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Clinical science

A proteomics study of rheumatoid arthritis patients on etanercept identifies putative biomarkers associated with clinical outcome measures

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Abstract

Objectives: Biologic DMARDs (bDMARDs) are widely used in patients with RA, but response to bDMARDs is heterogeneous. The objective of this work was to identify pretreatment proteomic biomarkers associated with RA clinical outcome measures in patients starting bDMARDs.

Methods: Sequential window acquisition of all theoretical fragment ion spectra mass spectrometry (SWATH-MS) was used to generate spectral maps of sera from patients with RA before and after 3 months of treatment with the bDMARD etanercept. Protein levels were regressed against RA clinical outcome measures, i.e. 28-joint DAS (DAS28) and its subcomponents and DAS28 <2.6 (i.e. remission). The proteins with the strongest evidence for association were analysed in an independent, replication dataset. Finally, subnetwork analysis was carried out using the Disease Module Detection algorithm and biological plausibility of identified proteins was assessed by enrichment analysis.

Results: A total of 180 patients with RA were included in the discovery dataset and 58 in the validation dataset from a UK-based prospective multicentre study. Ten individual proteins were found to be significantly associated with RA clinical outcome measures. The association of T-complex protein 1 subunit η with DAS28 remission was replicated in an independent cohort. Subnetwork analysis of the 10 proteins from the regression analysis identified the ontological theme, with the strongest associations being with acute phase and acute inflammatory responses.

Conclusion: This longitudinal study of 180 patients with RA commencing etanercept has identified several putative protein biomarkers of treatment response to this drug, one of which was replicated in an independent cohort.

Keywords: RA, biologics, anti-TNF, etanercept, treatment response, proteomics, genetics, biomarkers

Rheumatology key messages

- There are currently no validated pretreatment protein biomarkers of RA treatment response.
- Identification of biomarkers of treatment response would allow more cost effective, informed decision making in RA treatment.
- We identified 10 protein biomarkers associated with clinical outcome measures in etanercept-treated patients with RA.

Introduction

In patients with RA in whom conventional synthetic DMARD (csDMARD) therapy fails to control disease activity, treatment can be increased to more costly biologic DMARDs (bDMARDs) such as adalimumab and etanercept. However, treatment response to bDMARDs is not universal and, in up to 40% of these patients, inflammation remains inadequately controlled, either due to primary inefficacy or loss

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of response [1, 2]. Identification of reliable biomarkers predictive of response to these agents is a research priority, as predictive biomarkers would enable clinicians and patients to make informed therapy selection.

Multi-omics studies of biomarkers of treatment response are starting to accumulate in the RA literature and have typically focused on genetic, transcriptomic, metabolomic and lipidomic markers, but few have investigated proteomic biomarkers [3–5]. Proteins have many features that make them ideal potential biomarkers, given that proteins carry out diverse biological processes, interact with drugs and capture information on post-translational modifications. Furthermore, many proteins are stable and conventional assays commonly used in healthcare rely on protein technology (e.g. ELISAs), so translation of laboratory findings can be rapidly accelerated into clinical practice. Finally, recent technological advances in high-throughput methods mean that it is now possible to analyse large numbers of proteins in patient samples; e.g. sequential window acquisition of all theoretical fragment ion spectra mass spectrometry (SWATH-MS) is a high-coverage shotgun proteomics technique with near-comprehensive proteome cover and excellent dynamic range and reproducibility [6]. This enables the study of theoretically all spectra present in a sample, with a permanent record of each spectral map, meaning that data can be re-interrogated for new peptides of interest *in silico* as interactions become clearer following analysis.

There have been previous reports of protein biomarkers obtained using large-scale shotgun proteomics techniques associated with treatment response in RA [7], however, these studies have been limited by heterogeneous treatment populations (i.e. patients were on more than one drug, but grouped together for analysis), small sample sizes and no comparisons to healthy controls (HCs). Proteins such as monocyte chemoattractant protein (MCP)-1 [8], epidermal growth factor (EGF) [8], vitamin K–dependent protein S (PROS) [9] and E3 ubiquitin-protein ligase carboxyl terminus of heat shock cognate 70-interacting protein (CHIP) [9] were identified, but these were small-scale studies with <35 patients included. Furthermore, none of these proteins have been consistently replicated in independent prospective validation cohorts with a large sample size.

The aim of the current study was to identify protein biomarkers predictive of treatment response to the bDMARD etanercept in patients with RA and to use network-based methods to identify relevant pathways.

Methods

Patient and public involvement

Prior to implementation, the study concept and design were discussed and developed in conjunction with the Research User Network (comprising patients with various rheumatological conditions, including RA), based within the Centre for Musculoskeletal Research (CfMR) at the University of Manchester.

Study participants

Patients with RA according to the 1987 ACR classification criteria were recruited to the Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate [BRAGGSS; Research Ethics Committee (REC) reference 04/Q1403/37], a prospective multicentre observational study based in the UK [10]. This study was in compliance with the Declaration of Helsinki and all participants gave written informed consent. The study cohort consisted of participants recruited to the prospective arm of BRAGGSS who were commencing etanercept or an etanercept biosimilar. Participants were Caucasian, bDMARD naïve, had a pretreatment 28-joint DAS (DAS28) >5.1 (indicative of high disease activity) and were ≥ 18 years of age. In order to be eligible for analysis, each participant required serum to be available at two time points, pretreatment (baseline) and following 3 months on the drug, in addition to RA clinical disease outcome measures available at baseline and 3 and 6 months. Participants were recruited between 2009 and 2016 from secondary care rheumatology departments in the 60 centres participating in BRAGGSS, with follow-up concluding 12 months after initial recruitment into the study for each participant. Participants were opportunistically recruited over several years and a sample size calculation was not applied.

In all RA participants, clinical data and DAS28 were available. DAS28 was calculated using a four-component algorithm consisting of tender joint count (TJC) and swollen joint count (SJC) of 28 joints, patient visual analogue scale of global health (VAS-GH, 0–100 mm) and high-sensitivity CRP (hsCRP) measured using ELISA at the National Institute for Health and Care Research (NIHR) National Biosample Centre (Milton Keynes, UK).

HCs were recruited from the National Repository Study (REC reference 99/8/084), a study consisting of healthy volunteers to provide samples for comparison cohorts and protocol, technique and method development.

Sample processing

All patient serum samples were processed at the CfMR and by CfMR laboratory staff. Blood tubes were spun at 1720 *g* for 10 min, then serum was extracted into aliquots before being frozen at -80°C. RA participant samples were received either on the same day (from centres close to the CfMR geographically) or mostly through the UK postal service (Royal Mail), with a median time between collection and sample processing of 2 days (interquartile range 1–3). All HC samples were collected onsite and processed on the same day at the CfMR.

Frozen serum aliquots were transferred to the Stoller Biomarker Discovery Centre (SBDC, Manchester, UK), where protein spectral maps were extracted for each sample using SWATH-MS, using techniques for sample processing and data acquisition as previously described [11, 12]. Samples were processed by the SBDC; SWATH-MS acquisition is discussed in more detail in the Supplementary Methods, available at *Rheumatology* online.

Statistical analysis

All analyses were carried out in R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria) [13]. Proteomics data were pre-processed, including imputation of missing protein values, as described in the Supplementary Methods, available at *Rheumatology* online. Peptide spectra were identified from samples using both a generic open-access plasma library, as well as a bespoke library of proteins curated from pre-existing literature on proteomics studies in patients with RA.

Differentially expressed proteins between cases and controls

Differential expression of proteins between RA patients (cases) at baseline and HCs was calculated using a Welch's *t*-test implemented by using the col_t_welch function in the MatrixTests [14] package. In order to reduce the high dimensionality of the dataset, only proteins that were statistically differentially expressed ($P < 0.05$) between patients with active RA and HCs, who represented a healthy physiological state, were retained, meaning that these proteins were significantly increased or decreased in cases compared with controls. Therefore, proteins for investigation were selected on the basis that they showed differential expression in samples from RA patients compared with HCs.

Association of protein expression with clinical outcome measures

Primary analysis was carried out in a discovery cohort of patients with RA. The R base package was used to carry out regression between expression of each protein and the following continuous RA disease outcomes:

- Primary outcome measures: EULAR response criteria [15] (poor *vs* good/moderate) and DAS28 remission (i.e. DAS28 <2.6) [16]—logistic regression.
- Secondary outcome measures: DAS28 and its subcomponents (TJC, SJC, VAS-GH, hsCRP)—linear regression.

In linear regression analysis, a positive β-coefficient indicated a positive association between a protein and the clinical outcome measure of interest and a negative coefficient indicated an inverse association, i.e. as a clinical outcome measure value increased, the protein value decreased. Both univariate and multivariable analyses were carried out for each protein. The following variables were included as potential confounding covariates: age at baseline, RA disease duration prior to starting etanercept, biological sex, concurrent csDMARD therapy, BMI, seropositivity of either RF or ACPA, pretreatment (baseline) DAS28, systemic corticosteroid use within ±12 weeks of starting etanercept (intramuscular and oral administration) and the presence of the following comorbidities: cardiovascular disease, respiratory disease, liver disease, renal disease, diabetes and malignancy; these are expanded on further in the Supplementary Methods, available at *Rheumatology* online. Adjustment for false discovery rate due to multiple testing was carried out using the Benjamini–Hochberg procedure [17]. Significantly associated proteins (following multiple testing adjustment with $P < 0.05$) were then added into a multivariable model adjusting for the same confounding covariates. Proteins at baseline (pretreatment) were compared with outcomes at 3 and 6 months and proteins at 3 months were compared with outcomes at 6 months.

For validation of the most relevant findings from the primary analysis, peptide spectra for prioritized proteins were extracted from SWATH-MS spectral maps generated in an independent cohort of patients with RA. Statistical analysis was repeated as detailed above. Technical validation with an orthogonal method was carried out using a Pearson correlation between log2-transformed hsCRP (measured by ELISA at the NIHR National Biosample Centre) against CRP measured using SWATH-MS in the main discovery cohort of patients.

Subnetwork analysis

Subnetwork analysis was carried out on all proteins from the regression analysis that remained significant following adjustment in multivariable models that included potential confounders. First, enrichment analysis was carried out using Enrichr [18], then potential interactions with these significant proteins were determined using the Disease Module Detection (DIAMOnD) algorithm [19]. The optimal parameters for the subnetwork construction were determined using a grid search over the number of subnetwork proteins and the α-value (weighting applied to seed proteins), where the parameters giving the lowest biological validation *P*-value were used to generate the overall subnetwork. Biological validation refers to validating the generated subnetwork against the list of significant proteins identified following enrichment analysis. Detailed methods are outlined in the Supplementary Methods, available at *Rheumatology* online.

Results

Study participants

Samples from 180 patients with RA were included in the discovery cohort and from 58 patients in the validation cohort; their summary characteristics are detailed in Table 1. Patients who were not on concurrent csDMARDs, as detailed in Table 1, had been commenced on etanercept monotherapy after failing conventional csDMARD escalation, as per National Institute for Health and Care Excellence guidance [20].

Differential expression of proteins between RA cases and HCs

Pretreatment samples reflecting high RA disease activity from the 180 RA patients were compared with HCs ($n = 14$) and 216 of 482 proteins were found to be significantly differentially expressed between the two groups. A total of 70 of the 216 proteins were down-regulated in RA patients with active disease compared with HCs and the remaining proteins were all up-regulated. The full results are presented in Supplementary Table S1 (available at *Rheumatology* online). These 216 proteins were then prioritized in subsequent analyses.

Logistic regression models of protein expression associated with EULAR response and DAS28 remission (primary outcome measures)

Following adjustment in multivariable models, no proteins were associated with EULAR response (Table 2). T-complex protein 1 subunit η (TCPH; UniProt identifier Q99832) at baseline was found to be associated with reduced odds of achieving DAS28 remission at 3 months [adjusted odds ratio (OR_{adj}) 0.32 (95% CI 0.11, 0.85), adjusted *P* (P_{adj}) = 2.91E-02]. The full results of confounder-adjusted and multivariable analyses are presented in Supplementary Tables S2 and S3 (available at *Rheumatology* online). Figures demonstrating whether models met the assumptions of regression are presented in Supplementary Figs S1–S3 (available at *Rheumatology* online).

Linear regression models of protein expression associated with RA disease outcome measures (secondary outcome measures)

Following adjustment of linear outcome measures in multivariable models, a number of proteins were found to be associated with clinical outcome measures (Table 2):

- Four proteins were associated with DAS28.
- Five proteins were associated with hsCRP measured using ELISA. Dual specificity mitogen-activated protein kinase 3 (MAP2K3) at both baseline and 3 months was associated with hsCRP at 6 months.
- Aspartyl/asparaginyl β-hydroxylase (ASPH; UniProt identifier Q12797) was associated with VAS-GH.

The full results of confounder-adjusted and multivariable analyses are presented in Supplementary Tables S4–S12 (available at *Rheumatology* online). Figures demonstrating whether models met the assumptions of regression are presented in Supplementary Figs S4–S12 (available at *Rheumatology* online).

Table 1. Baseline characteristics of patients recruited to the study

Characteristic	Value
Discovery cohort ($n = 180$)	
Female, *n* (%)	134 (74.44)
Age, years, median (IQR)	56.90 (49.96–64.93)
Disease duration prior to starting bDMARD, years, median (IQR)	6 (2–14)
Body mass index, kg/m^2, median (IQR)	27.56 (23.86–32.54)
Concurrent csDMARD, *n* (%)	147 (81.67)
DAS28, median (IQR)	5.9 (5.3–6.4)
Ever seropositive (RF and/or ACPA), *n* (%)	120 (66.67)
Validation cohort ($n = 58$)	
Female, *n* (%)	44 (75.86)
Age, years, median (IQR)	58.18 (51.65–66.43)
Disease duration prior to starting bDMARD, years, median (IQR)	6 (3–10)
Body mass index, kg/m^2, median (IQR)	28.38 (23.51–35.06)
Concurrent csDMARD, *n* (%)	52 (89.66)
DAS28, median (IQR)	5.97 (5.49–6.77)
Ever seropositive (RF and/or ACPA), *n* (%)	39 (67.24)

IQR: interquartile range.

Validation in an independent cohort (SWATH-MS acquisition)

Validation of significant proteins identified from the previous section was carried out in an independent cohort using the original multivariable models from the discovery cohort. TCPH measured at baseline remained significantly associated with reduced odds of DAS28 remission at 3 months [OR_{adj} 0.06 (95% CI 0.00, 0.50), $P_{adj} = 2.71E\text{-}02$]. MAP2K3 measured after 3 months of treatment also remained significantly associated with hsCRP measured using ELISA after 6 months of treatment [β-coefficient$_{adj}$ 9.39 (95% CI 0.44, 18.33), $P_{adj} = 4.83E\text{-}02$]. The remaining proteins did not replicate in this smaller cohort. Full results are available in Supplementary Table 13 (available at *Rheumatology* online).

Technical validation of CRP measured by SWATH-MS using an orthogonal method

At baseline, CRP measured using SWATH-MS was significantly correlated with log2-transformed CRP measured using ELISA [Pearson's correlation coefficient 0.88 (95% CI 0.83, 0.91), $P \leq 2.2E\text{-}16$]. This was also the case after 3 months of treatment [Pearson's correlation coefficient 0.80 (95% CI 0.73, 0.86), $P \leq 2.2E\text{-}16$]. See Supplementary Figs S13 and S14 (available at *Rheumatology* online) for scatter plots of these data. This demonstrates close agreement between protein quantification using both SWATH-MS and ELISA.

Subnetwork analysis

Four of the ten proteins correlated with RA clinical disease outcomes were present in the Human Interactome [21], to which the DIAMOnD algorithm was applied. The most parsimonious network giving the lowest biological validation *P*-value was chosen, consisting of 157 genes and $\alpha = 3$ (Fig. 1). Aspartyl/asparaginyl β-hydroxylase (ASPH; one of the seed nodes) was found to be a hub/influential node within the subnetwork. Fig. 2 summarizes the ontological themes of the 157 genes following

Table 2. Proteins associated with RA clinical outcome measures after treatment with etanercept, adjusted in multivariable models

DAS28 remission (<2.6)				
Protein	**Protein measurement time point**	**Outcome measure time point**	**OR_{adj} (95% CI)**	**Adjusted *P*-value**
TCPH (Q99832)	Baseline	3 months	0.32 (0.11, 0.85)	2.91E-02
Protein	**Protein measurement time point**	**Outcome measure time point**	**β-coefficient$_{adj}$ (95% CI)**	**Adjusted *P*-value**
DAS28				
EHD1 (Q9H4M9)	Baseline	3 months	0.21 (0.05, 0.37)	9.49E-03
TCPH (Q99832)	Baseline	3 months	0.62 (0.16, 1.08)	9.59E-03
CRP (P02741)	3 months	6 months	0.16 (0.01, 0.30)	3.93E-02
C9 (P02748)	3 months	6 months	0.38 (0.01, 0.76)	4.74E-02
hsCRP measured using ELISA				
SELENOP (P49908)	Baseline	6 months	−5.68 (−9.19, −2.17)	1.87E-03
MAP2K3 (P46734)	Baseline	6 months	7.85 (3.49, 12.22)	5.79E-03
	3 months	6 months	6.30 (1.72, 10.89)	7.93E-03
CLTC (Q00610)	Baseline	6 months	4.76 (0.77, 8.75)	2.08E-02
SAA1 (P0DJI8)	3 months	6 months	3.24 (1.22, 5.26)	2.00E-03
MYLK (Q15746)	3 months	6 months	8.92 (1.13, 16.72)	2.64E-02
VAS-GH				
ASPH (Q12797)	3 months	6 months	−0.01 (−0.02, −0.01)	3.14E-02

UniProt identifiers are included in parentheses after each protein abbreviation.
CLTC: clathrin heavy chain 1; C9: complement component C9; EHD1: EH domain-containing protein 1; MCID: minimally clinically important difference; MYLK: myosin light chain kinase; SELENOP: smooth muscle; selenoprotein P; SAA1: serum amyloid A-1 protein.

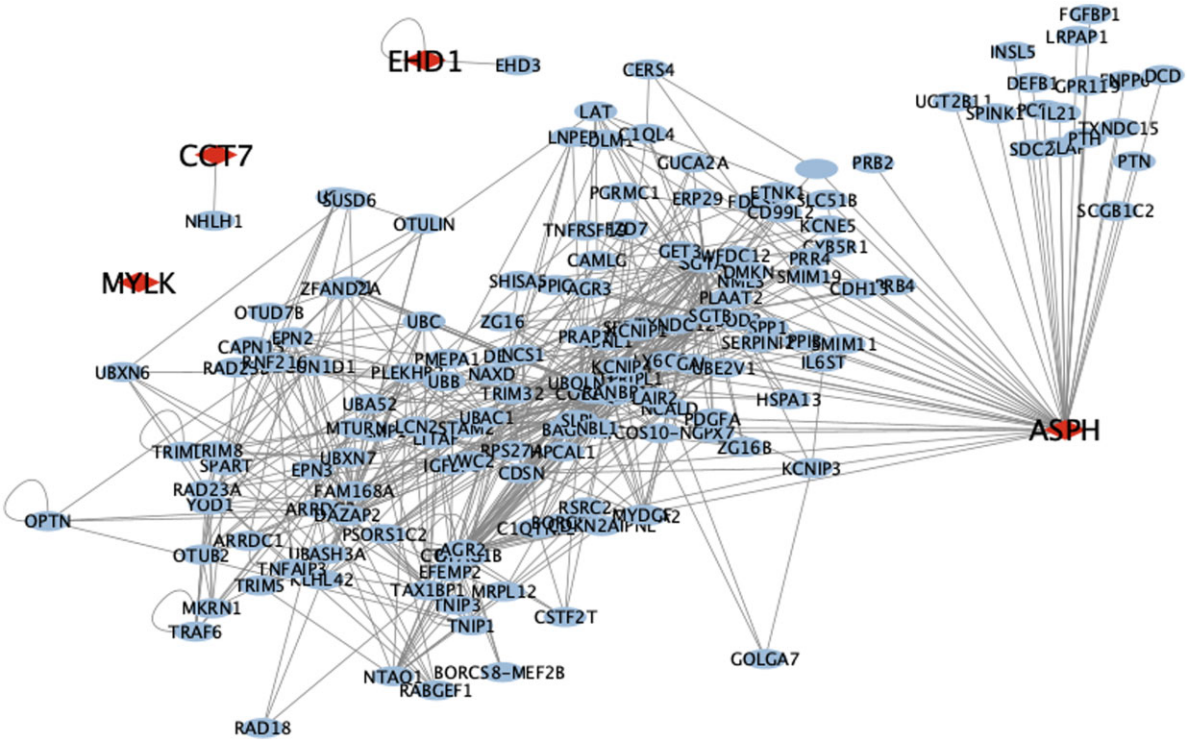

Figure 1. Diagrammatic representation of selected protein subnetwork based on the 10 significant proteins from regression analysis. The diamonds represent significant proteins from the regression analysis that are also present in the Human Interactome, which have been used as input nodes for the sub-network analysis. The ovals represent additional proteins identified from sub-network analysis using the Human Interactome

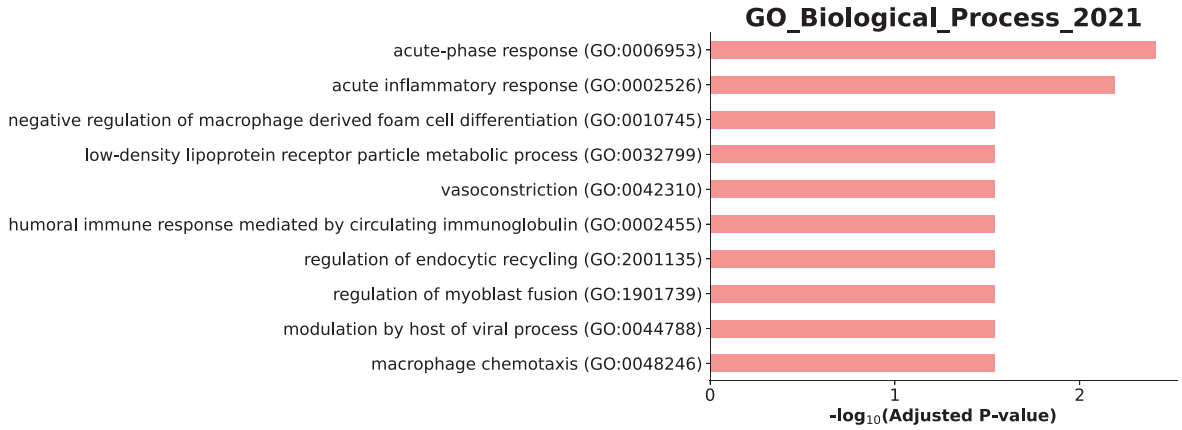

Figure 2. Summary of the ontological themes of the 157 subnetwork genes following enrichment analysis

enrichment analysis, with the strongest associations being with acute-phase response and acute inflammatory response.

Discussion

In the largest study of proteomic biomarkers compared with clinical outcome measures in patients with RA treated with etanercept to date, we report that four protein markers measured pretreatment associate with one or more measures of outcome by 3 or 6 months and six protein markers measured after 3 months of treatment associate with one or more measures of outcome by 6 months. Subnetwork analysis found 157 genes associated with the proteins identified, with enrichment analysis giving acute-phase response and acute inflammatory response as the most significantly associated pathways.

TCPH is a protein of note in this study, as its measurement at baseline was associated with DAS28 remission after 3 months of treatment, and this finding was replicated in an independent cohort. TCPH is involved in processes during adenosine triphosphate (ATP) hydrolysis [22, 23]. Pretreatment levels of TCPH were also associated with DAS28 by 3 months. ATP hydrolysis is the process by which energy is released in the conversion of ATP to adenosine diphosphate. Given that active inflammation is a high-energy state, this putative pretreatment biomarker may simply reflect the level of pretreatment inflammation, which is known to correlate with response to treatment, i.e. a higher pretreatment DAS28 correlates with a greater improvement due to regression to the mean. However, TCPH [β-coefficient 0.01 (95% CI 0.00, 0.01), $P_{adj} = 0.28$] was not correlated with pretreatment CRP levels, and further research will be required to determine whether the

association of TCPH with clinical outcome measures is specific to etanercept response in RA or is a general theragnostic indicator.

MAP2K3 is also an interesting protein in this study, as its measurement at both baseline and 3 months was associated with future hsCRP at 6 months. MAP2K3 is a dual-specificity kinase that is activated via cytokines and environmental stress [24]. Its association with future hsCRP could implicate it as a potential biomarker of systemic inflammation despite treatment with etanercept; it could be hypothesized that patients with increased levels may be prone to uncontrolled inflammation (measured by hsCRP as a proxy) despite treatment. Its lack of association with EULAR response or DAS28 could be because of inclusion of DAS28 subcomponents (TJC, VAS-GH) [25] that do not reflect systemic inflammation as well as hsCRP.

Findings from this study have not replicated previously identified proteins that were reported to show an association with treatment response to etanercept in patients with RA [8, 9]. This failure to replicate previous findings of other proteomics studies may be due to a number of reasons, such as heterogeneous populations of study (e.g. patients with different disease duration, disease severity, therapy prior to commencing bDMARD therapy, ethnicity etc.), as well as varying methods of proteomics acquisition. There are advantages and disadvantages to the use of various proteomics acquisition techniques, and some higher-throughput methods may not capture the full proteome during sample processing [26].

Findings from the subnetwork analysis showed that ASPH was an influential node in the overall subnetwork. It is interesting that this protein was associated with patient global health, indicating that there might be a biological component underlying this patient-reported outcome measure. ASPH (UniProt identifier Q12797) is a protein with two known isoforms: isoform 1 is involved in hydroxylation of Asp/Asn residues in specific epidermal growth factor–like domains [27] and isoform 8 is a membrane-bound calcium ion–sensing protein that is part of the endoplasmic reticulum (ER) [28]. The ER is the major protein synthesis site of the cell and disruption of normal ER homeostasis leads to a condition of physiological stress called ER stress [29]. ER stress precipitates an intracellular process termed unfolded protein response (UPR), which has the aim of re-establishing ER homeostasis and can result in either cell survival or death. Triggers for UPR activation include hypoxia, hypoglycaemia and genome instability, which are all physiological conditions that can be present during an active systemic inflammatory response [30–32], such as that of active RA. Subnetwork analysis also identified acute-phase response and acute inflammatory response as the most significant pathways implicated by the protein subnetwork, which would agree with current knowledge on RA pathophysiology [34] and would explain the significant associations between five proteins and future hsCRP measurements.

This study has a number of strengths, including the fact that it is a large, prospectively recruited cohort on a single bDMARD and analysis included 216 proteins shown to be differentially expressed between RA patients and HCs. SWATH-MS is a stable and reproducible method of proteomics acquisition, as it relies on destructive enzymatic digestion of proteins prior to MS and it is not limited by pre-selection of proteins of interest in the same way as proprietary multiplexed panels. The agreement with hsCRP values acquired using ELISA has been demonstrated from our data. A further strength of this study is the replication of associations of the proteins TCPH and MAP2K3 in an independent cohort.

However, there are a number of limitations. Multiple comparisons were performed, making the chance of false-positive findings higher, but significance thresholds were adjusted using the Benjamini–Hochberg correction in order to mitigate this. Only the 216 differentially expressed proteins were included in the analysis, but there may be proteins that are not significantly differentially expressed that correlate with treatment response. Given that SWATH-MS provides a permanent spectral map of all theoretical proteins in a biological sample, further proteins could be selected for testing in the future, based on previous or emerging reports of an association with treatment response, e.g. following subnetwork analysis. Finally, there was delay in sample processing for the majority of RA postal samples, but not HC samples. However, analysis of the delay to processing (data not presented) showed that inclusion of delay as a confounding variable did not affect the results. This may demonstrate utility in our findings, as they may be more likely to translate into the National Health Service (NHS), as our study protocol reflects delays in processing within the health service. Future replication of findings in an independent prospective cohort may lead to translation of predictive biomarkers of treatment response to etanercept into clinical practice.

In conclusion, in this longitudinal study of patients with RA we have identified candidate protein biomarkers of treatment response to etanercept measured using SWATH-MS, two of which were replicated in an independent dataset. Further validation and assessment of predictive utility will be required before translation into clinical practice.

Supplementary material

Supplementary material is available at *Rheumatology* online.

Data availability

Data are available upon request to the corresponding author.

Authors' contributions

S.L., J.B., A.W.M., J.D.I., A.G.W., K.L.H., A.B. and D.P. conceptualized and designed the study. S.L., C.F.Y. and N.N. analysed the data and wrote the manuscript with substantial contribution from all co-authors.

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Consistent safety profile with over 8 years of real-world evidence, across licensed indications[1–3]

Cosentyx® secukinumab

1,000,000 patients treated globally, and counting*[4]

100+ clinical trials*[5]

8+ years of real-world evidence[1–3]

8 indications[1–3]

Click here to visit our HCP portal and learn more

Real-world evidence shows a consistent safety profile over 6 years[6,7]

No trend toward increased AE rates over time (pooled PsA, AS, PsO):†[6]

AEs of select interest (EAIR per 100 PY)	1 year	2 years	3 years	4 years	5 years	6 years	Cumulative rate
Serious infections Cases	2.0 n=149	1.7 n=475	0.7 n=649	1.3 n=1,841	1.3 n=2,285	1.1 n=2,226	1.3 n=8,719
Malignant or unspecified tumours Cases	0.2 n=15	0.2 n=50	0.2 n=225	0.3 n=422	0.3 n=520	0.3 n=573	0.3 n=1,896
MACE Cases	0.2 n=15	0.1 n=39	0.2 n=151	0.2 n=238	0.2 n=264	0.1 n=287	0.2 n=1,031
Total IBD Cases	0.2 n=12	0.2 n=46	0.2 n=185	0.3 n=340	0.2 n=312	0.1 n=261	0.2 n=1,291
Exposure (PY)	7450	28,549	93,744	137,325	182,024	212,636	680,470

No trend towards increased rates of malignancy, MACE or IBD over time[6]

The most frequently reported adverse reactions are upper respiratory tract infections (17.1%) (most frequently nasopharyngitis, rhinitis).[1,2] Refer to the prescribing information for a summary of adverse events.

Adapted from Novartis Data on File. 2021.[6]

Refer to the Cosentyx Summary of Product Characteristics for full details, dosing and administration, including special populations.

Cosentyx® (secukinumab) licensed indications in rheumatology: Cosentyx, alone or in combination with methotrexate, is indicated for the treatment of active **psoriatic arthritis** in adult patients when the response to previous disease-modifying anti-rheumatic drug therapy has been inadequate; active **ankylosing spondylitis** in adults who have responded inadequately to conventional therapy; active **non-radiographic axial spondyloarthritis** with objective signs of inflammation as indicated by elevated C-reactive protein and/or magnetic resonance imaging evidence in adults who have responded inadequately to non-steroidal anti-inflammatory drugs; active **enthesitis-related arthritis** in patients 6 years and older (alone or in combination with methotrexate) whose disease has responded inadequately to, or who cannot tolerate conventional therapy; active **juvenile psoriatic arthritis** in patients 6 years or older (alone or in combination with methotrexate) whose disease has responded inadequately to, or who cannot tolerate, conventional therapy.[1,2]

Prescribing information, adverse event reporting and full indication can be found on the next page.

*Patients prescribed Cosentyx for any indication since launch.

†Successive time periods of PSUR shown with cumulative rate: 26 Dec 2014 to 25 Dec 2015; 26 Dec 2015 to 25 Dec 2016; 26 Dec 2016 to 25 Dec 2017; 26 Dec 2017 to 25 Dec 2018: 26 Dec 2018 to 25 Dec 2019; 26 Dec 2019 to 25 Dec 2020.[6]

Abbreviations: AE, adverse event; AS, ankylosing spondylitis; EIAR, exposure-adjusted incidence rate; HCP, healthcare professional; IBD, inflammatory bowel disease; MACE, major adverse cardiac event; PsA, psoriatic arthritis; PsO, plaque psoriasis; PY, patient year.

References: 1. Cosentyx® (secukinumab) GB Summary of Product Characteristics; **2.** Cosentyx® (secukinumab) NI Summary of Product Characteristics; **3.** European Medicines Agency. European public assessment report. Available at: https://www.ema.europa.eu/en/documents/overview/cosentyx-epar-medicine-overview_en.pdf [Accessed February 2024]; **4.** Novartis Data on File. Secukinumab – Sec008. 2023; **5.** Novartis. Novartis Cosentyx® positive 16-week PREVENT results advance potential new indication for patients with axial spondyloarthritis. Available at: https://www.novartis.com/news/media-releases/novartis-cosentyx-positive-16-week-prevent-results-advance-potential-new-indication-patients-axial-spondyloarthritis [Accessed February 2024]; **6.** Novartis data on file. Cosentyx Periodic Safety Update Report (PSUR); 26 December 2019 – 25 December 2020. 22 February 2021; **7.** Deodhar A, et al. Arthritis Res Ther 2019;21(1):111.

NOVARTIS

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Cosentyx® (secukinumab) Northern Ireland Prescribing Information.

Please refer to the Summary of Product Characteristics (SmPC) before prescribing.

Indications: Treatment of: moderate to severe plaque psoriasis in adults, children and adolescents from the age of 6 years who are candidates for systemic therapy; active psoriatic arthritis in adults (alone or in combination with methotrexate) who have responded inadequately to disease-modifying anti-rheumatic drug therapy; active ankylosing spondylitis in adults who have responded inadequately to conventional therapy; active non-radiographic axial spondyloarthritis (nr-axSpA) with objective signs of inflammation as indicated by elevated C-reactive protein (CRP) and/or magnetic resonance imaging (MRI) evidence in adults who have responded inadequately to non-steroidal anti-inflammatory drugs; active enthesitis-related arthritis and juvenile psoriatic arthritis in patients 6 years and older (alone or in combination with methotrexate) whose disease has responded inadequately to, or who cannot tolerate, conventional therapy; active moderate to severe hidradenitis suppurativa (acne inversa) in adults with an inadequate response to conventional systemic HS therapy. **Presentations:** Cosentyx 150 mg solution for injection in pre-filled pen; Cosentyx 300 mg solution for injection in pre-filled pen. **Dosage & Administration:** Administered by subcutaneous injection at weeks 0, 1, 2, 3 and 4, followed by monthly maintenance dosing. Consider discontinuation if no response after 16 weeks of treatment. Each 150 mg dose is given as one injection of 150 mg. Each 300 mg dose is given as two injections of 150 mg or one injection of 300 mg. If possible avoid areas of the skin showing psoriasis. *Plaque Psoriasis:* Adult recommended dose is 300 mg monthly. Based on clinical response, a maintenance dose of 300 mg every 2 weeks may provide additional benefit for patients with a body weight of 90 kg or higher. Adolescents and children from the age of 6 years: if weight ≥ 50 kg, recommended dose is 150 mg (may be increased to 300 mg as some patients may derive additional benefit from the higher dose). If weight < 50 kg, recommended dose is 75 mg. However, 150mg solution for injection in pre-filled pen is not indicated for administration of this dose and no suitable alternative formulation is available. *Psoriatic Arthritis:* For patients with concomitant moderate to severe plaque psoriasis see adult plaque psoriasis recommendation. For patients who are anti-TNFα inadequate responders, the recommended dose is 300 mg, 150 mg in other patients. Can be increased to 300 mg based on clinical response. *Ankylosing Spondylitis:* Recommended dose 150 mg. Can be increased to 300 mg based on clinical response. *nr-axSpA:* Recommended dose 150 mg. *Enthesitis-related arthritis and juvenile psoriatic arthritis:* From the age of 6 years, if weight ≥ 50 kg, recommended dose is 150 mg. If weight < 50 kg, recommended dose is 75 mg. However, 150mg solution for injection in pre-filled pen is not indicated for administration of this dose and no suitable alternative formulation is available. *Hidradenitis suppurativa:* Recommended dose is 300 mg monthly. Based on clinical response, the maintenance dose can be increased to 300 mg every 2 weeks. **Contraindications:** Hypersensitivity to the active substance or excipients. Clinically important, active infection. **Warnings & Precautions:** *Infections:* Potential to increase risk of infections; serious infections have been observed. Caution in patients with chronic infection or history of recurrent infection. Advise patients to seek medical advice if signs/symptoms of infection occur. Monitor patients with serious infection closely and do not administer Cosentyx until the infection resolves. Non-serious mucocutaneous candida infections were more frequently reported for secukinumab than placebo in the psoriasis clinical studies. Should not be given to patients with active tuberculosis (TB). Consider anti-tuberculosis therapy before starting Cosentyx in patients with latent TB. *Inflammatory bowel disease (including Crohn's disease and ulcerative colitis):* New cases or exacerbations of inflammatory bowel disease have been reported with secukinumab. Secukinumab, is not recommended in patients with inflammatory bowel disease. If a patient develops signs and symptoms of inflammatory bowel disease or experiences an exacerbation of pre-existing inflammatory bowel disease, secukinumab should be discontinued and appropriate medical management should be initiated. *Hypersensitivity reactions:* Rare cases of anaphylactic reactions have been observed. If an anaphylactic or serious allergic reactions occur, discontinue immediately and initiate appropriate therapy. *Vaccinations:* Do not give live vaccines concurrently with Cosentyx; inactivated or non-live vaccinations may be given. Paediatric patients should receive all age appropriate immunisations before treatment with Cosentyx. *Latex-Sensitive Individuals:* The removable needle cap of the 150mg pre-filled pen contains a derivative of natural rubber latex. *Concomitant immunosuppressive therapy:* Combination with immunosuppressants, including biologics, or phototherapy has not been evaluated in psoriasis studies. Cosentyx was given concomitantly with methotrexate, sulfasalazine and/or corticosteroids in arthritis studies. Caution when considering concomitant use of other immunosuppressants. **Interactions:** Live vaccines should not be given concurrently with secukinumab. No interaction between Cosentyx and midazolam (CYP3A4 substrate) seen in adult psoriasis study. No interaction between Cosentyx and methotrexate and/or corticosteroids seen in arthritis studies. **Fertility, pregnancy and lactation:** *Women of childbearing potential:* Use an effective method of contraception during and for at least 20 weeks after treatment. *Pregnancy:* Preferably avoid use of Cosentyx in pregnancy. *Breast feeding:* It is not known if secukinumab is excreted in human breast milk. A clinical decision should be made on continuation of breast feeding during Cosentyx treatment (and up to 20 weeks after discontinuation) based on benefit of breast feeding to the child and benefit of Cosentyx therapy to the woman. *Fertility:* Effect on human fertility not evaluated. **Adverse Reactions**: *Very Common* (≥1/10): Upper respiratory tract infection. *Common* (≥1/100 to <1/10): Oral herpes, headache, rhinorrhoea, diarrhoea, nausea, fatigue. *Uncommon (≥1/1,000 to <1/100):* Oral candidiasis, lower respiratory tract infections, neutropenia, inflammatory bowel disease. *Rare (≥1/10,000 to <1/1,000)*: anaphylactic reactions, exfoliative dermatitis (psoriasis patients), hypersensitivity vasculitis. *Not known:* Mucosal and cutaneous candidiasis (including oesophageal candidiasis). *Infections:* Most infections were non-serious and mild to moderate upper respiratory tract infections, e.g. nasopharyngitis, and did not necessitate treatment discontinuation. There was an increase in mucosal and cutaneous (including oesophageal) candidiasis, but cases were mild or moderate in severity, non-serious, responsive to standard treatment and did not necessitate treatment discontinuation. Serious infections occurred in a small proportion of patients (0.015 serious infections reported per patient year of follow up). *Neutropenia:* Neutropenia was more frequent with secukinumab than placebo, but most cases were mild, transient and reversible. Rare cases of neutropenia CTCAE Grade 4 were reported. *Hypersensitivity reactions*: Urticaria and rare cases of anaphylactic reactions were seen. *Immunogenicity:* Less than 1% of patients treated with Cosentyx developed antibodies to secukinumab up to 52 weeks of treatment. *Other Adverse Effects:* The list of adverse events is not exhaustive, please consult the SmPC for a detailed listing of all adverse events before prescribing. **Legal Category:** POM. **MA Number & List Price:** EU/1/14/980/005 - 150 mg pre-filled pen x2 £1,218.78; EU/1/14/980/010 – 300 mg pre-filled pen x 1 £1218.78. **PI Last Revised:** May 2023. Full prescribing information, (SmPC) is available from: Novartis Pharmaceuticals UK Limited, 2nd Floor, The WestWorks Building, White City Place, 195 Wood Lane, London, W12 7FQ. Telephone: (01276) 692255.

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Adverse Event Reporting:

Adverse events should be reported. Reporting forms and information can be found at www.mhra.gov.uk/yellowcard. Adverse events should also be reported to Novartis via uk.patientsafety@novartis.com or online through the pharmacovigilance intake (PVI) tool at www.novartis.com/report

If you have a question about the product, please contact Medical Information on 01276 698370 or by email at medinfo.uk@novartis.com

Cosentyx® (secukinumab) Great Britain Prescribing Information.

Please refer to the Summary of Product Characteristics (SmPC) before prescribing.

Indications: Treatment of: moderate to severe plaque psoriasis in adults, children and adolescents from the age of 6 years who are candidates for systemic therapy; active psoriatic arthritis in adults (alone or in combination with methotrexate) who have responded inadequately to disease-modifying anti-rheumatic drug therapy; active ankylosing spondylitis in adults who have responded inadequately to conventional therapy; active non-radiographic axial spondyloarthritis (nr-axSpA) with objective signs of inflammation as indicated by elevated C-reactive protein (CRP) and/or magnetic resonance imaging (MRI) evidence in adults who have responded inadequately to non-steroidal anti-inflammatory drugs; active enthesitis-related arthritis and juvenile psoriatic arthritis in patients 6 years and older (alone or in combination with methotrexate) whose disease has responded inadequately to, or who cannot tolerate, conventional therapy; active moderate to severe hidradenitis suppurativa (acne inversa) in adults with an inadequate response to conventional systemic HS therapy. **Presentations:** Cosentyx 75 mg solution for injection in pre-filled syringe; Cosentyx 150 mg solution for injection in pre-filled syringe; Cosentyx 150 mg solution for injection in pre-filled pen; Cosentyx 300 mg solution for injection in pre-filled pen. **Dosage & Administration:** Administered by subcutaneous injection at weeks 0, 1, 2, 3 and 4, followed by monthly maintenance dosing. Consider discontinuation if no response after 16 weeks of treatment. Each 75 mg dose is given as one injection of 75 mg. Each 150 mg dose is given as one injection of 150 mg. Each 300 mg dose is given as two injections of 150 mg or one injection of 300 mg. If possible avoid areas of the skin showing psoriasis. *Plaque Psoriasis:* Adult recommended dose is 300 mg. Based on clinical response, a maintenance dose of 300 mg every 2 weeks may provide additional benefit for patients with a body weight of 90 kg or higher. Adolescents and children from the age of 6 years: if weight ≥ 50 kg, recommended dose is 150 mg (may be increased to 300 mg as some patients may derive additional benefit from the higher dose). If weight < 50 kg, recommended dose is 75 mg. *Psoriatic Arthritis:* For patients with concomitant moderate to severe plaque psoriasis see adult plaque psoriasis recommendation. 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If you have a question about the product, please contact Medical Information on 01276 698370 or by email at medinfo.uk@novartis.com