T lymphocytes have been considered the most relevant cell types in the initiation and maintenance of psoriasis and PsA. Skin-resident memory T cells have been demonstrated to have a key role in psoriatic lesion development in mice models \parencite{Boyle2004}. In human case reports, bone marrow transplantation has shown to cause initiation or termination of psoriasis \parencite{Gardembas1990; Eedy1990}. \textit{In vivo} studies demonstrated that transition to psoriatic lesions following engrafted human pre-lesional skin in immune-deficient mice was only dependent on T cells requiring injection of autologous activated CD4$^+$ not CD8$^+$ cells \parencite{Wrone-Smith1996}. Nevertheless, preferential migration into the epidermis and clonal populations T cells have only been isolated for CD8$^+$ cells \parencite{Wrone-Smith1996, Chang1994}. %Altogether, this may suggest that CD4$^+$ are drivers of T cell activation but resident CD8$^+$ are the main effector cells in the dysregulated psoriasis immune response.

In psoriasis and PsA, IL-23 together with other cytokines, including IL-1$\beta$ and IL6, induce activation and differentiation of na\”{i}ve CD4$^+$ and CD8$^+$ into pathogenic Th-17 and Tc17 cells producing IL-17 \parencite{Weaver2007}. IL-17$^+$ CD8$^+$ cells have been found in psoriatic skin and are enriched in PsA synovial fluid when compared to peripheral blood, showing correlation with markers of inflammation and structural changes in the joint \parencite{Menon2014,Ortega2009). Likewise, Th-17 infiltrated cells have been found in the epidermis of psoriatic lesions \parencite{Lowes2008, Pene2008}. Additionally, IL-12 and IFN-$\gamma$ lead *to* expansion ofTh-1 and Tc-1 cells, which contribute to perpetuation of the immune response through IFN$\gamma$ and IL-18 production in psoriasis and PsA \parencite{Austin1999, Perera2012, Cai2012, Dolcino2015}.

%Elevated IL-17 mRNA and protein levels have also been reported in psoriasis and PsA patients compared to controls \parencite{Cai2012; Dolcino2015).

NK

NK cells are lymphoid-derived innate immune cells identified as CD3$^-$ CD56$^+$. The majority of circulating NK cells (90\%) are CD56$^{dim}$ and show strong cytotoxicity driven by high content of perforin and granzymes \parencite{Mandal2014}. In contrast, CD56$^{bright}$ commonly infiltrate into second lymph organs and other tissues, where they are activated by DCs and produce immunoregulatory cytokines such as IFN-$\gamma$, promoting Th-1 expansion and the adaptive immune response \parencite{Martin-Fontecha2004,Ferlazzo2004}. In psoriasis, significant increase of cells expressing NK markers have been found in lesional compared to uninvolved skin \parencite{Cameron2002,Ottaviani2006}. %with NK CD56$^{bright}$ cells isolated from acute plaque lesions producing abundant IFN-$\gamma$ upon activating stimuli \parencite{Cameron2002,Ottaviani2006} . Expansion of NK CD3$^-$ CD56$^{bright}$ cells in inflamed joints was observed in a cohort including RA, PsA and AS patients \parencite{Dalbeth2002}. Moreover, NK cells in RA have shown to trigger osteoclastogenesis and bone destruction in vitro and in mice models \parencite{Soderstrom2010} . %Moreover, the cytokine IL-15, which is highly present in the the joint microenvironment can prime NK cells isolated from PsA peripheral blood to kill via activation of the receptor NKG2D and cPLA2.82 \parencite{Tang2013}.

Amongst the target cells receptors regulating NK cells function, the killer immunoglobulin-like receptor (KIR) family includes activating and inhibitory members. The inhibitory receptor KIR2DL1 and the activatory receptor KIR2DS1 recognise HLA- Cw\*06:02, strongly associated with psoriasis and PsA \parencite{Tobin2011}. Interestingly, gene based studies have shown genetic variability in \textit{KIR2DS1} gene, associated with psoriasis and PsA susceptibility and also reported for AS and RA \parencite{Luszczek 2004, Williams2005,Carter2007, Yen2001).

The role of B cells in the pathophysiology of psoriasis and PsA has remained unclear. B cells are mainly known as key players of the humoral adaptive immune response through antibody production. However, they also act as APCs, regulate CD4$^+$ activation and differentiation into Th effector cells by providing co-estimulatory signals and actively secrete cytokines \parencite{Bouaziz2007,Constant1995,Harris2000,Linton2003}. Recent studies in the imiquimod-induced psoriasis mice model have demonstrated more severe inflammation in CD19 knock-out mice a regulatory B cell subset producing IL-10 \parencite{Yanaba2013,Alrefai2016}. Furthermore, different B cell subsets have been found in PBMCs from psoriasis patients and in lesional skin and correlation with disease severity has been identified for some clinical subtypes \parencite{Lu2016}.

GWAS

GWAS represent a biologically unbiased approach to shed some light on pathophysiological relevant cell types and molecular pathways associated with disease. GWAS have underlined some of the most important cell types for which genetic variation may be functionally relevant by overlapping them with epigenetic features mapped in cell lines or primary cells isolated from healthy control \parencite{}. In psoriasis and PsA, enrichment of associated variants has been found for regulatory elements in several cell types and the majority of GWAS risk loci have been linked to genes that belong to a limited number of pathways, as detailed below (Capon 2017). These genes are selected by proximity to the associated variant and genes unknown to be regulated at a distance fail to be include, a limitation when interpreting GWAS results.

Systematic comparison of the genetic architecture across different conditions has revealed associated psoriasis and PsA risk loci shared, in the same or opposite directions, with AS, Crohn’s disease (CD), multiple sclerosis (MS), RA and type 1 diabetes (T1D) (ImmunoBase). This has supported the use of therapeutic interventions such as anti- IL-23 and anti- IL-17 antibodies to treat a number of immune-related phenotypes including psoriasis, PsA, AS and IBD, amongst others (Visscher et al. 2017).

GWAS have highlighted keratinocyte-specific genes such the *LCE* gene cluster and genes with a key role in skin biology such as *CARD14*. Further studies in the *PSORS4* region have revealed that association with disease is driven by a deletion in two of the genes within this family, *LCE3B* and *LCE3C* (*LCE3C LCE3B del*)(Cid et al. 2009). The lack of *LCE3B* and *LCE3C* expression in psoriasis patients has been hypothesised to impair the repair following skin disruption, potentially facilitating microorganism infection and triggering a dysregulated immune response (Bergboer et al. 2011). *C*ommon and rare pathogenic mutations of *CARD14* in keratinocyte cell lines leadk to increased activation of NF-kB as well as overexpression of psoriasis-associated genes including *IL6*, *TNFA* and *TNFAIP2*, among others (Jordan et al. 2012b).

Tervaniemi study showed a total of 2,589 DEGs (filtering criteria fold change$<$0.75 or fold change$>$1.5 and FDR$<$0.05), a larger number of differentially expressed genes than the identified in my study., reporting a larger number of up-regulated than down-regulated hits (Figure \ref{figure:Skin\_venn\_diagrams\_comparison\_other\_studies} bottom panel). A total of 359 out of the 1,227 DEGs (29.25\%) identified by the in-house study were shared with the Tervaniemi results, of which 239 and 75 were up- and down-regulated, respectively (Figure \ref{figure:Skin\_venn\_diagrams\_comparison\_other\_studies} bottom panel). Some examples of this overlap included up-regulation of \textit{STAT1}, genes from the \textit{S100} family (e.g \textit{S100A9} and \textit{S100A12}) and genes nearby psoriasis GWAS loci such as \textit{STAT3} and \textit{IFIH1}. Notably, 45 genes were differentially expressed in both studies but showed opposite direction. For example \textit{SERPINB2} was down-regulated in the in-house data and up-regulated in the Tervaniemi results.

\subsubsection{Tissue and disease specificity in gene expression modulation and relevant biological pathways}

Gene expression in peripheral blood of 3 healthy controls was compared to peripheral blood of the 3 PsA patients in CD14$^+$ monocytes, mCD4$^+$ and mCD8$^+$ cells. The fold change was calculated for the mean peripheral blood expression across the 3 PsA patients compared to the mean expression of the 3 healthy controls (as detailed in Chapter \ref{ch:Mat}). P-values for the significance of the change were also calculated for each particular gene. Integration of the differentially expressed genes between synovial fluid and peripheral blood in PsA and the modulated genes in peripheral blood between PsA and healthy controls allowed the identification of 3 group of genes (Figure \ref{figure:PSA\_PCR\_array\_HC\_FC\_correlation}). First, the genes only significantly modulated (based on p-value and fold change threshold criteria) in peripheral blood between controls and PsA were designated as systemic genes (Figure \ref{figure:PSA\_PCR\_array\_HC\_FC\_correlation} green dots). Those genes were not significantly modulated between synovial fluid and peripheral blood in PsA patients and could then be considered as the circulating disease "footprint". For example\ textit{CCL24} and \textit{CCL27} in CD14$^+$ monocytes. A second group of genes were designated as tissue-specific, since they were significantly modulated between synovial fluid and peripheral blood in PsA patients but did not show significant changes between controls and PsA at the circulating level (Figure \ref{figure:PSA\_PCR\_array\_HC\_FC\_correlation} red dots). This group included \textit{SPP1} and \textit{FN1} in the 3 cell types, the two genes presenting the greatest fold change between synovial fluid and peripheral blood with no significant changes in peripheral blood between PsA and healthy controls . The third category comprised genes significantly modulated for each cell type between controls and PsA patients in peripheral blood as well as between synovial fluid and peripheral blood in PsA patients. These genes were defined as putative disease-specific genes (Figure \ref{figure:PSA\_PCR\_array\_HC\_FC\_correlation} blue dots). For example, \textit{GPR68} in mCD4$^+$ cells was up-regulated in PsA peripheral blood mCD4$^+$ when compared to the control counterparts and further up-regulated in synovial fluid when compared to peripheral blood in PsA individuals (Figure \ref{figure:PSA\_PCR\_array\_HC\_FC\_correlation} B).

Pathway enrichment analysis using the significantly modulated genes between peripheral blood of healthy controls and PsA patients, only the Reactome immune system pathway appeared as significant for CD14$^+$ monocytes and mCD4$^+$ cells. This result reinforced the tissue-specificity of the pathways enriched for the modulated genes between synovial fluid and peripheral blood in CD14$^+$ monocytes PsA patients and clearly suggest a more pronounced inflammatory phenotype of the pathological CD14$^+$ monocytes in synovial fluid compared to peripheral blood.

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

The pilot study presented in this chapter has characterised the expression of relevant immune genes in CD14$^+$ monocytes, mCD4$^+$ and mCD8$^+$ cells isolated from synovial fluid and peripheral blood. In this study, CD14$^+$ monocytes and mCD8$^+$ presented the largest number of statistically significant DEGs between synovial fluid and peripheral blood. Integration of transcriptome profiling with paired-ATAC data in CD14$^+$ monocytes, mCD4$^+$ and mCD8$^+$ revealed that a small number of the DEGs between synovial fluid and peripheral blood were nearby DARs showing changes in the same direction. This overlap only presented significant enrichment for CD14$^+$ monocytes.

Pro-inflammatory genes, including \textit{TNFA}, \textit{CXCL13} or \textit{CCL18}, previously reported by Dolcino and colleagues as DEGs between synovial membranes and peripheral blood from PsA patients \parencite{Dolcino2015}, were found to be up-regulated in synovial fluid compared to peripheral blood in at least one of the cell types. Other genes were only found to be differentially expressed between synovial fluid and peripheral blood in CD14$^+$ monocytes, for example \textit{CXCL10}, \textit{CCL7}, and \textit{CCL17}, which play a critical role in the migration of monocytes from bone marrow to tissues \parencite{Tsou2007}. Other genes involved in Th-17 cell response, such as \textit{CXCL13} and \textit{IL26}\parencite{Takagi2008} only showed up-regulation in synovial fluid mCD4$^+$ and/or mCD8$^+$ when compared to peripheral blood, no changing expression in CD14$^+$ monocytes

Comparison of DEGs in peripheral blood between PsA patients and controls with the DEGs between synovial fluid and peripheral blood in patients identified a number of systemic, tissue-specific and putative-disease specific DEGs, all based on the significance of the changes in gene expression found in this data. In CD14$^+$ monocytes, a much larger number of immune-relevant genes were found to be differentially expressed between the two tissues of patients when compared to those changing expression between PsA patients and healthy controls in peripheral blood. This is likely due to the highly inflammatory environment in the synovium of PsA patients as well as to the plasticity of monocytes and their transition into macrophage differentiation at the site of inflammation \parencite{Yoon2014, Park2016}. A particularly interesting systemic DEG in mCD8$^+$ cells was \textit{CCR10}, a chemokine receptor co-expressed by a subset of memory cells that preferentially migrate to skin, which has also been identified as an up-regulated gene in CD8$^+$ cells in the psoriasis cohort when compared to healthy controls (Chapter \ref{ch:Results2} and in patients with atopic dermatitis \parencite{Hijnen2005}. In mCD4$^+$, \textit{GPR68}, a G protein-coupled receptor (GPCR) was found to be a putative disease-specific gene up-regulated in peripheral blood of PsA patients when compared to controls and further increasing its expression in synovial fluid. \textit{GPR68} undergoes activation through pH acidification, which is a characteristic feature of synovial tissues under inflammation \parencite{Biniecka2016, Saxena2011},. Interestingly, \textit{GPR65}, another member of the acid-sensing GPCR family, has been associated with a number of immune mediated diseases, including AS, CD and MS and found to be a marker of pathogenic Th17 cells in the murine and human systems \parencite{Cortes2013,Lassen2016,Wirasinha2018,Gaublomme2015, Al-Mossawi 2017}.

In the 3 cell types \textit{SPP1} and \textit{FN1} were the most significantly DEGs between synovial fluid and peripheral blood of PsA patients, in line with the finding from Dolcino and colleagues. Significant up-regulation of both genes in this data was tissue-specific in the 3 cell types, without significant changes in peripheral blood between PsA patients and controls. This was consistent with Dolcino and colleagues results for \texit{FN1} but not for \textit{SPP1}, where moderate up-regulation in peripheral blood from PsA patients compared to controls was also detected. Moreover, in CD14$^+$ monocytes ATAC data has also revealed two synovial fluid open DARs at the promoter and downstream \textit{FN1} only. \textit{FN1} encodes fibronectin-1, a main component of the cartilage matrix, involved in cell adhesion, migration, growth and differentiation and found to be highly expressed in RA inflamed synovium \parencite{Chang2005}. \textit{FN1} has also shown to induce bone resorption mediated by pro-inflammatory mediators, such as nitric oxyde and IL-1$\beta$ \parencite{Gramoun2010}.

Pathway analysis using significant DEGs between synovial fluid and peripheral blood in this data revealed enrichment for chemokine, TLR and NOD-like signalling pathways in CD14$^+$ monocytes. This was consistent with the well-known role of TLR and NOD-like receptors for rheumatic diseases \parencite{McCormack2009}. The significant up-regulation of the receptors \textit{TLR1} and \textit{TLR2} in synovial fluid CD14$^+$ monocytes compared to peripheral blood contributed to the enrichment of the TLR signaling pathway. Other studies have also reported increased \textit{TLR-2} and \textit{TLR-4} expression in SFMCs compared to PBMCs in patients with juvenile idiopathic arthritis \parencite{Myles2011}. NOD-like signaling has also been highlighted in the genome-wide trancriptomic analysis in lesional and uninvolved psoriatic skin presented in Chapter \ref{ch:Results2}. The nicotinamide enzyme coded by \textit{NAMPT} was up-regulated in synovial fluid compared to peripheral blood and its expression by pro-inflammatory monocytes mediates the production of pro-inflammatory cytokines and Th-17 cell differentiation, showing potential as a drug target in an arthritis mouse model \parencite{Presumey2013.} The cross-talk between the TLR and NOD-like signalling pathways was further evidenced by network-based analysis, where the functional interaction between members of both pathways may contribute to activation of NF$\kappa$B TF in synovial fluid, particularly in CD14$^+$ monocytes. The enrichment of synovial fluid open DARs in the proximity of genes within the NF-$\kappa$B pathway and could potentially lead to the transcriptional up-regulation of downstream genes such as \textit{TNFA}, textit{CCL2} and \textit{CCL5} and the enrichment of chemokine signalling in CD14$^+$ monocytes DEGs between synovial fluid and peripheral blood. In mCD4$^+$ , enrichment of the IL-10 signalling pathway was particularly interesting in the context of the expansion of Tregs in synovial fluid revealed by colleagues’ flow cytometry analysis in the same samples. Tregs are characterised by the expression of anti-inflammatory cytokines, including IL-10 \parencite{Garra2004} and qPCR transcriptomic data showed in a significant increase in expression of the IL-10 receptor subunit $\alpha$ \textit{IL10RA} and a similar trend for \textit{IL10} (p-value=0.004 and 0.14, respectively) in synovial fluid mCD4$^+$ and mCD8$^+$ compared to peripheral blood. This may suggest that inflammation in PsA is refractory to the immunomodulatory effects for IL-10 signalling in synovial fluid or counterbalanced by the its immunostimulatory properties, in line with failure of IL-10 agonist therapy in the treatment of CD and psoriasis \parencite{Marlow2013, Kimball2002}.

Osteopontin, \textit{SPP1} protein product, is a cytokine and chemokine expressed by many cell types, including monocytes/macrophages and T cells. It is involved in cell migration, adhesion and cell-mediated immune response through regulation of T cells, importantly in Th-17 and plays a role in a number of chronic inflammatory and autoimmune diseases, including MS, RA and SLE arthritis \parencite{Morimoto2010,Rittling2015}.

~~Although additional samples would be required to ensure the tissue-specificity of differential expression observed for \textit{SPP1} and \textit{FN1} in this study,~~ the up-regulation both genes in synovial fluid highlights activation of chemotaxis, immune cell infiltration, activation of the Th-17 immune response and dysregulation of osteoclast bone remodeling , all hallmarks of the PsA pathophysiology \parencite{Durham2015, Mensah2008}.

\subsection{Integration of protein expression data with chromatin accessibility and transcriptomic data}

Single-cell mass cytometry data generated in the 4 patients with qPCR and/or ATAC data available and additional 6 patients investigated the expression of a number of cytokines produced by CD14$^+$ monocytes in synovial fluid and peripheral blood. The increased percentage of monocytes producing TNF-$\alpha$, MCP-1, osteopontin and IP-10 was consistent with the 4 genes being transcriptionally up-regulated in PsA CD14$^+$ monocytes from synovial fluid compared to peripheral blood. An study using enzyme-linked immunosorbent assay (ELISA) has reported an increased production of TNF-$\alpha$ in PsA synovial fluid compared to OA \parencite{Partsch1997}. The importance of osteopontin has already been highlighted as its coding gene \textit{SSP1} coding for osteopontin was one of the top two DEGs between synovial fluid and peripheral blood of PsA, with particularly large fold changes in CD14$^+$ monocytes. Osteopontin is a cytokine and chemokine expressed by many cell types, including monocytes/macrophages, involved in cell migration, adhesion and cell-mediated immune response through Th-17 differentiation, playing a role in a number of chronic inflammatory and autoimmune diseases \parencite{Morimoto2010,Rittling2015}.

In CD14$^+$ monocytes, \textit{CCL2}/MCP-1 and \textit{CXCL10}/IP-10 were examples for pro-inflammatory molecules showing correlating differences between synovial fluid and peripheral blood at the chromatin accessibility, transcriptomic and protein expression levels. Dolcino’s study did not identify up-regulation of \textit{CCL2} or \textit{CXCL10} expression in PsA synovial membranes when compared to OA, likely due to the mixed composition of cell in the synovial membranes dominated by connective and adipose tissue. The open synovial fluid DARs upstream \textit{CCL2} and \textit{CXCL10} in CD14$^+$ monocytes highlighted changes in chromatin accessibility that are likely driven by the pro-inflammatory environment in the joint. These two differential accessible sites were not found to interact with distal promoters in unstimulated monocytes \parencite{Javierre2016} and, given the proximity to each of the genes, both regions may exert a local regulatory effect in the expression of \textit{CCL2} and \textit{CXCL10}, respectively.

In terms of pathophysiology, MCP-1 is one of the key cytokines involved in monocytes migration from peripheral blood into tissues but it also has a role in chemotaxis of T cells to the site of inflammation \parencite{Tsou2007,Shadidi2003}. Up-regulation of MCP-1 expression in plasma compared to controls has previously been demonstrated and shown to correlate with the infiltrated levels of T cells in the synovium \parencite{Ross2000}. Nevertheless, CD14$^+$ monocytes-specific gene and protein expression of \textit{CCL2}/MCP-1 in PsA had not been investigated. The transcriptional up-regulation of \textit{CCR2}, the receptor of MCP-1, in PsA synovial fluid mCD8$^+$ compared to peripheral blood in my data reinforces the biological role of \textit{CCL2}-\textit{CCR2} in mediating T cell infiltration in the synovium and the subsequent mCD8$^+$ T cell expansion in this tissue. Nevertheless, the potential therapeutic effect of \textit{CCL2}-\textit{CCR2} agonists has failed in RA using a human anti-CCR2 blocking antibody \parencite{Vergunst2008}. \texit{CXCL10} is also a pro-inflammatory chemokine secreted by several immune and non-immune related cells upon IFN-$\gamma$ and TNF-$\alpha$ stimulation and participates into the recruitment of T cells, monocytes and NK, amongst others, to sites of inflammation \parencite{Antonelli2014}. Significant up-regulation of \textit{CXCL10} by qPCR was only found in CD14$^+$ monocytes between synovial fluid and peripheral blood and in PsA peripheral blood vs healthy controls. A recent conference abstract studying immune cell populations isolated from PsA has also demonstrated greater expression levels of \texit{CXCL10} in monocytes compared to T cells \parencite{Muntyanu2017}. Interestingly, mass cytometry data of mCD4$^+$ and mCD8$^+$ cells from the same 10 PsA patients did not show significant differences in the percentage of cells producing this chemokine between synovial fluid and peripheral blood, reinforcing CD14$^+$ monocyte specificity of \texit{CXCL10} dysregulation between the two tissues in PsA. Lastly, increased transcript levels of \textit{CXCL10} in PsA peripheral blood compared to psoriasis vulgaris have highlighted the role of this chemokine as a potential biomarker and key player in the initiation and progression of joint inflammation \parencite{Abji2016}.

CXCL10 is secreted by lymphocytes, monocytes, keratinocytes, fibroblasts, and endothelial cells in response to interferon‐γ (IFNγ) and tumor necrosis factor. CXCL10 functions primarily in recruiting leukocytes such as T cells, eosinophils, monocytes, and natural killer cells to sites of inflammation, and also has angiostatic properties [5](https://onlinelibrary.wiley.com/doi/full/10.1002/art.39800#art39800-bib-0005).

-marker for PsA

-skin?

-observation in PB

\subsection{Limitations of the study}

\label{Discussion\_scRNAseq}

The work presented in this chapter is an exploratory study and a proof of principle for the implementation of a multi-omics approach in PsA clinical samples from affected joints, a very powerful strategy to dissect disease pathophysiology in a cell type specific manner. Nevertheless, a number of limitations and challenges were encountered and need to be taken into account to contextualise these results. The small sample size (n=3) and the lack of paired data across all the techniques represent one of the limitations. Moreover, RNA-seq would allow a genome-wide overview of transcriptomic differences between tissues and the identification of additional cell type specific relevant pathways that have not yet been unveiled by the set of genes interrogated in the qPCR array. Importantly, this study lacks of additional controls, including peripheral blood from healthy individuals or synovial fluid from another autoimmune or non-inflammatory joint disease, as incorporated by other studies \parencite{Fumitaka2018, Dolcino2015,Zhang2018}. This would allow pinpointing changes in chromatin accessibility, transcriptomic and protein expression specific for the PsA inflamed synovium and will be conducted in the next stages of the project.

Another challenge in this study relates to the analysis and integration of scRNA-seq and mass cytometry data. Both techniques still represent emerging fields where there is no “gold” standard on the strategy to combine samples across patients to account for batch effect or in downstream data analysis. In this exploratory study, monocytes were identified from each SFMCs and PBMCs scRNA-seq sample and combined using CCA for further subpopulation identification, which compared to other methods of merging and normalising data preserves smaller differences between samples. Cluster identification is another sensitive aspect in scRNA-seq data analysis and standard algorithms incorporated in Seurat failed to identify robust subpopulations of monocytes in the combined tissuesin this particular data. This may be the result of the differences between monocytes of synovial fluid and peripheral blood being larger than the variability expected by the clustering algorithm, and therefore alternative methods for identifying clusters in monocytes from the combined tissues should be investigated. The identification of robust and stable subpopulations through cluster analysis will also benefit from the implementation of algorithms designed for cluster validation such as Silhouettes, which has recently been used successfully in the field of single-cell \parencite{Rousseeuw1987, Zhang2018}. Moreover, incorporating RNA-seq from CD14$^+$ monocytes will help in the interpretation and validation of the scRNA-seq results. Similarly, different methodologies for cluster identification and annotation in mass cytometry are being explored and additional data analysis is in progress to identify distinct subpopulations of monocytes based on cytokine and chemokine expression. In terms of data integration, this pilot study used relatively simplistic multi-omics integration, limited by sample size, technical aspects and time scale, providing a platform for future validation studies, which will benefit of a more systematic approach.