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A small-scale preliminary study utilizing mass cytometry to distinguish two forms of arthritis

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Abstract

Objectives Spondyloarthritis (SpA) and rheumatoid arthritis (RA) are hallmarked by immune cell infiltration in synovial joints. Although, in general, different sites are affected, misclassification or delayed diagnosis due to overlapping clinical manifestations is not uncommon. Here, we investigated the diagnostic potential of mass cytometry (MC) in early peripheral SpA (pSpA) and RA patients in a small pilot study.

Methods Peripheral blood and synovial fluid mononuclear cells (PBMC and SFMC) of 4 pSpA, 7 RA, and 1 undifferentiated arthritis (UA) patient(s) were evaluated using a 37-marker MC panel. Data were analyzed through Visuallyte services, including dimension reduction, clustering, and Cytofast workflow.

Results PBMC data indicated naive CD4 T cell, B cell, and monocyte subsets to be differentially present in RA as compared with SpA. CD4 + Tem cell and NK cell subsets appeared more prominently present in pSpA SFMC. Merged PBMC and SFMC data showed overlapping immune profiles of an UA patient with pSpA patients. These results were in accordance with the formal clinical pSpA diagnosis the UA patient received after this study.

Conclusions Utilizing MC, several differences in immune cell composition in both SFMC and PBMC between RA and pSpA patients were observed. Combining PBMC and SFMC data in an unsupervised analysis resulted in the correct classification of the UA patient as pSpA patient prior to formal clinical pSpA diagnosis. This pilot study provides an example of how deep phenotyping with MC aids in differentiating arthritis patients and offers a rationale to further explore these findings.

Key points

- Due to overlapping symptoms and the absence of disease-specific biomarkers, the clinical diagnosis of peripheral spondyloarthritis (pSpA) and rheumatoid arthritis (RA) can be difficult.
- Visualizing immune cell profiles of peripheral blood (PB) and synovial fluid (SF) by mass cytometry (MC) suggests differences in immune cell composition between pSpA and RA patients.
- Based on immune profiles of combined PB and SF data, we could correctly predict the formal clinical pSpA diagnosis of an undifferentiated arthritis (UA) patient received later.
- This pilot study gives an example of how MC might contribute to faster clinical diagnosis of pSpA patients in the absence of biomarkers.

Keywords Immune cell profiles · Mass cytometry · Rheumatic autoimmune diseases · Rheumatoid arthritis · Spondylarthritis

Hester Koppejan and Sophie-Anne I. Smith contributed equally to this work.

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Introduction

Rheumatic diseases are characterized by joint inflammation, leading to irreversible joint damage if left untreated. Rheumatoid arthritis (RA) and spondyloarthritis (SpA) are two common rheumatic autoimmune diseases, with incidence rates of RA increasing in the last decades [1–4]. RA is hallmarked by inflammation in the synovial tissue of the

joints, cartilage, and bone. Several autoantibodies can be detected in RA patients, such as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), but a substantial part of the RA patients is seronegative [5, 6]. SpA is an umbrella term for a group of chronic immune-mediated diseases mainly involving the spine and peripheral joints. Other than HLA-B27 and C reactive protein (CRP), there are currently no reliable SpA diagnostic biomarkers used in clinical practice [7, 8]. The lack of biomarkers for SpA in combination with the shared clinical manifestations between SpA and other rheumatic diseases, such as RA, can make SpA diagnosis difficult and may lead to delayed diagnosis or even misclassification [9, 10].

The aim of this study is to pilot the diagnostic potential of a mass cytometry (MC) panel to distinguish rheumatic patients. Therefore, we created high-dimensional MC immune profiles of peripheral blood mononuclear cells (PBMC) and synovial fluid mononuclear cells (SFMC) of RA and peripheral SpA (pSpA) patients. We applied these profiles to predict the diagnosis of one undifferentiated arthritis (UA) patient, who did not receive a definite clinical diagnosis at the time of the study.

Materials and methods

Patient material

Paired heparin blood and synovial fluid (SF) was collected from seven patients with active RA (3 ACPA – RA, 4 ACPA + RA) and four patients with active pSpA visiting our outpatient clinic at the Department of Rheumatology, Leiden University Medical Centre, Leiden, the Netherlands. In addition, peripheral blood (PB) and SF were also obtained from a patient with arthritis with an unknown diagnosis. At the moment of sample collection, this patient was diagnosed as undifferentiated arthritis (UA) due to lack of biomarkers for either RA or pSpA. All patients had active arthritis at the moment of inclusion, so SF could be collected from the knee joint. Patient characteristics are summarized in Table 1.

Sample preparation

Peripheral blood mononuclear cells (PBMCs) and synovial fluid mononuclear cells (SFMCs) were isolated through Ficoll-Paque gradient centrifugation from fresh collected paired PB and SF samples. Prior to Ficoll-Paque gradient centrifugation, SF was treated with hyaluronidase as described previously [11]. After isolation, all PBMCs and SFMCs were treated as previously mentioned [12]. Briefly, freshly isolated cells were stained with 103-Rhodium (viability; Standard BioTools, formerly known as Fluidigm, South San Francisco, CA, USA), fixed, and stored at 4 °C

Table 1 Characteristics of patients with arthritis, including active knee arthritis

Sample ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Age in years	35	62	70	38	50	65	45	54	19	57	36	69
Disease duration in years	3	3	4	2	9	7	14	5	12	7	6	2
Female sex	Yes	No	No	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes
Clinical diagnosis	SpA	UA	RA	SpA	SpA	RA	SpA	RA	RA	RA	RA	RA
ACPA/RF	Neg/Neg	Neg/Neg	Neg/Neg	Neg/Neg	Neg/Neg	Pos/Pos	Unknown/Neg	Pos/Neg	Neg/Neg	Pos/Pos	Pos/Pos	Neg/Neg
SpA feature 1	HLA-B27 +	N/A	N/A	Enthesitis	Psoriasis	N/A	Psoriasis	N/A	N/A	N/A	N/A	N/A
SpA feature 2	N/A	N/A	N/A	Family history	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Classification criteria*	Y	N/A	Y	Y	Y	Y	Y	Y	Y	Y	Y	N
SJC at assessment	2	5	1	1	1	4	2	1	1	2	3	3
Current treatment	SSZ	N/A	LEF, PRED	LEF	SSZ	N/A	SSZ	MTX	MTX	BARI	MTX, HCQ	PRED
Previous treatment 1	MTX	HCQ	MTX, SSZ		MTX, LEF	MTX	MTX	N/A	SSZ, MTX	MTX, HCQ	N/A	N/A
Previous treatment 2						TOCI			ETN	LEF, ETN		
Previous treatment 3									ADA			

Legend: SpA spondyloarthritis, RA rheumatoid arthritis, UA undifferentiated arthritis, ACPA anti-citrullinated protein antibodies, RF rheumatoid factor, SJC swollen joint count, SSZ sulfasalazine, MTX methotrexate, LEF leflunomide, HCQ hydroxychloroquine, PsO psoriasis, PRED prednisone, BARI baricitinib, TOCI tocilizumab, ETN etanercept, ADA adalimumab

*Classification criteria: for SpA, the ASAS peripheral SpA criteria are used, and for RA, the 2010 ACR EULAR criteria are used

for a maximum of three days. Next, cells were stained with a 37-marker mass cytometry panel and acquired on a Helios CyTOF system (Standard BioTools) with a high throughput (HT) injector as reported before [12]. Antibodies used for staining are listed in Supplementary Table S1. Each acquisition included an internal reference control as described previously [12].

Data analysis

Obtained flow cytometry standard (FCS) files were normalized and gated for single live cells as reported formerly [12]. Hierarchical stochastic neighbor embedding (HSNE) was performed in Cytosplore to visualize high-dimensional data in two-dimensional space [13]. A 7-level HSNE based on the expression of all 37 markers of the panel was used to identify the major immune subsets. Secondly, data was submitted for analysis to the Visualyte analysis platform. This included HSNE, Gaussian Mean shift clustering, and Cytofast analysis [13–15]. Due to the small sample size, no statistical comparisons were done, and data trends were described.

Results

HSNE dimension reduction of paired PBMC and SFMC samples separated the data into major immune cell clusters, such as B cells, T cells, innate cells, and myeloid cells (Fig. 1a and b). Clustering in Cytosplore resulted in 30 different clusters (Fig. 1c and Supplementary Fig. 1). Cytofast analysis indicated clusters 1.11, 1.14, 1.15, and 1.16 as the main “drivers” separating PBMCs from SFMCs (Fig. 1d). Cluster 1.11 represented a CD56+high NK cell subset based on the high expression of CD56, low expression of CD16, and the absence of CD3 [16, 17]. Cluster 1.14 represented CD4+effector memory T (Tem) cells and cluster 1.15 could be characterized as CD4+naïve T cells, based on the classical CD4 T cell markers, CD45RO, CD45RA, CCR7, and CD27. Cluster 1.16, positive for CD19, CD20, and HLA-DR, represented a CD19+B cell subset. Numerically, the frequency of the clusters suggested clusters 1.11, 1.15, and 1.16 to be more prominent in PBMCs, whereas cluster 1.14 was more frequently present in SFMCs (Fig. 1d).

Next, we aimed to explore the diagnostic potential of this MC panel and investigated whether MC can be used for the prediction of undiagnosed patients. Therefore, a principal component analysis (PCA) was performed to predict the diagnosis of an UA patient based on the RA and pSpA immune profiles. For this PCA, the combined data of PB and SF cells were used. The PCA showed that the immune profile of the UA patient overlapped with the pSpA patients (Fig. 1e), indicating the high likelihood that the UA patient

would receive a pSpA diagnosis. Interestingly, although the treating physician was not aware of this analysis, 1 year after study completion, this UA patient received the formal clinical diagnosis pSpA, in line with our results.

For further analysis, the PBMC and SFMC samples were analyzed separately. We merged the datapoints of the UA patient together with the pSpA group for these analyses, resulting in a total of 5 pSpA patients. To improve the resolution of the PBMC data, the data was stratified based on CD3 expression (Fig. 2 and Supplementary Fig. 2). Analyses of PBMC resulted in 20 different CD3+T cell clusters (Fig. 2a and b) and 21 different CD3-negative non-T cell clusters (Fig. 2c and d). Numerically, cluster 2.13 was more prominently present in RA as observed after Cytofast analysis (Fig. 2b). Cluster 2.13 represented a naïve CD4+T cell cluster based on CD45RA+ and CD45RO- expression.

For the CD3-negative data, clusters 3.8, 3.12, and 3.20 showed numerical variation between the patient groups (Fig. 2d and Supplementary Fig. 3). The presence of cluster 3.8, a CD56+low NK cell cluster, was higher in 2 out of 3 of ACPA-RA patients, compared with ACPA+RA and pSpA patients. The presence of cluster 3.12 was numerically high in RA and low in pSpA patients. Cluster 3.12 represented a B cell cluster, based on the lack of CD14 and CD3 and the expression of CD19 and CD20. Cluster 3.20 displayed a skewing toward ACPA+RA. This cluster consisted of classical monocytes, based on the expression of CD14, HLA-DR, and CD38 combined with a lack of CD16.

The SFMC data was subjected to a similar approach, stratifying the data based on CD3 expression, resulting in 13 CD3+T cell clusters and 18 CD3-non-T cell clusters (Fig. 3 and Supplementary Fig. S4). Comparing the T cell cluster frequencies, clusters 4.1, 4.6, and 4.7 showed numerical variation between RA and pSpA patients (Fig. 3b). The presence of clusters 4.1 and 4.7 both seemed to be upregulated in pSpA compared with RA and could be classified as a CD4+Tem subset, based on the strong expression of CD45RO and lack of CD45RA and CCR7. Although they are both Tem clusters, cluster 4.7 expressed markers CD161 and CD196, also known as CCR6. These two markers indicated the presence of Th17 T cells in this cluster, however, not exclusively, as also non-classical Th1 T cells can be present within this phenotype. Cluster 4.6, in contrast to clusters 4.1 and 4.7, was numerically upregulated in RA compared with pSpA and contained CD8+Tem cells.

Within the CD3-negative-cell stratum, cluster 5.3 was numerically upregulated in pSpA patients compared with ACPA+ and ACPA-RA (Fig. 3d and Supplementary Fig. S5). Cluster 5.3 was negative for all the lineage markers and the ILC marker CD127, but highly positive for CD56 and therefore classified as CD56+high NK cells.

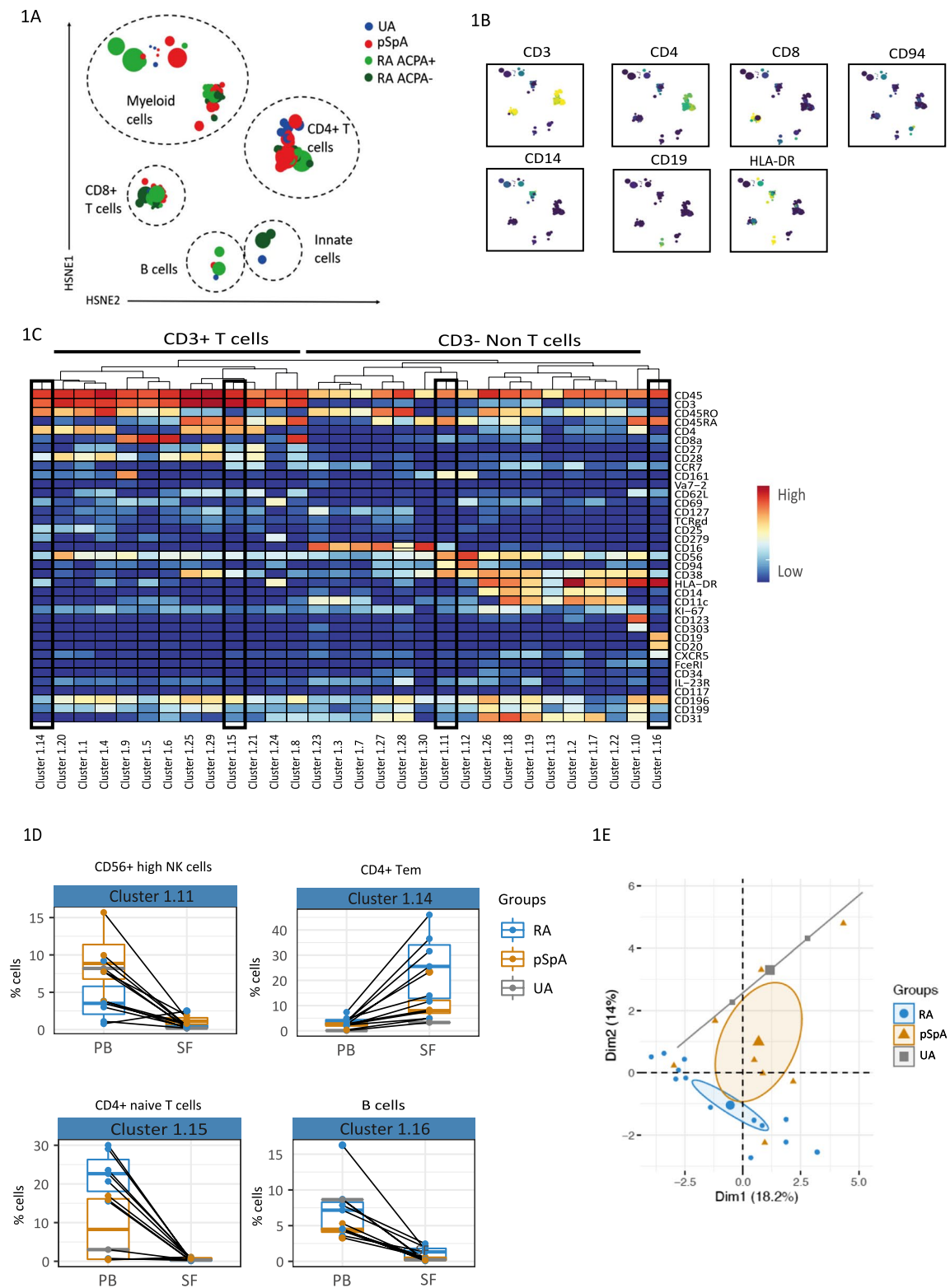
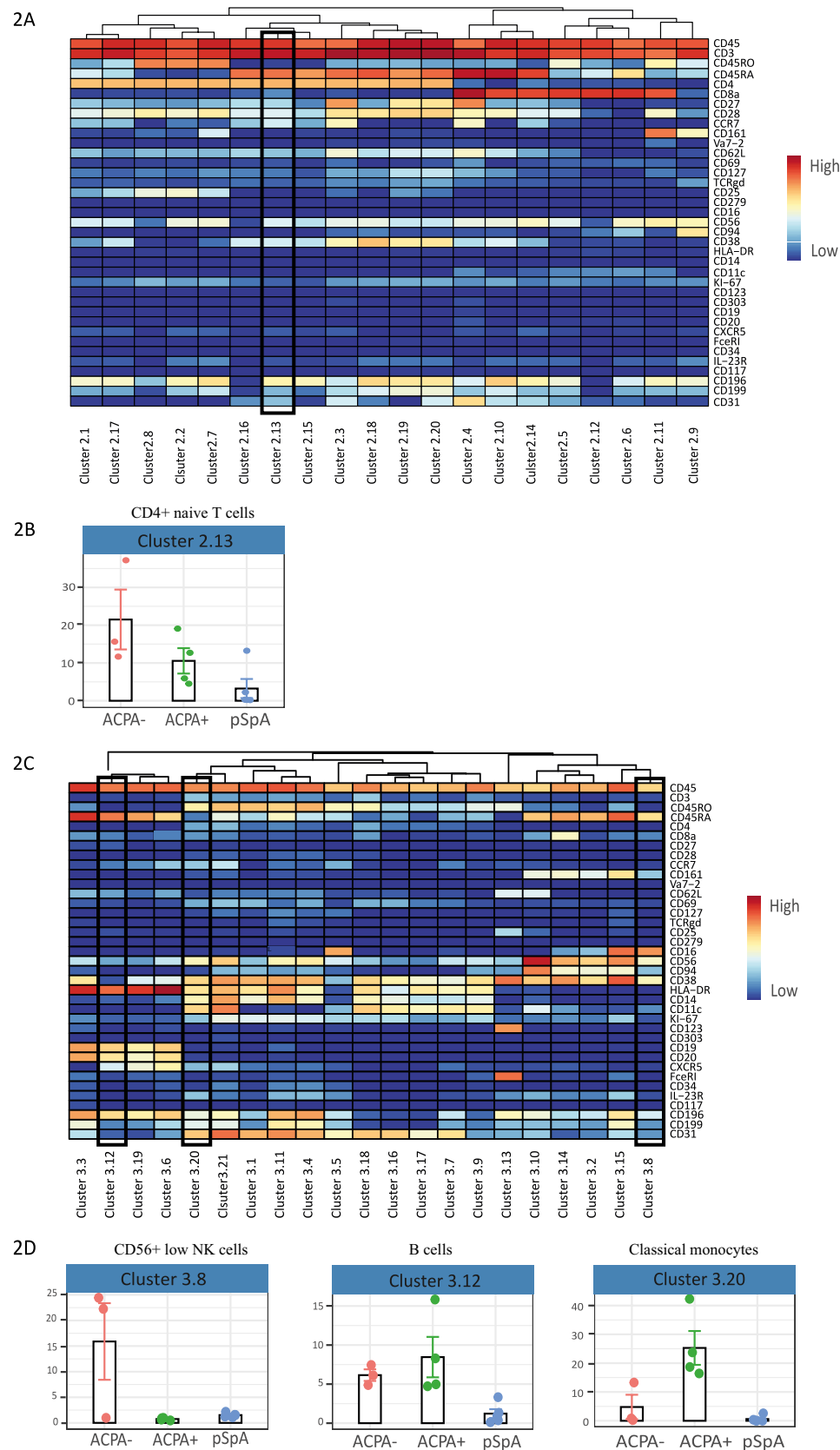
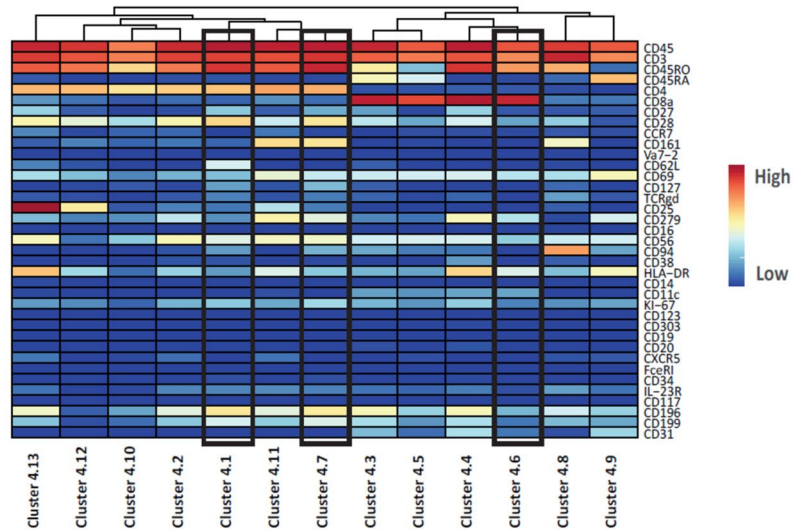


Fig. 1 **A** A seven-level HSNE visualization of UA, SpA, RA ACPA+, and RA ACPA- for PB and SF data combined, separating the main immune subsets, **B** A seven-level HSNE plot of PB and SF data showing the expression of main lineage markers. **C** Overview heatmap of mean marker expression in the 30 clusters identified by Cytosplore (Gaussian mean shift) for PB and SF data combined. **D** Percentage of cells in cluster 1.11 (CD56+ high NK cells), 1.14 (CD4+ Tem cells), 1.15 (CD4+ naive T cells), and 1.16 (B cells) for PB and SF data. **E** Principal component analysis (PCA) of RA, SpA, and UA with PB and SF data combined

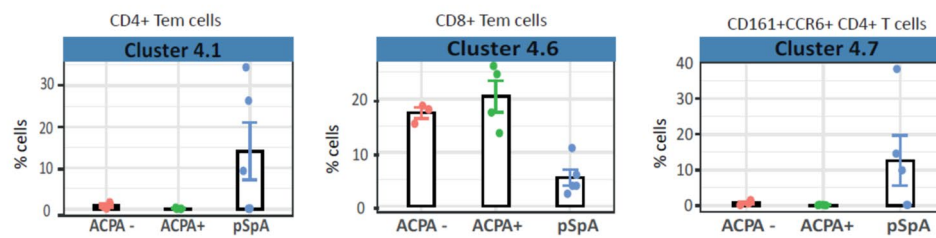
Fig. 2 **A** Heatmap of mean marker expression of the 20 CD3 + T cell clusters identified by Cytosplore (Gaussian mean shift) for PB data only. **B** Percentage of cells in CD3 + PB cluster 2.13 (naive CD4 + T cells) for the different patient groups (ACPA + RA, ACPA – RA, and SpA). **C** Heatmap of mean marker expression in the 21 CD3 – non-T cell clusters identified by Cytosplore (Gaussian mean shift) for PB data only. **D** Percentage of cells in CD3 – PB cluster 3.8 (CD56 + low NK cells), cluster 3.12 (B cells), and cluster 3.20 (classical monocytes) for the different patient groups (ACPA + RA, ACPA – RA, and SpA)



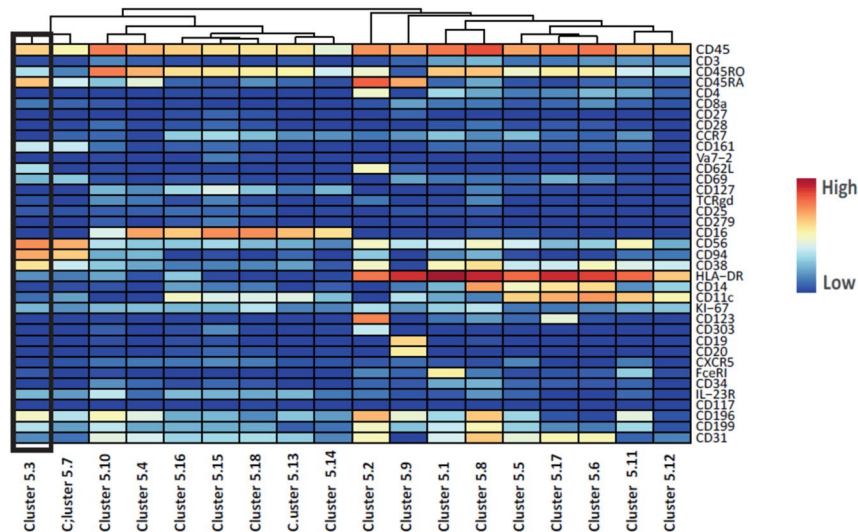
3A



3B



3C



3B

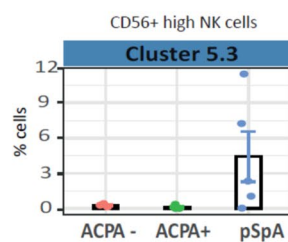


Fig. 3 **A** Heatmap of mean marker expression of the 13 CD3+T cell clusters identified by Cytosplore (Gaussian mean shift) for SF data only. **B** Percentage of cells in CD3+SF cluster 4.1 (CD4+Tem cells), cluster 4.6 (CD8+Tem cells), and cluster 4.7 (CD161+CCR6+CD4+T cells) for the different patient groups (ACPA+RA, ACPA–RA, and SpA). **C** Heatmap of mean marker expression in the 18 CD3–non-T cell clusters identified by Cytosplore (Gaussian mean shift) for SF data only. **D** Percentage of cells in CD3–SF cluster 5.3 (CD56+ high NK cells) for different patient groups (ACPA+RA, ACPA–RA, and SpA)

Discussion

Diagnosis of RA and pSpA patients can be difficult due to overlapping disease symptoms, absence of detectable autoantibodies in a substantial part of RA patients, and lack of pSpA biomarkers. Therefore, there is a need for more precise diagnostic tools. In this small pilot study, we visualized high-dimensional immune profiles of RA and pSpA patients with a 37-marker MC panel and unsupervised analysis tools. By comparing immune profiles of RA and pSpA patients to the immune profile of an undiagnosed rheumatic patient, we aimed to probe the diagnostic potential of MC for the diagnosis of rheumatic diseases. The results showed a clear overlap of the immune cell profile of the undiagnosed rheumatic patient with the immune cell profile of pSpA patients. This was in agreement with the formal clinical pSpA diagnosis the patient received later. Therefore, this study provides an example how MC and unsupervised analysis tools could contribute to faster and more precise diagnosis of rheumatic patients in the clinic.

Next to the diagnostic potential of MC, we also more closely investigated the high dimensional immune cell profiles of the RA and pSpA patients. Although no statistical analysis was performed due to a small sample size, we did observed several divergences between the patient groups after unsupervised analysis. The PB data revealed a NK cell subset to be more specific for ACPA–RA. Our previous study already suggested a role for NK cells in ACPA–RA [12], although further elaborate experiments would be required to investigate this in more depth. Furthermore, the higher frequency of CD8+ T cells in the SF data of RA patients is in accordance with a previous study in which the differentially expressed genes between SpA and RA for SF were investigated [18]. In the same study, resting NK cells were found to be upregulated in SpA compared with RA, in agreement with our SF results. Obviously, our data need further replication and confirmation given the number of patients analyzed. However, they do point to potential differences in immune cell composition between the different rheumatic diseases.

By including both PB and SF data in the analysis, our data not only reflect the immune cell composition in blood, but also describe the situation in situ in the inflamed joint

as well. Interestingly, the SF data showed different patterns compared to the PB data. This difference between PB and SF was mainly driven by CD4+naive T cells, CD4+Tem, and NK cells. In accordance with our data, literature shows this higher frequency of CD4+memory T cells and lower number of naive CD4+T cells in the SF of patients with SpA compared with PB [19]. Similarly, the differences in immune cell composition between SF and PB also warrant further study to confirm the observations made.

As already indicated, a clear limitation of this study is that no statistical analysis was done due to the small number of subjects included. Therefore, this study only serves as a proof of concept and does not allow to draw any conclusions whether there are true significant differences between the patient groups. Furthermore, it is important to consider that the patients received different medications at the time of sampling. The precise effects of these treatments on the MC immune cell profiles or on the outcome of this study are not known.

In summary, although the clinical manifestations of RA and pSpA can be very similar, our data point to a difference in immune cell composition between these two inflammatory rheumatic diseases. By visualizing these differences with MC and comparing them with the immune cell profile of an undiagnosed patient, we showed the feasibility to use MC to aid clinical diagnosis. Thereby, our pilot study offers a rationale for follow-up studies into the immune cell subset composition of rheumatic patients to value the use of MC and high dimensional data in clinical decision-making.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10067-024-07233-7>.

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Author contribution FvG, HK, MH, and RT contributed to the study conception and design. Material preparation, data collection, and processing were performed by HK and MH. The first draft of the manuscript was written by FvG, HK, SS, and RT, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets used for the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate Ethics approval was granted by the Leiden University Medical Center research ethics board, and

informed consent was obtained from each participant according to the Declaration of Helsinki. All samples and data were coded to maintain anonymity of the patients.

Disclosures HK has performed this work as part of her PhD studies and is currently an employee of Standard BioTools (formerly known as Fluidigm). The other authors have disclosed no conflicts of interest.

References

1. Silman AJ, Pearson JE (2002) Epidemiology and genetics of rheumatoid arthritis. *Arthritis Res* 4(Suppl 3):S265–272. <https://doi.org/10.1186/ar578>
2. Safiri S et al (2019) Global, regional and national burden of rheumatoid arthritis 1990–2017: a systematic analysis of the Global Burden of Disease study 2017. *Ann Rheum Dis* 78:1463–1471. <https://doi.org/10.1136/annrheumdis-2019-215920>
3. Dougados M, Baeten D (2011) Spondyloarthritis. *Lancet* 377:2127–2137. [https://doi.org/10.1016/s0140-6736\(11\)60071-8](https://doi.org/10.1016/s0140-6736(11)60071-8)
4. Stolwijk C, van Onna M, Boonen A, van Tubergen A (2016) Global prevalence of spondyloarthritis: a systematic review and meta-regression analysis. *Arthritis Care Res (Hoboken)* 68:1320–1331. <https://doi.org/10.1002/acr.22831>
5. Scherer HU, Huizinga TWJ, Krönke G, Schett G, Toes REM (2018) The B cell response to citrullinated antigens in the development of rheumatoid arthritis. *Nat Rev Rheumatol* 14:157–169. <https://doi.org/10.1038/nrrheum.2018.10>
6. Matthijssen XME, Niemantsverdriet E, Huizinga TWJ, van der Helm-van Mil AHM (2020) Enhanced treatment strategies and distinct disease outcomes among autoantibody-positive and -negative rheumatoid arthritis patients over 25 years: a longitudinal cohort study in the Netherlands. *PLoS Med* 17:e1003296. <https://doi.org/10.1371/journal.pmed.1003296>
7. Sharip, A. & Kunz, J (2020) Understanding the pathogenesis of spondyloarthritis. *Biomolecules* 10. <https://doi.org/10.3390/biom10101461>
8. Maksymowych WP (2015) Biomarkers in axial spondyloarthritis. *Curr Opin Rheumatol* 27:343–348. <https://doi.org/10.1097/bor.0000000000000180>
9. Mease PJ et al (2022) Comparison of clinical manifestations in rheumatoid arthritis vs. spondyloarthritis: a systematic literature review. *Rheumatol Ther* 9:331–378. <https://doi.org/10.1007/s40744-021-00407-8>
10. Molto A, Sieper J (2018) Peripheral spondyloarthritis: concept, diagnosis and treatment. *Best Pract Res Clin Rheumatol* 32:357–368. <https://doi.org/10.1016/j.berh.2019.02.010>
11. Brouwers H et al (2022) Hyaluronidase treatment of synovial fluid is required for accurate detection of inflammatory cells and soluble mediators. *Arthritis Res Ther* 24:18. <https://doi.org/10.1186/s13075-021-02696-4>
12. Koppejan H et al (2021) Immunoprofiling of early, untreated rheumatoid arthritis using mass cytometry reveals an activated basophil subset inversely linked to ACPA status. *Arthritis Res Ther* 23:272. <https://doi.org/10.1186/s13075-021-02630-8>
13. van Unen V et al (2017) Visual analysis of mass cytometry data by hierarchical stochastic neighbour embedding reveals rare cell types. *Nat Commun* 8:1740. <https://doi.org/10.1038/s41467-017-01689-9>
14. Beyrend G, Stam K, Höllt T, Ossendorp F, Arens R (2018) Cytosfast: A workflow for visual and quantitative analysis of flow and mass cytometry data to discover immune signatures and correlations. *Comput Struct Biotechnol J* 16:435–442. <https://doi.org/10.1016/j.csbj.2018.10.004>
15. Beyrend G, Stam K, Ossendorp F, Arens R (2019) Visualization and quantification of high-dimensional cytometry data using Cytosfast and the upstream clustering methods FlowSOM and Cytosplore. *J Vis Exp*. <https://doi.org/10.3791/60525>
16. Carson WE, Fehniger TA, Caligiuri MA (1997) CD56bright natural killer cell subsets: characterization of distinct functional responses to interleukin-2 and the c-kit ligand. *Eur J Immunol* 27:354–360. <https://doi.org/10.1002/eji.1830270203>
17. Inggjerdigen M, Kveberg L, Naper C, Vaage JT (2011) Natural killer cell subsets in man and rodents. *Tissue Antigens* 78:81–88. <https://doi.org/10.1111/j.1399-0039.2011.01714.x>
18. Wang J, Xue Y, Zhou L (2022) Comparison of immune cells and diagnostic markers between spondyloarthritis and rheumatoid arthritis by bioinformatics analysis. *J Transl Med* 20:196. <https://doi.org/10.1186/s12967-022-03390-y>
19. Dejaco C, Duftner C, Klauser A, Schirmer M (2010) Altered T-cell subtypes in spondyloarthritis, rheumatoid arthritis and polymyalgia rheumatica. *Rheumatol Int* 30:297–303. <https://doi.org/10.1007/s00296-009-0949-9>

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