nr-axSpA). This classification is based on the presence, for r-axSpA, or absence, for nr-axSpA, of structural damage in the SI joints detectable on plain radiographs, typically from the pelvis. These structural changes in the SI joints are called sacroiliitis and are manifested in the radiographs as sclerosis, erosions, narrowing of the joint space and partial or total ankylosis. The sacroiliitis grading was described in the consensus of the modified New York (mNY) criteria in 1984, where definite radiographic sacroiliitis was defined by the presence of sacroiliitis at least grade 2 bilaterally or grade 3-4 unilaterally(3) – Figure 2. This is an important and historical classification, nevertheless the development of a new classification criteria in 2009 by the Assessment of SpondylArthritis International Society (ASAS) had a pivotal influence in the understanding of axSpA, viewing that axSpA presented two different stages (nr-axSpA and r-axSpA) in a single disease entity(4).

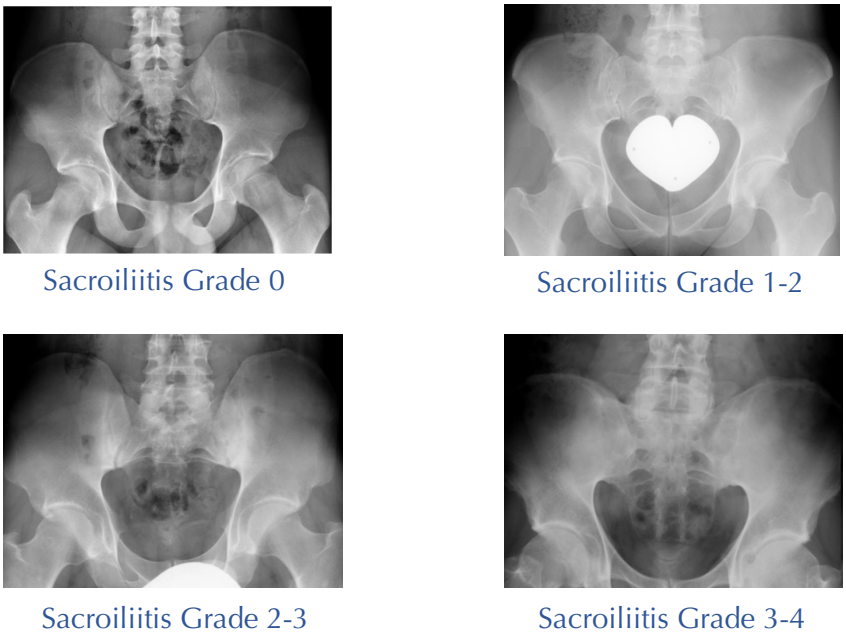


Figure 2. Sacroiliitis grading according to the modified New York criteria of 1984 by van der Linden.

3.2 Current Treatment Options for axSpA

The management of axSpA involves a combination of non-pharmacological and pharmacological approach. Each treatment is specific to the needs of each patient based on the clinical presentation including axial, peripheral and extra-musculoskeletal manifestations, as well as any existing comorbidities and psychosocial factors(5-7).
Non-pharmacological interventions are crucial in the axSpA management. Patients are educated about their condition, understanding the nature and symptoms of the disease, its course, treatment options and prognosis. This educational approach targets to empower and involve patients actively in their own care being part of the shared decision-making together with their physician. Along with patient education, regular exercise and physiotherapy also serve as keystone of non-pharmacological treatment(6).

Pharmacological treatment for axSpA has developed relevant advancements, expanding the therapeutic options available to patients in the last years. Nonsteroidal anti-inflammatory drugs (NSAIDs) remain as first-line treatment, reducing inflammation and frequently succeeding in symptom control(6).

For those patients who present high disease activity despite full-dose of at least two different NSAIDs, advanced therapy should be considered including Tumor Necrosis Factor (TNF) inhibitors, Interleukin (IL)-17 inhibitors, or Janus Kinase (JAK) inhibitors. According to the 2022 EULAR/ASAS recommendations for the management of axSpA, the decision between initiation TNF or IL-17 inhibitors should be guided by individual factors such as comorbidities and extra-musculoskeletal manifestations in the discretion of the patient and their rheumatologist(6).

TNF inhibitors belong, together with IL-17, to a type of biologic disease-modifying anti-rheumatic drug (bDMARD) and used primarily to treat conditions that involve the immune system such as rheumatoid arthritis (RA) or SpA. These drugs are designed to target specific parts of the immune system that trigger inflammation(8, 9). TNF inhibitors work by blocking the action of TNF, a cytokine protein that plays a significant role in promoting inflammation. There are several types including: adalimumab, certolizumab pegol, etanercept, golimumab and infliximab. Their administration is through

subcutaneous injections (except infliximab that can be also administrated by intravenous infusions) administered once, twice or four times per months depending on the specific drug. Each of the currently available TNF inhibitors have a strong and similar clinical efficacy in active axSpA with clear improvement of symptoms as well as reduction of active inflammation present in Magnetic Resonance Imaging (MRI)(10-14). While NSAIDs and bDMARDs have clearly proven successful in reducing symptoms and even preventing structural damage in axSpA, up to 30-40% of patients from most of the clinical trial data available with active axSpA do not reach an adequate clinical response, considered as non-responders. While predictors such as shorter disease duration, younger age, better functional status, elevated levels of serum CRP, and active inflammation on MRI are predictors of a good clinical response, the limited availability of such predictors highlight the need for more targeted therapeutic strategies.

3.3 Importance of gut microbiota in axSpA

Historically, the concept that bacterial infections may serve as a trigger for disease onset in genetically predisposed individuals(15) has long been a subject of research and debate. Particularly in the case of reactive arthritis (one subgroup of the SpA spectrum), there is evidence to suggest that certain bacterial infections in the gut, cause by specific enteropathogens such as Salmonella, Shigella, Yersinia and Campylobacter among other infections such as Chlamydia or, can precipitate arthritic symptoms(16, 17).

The observation that approximately 70% of patients with axSpA have increased gut permeability and signs of intestinal inflammation is highly significant, as it points to a likely interaction between gut health and SpA. This increased gut permeability, often referred to as “leaky gut”, could allow bacteria or bacterial products to translocate from the intestinal lumen into the systemic circulation, triggering an immune response that may contribute to SpA symptoms(18). This finding takes on added relevance when considered alongside the increased prevalence of SpA in patients with inflammatory bowel disease (IBD), such as Crohn’s disease (CD) and ulcerative colitis (UC), compared to the general population(19). This concomitance between SpA and IBD suggests a shared pathological mechanism, possibly rooted in the immune response to intestinal inflammation as well as to the changes in the structure of the gut microbiota(20).

The gut microbiota, the complex community of microbes that coexist in our intestines, is increasingly recognized for its critical role in health and disease. Particularly in axSpA, recent research has pointed the importance of gut microbiota in its pathogenesis, suggesting that an imbalance or "dysbiosis" in the gut microbiota together with alterations in the immune responses, and metabolic pathways may contribute to the development and maintenance of the disease.

The observation that HLA-B27 and ß2 microglobulin transgenic rat models can develop a disease phenotype similar to human SpA, but not in a germ-free environment, is a fundamental finding(21, 22). *Taurog et al.* in 1994 observed that these transgenic rats who suffered colitis and arthritis in normal conditions, did not develop them when they were raised in germ-free conditions, and interestingly after gastrointestinal colonization even with a few commensals they would present arthritis and colitis(22). This strongly suggests that the microbiome plays a critical role in the initiation or progression of SpA disease, at least in these animal models. Research in patients with SpA has grown since then, exploring the relationship between the human gut microbiota and axSpA.

These microbial changes have two implications. Firstly, they may be critical to the development of axSpA. A main example is the study by *Asquith et al.* in 2014, which identified changes in the composition of the gut bacteria in patients with axSpA(23). The study was focused on the effects of HLA (B27 for axSpA and DRB1 for RA) on the gut microbiota suggesting that it may play a role in the pathogenesis of the SpA. Nevertheless, they observed a decrease in the abundance of family *Lachnospiraceae* and an increase in the genus *Collinsella*. The lower abundance in *Lachnospiraceae*, generally considered beneficial bacteria in the maintenance of gut health, could result in increased local inflammation and susceptibility to disease. On the other hand, the increase in *Collinsella* could potentially lead to more pro-inflammatory environment, and thereby acting as a trigger or more likely as a contributing factor to the initiation of axSpA symptoms. Another key study was performed by *Costello et al.,* published in 2015, where they compared the gut microbiota composition from patients with AS with healthy controls. They found that patients with AS had a specific dysbiosis characterized by the

decrease in the abundance of several taxa such as *Faecalibacterium prausnitzii*, *Bificobacterium adolescentis*, and *Bifidobacterium longum*, and an increase in the abundance of *Ruminococcus gnavus*. This last taxon already implicated in the alterations of other rheumatic diseases such as RA, and more recently reclassified in the genus of *Blautia(24)*. The study also found that the gut dysbiosis was associated with disease activity.

Secondly, these alterations in the gut microbiota may play a role in the progression of the disease. A disrupted microbiota could further exacerbate the immune response and the inflammatory pathways of axSpA. Therefore, not only could these bacterial shifts potentially serve as a biomarker of disease onset, but they could also be actively implicated in the severity and course of the disease over time(25-27). Such observations have broad implications for both diagnostics and therapeutics. For example, finding microbial signatures in axSpA could serve as biomarkers for early diagnosis, allowing for more timely and targeted interventions. They could also point toward the future potential of "microbiome modulation" as a therapeutic approach in axSpA, raising the possibility that treatments aimed to restoring a balanced gut microbiota could complement existing pharmaceutical approaches to reduce disease activity.

3.4 Current knowledge on how bDMARDs affect the gut microbiota

bDMARDs including TNF inhibitors, IL-17 inhibitors among other advanced therapies, have been extensively used for the treatment of many immune-mediated diseases such as RA and SpA. However, their impact on the gut microbiota ecosystem is an area of new investigation, where preliminary data suggest that these drugs could influence the microbial composition and in consequence have an impact in treatment response. *Vallier et al*. examined how TNF inhibitor treatment modulated the gut microbiota in axSpA suggesting that this treatment can influence the composition of gut microbiota and potentially improve the symptoms and disease activity. Aligned with this work, *Ditto et al*. found results, specifically on patients with IBD-related SpA, where both conditions share similarities in their gut dysbiosis(28).

These changes in the microbiota may not necessarily be a direct effect of the drug on the microbial communities; instead, they may reflect a less inflamed gut environment as bDMARDs effectively control disease activity. In addition, microbiota composition may serve as a predictive biomarker of treatment response, opening the door to more personalized medicine(29). Despite these findings, understanding this intricate relationship remains challenging due to the complexity of the gut microbiota and the lack of large-scale studies. Deepening our understanding could help optimize treatment strategies and provide insight into mechanisms of drug action and resistance. Therefore, it is not yet possible to draw clear conclusions about the role of bDMARDs in modulating the gut microbiota and further research is needed.

3.5 Aim of the project

To explore the changes in the gut microbiota of patients with axSpA after receiving one year treatment with bDMARDs.

4 Methods

4.1 Patient selection and study design

Patients with radiographic axSpA (also known as AS) were recruited between 2015 and 2019 in an extension of the prospective German Spondyloarthritis Inception Cohort (GESPIC) before beginning bDMARD therapy. Briefly, GESPIC is an ongoing prospective cohort initiated to study the course and log-term outcomes of SpA across its whole spectrum of clinical presentation, including but not limited to gut microbiome composition. Patients recruited in the AS arm of this cohort were required: 1) to be at least 18 years old; 2) fulfill the mNY criteria and to be eligible to start a bDMARD therapy, presenting high disease activity (BASDAI ≥ 4 and/or ASDAS ≥ 2.1) despite previous treatment with NSAIDs; 3) to be naïve to or not received treatment with bDMARDs for at least three months before the enrollment in the study. There were no other restrictions regarding therapy, and the choice of bDMARD was left to the discretion of the clinical rheumatologists in accordance with standard practice.

Clinical and biological samples were collected every six months alongside with patient metadata (demographic information and clinical characteristics such as disease activity parameters BASDAI, CRP and ASDAS). Stool samples were collected from all patients at baseline prior to treatment and after one year. The definition of response to bDMARD treatment was a clinically important improvement in ASDAS, defined as the decrease of ASDAS of at least 1.1 points compared to baseline.

Control individuals were obtained from the Optiref study(30), which included patients with chronic back pain who the diagnosis of SpA was ruled out after a standardized rheumatologic examination. Individuals with a diagnosis of CD, anterior acute uveitis or psoriasis were excluded from the control group.

All patients gave and signed their written informed consent. The study was approved by the ethical committee (Charité- Universitätsmedizin Berlin, Berlin, Germany) and it was conducted in accordance with the declaration of Helsinki and Good Clinical Practice.

4.2 Sample preparation

Samples were collected in the stool collection tube with DNA Stabilizer from Stratec-PSP® Spin Stool DNA Plus Kit / PSP® Spin Stool DNA Basic Kit by patients and send it back to the site in a window of up to two weeks to collect and return from the site visit. When the samples arrived at the site were stored in the lab at -80°C until DNA extraction.

4.3 16S rRNA Sequencing

After stored at -80°C, fecal sample were defrosted on ice before processing. A 1 ml aliquot of each fecal sample, resuspended in RNALater (to stabilize and protect DNAR and RNA from degradation), was centrifuged and washed once with water to remove excess fixative and salt. DNA was extracted then using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research), following manufacturer's guidelines. Bead beating, a mechanical lysis process where the cells brake to release their DNA, was performed four times, each for a duration of 5 minutes. The V4 region of the 16S rRNA gene was amplified using 25ng of DNA per 30µl PCR reaction, employing Q5 polymerase (NEB Biolabs). Each sample was subjected to triplicate PCR amplifications which were later pooled. Post-amplification PCR amplicons were normalized and sequence using an Illumina MiSeq platform (PE300).

Data analysis was performed using the LotuS (Low-Input Tool for Sequencing) pipeline (v1.62), including steps for sequence quality filtering, read merging, adapter and primer removal, chimera identification and removal, as well as Operational Taxonomic Unit (OTU) clustering and taxonomic classification, which was based on the SILVA (v138), Greengenes (v13.5), and HITdb (v1.0.0) databases.

4.4 Statistical Analysis

Analysis was performed using R statistical programming environment, version 4.0.3. Raw counts were rarefied to 5,000 reads and filtered to exclude OTUs not present in at least 3% of the samples. The *phyloseq* package (version 1.34.0) was used for Shannon entropies and OUT binning calculations at the genus and phylum level. Beta diversity was performed using the vegan (version 2.5.7) and stats packages to calculate pariwise

Bray-Curtis dissimilarities and to perform a principal coordinates ordination analysis (PCoA). To compare abundances of OTUs and to test for significance independent of the clinical parameters Wilcoxon tests were performed and adjusted for multiple testing by Benjamini-Hochberg procedure.

5 Results

5.1 Patient characteristics

From the total 129 patients included in the initial cohort, we selected 99 patients with a diagnosis of AS and 63 individuals from the control group for our analysis based on the availability of clinical and stool samples. For the AS group, the average age (mean ± SD) was 36.4 ± 10.4 years with symptom duration of 11.5 ± 11.3 years and male predominance, representing the 64.4% of the total group.

A higher prevalence of HLA-B27 positivity was observed among AS patients with 89.9% compared with 7.9% of the controls. Regarding extra-musculoskeletal manifestations nearly a quarter of patients had history of anterior acute uveitis (22.2%), 17.2% had psoriasis, and 7.1% were diagnosed of IBD. Patients with AS presented high disease activity, with average levels of CRP of 12.9 ±19.3 mg/L, BASDAI 5.7 ± 1.4, and ASDAS index of 3.4 ± 0.8. Most of patients with AS did not received previous csDMARDs, and 75.8% were bDMARDs naïve. For details, please see Table 1 with all demographic and clinical characteristics.

Table 1. Baseline demographic and clinical characteristics of the patients with axSpA in the GESPIC study and controls who were included in the current analysis

	AS patients n=99	Control individuals n=63
Age, years, mean ± SD	36.4 ± 10.4	38.2 ± 10.4
Male sex, n (%)	64 (64.4)	26 (41.3)
Smoking status: current smoker, n (%)	34 (34.3)	11 (17.5)
Symptom duration, years, mean ± SD	11.5 ± 11.3	-
HLA-B27 positive, n (%)	89 (89.9)	5 (7.9)
BMI, kg/m^2, mean ± SD	25.2 ± 4.3	26.2 ± 5.7
Uveitis ever, n (%)	22 (22.2)	-
Psoriasis ever, n (%)	17 (17.2)	-
IBD ever, n (%)	7 (7.1)	-
CRP, mg/L, mean ± SD	12.9 ± 19.3	1.2 ± 1.8
BASDAI, range (0-10), mean ± SD	5.7 ± 1.4	-
ASDAS, mean ± SD	3.4 ± 0.8	-
BASFI, range (0-10), mean ± SD	4.6 ± 2.2	-

Current NSAID treatment, n (%)	97 (98.0)	39 (62.9)
csDMARDs intake, current, n (%)	4 (4.0)	-
csDMARDs intake, ever, n (%)	12 (12.1)	-
Naïve to bDMARD treatment, n (%)	75 (75.8)	-

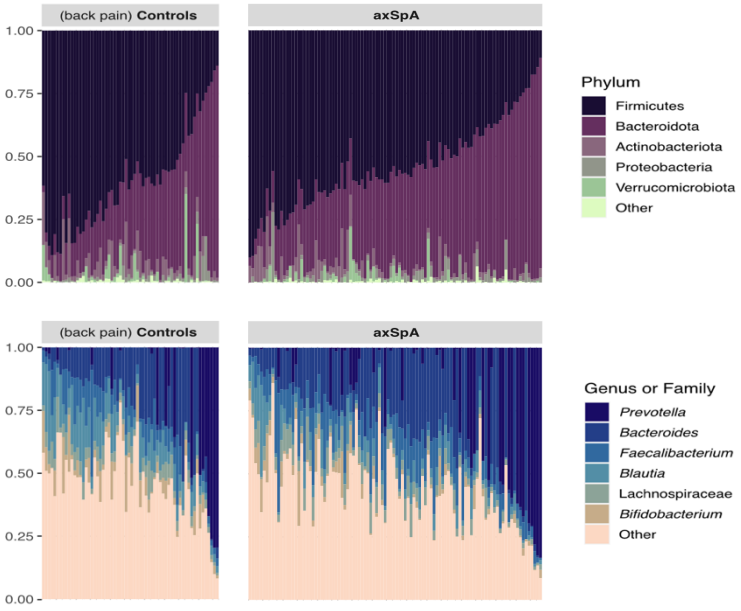
AS, ankylosing Spondylitis; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; bDMARDs, biologic disease-modifying antirheumatic drugs; BMI, body mass index; CRP, C-reactive protein; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; IBD, inflammatory bowel disease; SD, standard deviation.

After baseline all included patients started a bDMARD therapy: 98 (98.9%) with TNF inhibitors and 1 (1.1%) with IL-17 inhibitors. From all patients, 67 (67.7%) of patients had a good treatment response according to ASDAS clinically important improvement (as it was defined in this study for clinical response) with an improvement of at least 1.1 points in the ASDAS index.

5.2 Microbiota composition of gut microbiota

We performed 16S rRNA sequencing and taxonomically profiled a total of 162 samples (99 patients and 63 controls). At the phylum level, both groups, patients and controls, were dominated by Firmicutes, followed by Bacteroidota, Actinobacteriota, and Proteobacteria (for details, please see Figure 3).

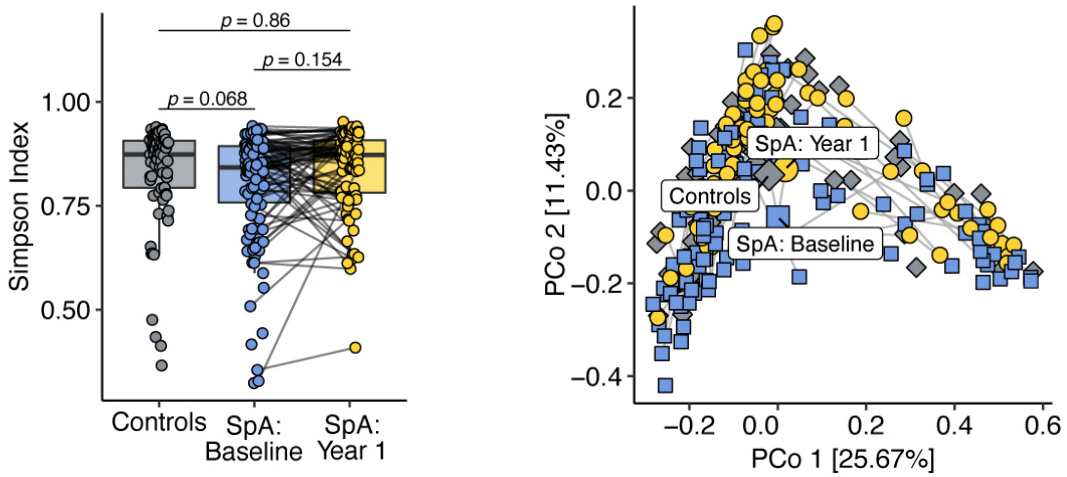
Figure 3. Microbiota composition at the phylum and genus level of patients with axSpA and control individuals at baseline.



To examine the variation and diversity of the microbiota composition in patients with axSpA before and after biological therapy, we performed alpha and beta analyses those communities and compared it with the control group.

To assess alpha diversity, we used the Simpson indices, suggesting a modest increase in microbial diversity in axSpA patients from their baseline to year 1 (Figure 4). Although this increase was not statistically significant (paired Wilcoxon p-value of 0.154), it brought the alpha diversity of the axSpA group closer to that of the control group. One possible explanation for this trend could be that the therapy influences the composition of the microbiota in patients with axSpA, making it more similar to the microbial landscape seen in the control individuals.

Figure 4. Alpha and beta diversity of patient with axSpA treated with bDMARDs and comparted with control at baseline.



Alpha (right) and beta (left) diversity analysis in patients with axSpA before and after 1 year of treatment with bDMARDs for a year. Points labeled in the beta diversity represents group means. PCo, principal coordinate; SpA, spondyloarthritis.

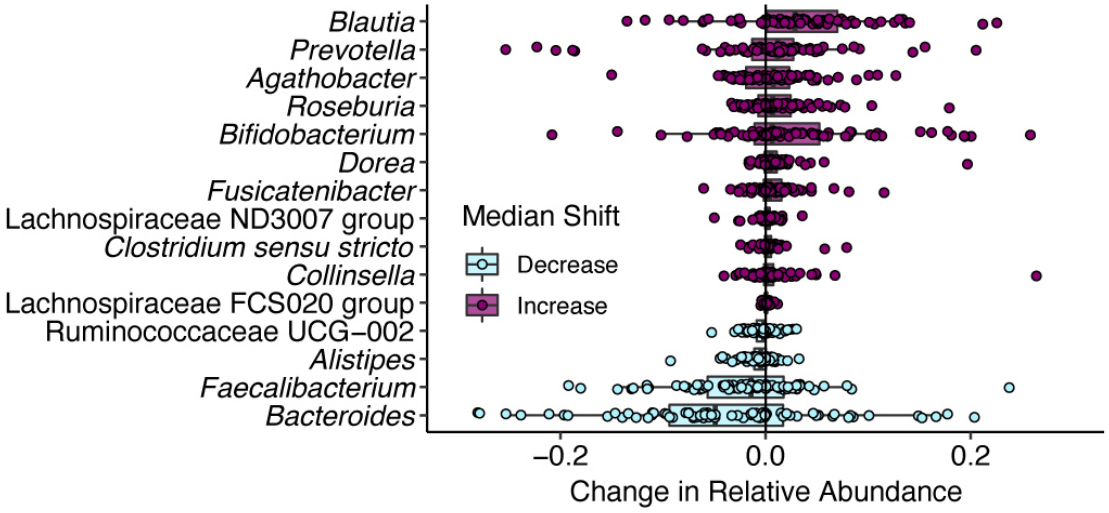
In addition to alpha diversity, beta diversity, which evaluates the differences in microbial diversity between samples, was assessed. Using Bray-Curtis dissimilarities, patients with axSpA seemed to show a qualitative normalization of their microbiota composition after treatment compared with control individuals. When the beta diversity data was visualized using principal coordinate space (PCoA) plot, it becomes clear the shift after treatment (Figure 2). At baseline, samples from patients with axSpA (blue squares) tend

to cluster away from controls (gray diamonds). However, after one year of treatment, there is a clear migration and distribution of the axSpA samples (yellow circles) toward the control cluster, showing a closer resemblance to the controls.

5.3 Taxonomy shifts of gut microbiota after treatment with bDMARDs

At the genus level, patients with axSpA presented differences in the microbiota composition when compared with the control group at baseline. There was a clear depletion of specific *Lachnospiraceae* taxa, primarily *Blautia*, *Roseburia*, and *Fusicatenibacter*, and an enriched relative abundance of *Collinsella* observed in the axSpA group (Figure 5).

Figure 5. Predominant mean relative abundance taxonomic shifts in patients with axSpA after one year of biological treatment.



After one year of biological treatment, we observed shifts in the relative abundance of these taxa in the majority of patients as it shown in Figure 5. An increase in the abundances of previously depleted *Lacnospiraceae* taxa was evident, with *Blautia* showing the most significant increase. Examining the dynamics of highly abundant taxa, significant correlations were observed between shifts in *Prevotella* and *Bacteroides* and changes in the ASDAS index at one year time point. This correlation remained robust even after controlling for intra-individual variance and global changes in alpha diversity. *Faecalibacterium*, which was particularly enriched at baseline in HLA-B27 positive

patients (adjusted Wilcoxon p<0.001), depleted after treatment. The relative abundance of *Collinsella* showed a modest median increase after treatment, despite its elevated presence at baseline. Looking at treatment response, there was no statistically significant difference in the change in *Collinsella* abundance between those who responded to biologics and those who did not (adjusted Wilcoxon p=0.33).

6 Discussion

Our study aimed to characterized fecal gut microbiota in patients with active axSpA compared to controls and to analyze their variation after 1 year of treatment with bDMARDs. We found that patients with axSpA treated with bDMARDs for one year shifted they gut microbiota to resemble more closely the controls with an increase in taxa from the *Lachnospiraceae* family, such as *Blautia, Roseburia* and *Fusicatenibacter*. *Collinsella* enrichment remained stable across time and treatment, suggesting it may be a disease biomarker.

In our previous work(31), were we characterized the shared and disease specific gut microbiota of patients with axSpA, IBD and anterior acute uveitis in a cross-sectional analysis, and compared them to controls. We observed a shared depletion of predominately *Lachnospiraceae* taxa, with the strongest differential abundance between patients and controls on *Blautia*, an anaerobic gram-positive bacteria inside the Clostridia class. Together with other members of the *Lacnospiraceae* family are producers of short chain fatty acid (SCFA), which are bacterial fermentation products of dietary fiber, mainly functioning as an energy source in the host intestine and as regulators of gene expression(32). Blautia is one of the most abundant gut genera and has been previously described to be depleted in inflammatory diseases(33, 34) such as rheumatoid arthritis, IBD and SpA with potential for dysregulated microbial carbohydrate metabolism in the gut. The enrichment of *Blautia* in the gut environment may have the potential to alleviate the inflammatory status by inducing the production of the immunosuppressive regulatory T cells – Tregs (35, 36).

In this study, we observed increased relative abundance of *Collinsella* in patients with axSpA compared with controls that persisted over time and treatment. Collinsella is a genus of anaerobic bacteria that belongs to the family *Coriobacteriaceae* inside the *Actinomycetota* phylum and is commonly found in the human gut. Previous research has shown that an enrichment of *Collinsella* is linked to the downregulation of tight junction proteins in enterocytes in vitro(37). This could potentially lead to increased gut

permeability, often referred to as “gut leakage”. In addition, elevated levels of *Collinsella* have been associated with excessive production of the pro-inflammatory cytokine IL-17A and the transcription factor NFkB1(37). In healthy individuals, IL-17A - along with other subtypes of IL-17 – plays a protective role, guarding the host against bacterial and fungal infections at both epithelial and mucosal barriers(38). However, in the context of SpA(39, 40), overactivation of IL-17A contributes to a chronic inflammatory environment. Specifically, increased IL-17a activity drives the expansion of Th17 cells, perpetuating a positive feedback loop that leads to sustained IL-17A production and exacerbates the inflammatory response. Therefore, the observed enrichment of Collinsella maintained through time and treatment may play a multi-faceted role in the pathogenesis of the disease, affecting probably both gut integrity and immune modulation.

The study has several limitations that should be considered when interpreting the results. The sample size is relatively small and all participants were recruited from a single center, which may limit the reproducibility or generalizability of the findings. The follow-up period of one year, where assessments were only done at baseline and year 1 could miss the dynamic changes that may occur at shorter intervals and do not control well for intra-individual variability. We did not control for variables such as nutrition or lifestyle, which can influence the microbiota composition, although we controlled the study for other medication such as metformin and antibiotics, which have previously shown to alter the microbiota composition. The 16S rRNA sequencing offers taxonomic insights, but lacks functional data, limiting our ability to understand how certain microbes may interact in the context of axSpA. That said, taxonomic profiles based on 16S rRNA sequencing remains still widely used in clinical studies and have shown to correlate well with whole metagenome sequencing. Another limitation is the lack of a control group receiving a placebo instead of biological treatment to isolate the effects of bDMARDs on the microbiota. Therefore further research needs to be performed, ideally with randomized clinical trials, to provide more comprehensive evidence.

7 Conclusions

In our study, patients with axSpA exhibited a shift in their gut microbiota following bDMARD treatment, which highlights the potential of microbial composition as both a therapeutic target and a marker of treatment efficacy. Particularly, the increase in abundance of several taxa of the Lachnospiraceae family, especially *Blautia*, known for its anti-inflammatory properties, suggests its role as a potential biomarker for effective biologic treatment and improved disease outcomes. On the other hand, the consistently relative enriched abundance of Collinsella, even after a long exposure to treatment, raises the possibility of it serving as a disease signature that remains present along the disease course or treatment. These findings should be further investigated, if possible, through metagenomic or metatranscriptomic analyses to identify functional pathways to help understanding the active interaction between microbial communities and the host. Moreover, microbial biomarkers may offer predictive or prognostic value into personalized treatment strategies, supporting the decision of which biological treatment would be the most suitable for each patient. Our study also raises the question whether modulating the gut microbiota, either through diet or pre/probiotics, could be used as a complementary therapy to the biological drugs in the treatment of axSpA. The current results open the door to a more detailed understanding of the pathogenesis of axSpA and their treatment outcomes, highlighting the need for further longitudinal studies to confirm the clinical relevance of these microbial shifts.

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9 Anexos

ANEXO IX

Título del Trabajo:

"Improvement Of The Gut Microbiota Balance In Patients With Axial Spondyloarthritis After One Year Of Biological Therapy"

"Mejora Del Equilibrio De La Microbiota Intestinal En Pacientes Con Espondiloartropatía Axial Después De Un Año De Tratamiento Biológico"

Este trabajo ha sido realizado en

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