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ORIGINAL RESEARCH

Characterisation of gut microbiota composition in patients with axial spondyloarthritis and its modulation by TNF inhibitor treatment

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ABSTRACT

Objective To assess whether gut microbiota composition is associated with patient characteristics and may have predictive value on the response to TNF inhibitor (TNFi) treatment in axial spondyloarthritis (AxSpA).

Methods The study involved 61 patients fulfilling the Assessment of SpondyloArthritis International Society classification criteria for AxSpA. All patients had active disease despite non-steroidal anti-inflammatory drugs intake and were eligible for treatment with a TNFi. At baseline, the mean Ankylosing Spondylitis Disease Activity Score was 2.9±1 and mean C reactive protein (CRP) level 9.7±11.4 mg/L. Bacterial 16S ribosomal RNA gene sequencing was performed on stool samples collected at baseline (month 0 (M0)) and 3 months after TNFi initiation (month 3 (M3)). Alpha and beta diversity metrics were calculated on the relative abundance of core operational taxonomic units (OTUs).

Results The HLA-B27 status affected at least in part the global composition of faecal microbiota at M0 as well as the abundance/prevalence of several anaerobic bacteria in the families *Oscillospiraceae*, *Lachnospiraceae* and *Bifidobacteriaceae*. In contrast, smoking affected the global composition of faecal microbiota at both M0 and M3. The prevalence/abundance of seven bacterial OTUs at M0 was associated with response to TNFi treatment. One of the candidates, present only in non-responders, is the genus *Sutterella*, and the other six candidates are in the class *Clostridia*.

Conclusions Several SpA patients' characteristics modulate the composition of gut microbiota as did TNFi treatment. Moreover, the abundance/prevalence of seven OTUs at baseline may be used as a novel non-invasive index that predicts the response to TNFi with greater accuracy than HLA-B27 status, CRP level and measures of disease activity.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Given that HLA-B27 regulates the diversity of the gut microbiota in rats, we sought to assess whether some specificities of its composition were associated with HLA-B27 status of axial spondyloarthritis (AxSpA) patients and may have a predictive value on the outcome of anti-TNF neutralising antibodies in AxSpA patients.

WHAT THIS STUDY ADDS

⇒ The impact of anti-TNF treatment on gut microbiota composition has been poorly studied. We show that TNFi treatment modulated the composition of intestinal microbiota of AxSpA patients. Moreover, the abundance/prevalence of several operational taxonomic units (OTUs) at baseline should predict the response to TNFi with greater accuracy than HLA-B27 status, C reactive protein level and measures of disease activity.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ We have shown that gut microbiota composition was associated with various characteristics of AxSpA patients, including disease duration, smoking, disease activity and HLA-B27 status. Using a random forest model, we identified seven specific OTUs that seem to accurately predict the response to TNFi treatment. One of those candidates, present only in non-responders, is in the genus *Sutterella*, which was previously found associated with non-response to faecal microbiota transplantation in ulcerative colitis. Further investigations are needed to validate these results and assess the potential of these bacterial taxa as a non-invasive index of treatment outcome in clinical settings.

INTRODUCTION

Spondyloarthritis (SpA) is characterised by axial involvement (sacroiliac joints and spine), peripheral joint damage and enthesitis, and is associated with extra-articular manifestations such as psoriasis, uveitis or, less frequently, inflammatory bowel disease (IBD).[1 2]

SpA is a complex condition, involving a genetic susceptibility background combined

with the contribution of environmental factors that need better understanding. Among them, bacterial infections have long been suspected to trigger the disease in genetically predisposed patients, as evidenced by some patients exhibiting reactive arthritis in response to a gut bacterial infection with specific enteropathogens.[3] Moreover, about 70% of SpA patients exhibit increased gut permeability and signs of intestinal inflammation.[4 5] This finding is consistent with increased prevalence of SpA among patients with IBD as compared with the general population. In addition, HLA-B27 and β2 microglobulin transgenic rat models feature a disease similar to human SpA but not in a germ-free environment, which suggests a key role for the microbiome.[6]

SpA patients commonly show alterations in gut microbiota composition.[7–11] However, the composition of the caecal flora from HLA-B27 or β2 microglobulin transgenic rats can differ from that of wild-type animals.[6] These findings raise the question of whether such a genetic background could indirectly predispose to SpA by controlling microbiota-dependent colonisation. Although a particular bacterial pathogen has not been reproducibly revealed in former studies, few reports suggest the presence of a gut dysbiosis in peripheral or axial SpA (AxSpA).[7–11]

The first-line treatment of peripheral and AxSpA manifestations is non-steroidal anti-inflammatory drugs (NSAIDs), but in the event of drug failure or severe structural damage, the treatment is based on several biological (b-) or targeted synthetic (ts-) disease-modifying antirheumatic drugs. In this context, the stratification of SpA patients to the most efficient therapy is of key importance, both for optimal clinical care and healthcare costs. The therapeutic choice is currently driven by clinical and biological parameters, such as sex (male), younger age (less than 45 years), smoking habits (no smoking), increased C reactive protein (CRP) level and positive HLA-B27 status, which are associated with better response to TNF inhibitor (TNFi) treatment for AxSpA.[12] Unfortunately, these parameters are poorly discriminant because they are also associated with disease diagnosis and/or disease activity. Therefore, there is an urgent medical need to develop biomarkers that are able to better guide treatment decisions for SpA.

Several lines of evidence have underlined the relative importance of the gut microbiome in the therapeutic management of SpA and SpA-related conditions. Some biomarkers derived from the gut microbiota that should be able to predict response to TNFi treatment have been discovered.[13–15] Given that the intestinal flora may contribute to the development of a competent immune system, the present work aimed to assess whether SpA patients' characteristics can impact the composition of gut microbiota and to what extent TNFi modulate gut microbiota composition over a 3-month treatment period.

METHODS

Study participants

In total, 62 patients with axSpA were recruited to assess whether gut microbiota composition was associated with patient characteristics at baseline and may have a predictive value on the response to TNFi.

Eligible participants were from 21 to 74 years and were eligible for a TNFi because of lack of response to NSAIDs and/or severe disease course. All patients were naïve of any biologics and fulfilled the Assessment of SpondyloArthritis International Society classification criteria for AxSpA.[16] Written informed consent was obtained from each participant.

Faecal samples were collected at baseline (M0) and after 3 months of TNFi treatment (M3). We specifically chose patients without IBD to ensure that any effect observed in this study was specific to AxSpA. Numerous clinical parameters were recorded (table 1) and their interactions tested (online supplemental table 1).

Sample preparation and 16S rRNA sequencing of faecal microbiota

Stool sampling was performed at enrolment (M0) and after 3 months of TNFi treatment (M3). Methods for 16S rRNA sequencing and bioinformatics processing are in online supplemental material.

Statistical analysis

Statistical analysis involved using R V.3.5.1. Briefly, alpha and beta diversity were calculated and tested with the packages vegan V.2.5–2 and stats V.3.5.1 on a core microbiota (697 rational taxonomic units (OTUs) representing >95% of the microbiota of each sample). To identify biomarkers, core OTU abundance and prevalence were compared with experimental parameters with Kruskall-Wallis, Spearman correlation or χ^2 test depending on the data type, by using Eta, Spearman's correlation coefficient r and Cramer's V as effect size estimations. All p values were adjusted for multiple testing by the false discovery rate. To explore the predictive value of the biomarkers, a random forest algorithm was used (see online supplemental material for detailed method).

RESULTS

Patient characteristics and response to treatment

Demographic and disease characteristics of patients were collected at inclusion (table 1). Patients mainly had axial disease (98%), and 79% carried the HLA-B27 allele. Most were males (77%) and 43% were current smokers. All included patients had active disease at baseline with increased CRP level and/or inflammatory lesions on a recent MRI of sacroiliac joints. Despite NSAIDs intake, all enrolled patients had active disease, as defined by measures appropriate for clinical use referred to as the Ankylosing Spondylitis Disease Activity Score (ASDAS)[17] and were eligible for a TNFi. In this cohort, 42.6% of patients showed failure to respond to the TNFi according to delta-ASDAS (table 1).

Table 1 Characteristics of the patients with axial spondyloarthritis included in the study

Clinical parameters	SpA patients (n=61)
Age, years (mean±SD)	40±13.3
Male, n (%)	47 (77)
Smoking (current smokers), n (%)	26 (43)
Disease duration, years (mean±SD)	6.4±9.9
Axial disease, n (%)	59 (97)
Peripheral disease, n (%)	9 (15)
Enthesitis, n (%)	29 (47.5)
Recurrent increased CRP (linked to the disease); n (%)	33 (54)
Uveitis, n (%)	18 (29.5)
IBD, n (%)	0 (0)
Psoriasis, n (%)	8 (13)
Familial SpA or SpA related condition, n (%)	28 (46)
Biologic variables	
HLA B27-positive, n (%)	48 (79)
Radiologic variables, n (%)	
mNY-SI-positive	38 (62)
MRI-SI-positive*	52 (85)
Current treatments, n (%)	
NSAIDs	55 (90)
Steroids	1 (1,6)
DMARDs	9 (15)†
Anti-TNF received during the study, n (%)	
Ada/Gol	8 (13)/14 (23)
Eta	39 (64)
Disease activity at M0, mean±SD	
ASDAS-CRP	2.9±1
CRP (mean±SD)	11.7±18.2
Disease activity at M3, mean±SD	
ASDAS-CRP	1.5±1
CRP (mean±SD)	2.7±5.6
Response to treatment, n (%)	
ASDAS (R/PR/NR)	10 (16)/25 (41)/26 (43)

*Four patients with unavailable MRI data.
†Methotrexate n=4; sulfasalazine n=5.
Ada, adalimumab; ASDAS, Ankylosing Spondylitis Disease Activity Score; CRP, C reactive protein; DMARDs, disease-modifying anti-rheumatic drugs; eta, etanercept; goli, golimumab; IBD, inflammatory bowel disease; mNY, modified New-York criteria; NR, non-response; NSAIDs, non-steroidal anti-inflammatory drugs; PR, partial response; R, response; SI, sacroilitis; SpA, spondyloarthritis.

Bacterial ecology at baseline (M0) and after treatment (M3)

To understand how a TNFi can modulate the intestinal microbiota in AxSpA, we used 16S rRNA sequencing of faecal samples taken at baseline (M0) and after 3 months of TNFi treatment (M3). Alpha and beta diversity analyses were performed on a core microbiome, composed of the 97% identity OTUs representing >95% of each community. We found no significant association between alpha diversity indices and any patient parameter in our cohort (table 2) but significant associations between Bray-Curtis beta diversity indices and HLA-B27 (after correcting for library and smoking) and smoking at baseline and M3, respectively (table 3). Notably, the type of TNFi used (Ada/Gol vs Eta) did not seem to influence the intestinal microbiota diversity (table 2) or composition (table 3). To investigate the relation between intestinal microbiota and patient characteristics in more detail, we used a biomarker analysis to identify individual OTUs correlated with various parameters at M0 (online supplemental table 2) and M3 (online supplemental table 3).

Correlation between faecal bacterial biomarkers and disease parameters at M0

The presence/absence of four OTUs was correlated with disease duration (figure 1). One of the OTUs are in the genus *Bifidobacterium*, and two in the family *Oscillospiraceae* showed a positive correlation, as their abundance/prevalence increased with disease duration. By contrast, the last OTU, Otu0048, is in the family *Lachnospiraceae*, and its abundance/prevalence decreased with disease duration. The abundance of this OTU was also negatively associated with uveitis and sacroiliitis on X-rays according to New York criteria (figure 2, online supplemental table 2). The abundance/prevalence of two additional OTUs was correlated negatively with sacroiliitis: one OTU is in the genus *Alistipes* and one in the *Oscillospiraceae* family (figure 2, online supplemental table 2). In addition, the abundance of several OTUs, most in the *Lachnospiraceae* and *Oscillospiraceae* families, was correlated with patient parameters such as age, sex, treatment and familial aggregation of the disease (online supplemental table 2).

Correlation of faecal bacterial biomarkers with smoking habits

The abundance/prevalence of five OTUs was correlated at baseline with smoking (figure 3, online supplemental table 2). Three OTUs are in the *Christensenellaceae* family and were less abundant in smokers than non-smokers. The abundance/prevalence of one OTU in the *Rikenellaceae* family followed the same pattern. By contrast, the last one, in the *Oscillospiraceae* family, was more abundant among smokers than non-smokers. The abundance of most of those OTUs was largely unaffected by the treatment and still significantly associated with smoking after 3 months of a TNFi (figure 3, online supplemental table 3).

Table 2 Pairwise association between alpha diversity indexes and patient and sample parameters

			Smoke	Gender	B27	Age	Disease duration	Family	Psoriasis	Uveitis	Radio	NSAIDs	AntiTNF	DMARDs	Library	CRP	ASDAS
M0	Chao	Effect size	9.4	1.0	7.1	9.3	−4.5	7.8	0.8	2.0	0.1	0.6	1.0	1.3	0.7	5.1	−5.1
		P value	0.2262	0.7817	0.2262	0.7817	0.7817	0.2262	0.7817	0.7817	0.7817	0.7817	0.7817	0.7817	0.7817	0.7817	0.7817
	invSimpson	Effect size	0.8	0.9	11.6	8.8	−10.8	0.7	0.9	4.3	1.0	1.4	6.7	2.5	2.3	−6.8	−18.9
		P value	0.6288	0.6288	0.1409	0.6288	0.6288	0.6288	0.6288	0.6288	0.6288	0.6288	0.3834	0.6288	0.6288	0.6365	0.6288
M3	Chao	Effect size	9.5	2.0	6.0	7.9	−5.1	14.3	0.9	1.0	0.0	0.5	0.0	0.9		−3.8	−16.8
		P value	0.1370	0.7717	0.3111	0.9390	0.9390	0.0543	0.9390	0.9390	0.9638	0.9390	0.9760	0.9390		0.9390	0.6698
	invSimpson	Effect size	0.4	1.1	0.5	−1.7	−2.1	2.3	0.4	0.0	1.0	1.3	0.6	4.0		2.5	−24.3
		P value	0.8243	0.8128	0.8243	0.9503	0.9503	0.8019	0.8243	1.0000	0.8128	0.8128	0.8243	0.8128		0.9503	0.8019

Chao index was used as a measure of diversity and invSimpson index as a measure of evenness. For comparisons between a numerical and a categorical variable, Kruskal-Wallis test was used with Eta for effect size; for comparisons between two numerical variables, Spearman correlation was used. P values were adjusted for multiple testing using the false discovery rate.
ASDAS, Ankylosing Spondylitis Disease Activity Score; CRP, C reactive protein; DMARDs, disease-modifying anti-rheumatic drugs; NSAIDs, non-steroidal anti-inflammatory drugs.

Table 3 Pairwise association between beta diversity indexes and patient and sample parameters, taking into account library, smoke and HLA-B27 status

			Library	Smoke	B27	Gender	Age	Disease duration	Family	Psoriasis	Uveitis	Radio	NSAIDs	AntiTNF	DMARDs	CRP	ASDAS
M0	Bray	Effect size	**3.8**	2.5	**3.2**	1.4	1.5	2.3	1.5	1.2	1.6	1.7	1.5	1.2	3.1	1.4	1.6
		P value	0.0198	0.2598	0.0387	0.7401	0.7400	0.2820	0.7401	0.8020	0.7400	0.7400	0.7400	0.8020	0.7400	0.7401	0.7400
	Jaccard	Effect size	3.3	2.9	2.8	2.3	1.4	1.7	2.0	1.4	1.9	1.5	1.3	1.4	3.0	1.5	1.3
		P value	0.0738	0.0873	0.0984	0.2079	0.8827	0.8713	0.4749	0.8827	0.4749	0.8827	0.8827	0.8827	0.8827	0.8827	0.8827
M3	Bray	Effect size		**3.8**	3.0	1.7	1.4	2.1	1.5	1.0	1.6	2.0	2.2	0.8	2.4	1.2	2.6
		P value		0.0306	0.1130	0.5987	0.7868	0.3653	0.7868	0.9719	0.6299	0.3653	0.3427	0.9833	0.9719	0.9719	0.1934
	Jaccard	Effect size		3.0	2.4	2.2	1.5	1.8	1.8	1.3	1.9	1.6	1.7	1.4	2.8	1.4	1.9
		P value		0.1275	0.3944	0.4006	0.8508	0.5404	0.5404	0.8508	0.5404	0.6918	0.5404	0.8508	0.8508	0.8508	0.5404

Bray-Curtis and Jaccard distances were used to compare composition and prevalence respectively. Adonis test from package Vegan was used to test correlations. P values were adjusted for multiple testing using the false discovery rate.
Bold correspond to significant parameters ($p<0.05$).
ASDAS, Ankylosing Spondylitis Disease Activity Score; CRP, C reactive protein; DMARDs, disease-modifying anti-rheumatic drugs; NSAIDs, non-steroidal anti-inflammatory drugs.

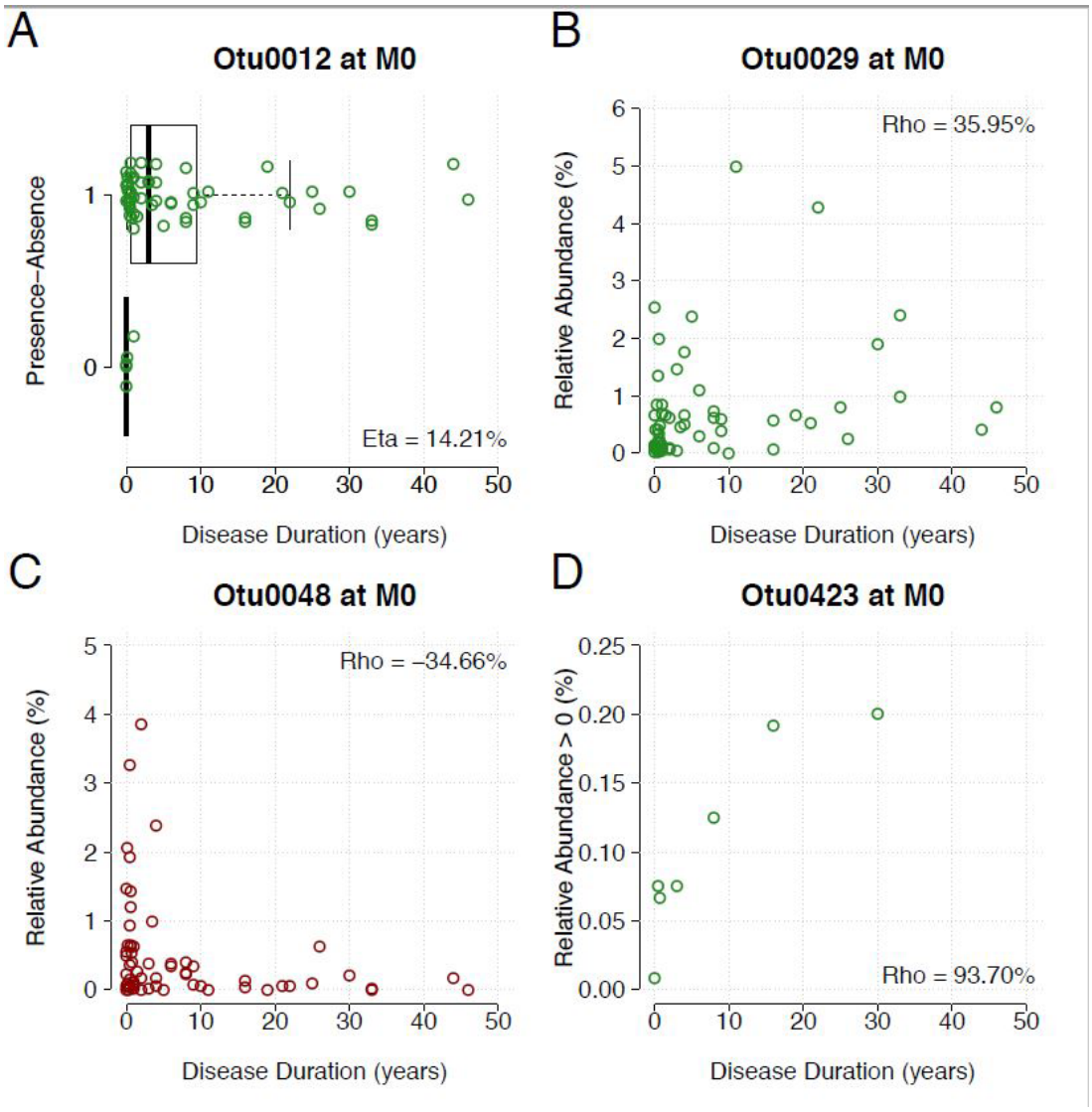

Figure 1 Indicator taxa for disease duration at baseline (month 0 (M0)). Effect size corresponding to the significant statistical test is shown on the graph.

Correlation of faecal bacterial biomarkers with HLA-B27 status at baseline

In line with the suspected effect of HLA-B27 status on the composition of the intestinal microbiota, the abundance/prevalence of five OTUs was correlated with HLA-B27 status at baseline (figure 4, online supplemental table 2). One OTU, belonging to *Bifidobacterium*, was more prevalent in HLA-B27-positive than HLA-B27-negative patients, and the remaining OTUs were more abundant/prevalent in HLA-B27-negative than HLA-B27-positive patients and were in three families: *Christensenellaceae*, *Oscillospiraceae*, *Anaerovoracaceae*. The abundance of none of these OTUs remained significantly associated with HLA-27 status at M3 (figure 4, online supplemental table 3).

Correlation of patients' microbiota with disease activity and response to TNFi

In addition to the abundance of the aforementioned bacterial biomarkers correlating with parameters known to influence AxSpA (HLA-B27 status, smoking, etc), the abundance/prevalence of four OTUs was directly correlated with disease activity as estimated by the ASDAS score at M0 (figure 5, online supplemental table 2); most are in *Lachnospiraceae* and *Oscillospiraceae* families.

We next tested each parameter for an association with response to treatment (online supplemental table 4). However, only ASDAS at M0 was significantly associated with treatment efficacy evaluated by ASDAS criteria, although both HLA-B27 status and smoking are known to influence response to a TNFi. However, the prevalence of two OTUs was associated with the response to treatment at baseline (online supplemental table 2). Otu0112, in the genus *Sutterella*, was associated with non-response according to the ASDAS criteria (figure 6A, online supplemental table 2). The abundance/prevalence of Otu0112 barely changed at M3 and was still significantly associated with the NR status at that time (figure 6B, online supplemental table 3).

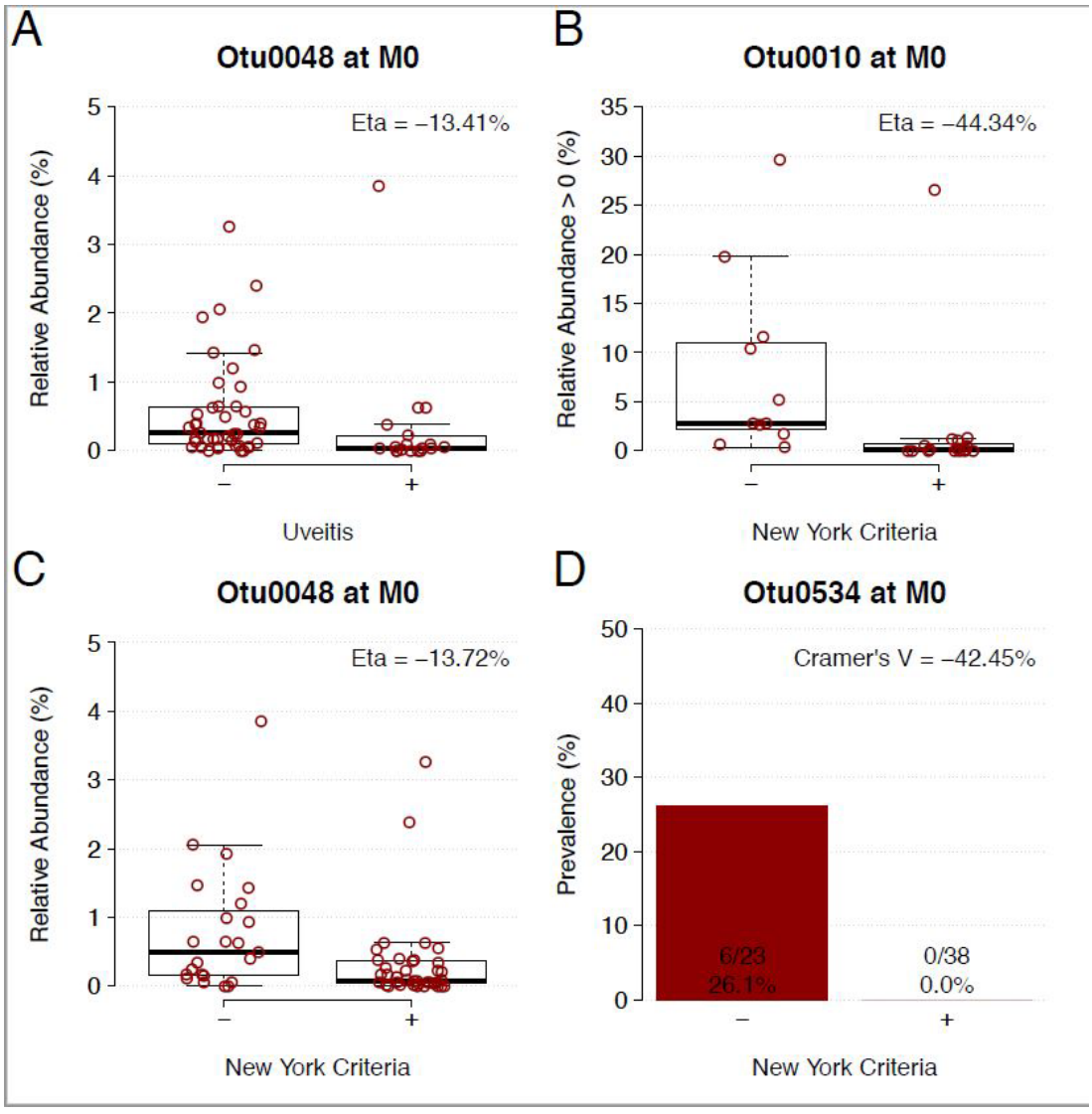

Figure 2 Indicator taxa for New York criteria and uveitis at baseline (M0). Effect size corresponding to the significant statistical test is shown on the graph.

To fully evaluate the predictive value of these OTUs as biomarkers of TNFi efficacy, we used a random forest analysis, implementing 500 iterations to gain confidence in the predictions (see Methods, online supplemental figure 3).

First, patient parameters and individual OTUs were evaluated separately for their ability to predict the response to treatment according to the ASDAS criteria. The median area under the receiver operating characteristic curve (AUC) reached values above 0.6 and 0.8, respectively, when using all variables in the models (figure 6C, 'all'). These AUC values increased slightly when using only the subset of the variables identified as important predictors in the full model (figure 6C, 'sub'). When combining the subset of patient parameters and OTUs identified as important, the median AUC further increased (figure 6C, 'combined'), which suggests that both types of variables play a role in explaining the response to the treatment, although microbial OTUs seem to play a greater role than patient parameters.

Consistently, when looking at the best subset and combined models of the 500 iterations (figure 6D), the model based on patient parameters performed rather poorly, with AUC 0.694, specificity 41.7% (10/24 true negatives) and sensitivity 82.9% (29/35 true positives), yielding many false positives. The model based on OTUs performed much better, with AUC 0.937, specificity 61.5% (16/26) and sensitivity 94.3% (33/35). Although the combined model did not improve the AUC much as compared with the OTU model (0.940 vs 0.937), it improved the specificity and sensitivity substantially, to 97.1% (34/35) and 75% (18/24), respectively, yielding very good confidence in the predictions.

Next, we selected OTUs that were found important in >90% of the models, which yielded seven good candidate predictors for response to treatment (figure 6E,F). Importantly, one of those candidates was Otu0112, already identified as a predictor in our biomarker analysis. Otu0112 was the only candidate to predict the response entirely based on its prevalence. In contrast,

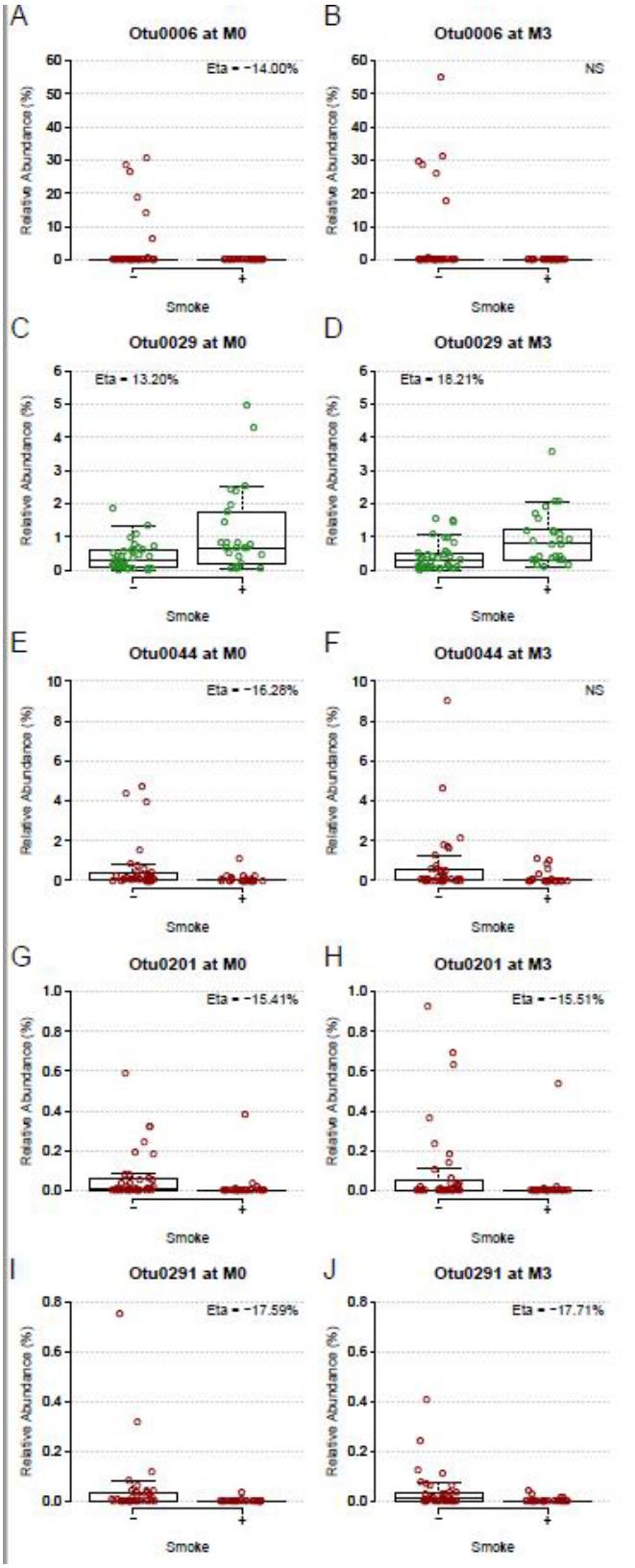

Figure 3 Indicator taxa for smoking status at baseline (M0) and after treatment (month 3 (M3)). Effect size corresponding to the significant statistical test is shown on the graph.

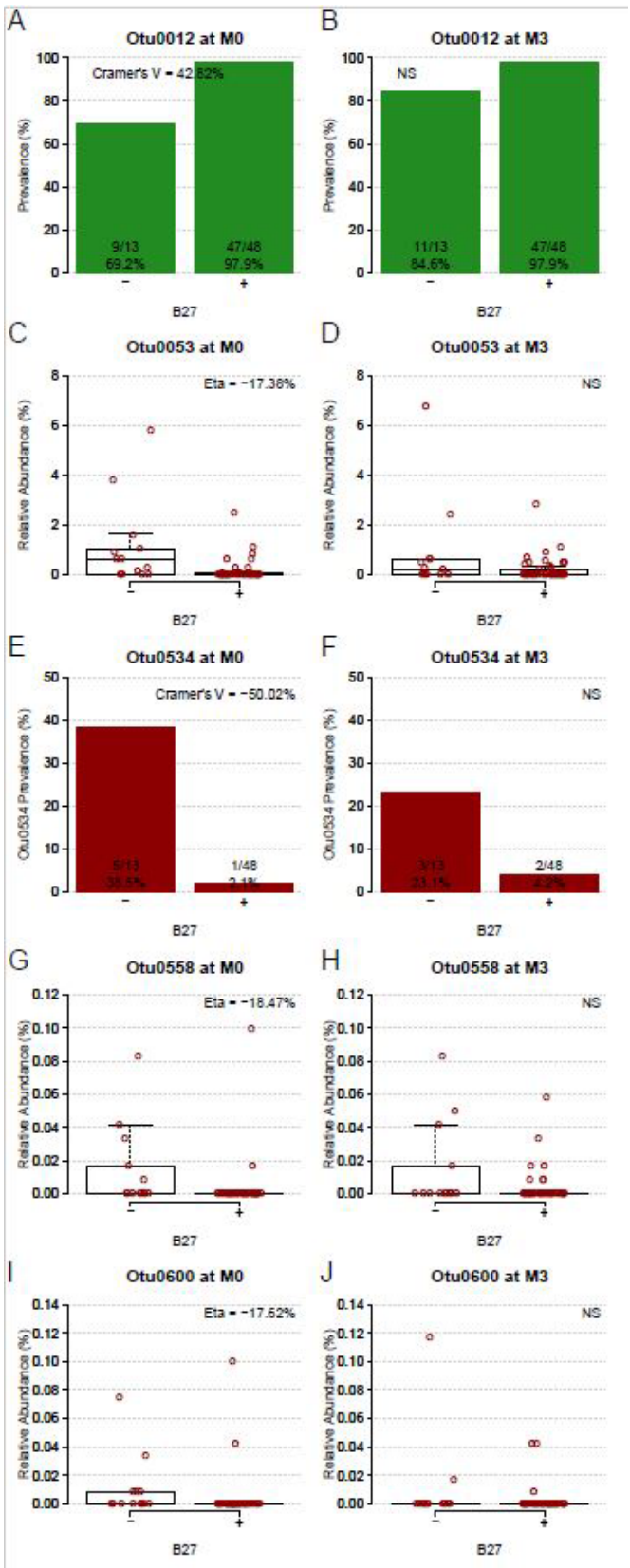

Figure 4 Indicator taxa for HLA-B27 status at baseline (M0) and after treatment (M3). Effect size corresponding to the significant statistical test is shown on the graph.

the remaining OTUs, in the class *Clostridia*, were present both in responders and non-responders but varied in their relative abundance.

Finally, the ranking of the explanatory variables in the best combined model showed that three OTUs were the strongest predictors across all parameters, followed by

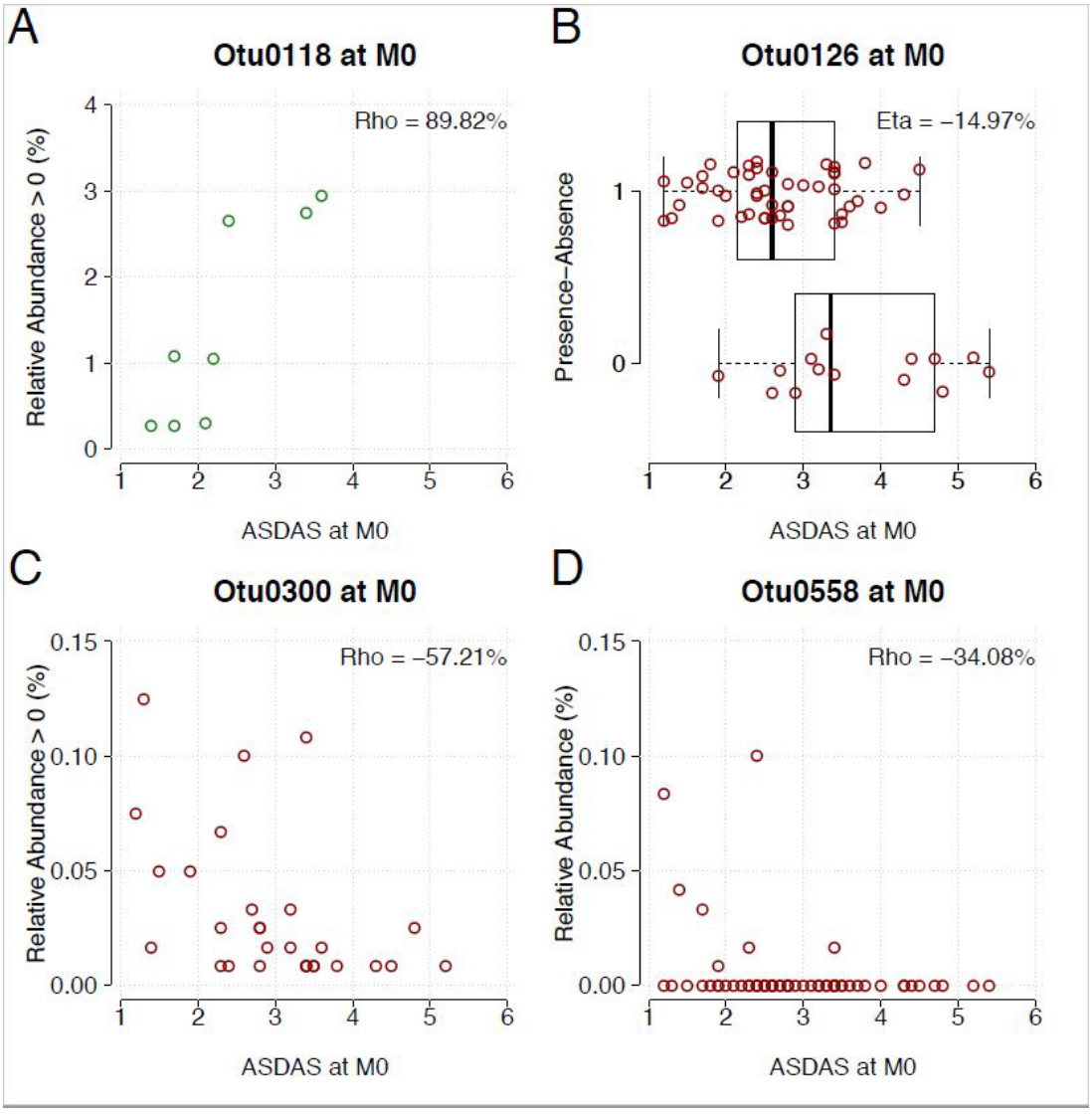

Figure 5 Indicator taxa for Ankylosing Spondylitis Disease Activity Score (ASDAS) at baseline (M0). Effect size corresponding to the significant statistical test is shown on the graph.

the ASDAS score at M0 (table 4). This finding further confirmed that OTUs were better predictors of the response to treatment than patient parameters in our cohort. Moreover, 80% (8/10) of the OTUs appearing in the best model are in the *Clostridia* class and 40% (4/10) the *Lachnospiraceae* family, but represent 71% (495/697) and 19% (132/697) of the core OTUs, respectively, which suggests an overrepresentation of these taxa among candidate predictors.

DISCUSSION

This study showed that baseline gut microbiota was globally affected by HLA-B27 status of AsSpA patients, whereas smoking was the only remaining contributor to microbiota composition at M3, thus indicating a change in microbiota composition during the 3 month treatment period. Moreover, using a random forest model, we identified seven specific OTUs that seem to accurately predict the response to treatment.

Over the past decades, high-resolution association mapping has identified a number of novel genes underlying the heritability of microbiota composition, including the HLA-B27 gene.[18] In line with these findings, we found that HLA-B27 status affected the abundance of several anaerobic bacteria belonging to the class *Clostridia*. In particular, the abundance of one OTU belonging to the *Christensenellaceae* family was decreased in HLA-B27-positive versus HLA-B27-negative patients. This specific taxon was previously reported to have high heritability.[19] Hence, some of this heritability could be related in part to HLA-B27 status. In addition, *Erap-1*-deficient mice, which exhibit spinal ankylosis and inflammation, were found deficient in *Christensenellaceae*.[20] Therefore, this bacterial genus could play a role in the skeletal phenotype observed in those mice, and by extension, *Christensenellaceae* could participate in the axial inflammatory phenotype of AxSpA patients. These results imply that HLA-B27 may affect the colonisation of

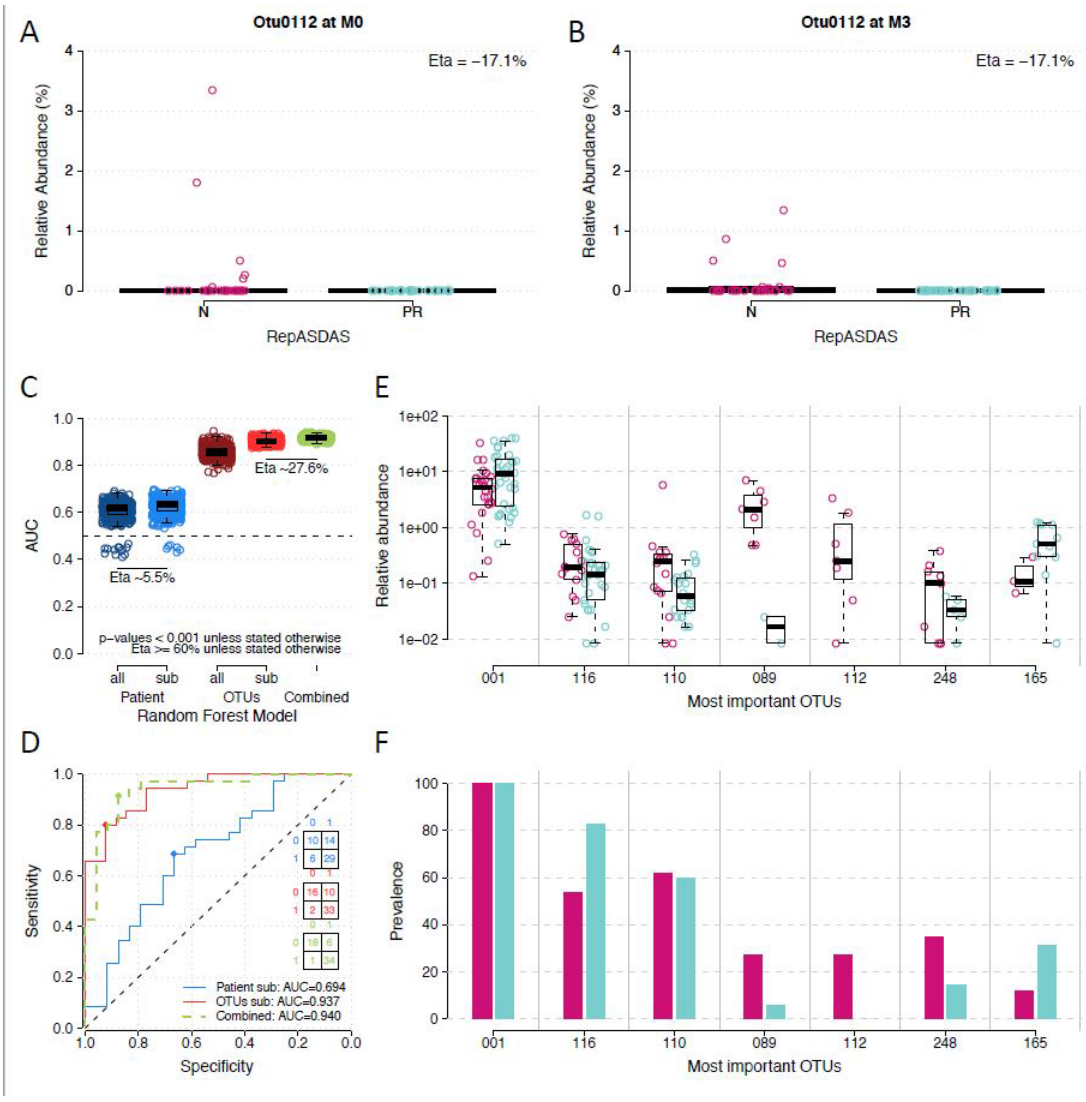

Figure 6 Microbial composition predicts response to treatment (ASDAS). (A, B) Indicator taxa for responder status (ASDAS) at baseline (M0, A) and after treatment (M3, B). Effect size corresponding to the significant statistical test is shown on the graph. (C–F) Random forest models explaining ASDAS response with patient parameters and operational taxonomic units (OTUs) at baseline. (C) Area under the receiver operating characteristic (ROC) curve (AUC) for 500 iterations of the random forest models, with only patient parameters, only OTUs or their combination. Model using all available parameters are 'all', and models containing only the important parameters identified in the full models are 'sub'. (D) ROC curve of the best models and their corresponding confusion matrices, with true state in rows and predicted state in columns. (E) Relative abundance of the most important OTUs identified in the random forest models. (F) Prevalence of the most important OTUs identified in the random forest models. ASDAS, Ankylosing Spondylitis Disease Activity Score; OTUs, operational taxonomic units; PR, partial response.

the intestinal tract with some of those bacteria and therefore indirectly participate in axial inflammation.

Of equal importance, we observed a significant decrease in the abundance and/or prevalence of three OTUs belonging to the *Christensenellaceae* family in smokers versus non-smokers, both at baseline and after 3 months of a TNFi. This finding is consistent with reports showing that nicotine alters the gut microbiome.[21] Therefore, smoking could participate in axial inflammation by a decrease in abundance of *Christensenellaceae* and consequently participate in disease severity and/or poor response to a TNFi.

Higher disease activity at baseline or after 3 months was found correlated with the abundance/prevalence of several OTUs belonging to *Oscillospiraceae* and *Lachnospiraceae* families, both in the *Clostridia* class. Bacteria from these families have been reported to participate in the degradation of the mucin protein backbone.[22] The consequence could be increased encroachment of bacteria into the inner mucus layer, gut barrier damage, increased gut permeability and participation in systemic inflammation. Translocation of intestinal microbes, caused by the increased permeability of the intestinal epithelial cell layer, or increased exposure to microbial products are two possible mechanisms connecting gut dysbiosis and joint pathology.[23]

Among the seven OTUs associated with response to treatment, half (n=4) belong to *Lachnospiraceae*, which are

Table 4 Ranking of the important parameters for the ASDAS response at M0

RepASDAS		Phylum	Class	Order	Family	Genus
Otu0089*	0.02604	Firmicutes	Clostridia	uncl. Clostridia	uncl. Clostridia	uncl. Clostridia
Otu0116*	0.02603	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	uncl. Lachnospiraceae
Otu0112*	0.02210	**Proteobacteria**	**Gammaproteobacteria**	**Burkholderiales**	**Sutterellaceae**	**Sutterella**
ASDAS	0.02040	--	--	--	--	--
Otu0110*	0.01537	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium sensu stricto 1
Otu0001*	0.01256	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	uncl. Lachnospiraceae
Otu0248*	0.00955	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Frisingicoccus
Otu0095	0.00897	Firmicutes	Clostridia	uncl. Clostridia	uncl. Clostridia	uncl. Clostridia
Otu0165*	0.00743	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	uncl. Lachnospiraceae
Otu0033	0.00606	Bacteroidota	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes
B27	0.00175	--	--	--	--	--
Uveitis	0.00168	--	--	--	--	--
Gender	0.00105	--	--	--	--	--
AntiTNF	0.00053	--	--	--	--	--
Smoke	−0.00011	--	--	--	--	--
Otu0225	−0.00025	Firmicutes	Clostridia	uncl. Clostridia	uncl. Clostridia	uncl. Clostridia
BASDAI	−0.00086	--	--	--	--	--
CRP	−0.00095	--	--	--	--	--
Age	−0.00175	--	--	--	--	--
Family	−0.00179	--	--	--	--	--

Mean decrease Gini of parameters used in the combined random forest in the optimal model. Stars denote the operational taxonomic units (OTUs) that were found important in >90% of the iterations. Detailed taxonomy is provided for each OTU.
ASDAS, Ankylosing Spondylitis Disease Activity Score ; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C reactive protein.

able to degrade complex polysaccharides into short-chain fatty acids (SCFAs). Thus, SCFAs could be conventionally used in combination with other disease parameters for assessing treatment efficacy in the clinic.

The abundance/prevalence of one OTU (Otu0112) belonging to the genus *Sutterella* was associated with non-response to treatment and seemed to be the most discriminating bacterial biomarker given that it was exclusively present in non-responders. Specifically, the presence of Otu0112 was detected in 27% of non-responders at baseline (M0) and post-treatment (M3) and was completely absent in responders at M0 and M3. It is the only OTU among the seven identified by the random forest analysis that is discriminatory in terms of presence/absence. From our results, the presence of the genus *Sutterella* (Otu0112) may be associated with failure of the treatment in almost one third of non-responders. This finding is reminiscent of what is observed in ulcerative colitis,[24] thus suggesting that some non-responders experience impaired gut barrier function. Hiippala *et al* showed *Sutterella* spp abundant in the duodenum of healthy adults, with a decreasing gradient toward the colon.[25] The ability of *Sutterella* spp to adhere to intestinal epithelial cells indicates that they may have an immunomodulatory role.[25] Moon *et al* observed a phenotype in a subset of their mice characterised by low faecal IgA levels, which was attributed to high levels of *Sutterella* within the gut microbiome.[26] Thus, rather than directly inducing inflammation, *Sutterella* could impair the functionality of the intestinal antibacterial immune response, particularly in its capacity to limit intracellular bacterial species,[24 27] including pathobionts such as *Fusobacterium* species.[27] Low levels of *Sutterella* within the gut microbiome are associated with gut immune homoeostasis and high level of IgA. High IgA level protects against bacterial invasion of epithelial cells by opportunistic pathobionts. Enrichment of *Sutterella* leads to degradation of IgA and consequently lower levels of IgA in the gut mucosa, a microenvironment promoting pathobiont invasion of epithelial cells and intracellular survival.[24] Overall, *Sutterella* appear to be prevalent commensals with intraepithelial cell adhesion properties, mild proinflammatory capabilities and immunomodulatory functions in the human gastrointestinal tract. Considering this evidence as well as our observations in this cohort, *Sutterella* may be a promising taxon for the development of new predictive biomarkers for specific use in the clinic.

In this study, we did not compare the composition of the baseline gut microbiota with that of healthy subjects in order to focus on the patient-specific characteristics that lead to success or failure of treatment. We excluded patients with an associated IBD so that the results obtained were specific to SpA without being influenced by IBD-specific dysbiosis. We did not take into account differing diets of patients. Indeed, the high inter-individual variability of the microbiota[28] that depends on dietary habits, age, sex, genetic background requires a large sample size to be properly exploited, so such comparisons are hazardous in a population of a few dozen participants. Therefore, we looked at the characteristics of the patients associated with the composition of the gut microbiota at baseline and after 3 months of TNFi treatment. This approach has been poorly used to date in SpA[13] and is robust because it limits many biases in that such analyses over a short period assumes the stability of environmental factors and diet, all in an identical genetic context for a given patient.

Despite those limitations, we found several SpA patient characteristics that modulated the composition of gut microbiota as did TNFi treatment. In addition, the abundance/prevalence of seven OTUs at baseline were able to predict the outcome of TNFi with greater accuracy than HLA-B27 status, CRP level and measures of disease activity.

Although replication studies with larger sample sizes are needed, our study found predictive bacterial biomarkers for the response to treatment that could open the way to new treatment strategies for AxSpA: targeting individual bacteria by reducing their abundance with specific antibiotics or increasing their abundance with prebiotics or probiotics, depending on their role in maintaining or establishing SpA. In addition, these bacterial biomarkers, if used as a diagnostic tool before treatment, would allow for the stratification of patients to provide them with a personalised therapeutic approach likely to be efficient, inexpensive and above all, responding to the specificity of each patient.

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Contributors CM-R, MD and MC designed the study, analyzed data, interpreted results and wrote the manuscript; SF performed experiments; MV, BS, EL, VM, CC, J-FD, MC, and CM-R performed data analysis; CM-R and MD had overall medical oversight, provided patient samples and clinical data, and performed clinical data analysis; all authors revised and approved the manuscript. Guarantor: CM-R.

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accession numbers SAMN26680282-SAMN26680499. Scripts and additional data used for bioinformatics analyses are accessible via Figshare: https://doi.org/10.6084/m9.figshare.18515264, https://doi.org/10.6084/m9.figshare.18515267, https://doi.org/10.6084/m9.figshare.18515276 and https://doi.org/10.6084/m9.figshare.18515279.

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