

Identification and Evaluation of Serum Protein Biomarkers That Differentiate Psoriatic Arthritis From Rheumatoid Arthritis

Angela Mc Ardle,¹  Anna Kwasnik,¹ Agnes Szentpetery,¹ Belinda Hernandez,² Andrew Parnell,¹ Wilco de Jager,³ Sytze de Rook,⁴ Oliver FitzGerald,¹ and Stephen R. Pennington¹

Objective. To identify serum protein biomarkers that might distinguish patients with early inflammatory arthritis (IA) with psoriatic arthritis (PsA) from those with rheumatoid arthritis (RA) and may be used to support appropriate early intervention.

Methods. The serum proteome of patients with PsA and patients with RA was interrogated using nano-liquid chromatography mass spectrometry (nano-LC-MS/MS) (n = 64 patients), an aptamer-based assay (SomaScan) targeting 1,129 proteins (n = 36 patients), and a multiplexed antibody assay (Luminex) for 48 proteins (n = 64 patients). Multiple reaction monitoring (MRM) assays were developed to evaluate the performance of putative markers using the discovery cohort (n = 60 patients) and subsequently an independent cohort of PsA and RA patients (n = 167).

Results. Multivariate machine learning analysis of the protein discovery data from the 3 platforms revealed that it was possible to differentiate PsA patients from RA patients with an area under the curve (AUC) of 0.94 for nano-LC-MS/MS, 0.69 for bead-based immunoassay measurements, and 0.73 for aptamer-based analysis. Subsequently, in the separate verification and evaluation studies, random forest models revealed that a subset of proteins measured by MRM could differentiate PsA and RA patients with AUCs of 0.79 and 0.85, respectively.

Conclusion. We present a serum protein biomarker panel that can separate patients with early-onset IA with PsA from those with RA. With continued evaluation and refinement using additional and larger patient cohorts, including those with other arthropathies, we suggest that the panel identified here could contribute to improved clinical decision making.

INTRODUCTION

Psoriatic arthritis (PsA) is a form of inflammatory arthritis (IA) affecting ~0.25% of the population (1–4). It is a highly heterogeneous disorder associated with joint damage, disability, disfiguring skin disease, and poor patient-related quality of life outcome measures (4). Inherently irreversible and frequently progressive, the process of joint damage begins at or before the clinical onset of disease. Indeed, structural joint damage, which is likely to result in joint deformity and disability, is present in 47% of patients within 2 years of disease onset (3,5). Reductions in

quality of life and physical function are comparable to those in rheumatoid arthritis (RA) and are compounded by the presence of chronic disfiguring skin disease (6–9). Direct and indirect health costs pose a significant economic burden on society and increase with severe physical dysfunction (9).

Early diagnosis and management of PsA leads to better long-term outcomes; however, with no diagnostic laboratory test available, the diagnosis is often delayed or missed, and this has significant consequences for individuals with PsA (10–12). At disease onset, PsA often resembles other forms of arthritis including RA. Despite the clinical similarities between PsA and RA, their distinctive

Supported by the European Commission under the EU FP7 Programme Monitoring Innate Immunity in Arthritis and Mucosal Inflammation (MIAMI) (project grant 305266) and the Health Research Board (grant HRA-HSR-2015-1284). The University College Dublin Conway Institute is supported by the Higher Education Authority of Ireland.

¹Angela Mc Ardle, PhD, Anna Kwasnik, PhD, Agnes Szentpetery, MD, Andrew Parnell, PhD, Oliver FitzGerald, MBBCh, BAO, MRCPI, MRCP, MD, Stephen R. Pennington, PhD: University College Dublin, Dublin, Ireland; ²Belinda Hernandez, PhD: Trinity College Dublin and University College Dublin, Dublin, Ireland; ³Wilco de Jager, PhD: Wilhelmina Children Hospital and University Medical Centre Utrecht, Utrecht, The Netherlands;

⁴Sytze de Rook, PhD: University Medical Centre Utrecht, Utrecht, The Netherlands.

Drs. FitzGerald and Pennington contributed equally to this work.

Dr. Pennington owns stock or stock options in Atturos. No other disclosures relevant to this article were reported.

Address correspondence to Oliver FitzGerald, MBBCh, BAO, MRCPI, MRCP, MD, or Stephen R. Pennington, PhD, University College Dublin, UCD Conway Institute of Biomolecular and Biomedical Research, Dublin, Ireland. Email: oliver.fitzgerald@ucd.ie or Stephen.Pennington@ucd.ie.

Submitted for publication December 8, 2020; accepted in revised form June 8, 2021.

pathologic manifestations often require different treatments. For example, drugs targeting the interleukin-12 (IL-12)/IL-23 and IL-17 pathways, which are highly effective in psoriasis and PsA, are ineffective in RA, while drugs targeting B cells such as rituximab are effective in RA but have not been proven beneficial in PsA (4,13).

PsA is most often diagnosed when a patient presents with musculoskeletal inflammation in the presence of psoriasis and in the absence of rheumatoid factor (RF). However, a clear diagnosis can be difficult, as up to 10% of PsA patients may have RF or anti-citrullinated peptide antibody (ACPA), and joint involvement may precede the development of skin or nail psoriasis in 15% of patients with PsA (14). The Classification of Psoriatic Arthritis (CASPAR) Study Group criteria are accepted as having high sensitivity (98.7%) and specificity (91.4%) in classifying patients with longstanding PsA (15). CASPAR criteria show reduced sensitivity in patients with early disease (87.4%), though specificity is improved (99.1%) (16). CASPAR criteria are valid when including patients in research studies or in clinical trials, but it is recognized that they should not be used for diagnosis and are of little value therefore in a primary care or dermatology setting where specialist rheumatologic expertise is very often not readily available (4,17). An effective clinical laboratory test is needed to improve diagnosis and clinical decision making in PsA.

Ideally, a clinical laboratory test should be based on an easily accessible biologic sample such as blood (10), and we therefore set out to discover serum-based biomarkers that could discriminate between patients with PsA and those with RA. With advances in multiplexed technologies, it has become possible to simultaneously measure multiple analytes. However, in complex biologic fluids such as serum, it is apparent that no single technological platform is capable of measuring the entire protein content of a given sample (3,4,18). For this reason, we undertook a comprehensive and complementary analysis of the serum proteome in a cohort of patients with early IA. We used unbiased nano-liquid chromatography mass spectrometry (nano-LC-MS/MS) for serum samples depleted of abundant proteins to identify differentially expressed proteins. In parallel, aptamer-based and bead-based multiplexed assays were used to target low-abundant proteins not easily detectable by nano-LC-MS/MS. Statistical analysis revealed that proteins identified by nano-LC-MS/MS were the most useful in differentiating individuals with PsA from those with RA. Therefore, in subsequent steps we prioritized these proteins for further investigation.

The translation of biomarkers from discovery to clinical use poses many challenges, not least because of the difficulty of confidently identifying suitable candidates from the discovery phase. Multiple reaction monitoring (MRM), a form of targeted MS, is a highly versatile approach that makes it relatively easy to develop and adapt assays that support the simultaneous measurement of multiple proteins. Assay development times are typically much shorter for MRM assays compared to enzyme-linked immunosorbent assays (ELISAs), and multiplexing of MRM assays

is significantly easier. We therefore exploited the advantages of MRM to undertake a 2-phase approach to progress the candidate protein biomarkers identified in the nano-LC-MS/MS discovery study. First, we undertook a verification phase in which MRM assays for a panel of 150 candidate biomarker proteins identified in the discovery cohort were developed and used to measure protein levels in patients from that cohort; in a second evaluation phase, we adapted the MRM assay to encompass an expanded panel of 173 proteins and used this to measure the proteins in an independent cohort. Figure 1 provides an overview of the study workflow.

PATIENTS AND METHODS

Patients. In the discovery and initial verification phases, a total of 64 patient samples were used, and the extensive clinical characterization of the cohort has previously been described in full by Szentpetery et al (19). Briefly, patients ages 18 to 80 years with recent-onset (symptom duration <12 months), treatment-naive PsA or RA with active joint inflammation were enrolled. PsA patients ($n = 32$) fulfilled the CASPAR criteria (15), and patients with RA ($n = 32$) met the American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) 2010 classification criteria (20). Baseline serum samples were obtained from each patient using standard methodology, aliquotted, and frozen at -80°C (see Supplementary Document 1 on the *Arthritis & Rheumatology* website at <http://onlinelibrary.wiley.com/doi/10.1002/art.41899/abstract>). The study was approved by St. Vincent's Healthcare Group Ethics and Medical Research Committee, and patients were enrolled only after agreeing to participate in the study and providing informed consent.

Samples from a total of 167 patients were used in the second verification phase. There were 95 patients recruited from a cross-sectional cohort of patients with established PsA who all met CASPAR criteria and 72 patients recruited from the RA Biologics Registry of Ireland who all met ACR/EULAR 2010 classification criteria and had similar levels of active disease as the PsA patients. Again, baseline serum samples were obtained, aliquotted, and frozen at -70°C .

Label-free nano-LC-MS/MS analysis. A detailed description of the unbiased LC-MS/MS workflow has previously been described (10). Briefly, serum samples ($1,700\ \mu\text{g}$) were depleted of the 14 most abundant serum proteins (albumin, transferrin, haptoglobin, IgG, IgA, α_1 -antitrypsin, fibrinogen, β_2 -macroglobulin, α_1 -acid glycoprotein, complement C3, IgM, apolipoprotein A-I, apolipoprotein A-II, and transthyretin) using the Agilent Multiple Affinity Removal System comprising a Hu-14 column (HuMARS14) ($4.6 \times 100\ \text{mm}$; Agilent Technologies, no. 5188-6557) on a Biocad Vision Workstation. Depleted fractions (containing $50\ \mu\text{g}$ protein) were reduced, denatured, and alkylated prior to trypsinization. The digested samples were desalted and purified using C18 resin

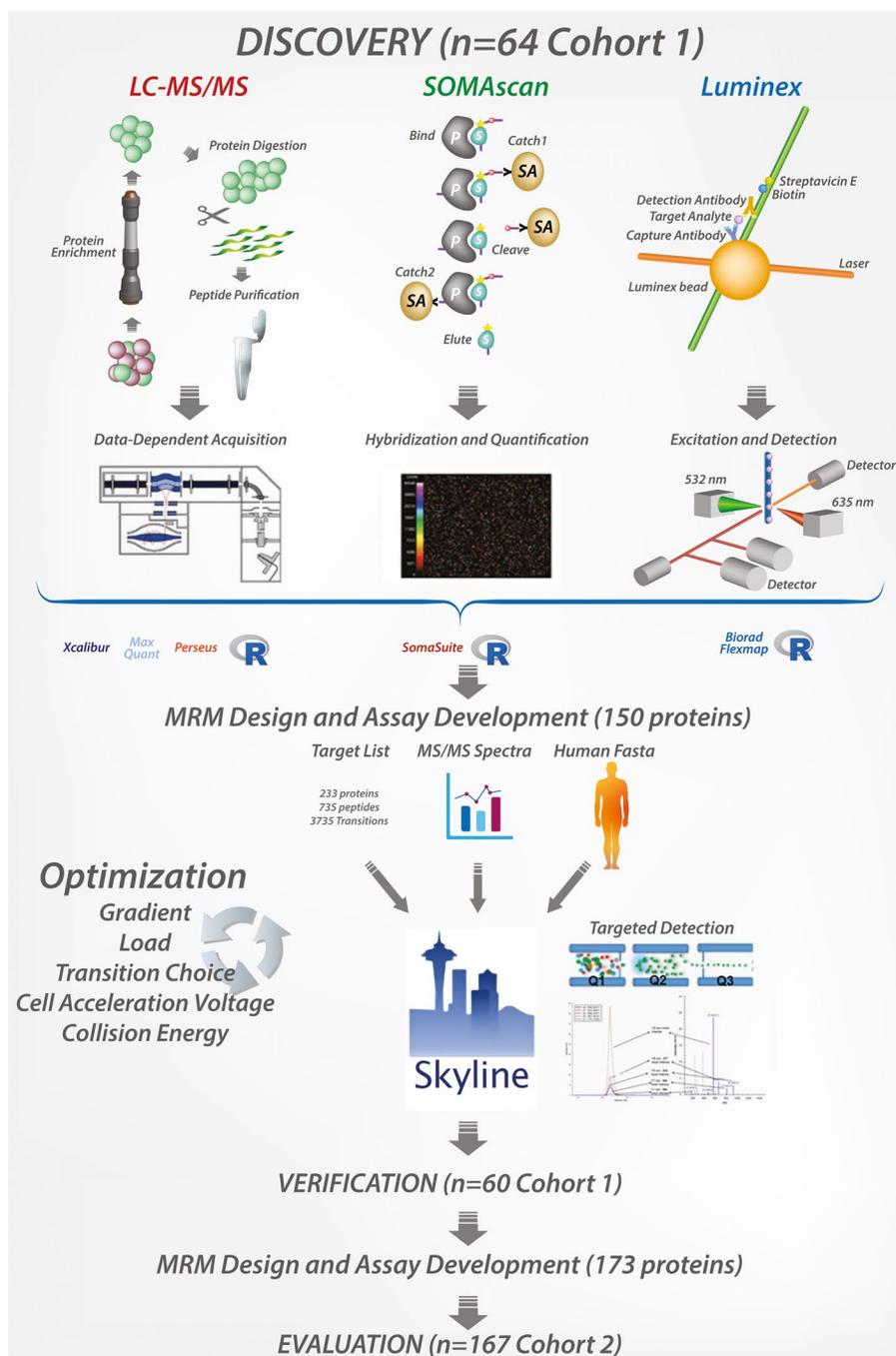


Figure 1. Overview of the experimental workflow. Three platforms were used: nano-liquid chromatography mass spectrometry (nano-LC-MS/MS), aptamer-based immunoassays, and bead-based immunoassays for biomarker discovery. Resulting data were analyzed by univariate and multivariate analysis. Putative biomarkers identified by nano-LC-MS/MS proteins were brought forward for multiple reaction monitoring (MRM) assay development, which was divided into 2 phases. During phase I, it was possible to develop an assay for 150 proteins which were measured in the discovery cohort. During phase II, an assay was developed for 173 proteins which were measured in an independent evaluation cohort. SA = streptavidin.

pipette stage tips. Purified samples were dried under vacuum and resuspended in MS-compatible buffer A (3% acetonitrile, 0.1% formic acid) (21,22). Label-free nano-LC-MS/MS analysis was performed on a Q Exactive mass spectrometer equipped with a Dionex Ultimate 3000 (RSLCnano) chromatography system (ThermoFisher Scientific). Two microliters (equivalent to 2 μ g of digested protein) of each sample were injected onto a fused

silica emitter separated by an increasing acetonitrile gradient over 101.5 minutes (flow rate 250 nl/minute) (10).

Bioinformatic data analysis. As previously reported, nano-LC-MS/MS data were visually inspected using XCalibur software (version 2.2 SP1.48). MaxQuant (version 1.4.12) was then used for quantitative analysis of the LC-MS/MS data, while

Perseus software (version 1.5.0.9) supported statistical analysis (10,23).

Aptamer-based analysis. Individual patient serum samples were subjected to a multiplexed aptamer-based assay developed by Gold et al to measure the levels of 1,129 proteins, as previously reported (10).

Bead-based immunoassay. Individual serum samples were subjected to in-house-developed and validated multiplexed immunoassays measuring 48 analytes. The assays and analyses were undertaken, as previously described, at the Multiplex Core Facility Laboratory of Translational Immunology at the University Medical Centre Utrecht (10).

MRM design and optimization. The development and optimization of MRM assays was performed using Skyline software (version 3.6.0.1062) (MacCoss Lab) (24). Assays for prototypic peptides were developed for all proteins of interest where peptides showed no missed cleavages or “ragged ends” and sequence length was between 7 and 25 amino acids. When possible, peptide sequences with reactive cysteine or methionine residues were avoided but not excluded. An MRM assay was deemed to be analytically validated when it demonstrated the following characteristics: dot product ≥ 0.8 , signal to noise ≥ 10 , data points under the curve ≥ 10 (25), and percentage coefficient of variance showing a retention time $\leq 1\%$ and area $\leq 20\%$ (26). The majority of MRM assays developed significantly exceeded these criteria.

Sample preparation for LC-MRM analysis. *Verification phase.* Crude serum (2 μ l) was added to the wells of 96-well deep well plates (ThermoFisher Scientific) and diluted at 1:50 with NH_4CO_3 (Sigma). RapiGest denaturant (Waters) was resuspended in 50 mM NH_4CO_3 to give a stock solution of 0.1% weight/volume, and 50 μ l of this stock solution was added to each sample so that the final concentration of RapiGest was 0.05%. Plates were covered with adhesive foil (ThermoFisher Scientific), and samples were incubated in the dark at 80°C for 10 minutes. After incubation, plates were centrifuged at 2,000 relative centrifugal force (rcf) at 4°C for 2 minutes to condense droplets. Subsequently, dithiothreitol (DTT) was added to each sample at a final concentration of 20 mM. Samples were then incubated at 60°C for 1 hour followed by centrifugation at 2,000 rcf at 4°C for 2 minutes.

Next, iodoacetamide was added to each sample to give a final concentration of 10 mM, and plates were incubated at 37°C in the dark for 30 minutes. Plates were again centrifuged at 2,000 rcf at 4°C for 2 minutes, and samples were then diluted with LC-MS/MS-grade H_2O to produce a final concentration of 25 mM NH_4CO_3 . Trypsin (Promega) was added to each sample so that the protein:enzyme ratio was 25:1. The reaction was stopped with

the addition of 2 μ l of neat trifluoroacetic acid (Sigma) to each sample and incubated for a further 30 minutes at 37°C. In order to pellet RapiGest, digests were transferred from 96-well plates to 1.5 ml low-bind Eppendorf tubes and centrifuged for 30 minutes at 12,000 rcf. Supernatants were removed and transferred into clean Eppendorf tubes and lyophilized by speed vacuum at 30°C for 2 hours. Lyophilized samples were stored at -80°C until further use.

Evaluation phase. The denaturant used previously (RapiGest) was substituted with 25 μ l denaturant solution comprising 50% trifluoroethanol in 50 mM NH_4HCO_3 with 10 mM DTT, and this mitigated the need for the high-speed spin and transfer of supernatant, which represented an additional processing step less compatible with 96-well plate workflows.

MRM analysis. MRM analysis was performed using an Agilent 6495A triple-quadrupole mass spectrometer with Jet-Stream electrospray source (Agilent) coupled to a 1290 Quaternary Pump HPLC system. Peptides were separated using analytical Zorbax Eclipse Plus C18 (rapid resolution HT 2.1 \times 50 mm, 1.8 μ m, 600-bar columns) (Agilent) before introduction to the triple-quadrupole mass spectrometer. A linear gradient of acetonitrile (99.9% acetonitrile, 0.1% formic acid) 3–75% over 17 minutes was applied at a flow rate of 0.400 μ l/minute with a column oven temperature of 50°C. Source parameters were as follows: gas temperature 150°C, gas flow 15 liters/minute, nebulizer psi 30, sheath gas temp 200°C, and sheath gas flow 11 liters/minute. Peptide retention times and optimized collision energies were supplied to MassHunter (B0.08; Agilent Technologies) to establish a dynamic MRM-scheduled method based on input parameters of 800-msec cycle times and 2-minute retention time windows. The percentage coefficient of variation (%CV) of biologic and technical replicates was used as a measure of variance and was calculated using the following standard calculation: $\%CV = (\text{SD}/\text{mean}) \times 100$.

ELISA analysis. C-reactive protein (CRP) levels were evaluated at St. Vincent's University Hospital using an automated CRPL3 Tina-quant assay (Roche Diagnostics).

Statistical analysis. GraphPad Prism software package (version 7.00) was used to investigate the statistical significance of bead-based immunoassay data, while SomaSuite (version 1.0) was used to analyze aptamer-based assay data. The ability of quantified proteins/peptides to predict the diagnosis (PSa or RA) for individual patients was assessed using the random forest package in R (version 3.3.2). The most important variables in providing the receiver operating characteristic (ROC) area under the curve (AUC) were selected using the variable importance index, and the Gini decrease in impurity was used to assess the importance of each variable. All AUC values were obtained using the ROC R package.

Table 1. Baseline demographic and clinical characteristics of patients in the discovery, verification, and evaluation cohorts*

	Discovery and biomarker verification cohort			Independent cohort for biomarker evaluation		
	Total (n = 64)	PsA (n = 32)	RA (n = 32)	Total (n = 167)	PsA (n = 95)	RA (n = 72)
Age	43.6 ± 13.3	39.6 ± 11.14†	47.7 ± 14.1	53 ± 8.1	52 ± 6.6	55 ± 9.6
Female, no. (%)	37 (58)	15 (47)	22 (69)	89 (53)	51 (54)	38 (53)
Anti-CCP positive, no. (%)	33 (52)	0	26 (81)	49 (29)	1 (1)	48 (67)
RF positive, no. (%)	25 (39)	0	25 (78)	50 (30)	3 (3)	47 (65)
ESR, mm/hour	19.4 ± 16.8	12.0 ± 8.1‡	26.7 ± 20.0	NA	NA	NA
CRP, mg/liter (normal <5)	14.4 ± 19.8	6.6 ± 8.3‡	22.2 ± 24.6	24.9 ± 30.6	28.2 ± 27.8§	20 ± 34.0
DAS28-CRP, median (IQR)	4.2 (1.66–6.88)	3.7 (2.1–5.8)	4.9 (1.7–6.9)	NA¶	NA¶	4.2 (1.1–7.6)
TJC, median (IQR) (range 0–28)	6 (0–23)	4 (0–20)#	8.5 (0–23)	NA¶	10.4 (0–38)¶	8.2 (0–28)
SJC, median (IQR) (range 0–28)	2 (0–12)	1 (0–5)‡	3.5 (0–12)	NA¶	7.2 (0–25)¶	5.2 (0–24)
Dactylitis, no. (%)	NA	10 (31)	NA	NA¶	44 (46.3)	NA
BMI, kg/m ²	28.1 ± 6.3	27.97 ± 6.3	28.24 ± 6.3	28.0 ± 8.6	30.0 ± 10.6‡	27.2 ± 5.1
PASI, median (range)	NA	3.35 (0–27.7)	NA	NA	2.2 (0–14)	NA

* Except where indicated otherwise, values are the mean ± SD. anti-CCP = anti-cyclic citrullinated peptide; RF = rheumatoid factor; ESR = erythrocyte sedimentation rate; NA = not available; IQR = interquartile range; BMI = body mass index; PASI = Psoriasis Area and Severity Index.

† $P < 0.05$ versus rheumatoid arthritis (RA) patients.

‡ $P < 0.01$ versus RA patients.

§ $P < 0.0001$ versus RA patients.

¶ For the validation cohort, 68 and 66 joints were counted for the tender joint count (TJC) and swollen joint count (SJC), respectively, in the psoriatic arthritis (PsA) group, and therefore the Disease Activity Score in 28 joints using the C-reactive protein level (DAS28-CRP) could not be calculated.

$P < 0.001$ versus RA patients.

RESULTS

Patient sample characterization and study design.

For the discovery of novel candidate protein biomarkers, serum samples were collected at baseline from patients with early-onset, treatment-naïve PsA ($n = 32$) and those with early-onset, treatment-naïve RA ($n = 32$). Samples from a second independent cohort (PsA, $n = 95$; RA, $n = 72$) were used to confirm the performance of the putative markers identified during discovery. While these PsA and RA patients may have been receiving treatment at the time of baseline serum sampling, there were similar levels of active disease (as reflected by CRP level, erythrocyte sedimentation rate [ESR], and joint counts) in both patient groups. Key demographic and clinical characteristics for all patients are summarized in Table 1.

Unbiased nano-LC-MS/MS-based protein analysis.

To investigate differential serum protein expression between patients with PsA and those with RA, individual serum samples that had been depleted of high-abundance serum proteins were analyzed by nano-LC-MS/MS using a Q Exactive Hybrid Quadrupole-Orbitrap mass spectrometer. A total of 451 proteins were identified, of which 121 were identified in all 64 individual serum samples. Univariate analysis was applied to the 121 commonly identified proteins, and multivariate analysis was applied to the complete data set. Univariate analysis (Student's t -test using a Benjamini-Hochberg false discovery rate of 0.01) showed that 66 proteins were significantly differentially expressed between PsA and RA (Supplementary Table 1, <http://onlinelibrary.wiley.com/doi/10.1002/art.41899/abstract>). Unsupervised hierarchical cluster and principal components analysis performed using these

66 proteins revealed the overall differences/similarities between serum protein levels in the individual PsA and RA patients; clear within-group clustering and between group separations were observed (Figure 2). Random forest analysis of data from 451 proteins identified in the 64 patient samples demonstrated that patients with PsA and those with RA could be differentiated with an AUC of 0.94 (Table 2) (ROC plot in Supplementary Figure 1A, <http://onlinelibrary.wiley.com/doi/10.1002/art.41899/abstract>).

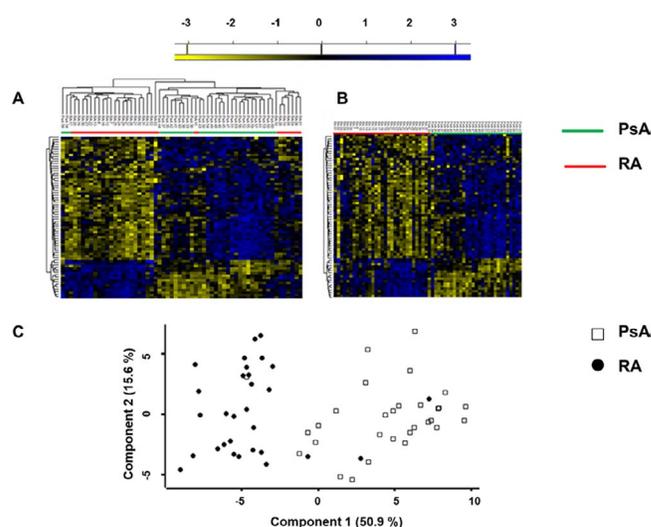


Figure 2. Association of protein signatures with diagnosis of psoriatic arthritis (PsA) or rheumatoid arthritis (RA). **A**, Unsupervised hierarchical cluster analysis. **B**, Supervised hierarchical cluster analysis. **C**, Principal components analysis. Plots were generated for differentially expressed proteins between PsA patients ($n = 30$) and RA patients ($n = 30$). $P < 0.01$ by Benjamin-Hochberg false discovery rate.

Table 2. Determination of protein signatures to predict diagnosis in patients with early PsA and those with RA*

Platform	No.	Correctly predicted/total	AUC
LC-MS/MS	60	55/60	0.94
Aptamer-based immunoassay	36	26/36	0.73
Bead-based immunoassay	64	43/64	0.69

* Area under the curve (AUC) values were generated using predicted probabilities from the random forest model used to discriminate between the groups. PsA = psoriatic arthritis; RA = rheumatoid arthritis; LC-MS/MS = liquid chromatography mass spectrometry.

Taken together, these data strongly suggest that there is a difference in the serum protein profiles between newly diagnosed PsA patients and RA patients. The top 50 proteins providing the AUC are listed in Supplementary Table 2 (<http://onlinelibrary.wiley.com/doi/10.1002/art.41899/abstract>).

Aptamer- and bead-based targeted protein analysis. To extend the breadth and depth of proteome coverage afforded by nano-LC-MS/MS, serum samples were subjected to analysis using 2 complementary protein measurement platforms. Aptamer-based analysis supported the quantification of 1,129 proteins in a subset of the patient samples for PsA ($n = 18$) and RA ($n = 18$). Univariate analysis revealed that 175 proteins were significantly differentially expressed between PsA and RA patients (Supplementary Table 3, <http://onlinelibrary.wiley.com/doi/10.1002/art.41899/abstract>). Multivariate analysis of the data obtained from the aptamer-based analysis revealed that it was possible to discriminate PsA from RA with an AUC of 0.73 (Table 2) (ROC plot in Supplementary Figure 1B, <http://onlinelibrary.wiley.com/doi/10.1002/art.41899/abstract>).

Based largely on their known importance in PsA and RA (3), 48 proteins were selected for analysis using in-house-developed multiplexed bead-based immunoassays (10). Of the 48 proteins targeted, 23 were identified in every sample. *T*-tests revealed that 4 proteins (IL-18 [$P \leq 0.001$], IL-18 binding protein [$P \leq 0.05$], hepatocyte growth factor [$P \leq 0.05$], and tumor necrosis factor receptor superfamily member 6 [$P \leq 0.05$]) were differentially expressed between PsA and RA samples (Supplementary Figure 2, <http://onlinelibrary.wiley.com/doi/10.1002/art.41899/abstract>). Random forest analysis of the bead-based immunoassay data showed that patients could be segregated with an AUC of 0.69 (Table 2 and Supplementary Figure 1C). Compared to the nano-LC-MS/MS analysis, the candidate protein biomarker discovery by both aptamer-based and bead-based assays yielded data sets with reduced predictive power, and therefore the subsequent evaluation process was streamlined to focus only on proteins identified by nano-LC-MS/MS.

LC-MRM verification of nano-LC-MS/MS-identified biomarkers. MRM is a targeted MS technology that is increasingly used to support candidate biomarker evaluation following LC-MS/MS and other protein discovery approaches. Both the cost

of MRM analysis and the time required to develop and optimize MRM assays are considerably less than antibody-based methods (27). For these and other reasons, MRM-based measurement of the nano-LC-MS/MS-identified proteins represents an attractive approach for verification and evaluation of their biomarker performance. The multiplexing capabilities afforded by MRM facilitated the development of an assay that included the top-ranking discriminatory candidate proteins from univariate and multivariate analysis of the nano-LC-MS/MS discovery data described above, but also allowed for the inclusion of additional proteins identified previously during studies of pooled patient samples (data not shown). A total of 233 proteins represented by 735 peptides and 3,735 transitions (5 per peptide) were brought forward for MRM assay development. Of the 233 proteins brought forward, it was possible to develop assays for 150 of them, represented by 299 peptides. The remaining candidates could not be detected reproducibly in crude serum. Of the 50 proteins listed in Supplementary Table 2, 33 were included in the assay.

This MRM assay panel was then used to measure the candidate proteins in 60 patient samples from the discovery cohort. It is noteworthy that to minimize any technical bias, both the pre-analytical processing and MRM analysis were undertaken in a randomized manner. Random forest analysis revealed that using this MRM assay panel it was possible to distinguish PsA from RA with an AUC of 0.79 (Figure 3A). While this initial work was in progress, we independently found an additional 23 candidate biomarker proteins to be capable of identifying other forms of IA (28). MRM assays for these proteins were developed and added to the initial MRM assay panel, yielding a new total number of proteins of 173 (represented by 334 peptides). This expanded panel was used to measure candidate proteins in an independent evaluation cohort of 95 PsA patients and 72 RA patients (Table 1). Seven synthetic isotopically labeled (SIL) peptides were incorporated into the assay to control for potential analytical variation. Summed intensity values from the SIL peptides were used to normalize patient data. Random forest analysis revealed that PsA patients could be separated from those with RA with an AUC of 0.85 (Figure 3B). The proteins ranked as most important in providing the AUC values are reported in Supplementary Table 4 (<http://onlinelibrary.wiley.com/doi/10.1002/art.41899/abstract>).

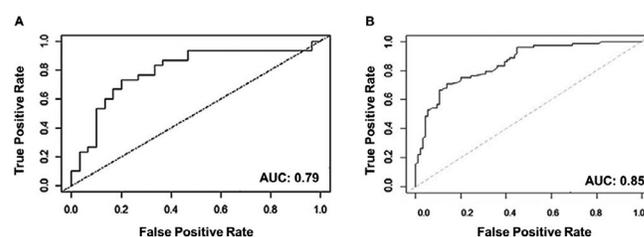


Figure 3. Receiver operating characteristic curve for performance of protein signatures in the discovery cohort ($n = 30$ psoriatic arthritis [PsA] patients and 30 rheumatoid arthritis [RA] patients) (A) and in the independent evaluation cohort ($n = 95$ PsA patients and 72 RA patients) (B). AUC = area under the curve.

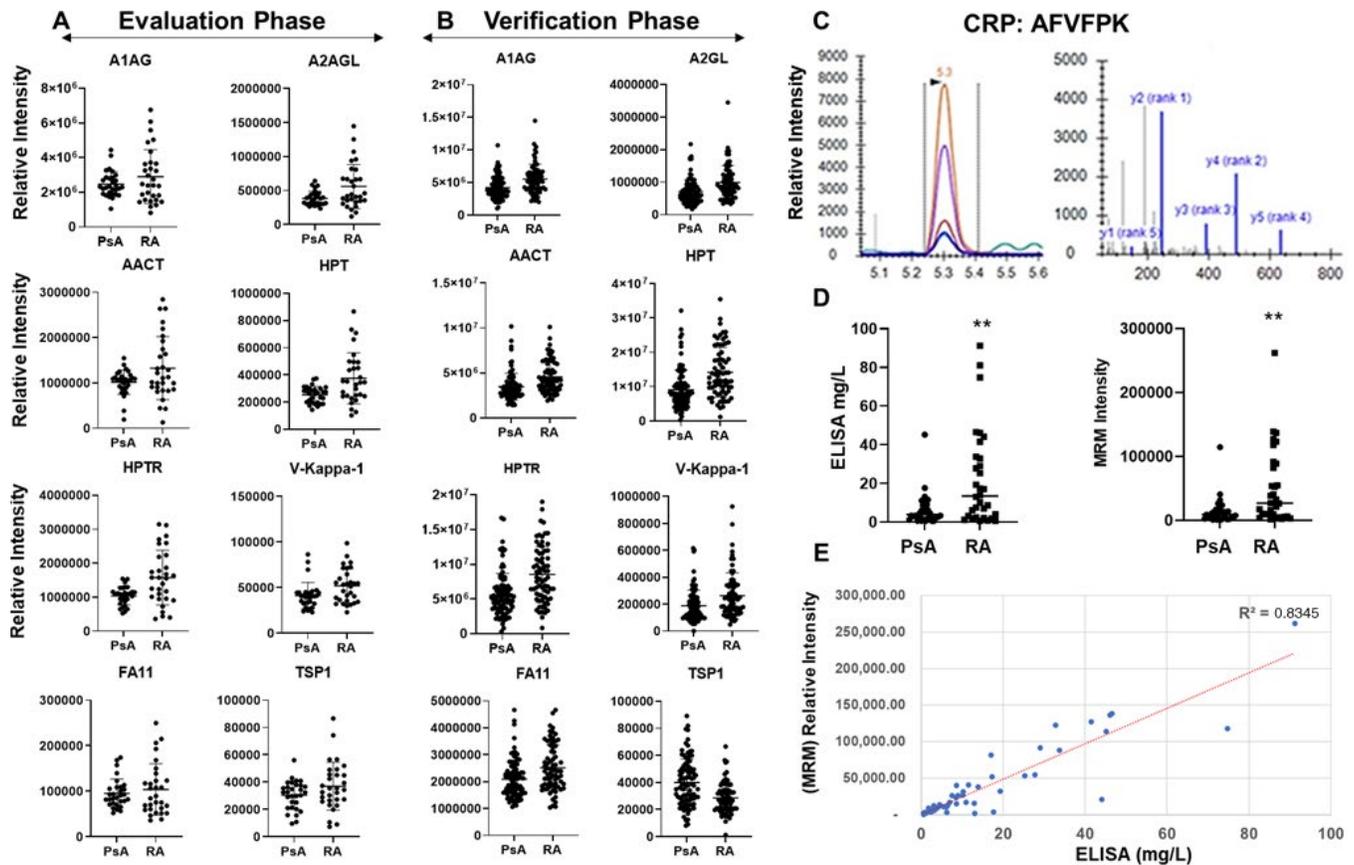


Figure 4. Protein expression changes in PsA and RA, as measured by multiple reaction monitoring (MRM). Eight proteins contributing to the AUC generated during target biomarker verification (AUC 0.79) and evaluation (AUC 0.85) show concordant expression changes in independent cohorts. **A**, During the initial verification phase, α 1-acid glycoprotein 1 (A1AG), coagulation factor XI (FA11), and thrombospondin 1 (TSP-1) were not significantly differently expressed between PsA and RA patients. Proteins α 2-glycoprotein (A2AGL) ($P < 0.006$), α 1-antichymotrypsin (AACT) ($P < 0.020$), haptoglobin (HPT) ($P < 0.001$), and haptoglobin-related protein (HPTR) ($P < 0.015$) were significantly up-regulated in RA. **B**, During a subsequent evaluation phase, α 1-acid glycoprotein 1 ($P < 0.00001$), α 2-glycoprotein ($P < 0.00001$), α 1-antichymotrypsin ($P < 0.00001$), haptoglobin ($P < 0.0001$), haptoglobin-related protein ($P < 0.00001$), $V_{\kappa}1$ ($P < 0.0001$), and coagulation factor XI were significantly up-regulated in RA, while TSP-1 was significantly up-regulated in PsA ($P < 0.00001$). **C**, MRM and mass spectrometry spectrum for C-reactive protein (CRP) levels are shown. **D**, CRP levels analyzed by enzyme-linked immunosorbent assay (ELISA) ($P \leq 0.009$) and MRM ($P \leq 0.006$) are shown. **E**, Pearson's correlation between ELISA and MRM measurements of CRP levels ($R^2 = 0.8345$) is shown. See Figure 3 for other definitions.

The data demonstrate clear overlap between proteins used to distinguish PsA patients from RA patients included in the discovery and verification cohorts. The differential expression levels of these overlapping proteins are illustrated in Figure 4. To this end, α 2-HS glycoprotein, α 1-antichymotrypsin, haptoglobin, haptoglobin-related protein, and RF C6 light chain ($V_{\kappa}1$) were found to be significantly up-regulated in RA patients compared to PsA patients when measured by MRM. Alpha-1-acid glycoprotein and coagulation factor XI were also found to be up-regulated in RA compared to PsA during both biomarker verification and the evaluation phase, but the observation only reached significance during the evaluation phase. This highlights the value in developing MRM assays for large panels of candidate proteins and evaluating them using additional independent patient cohorts. In the case of thrombospondin 1 (TSP-1), the protein was found to be slightly up-regulated in RA patients during verification in the initial discovery

cohort but was significantly up-regulated in PsA patients during the subsequent validation stage. It is evident that the potential PsA versus RA discriminatory role of this protein will require continued evaluation using additional independent cohorts.

Taken together, these observations provide support for the strategy we adopted, i.e., to use discovery experiments to generate an extensive panel of candidates and to use analytically robust MRM assays to verify their performance (using the initial discovery cohort), with a separate cohort of patients for evaluation. It is noteworthy that all samples used here were from patients who underwent detailed and expert clinical evaluation. It is also apparent that the strategy can be used to develop an initial classifier which can be tested and further developed to improve the performance of the predictive algorithm. This ongoing evolution of the MRM assay panel and associated machine learning algorithms represent a new and powerful approach to biomarker development.

Finally, there are at least 2 potential routes to implementing a multiplexed protein biomarker panel in the clinical setting. One is to use MRM assays and the other to develop antibody-based assays for the proteins of interest. To explore the extent to which MRM data may align with ELISA, we compared our MRM data on CRP levels with results obtained by standard clinical laboratory ELISA. MRM measurements were compared to the ELISA measurements in the 60 samples from the discovery set. It was not surprising to find that serum CRP levels were significantly up-regulated in patients with RA compared to those with PsA when measured by both ELISA ($P \leq 0.005$) and MRM ($P \leq 0.001$) (Figure 4D). Interestingly, the CRP values from both platforms were strongly correlated ($R^2 = 0.8345$) (Figure 4E), indicating that protein (peptide) measurements obtained by MRM can provide values similar to those obtained by existing immunoassays.

DISCUSSION

PsA is a complex disease with diverse manifestations; the clinical features observed in individuals with PsA often vary substantially but can overlap with other diseases. Differentiating between PsA and RA can be clinically challenging because of the similarities in their clinical presentation (29). It is increasingly evident that making an accurate diagnosis is important in order to determine which therapeutic strategy to adopt to optimize clinical and radiographic outcomes (30). With no diagnostic laboratory test available, the diagnosis is clinical: it depends on the skills and knowledge of the assessor and is commonly based on the presence of inflammatory musculoskeletal disease in a patient with skin/nail psoriasis and in the absence of RF (31). However, the lack of clear definitions for dermatologists and general practitioners for inflammatory musculoskeletal disease, coupled with inadequate training in musculoskeletal examination techniques, leads to diagnostic uncertainty and delay. As many as 30% of psoriasis patients visiting dermatology practices may have undiagnosed PsA (32). A diagnostic delay of >6 months is not uncommon, and this contributes to poor radiographic and functional outcomes (33,34).

There is a critical need to differentiate PsA from other forms of IA, including RA, and to develop and disseminate new approaches for the objective and sensitive diagnosis of PsA. This is especially important at the early stages of less differentiated disease, when a clear diagnosis and the establishment of disease-appropriate therapy may have the most impact in improving outcomes. Only a few studies have investigated whether there are biomarkers which discriminate between PsA and RA. In one study involving synovial tissue, messenger RNA for vascular endothelial growth factor and angiopoietin 2 were elevated in PsA patients compared to RA patients (35). However, obtaining a synovial biopsy specimen is an invasive procedure, and the discomfort, time, and cost associated with tissue sampling makes it highly undesirable for use in routine clinical practice (35,36). More recently, Siebert et al identified 170

urinary peptides that discriminated between patients with longstanding PsA and those with other arthropathies, including early RA, with an AUC of 0.97 (37). These findings are very promising, but urine collection is especially vulnerable to physiologic variation arising from diet and liquid intake. Additionally, urine tends to be a very diluted matrix high in salt and low in protein concentration. Thus, in the absence of stepwise workflows for sample concentration and clean-up, the quantification of proteins in urine can prove difficult as a result of interfering signals present in the matrix (38).

Serum is well recognized as a suitable sample for biomarker discovery, not least because proteins are shed from relevant affected tissues into the circulation, but also because it is readily obtained under standardized operating procedures (39). Thus, we used serum samples analyzed by 3 proteomic platforms (nano-LC-MS/MS, aptamer-based assays, and bead-based assays). Each platform is capable of measuring a limited but complementary range of proteins present at different abundance levels. This approach was adopted in order to maximize coverage of the serum proteome, and to date it is the most comprehensive analysis of the serum proteome in patients with PsA and those with RA. Although 3 platforms were used to identify putative biomarkers, the data from the unbiased nano-LC-MS/MS analysis proved to be more discriminatory compared to the data from the bead-based and aptamer-based platforms. A potential reason for this is that LC-MS/MS analysis allows for unbiased discovery of biomarkers, whereas the other approaches are limited by having fixed panels of protein markers. Furthermore, the aptamer-based platform uses a single aptamer to capture proteins, thus potentially reducing the specificity of readouts (40). It is also possible that the smaller number of patient samples used in the aptamer-based experiments may have constrained the statistical power of the analysis. With respect to the bead-based immunoassay, the 48 carefully selected proteins we measured may not have included key candidate cytokines and chemokines which could support the differentiation between PsA and RA. The proteins were selected based on their known importance in the pathogenesis of PsA and RA, but the panel was limited by the availability of proteins measurable with the in-house assay.

With no compelling evidence to justify the time and cost required to develop further multiplex antibody-based and/or aptamer-based assays, we instead focused on the nano-LC-MS/MS data and performed follow-up studies using MRM. MRM is an excellent tool for supporting large-scale, multiprotein biomarker studies. It is typically used to narrow an initial list of candidate proteins derived from discovery experiments to the subset that may truly address the clinical question under study (41). MRM analysis is performed using triple-quadrupole mass spectrometers, which inherently have higher sensitivity and greater linear dynamic range than the Orbitrap mass spectrometer used in the discovery experiments here. This boost in sensitivity facilitates the detection of low-abundant proteins in complex samples and therefore reduces

the need for sample pre-enrichment steps. Thus, MRM supports more robust workflows as well as time- and cost-effective assay development compared to traditional antibody-based approaches. MRM is frequently less sensitive than an equivalent immunoassay, and it was for this reason that we did not initially attempt to develop MRM assays for putative markers identified only by the aptamer-based or the bead-based analysis (17,42). The development of MRM immunoassays for these candidate biomarker proteins represents an obvious way in which improving the performance of the existing panel could be explored (43).

In the 2 phases of MRM analysis described here, it was especially interesting to note that a subpanel of 8 proteins (leucine-rich α_2 -glycoprotein, α_1 -antichymotrypsin, haptoglobin, haptoglobin-related protein, RF C6 light chain, α_1 -acid glycoprotein 1, coagulation factor XI, and TSP-1) that were identified as highly discriminatory during the initial verification phase were again confirmed as highly discriminatory during the second evaluation phase. Follow-up *t*-test analysis was performed on this set of proteins, and 7 of 8 proteins were found to be up-regulated in RA compared to PsA during both phases of analysis. TSP-1 was found to be significantly up-regulated in PsA compared RA during the second phase, whereas no significant difference was observed in initial verification. This discordance may relate to differences in the number of patients included in the 2 phases, or it may relate to the differences in the patients included; patients in the initial phase had early-onset, treatment-naïve disease, while those included in the second phase had longer-standing disease and were receiving therapy. This highlights, in part, the advantage of maintaining large panels of proteins for ongoing evaluation in patient cohorts.

Further analysis of this 8-protein subpanel was carried out using a web-based resource "Search Tool for the Retrieval of Interacting Genes/Proteins" (<https://string-db.org/cgi/network.pl>), revealing the biologic functions of these 8 markers of interest (Supplementary Table 5, <http://onlinelibrary.wiley.com/doi/10.1002/art.41899/abstract>). It is interesting to note that this panel is enriched for proteins functionally involved in structural remodeling, angiogenesis, homeostasis, and transportation. This perhaps is not surprising since PsA and RA are characterized by an increase in bone turnover and dysregulated angiogenesis. The radiographic features in PsA and RA can be quite different, with bony erosion observed in both conditions but osteoproliferation only seen in PsA (3). In the context of this investigation, it was not unanticipated that markers of structural remodeling contributed to an algorithm discriminating between individuals with PsA and those with RA. Here, we demonstrated that a major advantage of using MRM is that it allows the investigator to rapidly adapt a panel to include new candidate biomarkers. Our CRP assay that was developed using MRM over a few days also showed values highly correlated with those generated by ELISA.

Our study has several strengths, including the comprehensive and logical approach to biomarker development. Limitations include the modest number of patient samples in both study

phases as well as the absence of healthy and disease controls. Differentiating between PsA and RA is the focus of the current study, but it is not the only challenge faced by clinicians, as it can also be challenging to distinguish PsA from other arthropathies and from patients who have skin psoriasis only (14). This certainly represents a future objective, and assessing this biomarker panel in the appropriate additional cohorts is a critical next step. It is noteworthy that the independent cohort included in the second phase of evaluation included patients that had longstanding disease compared to the discovery cohort, which included those with early-onset disease. Despite this, the 2 cohorts shared similar levels of active disease, as reflected by CRP level, ESR, and joint counts (Table 1). However, it should be noted that the Disease Activity Score using the CRP level (DAS28-CRP) (44) was used as a disease activity measure in the PsA discovery cohort. This is not recommended, since it does not reflect the 68-joint counts recommended for the disease. Notwithstanding this, the DAS28-CRP results show that while lower in PsA, the mean values are not significantly different between the 2 diseases.

It is fair to say that the patients included in this study are representative of those attending IA clinics. We believe that obtaining data and samples from real-world conditions is critically important if our assay is to consistently segregate PsA from RA regardless of disease duration, disease activity, treatment, or comorbidities. The performance of the biomarker panel may reflect a genuine difference in the protein profile between PsA and RA patients, but further work in a larger number of patient samples is needed. It will also be necessary to examine the performance of the panel in distinguishing PsA from other forms of IA and from healthy individuals.

It should be noted that all PsA patients included in both the discovery and verification cohorts met the CASPAR criteria, which was required for inclusion. Therefore, it was not possible in this study to compare the performance of the biomarker panel to that of CASPAR criteria or to test whether a combination of CASPAR criteria and biomarkers is more useful. We intend to address this in a prospective study of psoriasis patients who are being followed up for the development of PsA or in a cohort of patients with early undifferentiated IA. Finally, although non-inflammatory disease controls were not included in our present analysis, it is worth highlighting research by Chandran et al that identified differences in serum proteins in patients with PsA compared to patients with osteoarthritis (45) and patients with psoriasis (46). The protein markers identified in these studies are prime candidates that should be included in future generations of MRM panel assays. At present, there is no diagnostic test for PsA and as a result, the diagnosis is often late or missed, resulting in functional consequences for the patient (12,47). With at least 20% of the patients referred to early arthritis clinics diagnosed as having PsA, there is an urgent need to develop a test to support early detection of this disease (31).

In conclusion, the work described here represents a significant contribution toward the development of such a test. Fundamental

next steps have been outlined, and the MRM approach is ideally suited to support the large-scale studies required to develop and validate a robust panel of distinguishing biomarkers. We believe that with further development it will be possible to establish a diagnostic test for PsA that will reduce diagnostic delay, inform treatment selection, and improve both short-term and long-term outcomes.

ACKNOWLEDGMENT

The authors would like to thank Prof. Anthony G. Wilson (Arthritis Ireland Chair of Rheumatology, UCD Centre for Arthritis Research, School of Medicine, Conway Institute, Belfield, Dublin 4). Prof. Wilson made a fundamental contribution to our study by providing the RA cohort used in our validation analysis.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Mc Ardle had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Mc Ardle, Szentpetery, Hernandez, Parnell, FitzGerald, Pennington.

Acquisition of data. Mc Ardle, Kwasnik, Szentpetery, de Jager, de Roock, FitzGerald, Pennington.

Analysis and interpretation of data. Mc Ardle, Kwasnik, Szentpetery, Hernandez, Parnell, de Jager, de Roock, FitzGerald, Pennington.

REFERENCES

- Ogdie A, Langan S, Love T, Haynes K, Shin D, Seminara N, et al. Prevalence and treatment patterns of psoriatic arthritis in the UK. *Rheumatology (Oxford)* 2013;52:568–75.
- Ogdie A, Weiss P. The epidemiology of psoriatic arthritis [review]. *Rheum Dis Clin North Am* 2015;41:545–68.
- Mc Ardle A, Flatley B, Pennington SR, FitzGerald O. Early biomarkers of joint damage in rheumatoid and psoriatic arthritis [review]. *Arthritis Res Ther* 2015;17:141.
- McArdle A, Pennington SR, FitzGerald O. Clinical features of psoriatic arthritis: a comprehensive review of unmet clinical needs [review]. *Clin Rev Allergy Immunol* 2018;55:271–94.
- Kane D, Stafford L, Bresnihan B, FitzGerald O. A prospective, clinical and radiological study of early psoriatic arthritis: an early synovitis clinic experience. *Rheumatology (Oxford)* 2003;42:1460–8.
- Husted JA, Gladman DD, Farewell VT, Cook RJ. Health-related quality of life of patients with psoriatic arthritis: a comparison with patients with rheumatoid arthritis. *Arthritis Rheum* 2001;45:151–8.
- Sokoll KB, Helliwell PS. Comparison of disability and quality of life in rheumatoid and psoriatic arthritis. *J Rheumatol* 2001;28:1842–6.
- Lindqvist UR, Alenius GM, Husmark T, Theander E, Holmström G, Larsson PT, on behalf of the Psoriatic Arthritis Group of the Society for Rheumatology. The Swedish Early Psoriatic Arthritis Register—2-year followup: a comparison with early rheumatoid arthritis. *J Rheumatol* 2008;35:668–73.
- Lee S, Mendelsohn A, Sarnes E. The burden of psoriatic arthritis: a literature review from a global health systems perspective. *P T* 2010;35:680–9.
- McArdle A, Butt AQ, Szentpetery A, de Jager W, de Roock S, FitzGerald O, et al. Developing clinically relevant biomarkers in inflammatory arthritis: a multiplatform approach for serum candidate protein discovery. *Proteomics Clin Appl* 2016;10:691–8.
- Ritchlin CT, Kavanaugh A, Gladman DD, Mease PJ, Helliwell P, Boehncke WH, et al. Treatment recommendations for psoriatic arthritis [review]. *Ann Rheum Dis* 2009;68:1387–94.
- Kirkham B, de Vlam K, Li W, Boggs R, Mallbris L, Nab HW, et al. Early treatment of psoriatic arthritis is associated with improved patient-reported outcomes: findings from the etanercept PRESTA trial. *Clin Exp Rheumatol* 2015;33:11–9.
- Yago T, Nanke Y, Kawamoto M, Kobashigawa T, Yamanaka H, Kotake S. IL-23 and Th17 disease in inflammatory arthritis [review]. *J Clin Med* 2017;6:81.
- Butt AQ, McArdle A, Gibson DS, FitzGerald O, Pennington SR. Psoriatic arthritis under a proteomic spotlight: application of novel technologies to advance diagnosis and management [review]. *Curr Rheumatol Rep* 2015;17:35.
- Taylor W, Gladman D, Helliwell P, Marchesoni A, Mease P, Mielants H, on behalf of the CASPAR Study Group. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. *Arthritis Rheum* 2006;54:2665–73.
- Coates LC, Conaghan PG, Emery P, Green MJ, Ibrahim G, MacIver H, et al. Sensitivity and specificity of the classification of psoriatic arthritis criteria in early psoriatic arthritis. *Arthritis Rheum* 2012;64:3150–5.
- Chandran V. Spondyloarthritis: CASPAR criteria in early psoriatic arthritis [review]. *Nat Rev Rheumatol* 2012;8:503–4.
- Gibson DS, Rooney ME, Finnegan S, Qiu J, Thompson DC, Labaer J, et al. Biomarkers in rheumatology, now and in the future [review]. *Rheumatology (Oxford)* 2012;51:423–33.
- Szentpetery A, Herffernan E, Haroon M, Kilbane M, Gallagher P, McKenna MJ, et al. Striking difference of periarticular bone density change in early psoriatic arthritis and rheumatoid arthritis following anti-rheumatic treatment as measured by digital X-ray radiogrammetry. *Rheumatology (Oxford)* 2016;55:891–6.
- Aletaha D, Neogi T, Silman AJ, Funovits J, Felson D, Bingham CO III, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010;62:2569–81.
- Rappsilber J, Ishihama Y, Mann M. Stop and go extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. *Anal Chem* 2003;75:663–70.
- Turriziani B, Garcia-Munoz A, Pilkington R, Raso C, Kolch W, von Kriegsheim A. On-beads digestion in conjunction with data-dependent mass spectrometry: a shortcut to quantitative and dynamic interaction proteomics. *Biology (Basel)* 2014;3:320–32.
- Cox J, Neuhauser N, Michalski A, Scheltema RA, Olsen JV, Mann M. Andromeda: a peptide search engine integrated into the MaxQuant environment. *J Proteome Res* 2011;10:1794–805.
- MacLean B, Tomazela DM, Shulman N, Chambers M, Finney GL, Frewen B, et al. Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics* 2010;26:966–8.
- Molecular and Cellular Proteomics. Targeted Mass Spec Guidelines. URL: <https://www.mcponline.org/guidelines-publication-manuscript-s-describing-development-and-application-targeted-mass>.
- Grant RP, Hoofnagle AN. From lost in translation to paradise found: enabling protein biomarker method transfer by mass spectrometry. *Clin Chem* 2014;60:941–4.
- Picotti P, Bodenmiller B, Aebersold R. Proteomics meets the scientific method. *Nat Methods* 2013;10:24–7.
- Gohar F, McArdle A, Jones M, Callan N, Hernandez B, Kessel C, et al. Molecular signature characterisation of different inflammatory phenotypes of systemic juvenile idiopathic arthritis. *Ann Rheum Dis* 2019;78:1107–13.

29. Haroon M, FitzGerald O. Psoriatic arthritis: complexities, comorbidities and implications for the clinic [review]. *Expert Rev Clin Immunol* 2016;12:405–16.
30. Merola JF, Espinoza LR, Fleischmann R. Distinguishing rheumatoid arthritis from psoriatic arthritis. *RMD Open* 2018;4:e000656.
31. Coates LC, Helliwell PS. Psoriatic arthritis: state of the art review [review]. *Clin Med (Lond)* 2017;17:65–70.
32. Haroon M, Kirby B, FitzGerald O. High prevalence of psoriatic arthritis in patients with severe psoriasis with suboptimal performance of screening questionnaires. *Ann Rheum Dis* 2013;72:736–40.
33. Haroon M, Gallagher P, FitzGerald O. Diagnostic delay of more than 6 months contributes to poor radiographic and functional outcome in psoriatic arthritis. *Ann Rheum Dis* 2015;74:1045–50.
34. Tillett W, Jadon D, Shaddick G, Cavill C, Korendowych E, de Vries CS, et al. Smoking and delay to diagnosis are associated with poorer functional outcome in psoriatic arthritis. *Ann Rheum Dis* 2013;72:1358–61.
35. Fearon U, Griosios K, Fraser A, Reece R, Emery P, Jones PF, et al. Angiopoietins, growth factors, and vascular morphology in early arthritis. *J Rheumatol* 2003;30:260–8.
36. Ilie M, Hofman P. Pros: can tissue biopsy be replaced by liquid biopsy? [editorial]. *Transl Lung Cancer Res* 2016;5:420–3.
37. Siebert S, Porter D, Paterson C, Hampson R, Gaya D, Latosinska A, et al. Urinary proteomics can define distinct diagnostic inflammatory arthritis subgroups. *Sci Rep* 2017;7:40473.
38. Chiu M, Lawi W, Snyder SI, Wong PK, Liao JC, Gau G. Matrix effects—a challenge toward automation of molecular analysis. *J Lab Autom* 2010;15:233–42.
39. Wilson R. Sensitivity and specificity: twin goals of proteomics assays. Can they be combined? [review]. *Expert Rev Proteomics* 2013;10:135–49.
40. Joshi A, Mayr M. In aptamers they trust: the caveats of the SOMAscan biomarker discovery platform from SomaLogic. *Circulation* 2018;138:2482–5.
41. Carr SA, Abbatiello SE, Ackermann BL, Borchers C, Domon B, Deutsch EW, et al. Targeted peptide measurements in biology and medicine: best practices for mass spectrometry-based assay development using a fit-for-purpose approach. *Mol Cell Proteomics* 2014;13:907–17.
42. Lange V, Picotti P, Domon B, Aebersold R. Selected reaction monitoring for quantitative proteomics: a tutorial. *Mol Syst Biol* 2008;4:222.
43. Yassine H, Borges CR, Schaab MR, Billheimer D, Stump C, Reaven P, et al. Mass spectrometric immunoassay and MRM as targeted MS-based quantitative approaches in biomarker development: potential applications to cardiovascular disease and diabetes [review]. *Proteomics Clin Appl* 2013;7:528–40.
44. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts: development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995;38:44–8.
45. Chandran V, Abji F, Perruccio AV, Gandhi R, Li S, Cook RJ, et al. Serum-based soluble markers differentiate psoriatic arthritis from osteoarthritis. *Ann Rheum Dis* 2019;78:796–801.
46. Chandran V, Cook RJ, Edwin J, Shen H, Pellett FJ, Shanmugarajah S, et al. Soluble biomarkers differentiate patients with psoriatic arthritis from those with psoriasis without arthritis. *Rheumatology (Oxford)* 2010;49:1399–405.
47. Sorensen J, Hetland ML, on behalf of all departments of rheumatology in Denmark. Diagnostic delay in patients with rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis: results from the Danish nationwide DANBIO registry. *Ann Rheum Dis* 2015;74:e12.