WORKING TITLE

Changes in inflammation-associated plasma proteins in patients with peripheral psoriatic arthritis treated with faecal microbiota transplantation: exploratory findings from the FLORA trial

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Introduction

BACKGROUND

Psoriatic arthritis (PsA) is a chronic, systemic, seronegative, immune-mediated disease of unknown aetiology belonging to the spectrum of spondyloarthropathies (SpA).[1] Patients with PsA often present with diverse disease manifestations including peripheral arthritis, enthesitis, dactylitis, sacroiliitis, and axial inflammation in addition to skin and nail psoriasis.[2] The mechanisms underlying the pathogenesis of PsA are complex involving genetics, immunological, and environmental factors. Even though infections and (micro)trauma have been proposed as exciting events,[3] no common trigger(s) underlying the dysregulated immunological cascade have been definitely implicated.[1]

PsA is mainly driven by the effector arm of the immune system responsible for the production of interleukin (IL)-17 and related cytokines. When immune activation has been provoked, pro-inflammatory mediators activate resident cells such as fibroblasts, chondrocytes, and osteoblasts, which in turn secrete more pro-inflammatory mediators, including tumor necrosis factor (TNF)-α, IL-1, IL-6, IL-12, IL-15, IL-18, interferon-γ and activate the IL-17/IL-23 axis, that further recruit immune cells into the joints, thereby creating a self-perpetuating inflammatory response.[1] A cross-sectional study evaluating more than 951 unique serum proteins found large proteomic disturbances in patients with PsA as compared with healthy controls and revealed that the strongest proteomic changes occurred in novel proteins not yet linked to the pathogenesis of PsA.[4] Interestingly, expression of ICAM-1 and CCL18 had the most significant correlation to joint disease activity. Moreover, a recent study identified a serum protein biomarker panel that separated patients with early PsA from those with rheumatoid arthritis.[5] More studies investigating the proteomic signature of the systemic inflammatory response and dynamics associated with fluctuation of disease activity in psoriatic arthritis are highly needed.[6,7]

For a century, the link between enteric infections and reactive arthritis[8] has motivated investigation into the proposed gut–joint axis implicating intestinal microorganisms in the aetiology of immune-mediated arthritic disease.[9] In recent years, this theory has gained renewed interest due to accumulating evidence of disease-related imbalance (dysbiosis) in the composition and function of the intestinal microbiota in chronic disorders.[10-12] Among these, PsA has been associated with decreased intestinal bacterial diversity displaying both disease specific patterns[13] and microbial abnormalities similar to those seen in other subtypes of spondyloarthritis, rheumatoid arthritis, and inflammatory bowel disease (IBD).[14] These findings have encouraged research into the host-microbiota interplay in the dysregulated immunological cascade underlying immune-mediated arthritis and the prospects of microbiota-targeted therapies.[15] Interestingly, in a small cohort of children with juvenile idiopathic arthritis, exclusive enteral nutrition for three to eight weeks seemed to have a systemic anti-inflammatory effect and resulted in a significant decrease in MMP-1, MCP-4, and 4E-BP1.[16]

Faecal microbiota transplantation (FMT) is currently considered the most efficient method to restore diversity of the gastrointestinal microbiota.[17,18] Indeed, the transfer of faeces containing minimally manipulated communities of microorganisms from a donor to a recipient has revolutionised the treatment of *Clostridioides difficile* infection.[19] FMT has also proven able to induce beneficial responses in patients with inflammatory bowel disease thereby demonstrating local therapeutic immune-modulating abilities.[20] However, the systemic immunological response following FMT has yet to be characterised in man.[21]

RATIONALE

In 2020, our research group completed the first-in-man randomised controlled trial in patients with chronic arthritis (the FLORA trial) evaluating efficacy and safety of FMT in patients with PsA.[22] The primary efficacy endpoint was the proportion of participants experiencing treatment failure (i.e., needing treatment intensification) through 26 weeks.[23] The clinical results indicated that FMT in patients with active PsA can affect disease activity. In addition, we observed that in line with previous FMT trials in patients with ulcerative colitis[24] some donations may have more immune-modulating effects than others (unpublished data). Consequently, in-depth mechanistic insight needs to be undertaken to reveal the inflammatory disease mechanisms underlying PsA and the subsequent inflammatory response following FMT. Taken together, research into changes in systemic inflammatory markers following FMT is highly needed to determine the FMT immune-modulating mode of action, if any, in patients with systemic inflammatory arthritis.[21]

AIM

1. To explore baseline differences in inflammation-associated plasma protein levels between healthy individuals (blood donors), healthy stool donors, and patients with peripheral PsA treated with methotrexate.
2. To look for associations between inflammation-associated plasma protein levels and a) clinical measures of PsA disease activity, and b) treatment outcome.
3. To explore differences in changes/dynamics of inflammation-associated plasma proteins from baseline to week 4, week 12, and week 26 between FMT-treated patients with PsA and sham-treated patients with PsA.
4. To investigate the impact of FMT and biological treatment (for at least four weeks) on levels of each inflammation-associated plasma protein from baseline to week 26.

HYPOTHESES

Ia. There are no differences in levels of inflammation-associated plasma proteins between healthy individuals (blood donors) and stool donors.

Ib. There are differences in levels of inflammation-associated plasma proteins between healthy individuals (age and sex-matched) and patients with peripherial PsA treated with MTX

II. Distinct combinations of inflammation-associated plasma proteins are associated with the following baseline clinical measures of PsA (a-d) and treatment outcome at week 26 (e):
a. Health Assessment Questionnaire Disability Index (HAQ-DI) at baseline.
b. Swollen joint count at baseline.
c. SPAARC enthesitis count at baseline.
d. Tender point count at baseline.

e. Treatment failure at week 26.

III. There are differences in inflammation-associated plasma protein dynamics between FMT-treated patients with PsA and sham-treated patients with PsA at
a) Week 4 following the experimental intervention.
b) Week 12 following the experimental intervention.
c) Week 26 following the experimental intervention.

IV. FMT and biological treatment modulate inflammation-associated plasma protein levels from baseline to week 26.

OBJECTIVES

Cross-sectional design: The primary objective is to investigate differences in inflammation-associated plasma protein levels between healthy individuals and patients with peripheral PsA treated with methotrexate. The secondary objectives are to investigate associations of inflammatory protein combinations and clinical measures of PsA disease activity and to look for protein cluster structure in the patient group.

Prospective design: The primary objective is to investigate differences in inflammation-associated plasma protein changes/dynamics between FMT-treated and sham-treated patients with PsA. The secondary objective is to explore the impact of FMT and biological treatment from baseline to week 26 on inflammation-associated plasma protein levels.

Methods

STUDY DESIGN

The analyses will be based on data from patient-reported outcomes, clinical disease measures, and biobank measurements collected at baseline, week 4, week 12, and week 26 of the FLORA trial cohort. Data on the healthy controls are only collected at baseline. The FLORA trial is a proof-of-concept, 26-week, 1:1 randomised, parallel-group, double-blind, placebo-controlled, single-centre, superiority trial. The aim of the FLORA trial was to investigate efficacy and safety of FMT in patients with active, peripheral PsA.[23] In the present exploratory study, we combine cross-sectional data with prospectively collected data to explore changes in inflammation-associated plasma proteins levels in FMT-treated patients with peripheral PsA.

SETTING

Blood samples from 31 FLORA trial patients were collected from May 2017 to June 2020.
Blood samples from 4 stool donors were collected from April to July 2017.
Blood samples from 31 blood donors were collected from February to March 2021.

PARTCIPANTS

The FLORA trial cohort includes 31 patients with PsA (15 treated with FMT and 16 treated with sham at baseline), 31 healthy age- and sex matched blood donors, and 4 stool donors.

Patients included in the FLORA trial were between 18 to 75 years of age; fulfilled the Classification for Psoriatic Arthritis (CASPAR) criteria;[25] and had active peripheral disease, defined as three or more swollen joints, despite ongoing treatment with methotrexate at the maximal tolerable dose (≥15 mg per week) for at least 3 months prior to study inclusion. A washout period of 12 weeks (26 weeks for biological agents) was required in patients previously treated with intra-articular or systemic glucocorticoids, non-methotrexate conventional synthetic, and biologic disease-modifying anti-rheumatic drugs. Key exclusion criteria were immune-mediated arthritis other than PsA; inflammatory bowel disease; cancer; severe chronic infection; and history of food allergy, severe food intolerance, or celiac disease

VARIABLES AND DATA SOURCE

Outcomes

Overall, 92 inflammation-associated plasma proteins available from the Olink inflammation panel (table S1). The plasma samples were collected from participants' venous blood. The plasma was treated with ethylenediaminetetraacetic acid (EDTA), separated and frozen within 3½ hour after sampling and stored at −80 °C until analyses. The samples had not previously been thawed until March 2021, where measurements took place at BioXpedia A/S laboratory.[26] Plasma protein levels were quantified using the PEA multiplex assays.[27] For each protein, a pair of oligonucleotide-labeled antibody probes bind to the targeted protein, and if the two probes are in close proximity, a polymerase chain reaction (PCR) target sequence is formed by a proximity-dependent DNA polymerization event and the resulting sequence is subsequently detected and quantified using standard real-time PCR. Data is then normalised and transformed using internal extension controls and inter-plate controls, to adjust for intra- and inter-run variation. The final assay read-out is given in Normalized Protein eXpression (NPX), which is an arbitrary unit on log2-scale where a high value corresponds to a higher protein expression. Each protein has specific lower limit of quantification and an upper limit of quantification between which a 1-unit increase in NPX correspond to a two-fold increase in protein concentration. Internal controls specify a run-specific lower limit of detection as 3 times the standard deviation over background. In the primary analyses, we will include actual protein concentration regardless of lower limit of detection. In the sensitivity analyses, protein concentration below lower limit of detection will be excluded. The freezer storage time, collection date (month and season), age and sex can induce either an increase or decrease in protein levels.[28]

Table S1. Olink inflammation panel (p_1-p_{92})

Adenosine Deaminase (ADA)
Artemin (ARTN)
Axin-1 (AXIN1)
Beta-nerve growth factor (Beta-NGF)
Caspase 8 (CASP-8)
C-C motif chemokine 4 (CCL4)
C-C motif chemokine 19 (CCL19)
C-C motif chemokine 20 (CCL20)
C-C motif chemokine 23 (CCL23)
C-C motif chemokine 25 (CCL25)
C-C motif chemokine 28 (CCL28)
CD40L receptor (CD40)
CUB domain-containing protein 1 (CDCP1)
C-X-C motif chemokine 1 (CXCL1)
C-X-C motif chemokine 5 (CXCL5)
C-X-C motif chemokine 6 (CXCL6)
C-X-C motif chemokine 9 (CXCL9)
C-X-C motif chemokine 10 (CXCL10)
C-X-C motif chemokine 11 (CXCL11)
Cystatin D (CST5)
Delta and Notch-like epidermal growth factor-related recep (DNER)
Eotaxin-1 (CCL11)
Eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1)
Fibroblast growth factor 5 (FGF-5)
Fibroblast growth factor 19 (FGF-19)
Fibroblast growth factor 21 (FGF-21)
Fibroblast growth factor 23 (FGF-23)
Fms-related tyrosine kinase 3 ligand (Flt3L)
Fractalkine (CX3CL1)
Glial cell line-derived neurotrophic factor (GDNF)
Hepatocyte growth factor (HGF)
Interferon gamma (IFN-gamma)
Interleukin-1 alpha (IL-1 alpha)
Interleukin-2 (IL-2)
Interleukin-2 receptor subunit beta (IL-2RB)
Interleukin-4 (IL-4)
Interleukin-5 (IL-5)
Interleukin-6 (IL-6)
Interleukin-7 (IL-7)
Interleukin-8 (IL-8)
Interleukin-10 (IL-10)
Interleukin-10 receptor subunit alpha (IL-10RA)
Interleukin-10 receptor subunit beta (IL-10RB)
Interleukin-12 subunit beta (IL-12B)
Interleukin-13 (IL-13)

Interleukin-15 receptor subunit alpha (IL-15RA)
Interleukin-17A (IL-17A)
Interleukin-17C (IL-17C)
Interleukin-18 (IL-18)
Interleukin-18 receptor 1 (IL-18R1)
Interleukin-20 (IL-20)
Interleukin-20 receptor subunit alpha (IL-20RA)
Interleukin-22 receptor subunit alpha-1 (IL-22 RA1)
Interleukin-24 (IL-24)
Interleukin-33 (IL-33)
Latency-associated peptide transforming growth factor beta 1 (LAP TGF-beta-1)
Leukemia inhibitory factor (LIF)
Leukemia inhibitory factor receptor (LIF-R)
Macrophage colony-stimulating factor 1 (CSF-1)
Macrophage inflammatory protein 1-alpha (CCL3)
Matrix metalloproteinase-1 (MMP-1)
Matrix metalloproteinase-10 (MMP-10)
Monocyte chemotactic protein 1 (MCP-1)
Monocyte chemotactic protein 2 (MCP-2)
Monocyte chemotactic protein 3 (MCP-3)
Monocyte chemotactic protein 4 (MCP-4)
Natural killer cell receptor 2B4 (CD244)
Neurotrophin-3 (NT-3)
Neurturin (NRTN)
Oncostatin-M (OSM)
Osteoprotegerin (OPG)
Programmed cell death 1 ligand 1 (PD-L1)
Protein S100-A12 (EN-RAGE)
Signaling lymphocytic activation molecule (SLAMF1)
SIR2-like protein 2 (SIRT2)
STAM-binding protein (STAMPB)
Stem cell factor (SCF)
Sulfotransferase 1A1 (ST1A1)
T-cell surface glycoprotein CD5 (CD5)
T-cell surface glycoprotein CD6 isoform (CD6)
T-cell surface glycoprotein CD8 alpha chain (CD8A)
Thymic stromal lymphopoietin (TSLP)
TNF-beta (TNFB)
TNF-related activation-induced cytokine (TRANCE)
TNF-related apoptosis-inducing ligand (TRAIL)
Transforming growth factor alpha (TGF-alpha)
Tumor necrosis factor (Ligand) superfamily, member 12 (TWEAK)
Tumor necrosis factor (TNF)
Tumor necrosis factor ligand superfamily member 14 (TNFSF14)
Tumor necrosis factor receptor superfamily member 9 (TNFRSF9)
Urokinase-type plasminogen activator (uPA)
Vascular endothelial growth factor A (VEGF-A)

Risk of bias assessment

Patients

Data collection was performed in accordance with the FLORA trial protocol.[22]
There is no risk of recall bias since all data were collected at the time of interest or verified from the patient records.
Demographics and clinical characteristics of the two intervention groups (FMT vs sham) were comparable at baseline, except for some random imbalance in sex (female sex 53% [FMT] vs 75% [sham]) and median disease duration (2.6 years [FMT] vs 5.6 years [sham]).
Patients and the treating rheumatologists (i.e., care-providers and outcome assessors) were unaware of treatment allocation and treatment.
One participant in the FMT group vomited during the gastroscopic-guided FMT and may not have received the full amount of donor transplant.
Patients who were classified as treatment failures received additional anti-inflammatory treatment, which can have affected inflammatory protein levels in blood samples collected following this visit (this issue is not relevant for samples collected at week 4 since no additional treatment was instigated before this time).[29]
Other treatments during follow-up that could introduce bias include antibiotics.
We do not expect any significant difference in the freezer storage time or collection date between interventional groups (FMT vs sham) because participants were assigned to FMT or sham transplantation using permuted blocks with varying sizes of four and six, according to computer-generated random numbers.

Controls

The healthy blood donors were age- (within 5-year intervals) and sex-matched with the FLORA trial patient cohort. No other characteristics are available for this cohort.
The stool donors are characterised by sex, exact age, weight, height, HLA-B27 status, and smoking status (they are all never smokers).
Blood donor control samples had been stored for significant shorter time at – 80 degrees (weeks) than patient and stool donor samples (months to years) before analyses.

Study size

To the best of our knowledge, this is the first study comparing 92 inflammation-associated plasma proteins (Olink inflammation panel) before and after treatment with FMT.

STATISTICAL METHODS

Comparing baseline distributions

Baseline characteristics of patients and healthy controls (HC) will be presented in **Table 1** without conducting any formal testing. Comparison of baseline demographic and disease activity measures between FMT- and sham-treated patients is available in the FLORA trial publication on the clinical data.[23]

I. Inflammation-associated plasma protein signatures in PsA and healthy controls at baseline

We will use t-test/Wilcoxon rank-sum test to compare baseline levels of each inflammation-associated plasma protein (p_1-p_{92}) between

a. HC vs stool donors.

b. HC vs patients with PsA.

All comparisons will be presented with p values. The order of the proteins will be according to their difference significance from very different between patients and controls, to not different at all. Results will be presented in **Table 2**.

In addition, we will group the samples (patients and HC/stool donors) and visualise the results in a dendrogram (Figure 1). We expect that the dendrogram will cluster patient samples together and HC and stool donor samples together, thereby indicating that the overall protein signature in patients with PsA is distinct from HC and stool donors.

In the patient group (n=31), we will perform a hierarchical clustering analysis to explore whether there is a cluster structure at baseline among patients and correlate these clusters, if any, with disease outcome (treatment failure vs success at week 26), independent of being in the sham or FMT group.

II. Relations between inflammation-associated plasma proteins and disease activity measures

We will perform a linear regression model to explore associations between distinct baseline protein combinations and each of the baseline disease activity measures/treatment outcome outlined below (A-E). In addition, we will employ random forest regression and compare the model accuracy between the cases to account for interactions between the proteins.

A) HAQ-DI at baseline (ranges from 0 to 3)
B) Swollen joint count at baseline (ranges from 0 to 66)
C) SPAARC enthesitis count at baseline (ranges from 0 to 16)
D) Tender point count at baseline (ranges from 0 to 18)
E) Treatment failure at week 26 (yes/no).

Side Remark: This investigation is, of course, highly speculative, since we only have 31 patients.

Based on the findings of the baseline disease measures (A-D), we will test the stability of findings by applying the model at week 26.

For each of the models (A-E), we will plot the standardised coefficients of the linear model as well as the decision trees of the random forest regression based on the Gini index. Skewed variables will be log-transformed before building the model. For each of the models, we will visualise the variable importance (VImp) values and variable interaction (VInt) values of the proteins by creating a heatmap using the leaf-sorting algorithm (**Figure 2**).[30] We will apply a purple colour scale on the off-diagonal showing the Friedman's H-statistic values (un-normalized) with deeper purple indicating a higher VInt. Similarly, we will apply a green colour scale on the diagonal representing the level of VImp using an embedded approach supplied by the random forest.

III. Comparing changes in protein levels between FMT and sham-treated patients

For each of the inflammation-associated proteins (p_1-p_{92}), we will use the mixed ANOVA to explore in-group factors (time points) combined with between-group factors (FMT vs. sham) across time points (BL, week 4, week 12, week 26). Then, we will use a paired t-test to look for changes from baseline to any other time point.

Based on these findings, we will visualise the dynamics in protein levels (average with 95% confidence intervals) across the four time points BL, week 4, week 12, and week 26 (**Figure 3**).

IV. Effects of FMT and add-on treatment with biologics on inflammation-associated plasma proteins

For each of the 92 proteins (p_1-p_{92}), we will use a mixed effect model to explore the effect sizes of
a. FMT
b. Biological drugs (treatment > 4 weeks)
on protein levels from baseline to week 26.
We will report the model parameters and effect sizes in **Table 3**.

MULTIPLICITY ISSUES

We anticipate that problems will arise when performing a significant amount of statistical significance tests in this study (i.e., 92 protein measures). Since this is a truly exploratory journey, statistical significance levels from this study will not be adjusted per default (i.e. we will consider a possible statistical significance based on the arbitrary threshold that an observed difference is unlikely [<5%] to occur due to chance alone). However, since we are planning to perform 92 statistical tests, the chance of falsely detecting a non-existent difference between intervention groups (FMT vs sham) will be implied in the interpretation (conditioning on the total amount of statistical tests performed). We will use the Hochberg sequential procedure and order the p-values from our 92 tests (multiple comparisons) from largest to smallest on a list. If the error rate is fixed at (the conventional) 5% and the largest observed P value is less than 0.05, then all the tests will be considered significant. Otherwise, if the next largest P value is less than 0.05/2 (0.025), then all the tests except the one with the largest P-value will be considered statistically significant. If not, and the third P-value in the list is less than 0.05/3 (0.017), then all the tests except those with the largest

2 P values will be considered significant. This will be continued until all the comparisons have been made.

MISSING DATA

In this study - working from secondary hypotheses (completely independent of the original FLORA trial objectives) - there is full control for ensuring that all relevant covariates and variables were measured, or at least neither the investigators nor the patients had any knowledge of this "association between variables study". As a consequence of the premises behind this study, we will assume for our primary analyses that missing data is '*Missing Completely At Random*' (MCAR): There are no systematic differences between the missing values and the observed values.

SENSITIVITY ANALYSES

As stated above, we will assume that missing data occurred completely at random (MCAR) and the results of our primary test analyses (for associations between variables) will be performed based on the 'data as observed'. However, to explore the robustness of our findings we will also use multiple imputation where missing values are predicted on basis of all other variables considered. We will create at least five datasets with identical known information but with differences in imputed values reflecting the uncertainty associated with imputations.[31] As described under Outcomes, we will also perform sensitivity analyses by excluding protein measurements with concentration below lower limit of detection.

TABLES

Table 1. Baseline characteristics of patients with PsA, age- and sex-matched healthy controls (HC), and healthy stool donors.

Characteristic	**PsA** (n=31)	**HC** (n=31)	**Stool donors** (n=4)
Female sex, no. (%)			
Age, yr.			
Height, cm		-	
Weight, kg		-	
Time since diagnosis, yr.[a]		-	-
Rheumatoid factor IgM negative, no. (%)[b]		-	-
Anti-citrullinated peptide antibody negative, no. (%)[b]		-	-
HLA-B27 negative, no. (%)		-	
C-reactive protein, mg/L		-	-
Swollen joint 66 count		-	-
Tender joint 68 count		-	-
SPARCC enthesitis index[d]		-	-
Score ≥1, no. (%)		-	-
Score in patients with a score ≥1		-	-
Dactylitis		-	-
Affected digit(s) ≥1, no. (%)		-	-
Digits affected in patients with affected digit(s) ≥1		-	-
Physician's global assessment of disease activity, VAS[e]		-	-
PASI[f]		-	-
Score >0, no. (%)		-	-
Score in patients with a score of more than 0		-	-
Presence of nail disease, no. (%)		-	-
HAQ-DI[c]		-	-
Patient's global assessment of disease activity, VAS[e]		-	-
Arthritis pain, VAS[e]		-	-
Fatigue, VAS[e]		-	-
DLQI score[g]		-	-
Score >0, no. (%)		-	-
Score in patients with a score of more than 0		-	-
Methotrexate		-	-
Oral administration route, no. (%)		-	-
Oral dose, mg/week		-	-
Subcutaneous administration route, no. (%)		-	-
Subcutaneous dose, mg/week		-	-
Previous use of biologic DMARD, no. (%)		-	-
Previous use of non-MTX conventional synthetic DMARD, no. (%)		-	-
Smoking status		-	
Current, no. (%)		-	
Previous, no. (%)		-	

Never, no. (%)			-	
Alcohol consumption, units per week			-	-

Data are mean (SD) or n (%) unless otherwise stated.

FMT, faecal microbiota transplantation; DMARD, disease-modifying anti-rheumatic drug.

[a] Time since diagnosis of psoriatic arthritis is presented as median and interquartile range (IQR).

[b] Presence of rheumatoid factor (IgM) and anti-citrullinated peptide antibody was not accessed in one patient from the FMT group.

[c] Scores on the Health Assessment Questionnaire Disability Index (HAQ-DI) range from 0 to 3, with higher scores indicating greater disability.

[d] Spondyloarthritis Research Consortium of Canada (SPAARC) Enthesitis Index range from 0 to 16, with higher scores indicating more severe disease.

[e] This evaluation is based on a Visual Analogue Scale (VAS) of 0 to 100, with higher scores indicating greater disease activity or pain.

[f] Psoriasis Area Severity Index (PASI) scores range from 0 to 72, with higher scores indicating more severe disease.

[g] Dermatology Life Quality Index (DLQI) score range from 0 to 30, with higher scores indicating more severe disease.

Table 2. Comparison of baseline levels of inflammation-associated plasma proteins between healthy controls (HC), stool donors, and patients with PsA.

Protein type	**PsA** (n=31)	**Stool donors** (n=4)	**HC** (n=31)	**Difference PsA vs HC** (95% CI)	**P value PsA vs HC**	**Difference stool donors vs HC** (95% CI)	**P value stool donors vs HC**

We will use t-test/Wilcoxon rank-sum test to compare baseline levels of each inflammation-associated plasma protein (p_1-p_{92}).

Table 3. Effects of FMT and add-on treatment with biologics on inflammation-associated plasma protein levels. The table presents the model parameters and effect sizes.

FIGURES

Figure 1. Dendrogram of grouped samples from patients and healthy controls (HC)/stool donors, respectively. We expect that the dendrogram will cluster patient samples together and HC and stool donor samples together, thereby indicating that the overall protein signature in patients with PsA is distinct from HC and stool donors.

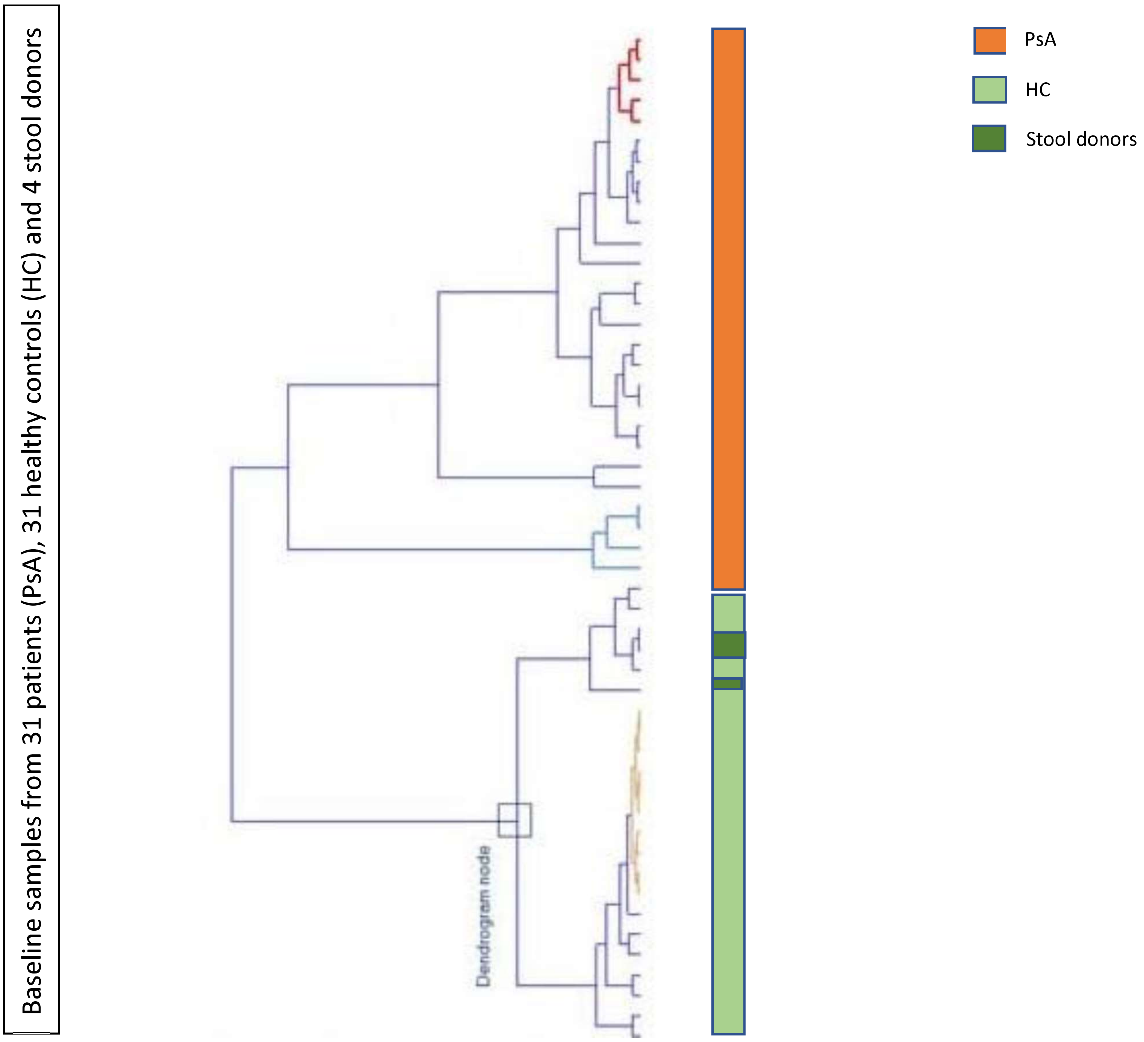


Figure 2. Heatmap of the random forest models with leaf sorting showing the variable importance (VImp) values and variable interaction (VInt) values of the 92 proteins measured at baseline. VImp and VInt are illustrated with green and purple colour scaling, respectively.

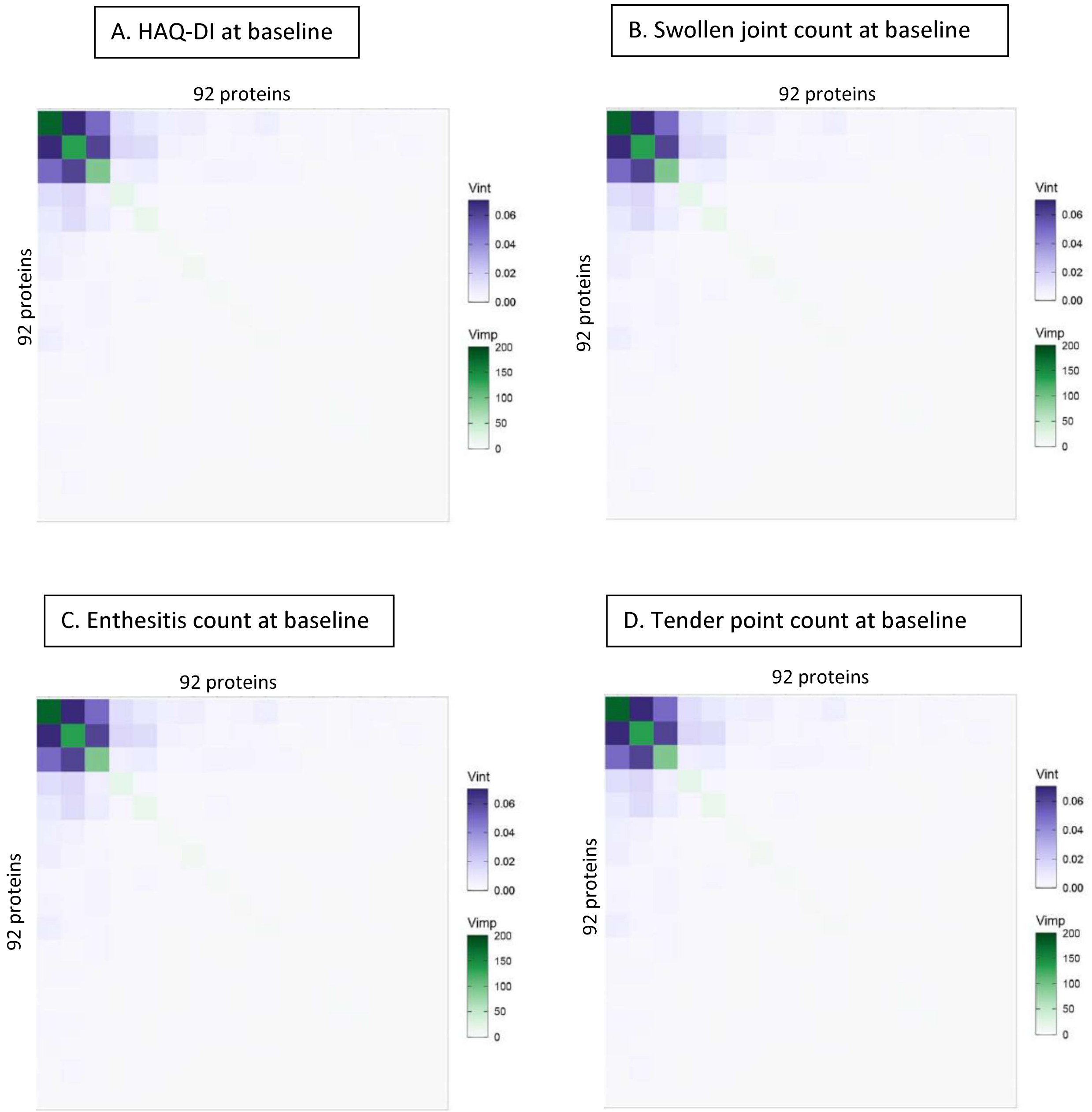


Figure 2 (continued)

E. Treatment outcome at week 26 (failure vs success)

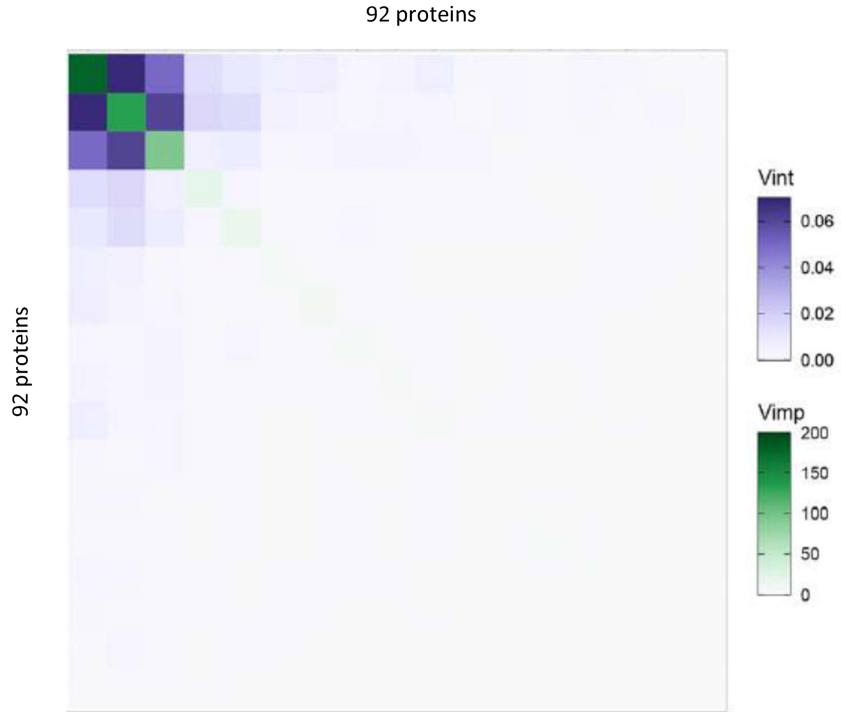
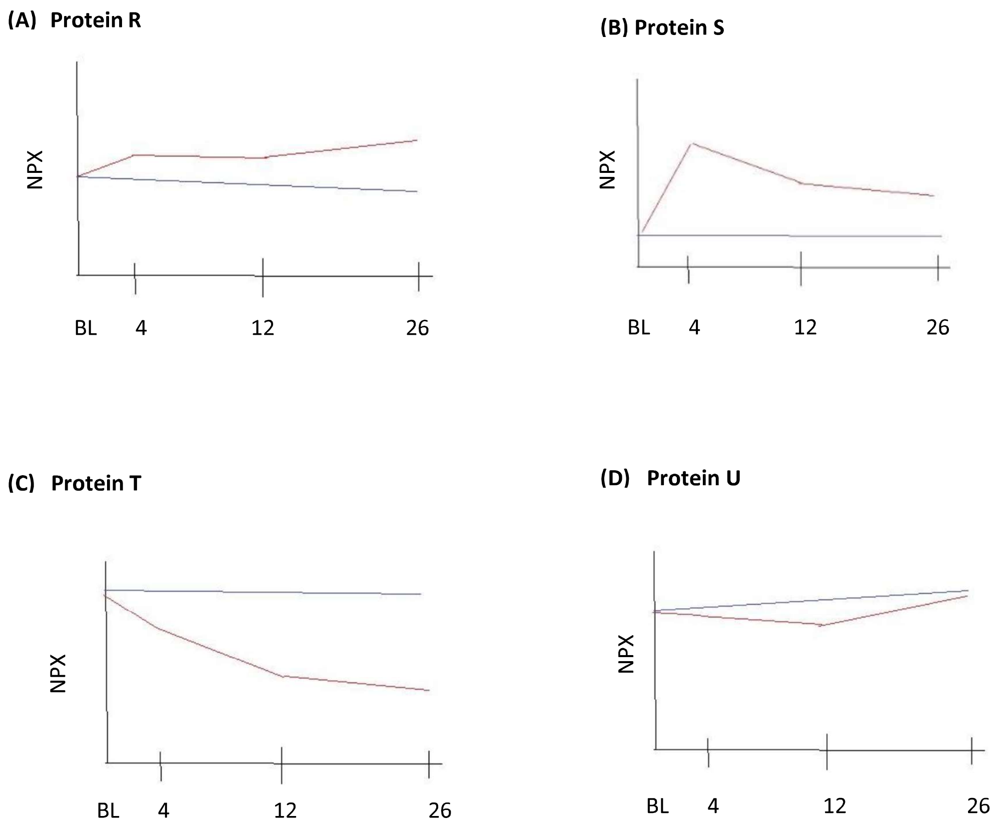


Figure 3. Protein levels by treatment group (FMT [red line] and sham [blue line]) from baseline to week 26. Here visualised with four arbitrary examples for Protein R, S, T, and U.



Mean with 95% CI, shading (e.g. light red [FMT] and light blue [sham]) around the lines (mean) will be used to mark 95% confidence intervals.
Normalised Protein eXpression (NPX) is an arbitrary unit on log2-scale where a high value corresponds to a higher protein expression.
FMT, faecal microbiota transplantation. FMT group: Red line. Sham group: blue line.

Panel A: Levels of protein R evaluated at BL, week 4, week 12, and week 26.
Panel B: Levels of protein S evaluated at BL, week 4, week 12, and week 26.
Panel C: Levels of protein T evaluated at BL, week 4, week 12, and week 26.
Panel D: Levels of protein U evaluated at BL, week 4, week 12, and week 26.

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