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PROTOCOL

Title: Assessment of 54 biomarkers in PsA patients initiating Tumour Necrosis Factor alpha inhibitor, Interleukin 17 inhibitor or Methotrexate: an exploratory study

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INTRODUCTION

Psoriatic Arthritis (PsA) is a chronic immune-mediated inflammatory disease with heterogenous symptoms that affect a variety of structures in a distinct individual way. Symptoms experienced by the individual patient might include clinical manifestations involving skin, joints, entheses, nails, and/or other connective tissues. Thus, PsA patients are diagnosed with the alleged same disease, they experience a diverse range of symptoms and manifestations which respond very differently to the available treatments.

Known immune pathological features of PsA include the induction of the interleukin (IL) 23 /IL-17 inflammatory pathway [1–3] with the differentiation and activation of IL-17-producing Th17 cells mediated by IL-23, believed to be the initial step, and cause for the development of the inflammatory response seen in PsA [3–5]. Though bringing the described clinical manifestations of PsA together with the current results of research, researchers still cannot explain why different PsA clinical phenotypes and responses to treatment are seen with such an individual difference when comparing PsA patients. Previous research indicates the important role of Tumour Necrosis Factor alpha (TNFα) and IL-17 in inflammatory joint disease [3], though these cytokines might only play a part in a unity to promote different clinical manifestations in PsA. The necessity of incorporating broader exploratory study setups become more apparent when studying previous biomarker research that describe various possible biomarkers. IL-1 has been mentioned as a biomarker in synovium and IL-1 mRNA has been found in increased amounts in joints in PsA patients, hence elevated IL-1 has been related to increased risk of PsA [6–8]. Treatment with IL-6 inhibitors has shown improvement of musculoskeletal manifestations, but minor improvement in skin lesions, and has been suggested as a biomarker useful as indicator for disease activity [9–11]. On the other hand, IL-6 has been found to induce IL-10 expression making Th17 non-pathogenic [12]. TNFα stimulates production of both of and IL-6 [8,13]. Moreover, researchers find that different cytokines seem to be involved in different clinical manifestations, thus proposing IL-19 to be involved in joint inflammation, whereas IL-20 and IL-24 mainly contribute to the systemic response [14]. Moreover, IL-17 is found both in skin lesions and joints, whereas IL-22, IL-6, and IL-1 are found in entheses [10,15,16].

Current treatment strategies include first line treatment with conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs) with little and/or unsatisfactory effect in PsA [17–19] . Upon failure of csDMARD, biological DMARD (bDMARD) will be initiated, including Tumor Necrosis Factor alpha inhibitors (TNFi) or Interleukin 17 inhibitors (IL-17i). bDMARDs have shown promising results in decreasing the inflammatory response in PsA, though, 20-30% of PsA patients still fail to respond to available biological agents [1,20]. This might be explained by different immune cellular response mechanisms to drugs due individual patient- and/or disease specific characteristics and mechanisms [21]. This demonstrate the importance in comparing immune pathological findings with patient- and disease-specific characteristics and treatment response.

To fully understand the complexity of the disease, and for future development of evidence-based treatment, it is highly relevant to further explore the undiscovered features of the PsA inflammatory pathways, including the underlying immune components' characteristics in patients suffering from PsA subsets [2–5,22–24]. With current knowledge we are left at a stage where PsA immune pathogenesis is still not fully understood, leaving us with unanswered questions on how to better stratify patients by their clinical manifestations in order to choose the optimal line of treatment and predict response to treatment. We believe that further understanding of PsA immune pathophysiology will be obtained by combining exploratory methods in a study of both cytokine-, immune cellular- and genetic characteristics in relation to clinical manifestations in PsA patients treated with MTX, TNFi or IL-17i.

OBJECVTIVES

The primary objective of the study is to assess individual the level of 54 biomarkers in 70 PsA patients in association with clinical phenotypes and disease outcome measures. Secondary, to evaluate effect of treatment on biomarker level in 50 PsA patients initiating new treatment e.g. TNFi, IL-17i or MTX and assess the difference between responders and non-responders. Further, to provide a thorough analysis of cytokine networks and interactions before and after treatment.

STUDY DESIGN

Initially, a search for literature ((psoriatic arthritis)AND(biomarker)AND(DMARD)) was conducted to explore previous research on biomarkers in PsA and effect of treatment on biomarker level. Abstracts and relevant articles from the past 5 years were screened.

In accordance with Danish law, the project has been approved by the Regional Committee on Health Research Ethics in the Capital Region of Denmark (J.no: H-15009080 / H-18024568) and is conducted in line with GDPR requirements and the Danish law on Data Protection approved by *Videnscenter for Dataanmeldelser* a part of the Capital Region of Denmark (J.no.: BFH-2015-043).

Study population

Seventy patients were recruited from Danish Departments of Rheumatology in Region Zealand and the Capital Region of Denmark; 20, 30, 20 initiating treatment with IL-17i, TNFi or MTX, respectively. PsA patients are included if following eligibility criteria are fulfilled

Inclusion criteria:

- age ≥18 years

- diagnosed with PsA fulfilling the CASPAR classification criteria
- initiation treatment with either IL-17 inhibitor, TNFα inhibitor, or Methotrexate

Exclusion criteria:

- other rheumatic inflammatory diseases than PsA
- prior exposure to IL-23- or IL-17- inhibitors
- previous treatment with cell-depleting therapies
- active systemic infection during the last two weeks (exception: common cold)

Clinical examination

Patients are recruited from the Parker Institute's consecutive PsA patient cohort (PIPA) [25]. Patients are included for a baseline visit adjacent to treatment initiation and a follow up visit four months after treatment initiation. A follow up visit prior to the four months visit was completed if patients experienced disease deterioration that would necessitate additional change of treatment. All visits consist of a clinical interview and examinations in line with table 1.

TABLE 1

	Baseline	4 months
Clinical interview		
Age	X	
Gender	X	
Disease duration	X	
Clinical examination		
PsA phenotype	X	X
Swollen joint count (66 joints)	X	X
Tender joint count (68 joints)	X	X
SPARCC enthesitis score (number)	X	X
PASI score	X	X
Patient reported outcome		
VAS (global, pain, fatigue),	X	X
HAQ	X	X
PsAID	X	X
Paraclinical examination		
Blood sampling	X	X
Ultrasonography (26 joints)	X	X

PsA; Psoriatic Arthritis, SPARCC; Spondyloarthritis Research Consortium of Canada, PASI; Psoriatic Arthritis Severity Index, VAS; Visual Analogue Scale, HAQ; Health Assessment Questionnaire, PsAID; Psoriatic Arthritis Impact of Disease

Composite outcome measures

Disease Activity for Psoriatic Arthritis (DAPSA) is included to evaluate PsA disease activity. DAPSA is calculated as the sum of swollen joints (66), tender joints (68), patient pain, patient global assessment and c-reactive protein (CRP) [26].

Responders and non-responders of treatment are characterized by DAPSA and PASI responses. Responders are defined by DAPSA50 / PASI50, reflecting ≥50% improvement in DAPSA and PASI, respectively, from baseline to four months follow up. Non-responders are defined by DAPSA50 / PASI50 <50%.

Handling of bio-samples

Peripheral blood has been collected in EDTA vacutainer tubes and centrifuged for 10 minutes at 2000 xg to collect plasma. Plasma aliquots of 1 ml are transferred immediately to a -80°C for storage until analysis.

Analysis of biomarkers

Plasma samples will be analyzed to measure biomarker levels on the MESO QuickPlex SQ 120 using the V-PLEX Human Biomarker 54-Plex Kit (Meso Scale Diagnostics, Rockville, MA, USA). Plasma duplicates will be assessed for all patient samples. Analysis is conducted in accordance with manufacturer's protocol available from their website:

https://www.mesoscale.com/en/products/v-plex-human-biomarker-54-plex-kit-k15248d/

EXPLORATORY OUTCOME MEASURES

Primary outcome:

- levels of 54 biomarkers (pg/ml) in 70 PsA patients prior to initiation of treatment

Secondary outcomes:

- baseline biomarker (level pg/ml) stratified by DAPSA50/PASI50 responders and non-responders
- correlation between baseline biomarkers levels and individual clinical and patient-reported measure
- change in biomarker level after four months of treatment stratified by DAPSA50/PASI50 responders and non-responders of treatment
- Component (correlation) coefficients to evaluate relationship between biomarkers in emerging cytokine networks

DATA ANALYSIS AND STATISTICAL ANALYSIS PLAN

Data analysis will be completed with *R Statistics* with additional relevant packages.

Plasma duplicate measures will be compared with coefficient of variation and intraclass correlation coefficients. Patient characteristics at baseline grouped by treatment mode of action (i.e. TNFi, IL-17i and MTX) will be presented as medians with corresponding interquartile range for continuous variables and number with corresponding percentages for categorical variables. Kruskal-Wallis test will be applied for the assessment of difference between TNFi, IL-17i and MTX treated patients. Primary outcome will be reported as biomarker

levels in pg/ml for all included patients. Spearman's rho correlation coefficient will be evaluated for the assessment of correlation between biomarkers and disease outcome measures at baseline and heatmaps for spearman's rho correlation will be generated. Baseline biomarker levels will further be examined, stratified by responders and non-responders of treatment. Differences in biomarker level between responders and non-responders will be evaluated with Mann-Whitney U-test for the comparison of unpaired measures. Change in disease outcome measures and biomarker level from baseline to follow up will be presented as mean change with corresponding standard deviation. Change in biomarker levels will further be illustrated in bar plots with error bars. Wilcoxon's signed rank test will be used for the comparison of paired measures. Three-way ANOVA, including type treatment, time point, and treatment response will be applied to identify biomarkers with global significance. Two-sided p-values <0.05 will be considered statistically significant during all analyses.

Table 1: Comparison of plasma duplicates
Table 2: Patients' characteristics grouped by type of treatment
Table 3: Baseline levels of 54 biomarkers
Figure 1: Correlation matrix/heat map for spearman rho correlations including biomarkers vs. disease outcome measures
Figure 2: Bar plot with error bars with baseline biomarker level stratified by responders and non-responders
Table 4: Change in clinical outcome from baseline to follow up
Figure 3: Bar plot with error bars of Δ biomarker level stratified by treatment type
Table 5: Three-way ANOVA results

Principal component analysis (PCA) will be applied for assessment of the interrelationship between cytokines as a part of descriptive analyses of cytokine networks. PCA is implemented for dimensionality reduction, exploring the variance and association between biomarkers. Number of components included for interpretation will be decided by the Eigenvalues (proportion of variation explained by each component) at the point where remaining eigenvalues will be of comparable sizes on the screen plot [27]. The contribution of each variable to the component will be considered significant/important if being above the expected average contribution, defined as the contribution of which variables were uniform e.g. 1/length of variables (number of biomarkers examined).

Figure 4: Component loading plots evaluating plasma biomarker levels at baseline for the individual treatment type and/or responders and non-responders
Table 6: Principal component matrix at baseline
Figure 5: Component loading plots evaluating plasma biomarker levels at follow up for the individual treatment type and/or responders and non-responders
Table 7: Principal component matrix at follow up

Depending on journal, guideline tables and figures may be included as supplementary files.

Dissemination plan and outreach

Initially, two publications are planned based on data retrieved from the study,/. i.e. 1; a descriptive analysis of biomarker levels before and after treatment and 2; a multivariate analysis including the principal component analysis to analyze cytokine networks.

Both will be reported in accordance with the STARD statement [28]. Results of the study will be disseminated through publication(s) in international peer-reviewed journals. All outcome, i.e. positive, negative or inconclusive results, will be published. Public outreach will be achieved by layman articles published on the Parker Institute's website (www.parkerinst.dk) and as public presentations in collaboration with the Parker Institute's Patient Association.

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