Title: Standardisation of Synovial Tissue Infiltrate Analysis: How far have we come? How much further do we need to go?

Authors: Malcolm D. Smith¹, Dominique Baeten², Ann-Kristin Ulfgren³, Iain B. McInnes⁴, Oliver Fitzgerald⁵, Barry Bresnihan⁶, Paul P. Tak⁶, and Douglas Veale⁵

Affiliated Institutions:
¹Rheumatology Research Unit, Repatriation General Hospital, Adelaide, South Australia
²Department of Rheumatology, Ghent University Hospital, Ghent, Belgium
³Department of Medicine Unit of Rheumatology, Karolinska University Hospital, Stockholm, Sweden
⁴Centre for Rheumatic Diseases, University of Glasgow, Glasgow, Scotland
⁵Department of Rheumatology, St. Vincent’s University Hospital, Dublin, Ireland
⁶Division of Clinical Immunology and Rheumatology, Academic Medical Centre, University of Amsterdam, The Netherlands

Keywords
Standardisation synovial tissue rheumatoid arthritis quantitation cellular infiltration cytokines
Abstract
There is increasing interest in the use of synovial tissue analysis as an outcome measure in randomised clinical trials of drug treatments in inflammatory arthritis, including Rheumatoid Arthritis. There is now good evidence that changes in the cellular infiltrate and expression of cytokines, chemokines and cell adhesion molecules can be demonstrated in the synovial membrane as a result of therapeutic interventions in Rheumatoid Arthritis. However, before synovial tissue analysis can be recommended as an outcome measure in such studies, a number of quality control issues related to site and method of synovial tissue acquisition, standardised methods of tissue processing and appropriate methods of detection and quantitation of cell lineage specific markers and relevant biological proteins need to be addressed. A special interest group was undertaken as part of Omeract 7 to examine the published evidence relating to the use of synovial membrane analysis as an outcome measure in Rheumatoid arthritis. This review is a distillation of that process and as such aims to summarise the literature, in particular to focus on obtaining synovial samples, processing and analyzing the inflammatory infiltrate. In addition, it highlights some of the novel technological scientific advances which are already being used for synovial analysis but which require further study. An important part of this paper is the future research agenda, which identifies significant research questions related to standardisation which are still to be addressed.
Introduction
There is increasing interest in the use of synovial membrane (SM) tissue analysis as an outcome measure in Phase 1b/Phase II treatment intervention trials in Rheumatoid Arthritis (RA), as a “proof of concept” relating to the efficacy of new therapeutic agents (1). The advantage of using SM analysis as an outcome measure is the ability to significantly reduce the patient numbers needed in studies undertaken early in the development of a new therapeutic agent when an accurate decision about whether to proceed with drug development to larger phase III and IV studies is required. Studies have already been published which have demonstrated the ability of sequentially acquired SM before and after treatment of RA patients to reflect change in clinical disease activity (2-21). In addition, several studies have shown lack of changes in relevant synovial biomarkers when ineffective treatment or placebo treatment is used (21-24).

However, before SM analysis can be utilized as an outcome measure in randomised clinical trials (RCTs), a number of issues need to be addressed: (i) standardisation and validation of the methods of acquisition (ii) how representative these samples are of the generalized disease process (iii) quantitation methods utilised to measure various parameters within these SM samples including correlation with changes in clinical disease activity. This paper will address each of these issues and also evaluate the quality of the evidence by applying the OMERACT (Outcome Measures in Rheumatoid Arthritis Clinical Trials) filter of truth, reliability and feasibility (25). While most of the published studies have been performed on synovial tissue obtained from patients with rheumatoid arthritis, there are limited studies looking at Osteoarthritis, Psoriatic Arthritis and other spondyloarthropathies and these will be referred to later in this paper.

Methods of acquisition of synovial membrane tissue
There are currently three methods of acquiring SM tissue from patients undergoing drug treatment for Rheumatoid Arthritis: blind needle biopsy, arthroscopic directed biopsy and biopsy under imaging (usually ultrasound) guidance. The imaging techniques are relatively new and there is little published data on the reliability of this technique so it will not be discussed further in this paper. However, there is considerable data on the use of the other two techniques - advantages and disadvantages of each will be discussed.

Blind needle biopsy has been used to sample SM for several decades and clearly has an established safety and feasibility record. It is not a costly procedure, requires no special facilities and can be undertaken in most rheumatology departments. The main disadvantages of this technique relate to the inability to directly visualise the site of biopsy and the tissue being biopsied. In addition, it is usually only successful in acquiring synovial tissue from the knee joint and in most cases, can only reliably sample synovial tissue from the suprapatellar bursa. There is also a concern about how often the blind needle biopsy technique may fail to obtain satisfactory SM samples, particularly if treatment results in a clinically quiescent joint, which is more difficult to take samples from using blind needle biopsy techniques. In one series with more than 800 Parker Pearson biopsy procedures, sufficient synovial tissue for histological examination was obtained in about 85% of the patients with various forms of arthritis (26). The procedure failed especially in joints which were not swollen. Within the context of a “proof of concept” phase IB or II RCT, such a failure rate to acquire adequate synovial tissue samples would not be acceptable.
While arthroscopic directed synovial biopsy does not have any of these potential deficiencies compared to the needle biopsy technique, it is more costly with restricted feasibility due to a requirement for skill acquisition (there is a significant “learning curve”) and the requirement for a specialised sterile area to undertake the procedure. There have been limited studies which directly compare the two synovial membrane biopsy techniques. Two studies have included SM obtained by both techniques and have concluded that there is generally good correlation between the analysis of synovial tissue taken by these two techniques (27, 28). However, it should be noted that these studies did not address the ability of the needle biopsy technique to sequentially acquire synovial tissue before and after drug treatment. In addition, the SM parameters analysed were limited to macrophage and T cell surface markers, lining layer thickness and vascularity so it is unclear whether these results can be generalised to other SM parameters of interest. Taken together, the available data indicate that blind needle techniques can be used to examine some major cell populations in cross-sectional studies, but arthroscopic sampling is preferable for serial biopsy in proof of concept clinical trials.

**How representative are the synovial membrane tissue samples of the disease process?**

How representative SM samples are may relate to a number of factors (a) does the SM in one joint (e.g. knee, wrist, MCP, etc) reflect that seen in other joints in inflammatory arthritis and (b) is there variability within an individual joint (eg non CP-J versus CP-J regions)? These questions have direct relevance in relation to the source of synovial tissue for SM analysis in RCTs and whether it is reliable to restrict the number of microscopic fields analysed when SM quantitation is performed.

Only one study has been published addressing how representative is SM from different joints. This study compared SM from the knee joints with SM from wrist or metacarpophalangeal joints (MCP) in RA patients (29). The authors examined the SM tissue from 9 patients who had undergone arthroscopic biopsy of an inflamed knee and small joint on the same day. Although patient numbers were small, numerous biopsy samples were analysed for markers of macrophages, T cells and the cytokine IL-6 and this study suggests there are good correlations for cell infiltration in the synovial sublining, the region where most inflammatory cells are found, between large (knee) and small (wrist or MCP) joints.

Various studies have addressed the differences in SM obtained from adjacent to the CP-J versus non CP-J regions within the same knee joint. The first study compared biopsies obtained by direct visualisation at arthroscopy and blind needle biopsies, therefore not directly comparing CP-J versus non CP-J tissue. The SM analysis was limited to staining for T lymphocytes and activated macrophages (28). Using the arthroscopic SM biopsies there were greater macrophage numbers in the CP-J SM specimens compared to non CP-J biopsies specimens, however this difference did not reach statistical significance. A second study looked at a wider range of SM parameters in a larger number of RA patients comparing CP-J with non CP-J regions and showed little difference between synovial tissue obtained from these two regions (30). A third study, involving small numbers (8) of RA patients, obtained synovial tissue at the time of joint replacement surgery (4 patients) or at knee arthroscopy (4 patients) and concluded that macrophage infiltration was greater at the CP-J and that expression of myeloid related proteins was seen predominantly at the CP-J (31). Other studies confirmed that there is on average no clear

cut difference in the features of synovial inflammation or the expression of mediators of inflammation and destruction at the CP-J compared with non CP-J regions (32, 33).

Several studies have addressed the variability of SM parameters within and between multiple biopsies taken from within a single joint. One study analysed 154 synovial biopsies from 29 RA patients for lining layer hyperplasia, fibrosis, proliferating blood vessels, perivascular lymphocyte infiltrates, focal aggregates and diffuse infiltrates of lymphocytes (34). The authors reported considerable homogeneity for these SM parameters within a single joint. Two studies have addressed the variability within and between synovial tissue biopsies with inter-patient variability looking at different immunohistochemical parameters. One study suggested that there was limited variability within and between synovial biopsies for cytokines and cell adhesion molecules, which was less than the variability seen in SM from different patients (35). Another study examined markers for bone erosion (RANKL and OPG) which are very relevant to RA. This study suggested marked inter- and intra-biopsy variability for RANKL expression, probably due to the variability in T cell infiltration, while OPG expression was more consistent within and between synovial biopsies (36). While this variability within SM probably accurately reflects the biological variability of expression, it does suggest that restricting the number of samples examined histologically may sacrifice reliability to improve feasibility, at least in the measurement of focally expressed SM parameters. For T cell infiltration and expression of activation antigens in RA synovium, a variance of less than 10% can be reached when at least six biopsy specimens are examined (37), suggesting that representative data can be obtained when a limited number of biopsy samples from different areas within one joint are investigated. Consistent with these data it has been demonstrated that using about six tissue samples allows for the detection of twofold differences in gene expression by quantitative PCR (38). Therefore, we recommend obtaining at least six biopsies for each technique used in research.

**Quantitation methods in SM analysis**

There are three common methods used in published studies to quantify immunohistochemical labeling of synovial tissues, looking at a variety of markers including cellular infiltrate (including phenotyping of cells), cytokine and chemokine expression, receptor expression on cell surfaces and cellular adhesion molecule expression (table 1).

**Manual Counting (MC)**

Manual cell counting is the time-honoured “gold standard” although little standardisation of this technique in terms of intra- and inter-variability or responsiveness to change in disease activity has been performed (figure 1). The results are usually recorded as the percentage of all cells counted in a high power field that are positive by immunohistochemical labelling for a particular parameter, corrected for area of tissue examined. Modifications of this technique can include restricting the region (ie lymphocyte aggregates) or cell type (ie T lymphocytes characterised by immunohistological staining) that is examined in each high power field. This technique is very time consuming but has face validity. It is subject to observer bias as well as field selection bias, unless all fields are analysed. While it is possible to restrict the number of fields analysed by manual counting without affecting reliability of the measurement, this has only been demonstrated for a limited number of SM parameters (27). This technique is not well suited to and has not been validated for quantitation of parameters which are
not always cell associated (e.g. cytokines).

**Semiquantitative Scoring (SQA)**

This technique is the fastest of the quantification techniques and represents a grading of biopsy staining at low to medium power magnification (figure 2). It does eliminate field selection bias but has some observer error, which may be minimised by observer training and standardisation as well as utilising two observers, with the final score being a consensus score. Although variations of this scoring method have utilised single scorers, with limited training or standardisation as well as variations in scoring and averaging of high power fields, none of these variations have been validated.

**Digital Image Analysis (DIA)**

This is the newest of the available quantification techniques, which may be the most sensitive to change, but also is the most expensive in terms of equipment required (figure 3). The results are usually expressed as the area of staining (in pixels), the density of staining (units) or a combined parameter of integrated optical density or IOD (pixel-units). Several validation studies have confirmed a direct correlation between cell numbers and area of staining as measured by DIA in various tissues. It is probably more time efficient than manual counting but there is considerable training involved for the observer. There is a potential for observer bias in the selection of thresholds and also in field selection. The former source of bias is minimised with most of the available software programs by automatic selection of the threshold when an image is scanned by the program. However, it is possible to manually over-ride this automatic threshold selection process. Automation of the field selection using a motorized microscope stage and scanning the entire tissue section field can minimise field selection bias. As with all measurement techniques, particularly MC and DIA, the observer needs to make a decision concerning how intact and representative the synovial tissue being analysed is and this can be a source of bias. This source of bias can be limited by analysing only SM samples that have an intact lining and contain sufficient sub-intimal tissue as well as improving the quality control of SM biopsy procedures. Automation of much of the procedures can reduce the time required to utilise this technique, making it attractive as a quantification method for large sample sizes.

Three studies have compared at least two of these quantitation methods using the same series of synovial tissue biopsies. One study utilized MC and SQA to quantitate T cell and macrophage content in sequential synovial biopsies taken during treatment studies using Methotrexate, Interleukin-10 and placebo (39). Both methods correlated well for lining and sublining macrophages and T-cell infiltration, with a suggestion that MC was more sensitive to change in disease activity in a subgroup of 9 patients with baseline and post-treatment synovial biopsies. Another study compared DIA with MC for intimal layer thickness, vascularity, CD3 and CD8 positive T cells in 78 synovial tissue sections (40). There was good correlation between the two methods for intimal layer thickness and CD8+ cell infiltration, while the two methods were less well correlated for CD3+ cell infiltration and poorly correlated for vascularity. Another study compared DIA and SQA for the quantitation of one cytokine (IL-1β) and one cell adhesion molecule (VCAM-1) in a series of synovial biopsies from 10 RA patients achieving an ACR remission with standard disease modifying agent (DMARD) treatment (41). This study showed good correlation between SQA and DIA although when the number of high power fields assessed was reduced from 20 to 6, there was some increase in variability. This study also
suggested that DIA was more sensitive to change than SQA. Another study compared all three methods of quantitation in a series of synovial biopsies taken from an involved and a non-involved knee joint from 9 patients with active RA (42). Parameters assessed were limited to T-cell (CD3+) and macrophage (CD68+) infiltrates. All three methods correlated well for both T-cells and macrophages and all three methods could distinguish between the inactive and active knee SM biopsies and the normal synovial tissue used as a control group in this study, although DIA and MC were more discriminatory than SQA. SQA was quicker to perform than DIA or MC, with MC being significantly slower to perform than either DIA or SQA (table 2).

**Responsiveness of synovial tissue analysis to treatment-induced changes in RA disease activity**

Several studies, using a range of quantitation methods, have demonstrated the responsiveness of SM analysis to changes in RA disease activity induced by treatment (table 3) with conventional DMARDs (2-9, 14, 15) or biological agents (10-13, 17, 18). A recent study was designed to identify the optimal synovial biomarker associated with clinical efficacy following a short treatment duration (20). This study demonstrated the status of sublining macrophages as an optimal biomarker associated with clinical response. Next this biomarker was tested across a range of discrete interventions and kinetics (21). Patients who participated in various randomized clinical trials were evaluated in the same centre, using standardized techniques. The treatments evaluated included methotrexate, leflunomide, prednisolone, infliximab, a specific CCR1 antagonist, and placebo. There was a significant correlation between the change in the number of sublining macrophages and the change in DAS28. The sensitivity to change of the biomarker was high in active treatment patients while no significant changes were detected in placebo treated patients.

**Conclusions**

Synovial membrane tissue acquisition is reasonably well standardized across rheumatology centers with preference for arthroscopic directed synovial biopsy techniques. Arthroscopic biopsy is more reliable than blind needle biopsy in accessing evaluable serial synovial tissue samples as well as accessing SM from small joints other than the knee joint. It can also allow SM biopsies from all regions within a joint under direct vision. It seems that SM acquired from actively inflamed large and small joints in RA patients is not significantly different. All three quantitation methods are well validated and standardized (table 1). However, methods which are both time efficient and sensitive to change in disease activity are probably more feasible, which favours DIA over SQA and MC methods of quantitative analysis. SM analysis reflects changes in RA disease activity in response to various therapies, including traditional DMARDs and biologic therapies. The lack of change in SM parameters in patients who fail to show a clinical response is further evidence of the reliability of SM analysis and suggests that it is an appropriate outcome measure for RCTs in RA.

**Synovial Tissue Analysis in Spondyloarthopathies and Osteoarthritis**

Although there have been limited studies on patients with inflammatory arthropathies other than rheumatoid arthritis, there are an increasing number of studies being published looking at the synovial tissue in psoriatic arthritis (44-49) and other spondyloarthopathies (50-55). Similar results, including the effect of standard DMARD treatment and biological therapies on the cellular infiltrate and cytokine production is
seen in these inflammatory arthropathies compared to that seen in the synovial tissue from patients with rheumatoid arthritis. Two of these studies (45, 47) have utilised a modification of the SQA method which has not been validated against other quantitation methods or the original SQA method and such validation studies are required if such modifications of the SQA method are to be used in future studies (see below). More recently, preliminary studies have also validated the use of synovial biopsies obtained by arthroscopy for other approaches such as microarray, which proved equally sensitive to change upon treatment (52, 55).

There are very limited studies on SM analysis in osteoarthritis (OA) which do not address the standardisation issues which are the main focus of this paper. While there appears to be an inflammatory component to the pathology of OA (57), it is unclear how relevant it is to the progression of the condition and at what stage in the progression of OA (pre-clinical, early symptoms or late disease) that SM pathology is important (58). Further studies of SM pathology in OA are clearly necessary, supported by the standardisation and validation studies which have currently been undertaken predominantly in SM from RA patients.

Although further studies are needed to assess specific standardisation issues such as intra- and inter-joint variability of the synovial histopathology in non-RA arthritides, these data indicate both the feasibility and the responsiveness to treatment. Since synovial histopathology reflects global disease activity in spondyloarthropathies, further exploration of synovial tissue analysis as a surrogate outcome measure in early phase clinical trials in all types of inflammatory arthritis are clearly warranted (56).
Further Studies Needed

Despite the fact that much work has already been done to validate and standardise SM analysis as an outcome measure, more research is required. Although not an exhaustive list, the following questions still need to be answered:

Do the published results concerning within and between biopsy variability apply to other synovial tissue parameters not measured in the published studies?

Can field analysis be limited to a manageable number of high power fields without introducing excessive variability and reducing reliability and does this apply to other synovial tissue parameters not measured in published studies looking at variability?

Is field selection for parameters, which do show regional variability (e.g. T cell aggregates) appropriate or does it introduce another bias into quantitation of synovial tissue analysis?

Given that there are differences between quantitation methods, should more than one method of quantitation be used in synovial tissue analysis and should a particular quantitation method be used depending on the SM parameter being measured (e.g. MC for quantitation of cell lineages in the inflammatory infiltrate, SQA or DIA for quantifying cytokines)?

Can modifications be made to the SQA method to restrict the number of observers and to average across measurements and does this correlate with the established method of SQA analysis?

Do all successful treatments for RA patients produce the same effects on the synovial tissue or are some SM changes unique to specific treatments?

Are the SM changes disease specific or can the results from RA studies be extrapolated to other inflammatory arthritides, including an asymmetric arthritis like Psoriatic Arthritis or a predominantly spinal condition like Ankylosing Spondylitis?

Most of the published studies on synovial tissue analysis have focussed on cellular markers, along with protein and mRNA analysis for a limited number of proteins of interest (e.g. cytokines, growth factors). With the development of more sophisticated techniques such as microarray, laser capture of sections of interest and novel proteomic approaches, it is likely that future synovial membrane studies will utilize these powerful instruments to further explore the biology of synovitis.

However, before there can be a wider application of arthroscopic synovial biopsies to clinical practice, basic research and RCTs, issues such as the availability of rheumatologists trained in arthroscopic techniques as well as the training and accreditation of rheumatological arthroscopists needs to be addressed. In addition, quality assurance in relation to the size of SM samples and how intact and representative these biopsies are need to be addressed. These issues need to be resolved before these techniques can be widely utilized as surrogate outcome measures in RCTs.

Statement

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in Annals of the Rheumatic Diseases editions and any other BMJPGL products to exploit all subsidiary rights, as set out in our licence.
References


30. Smeets TJM, Kraan MC, Galjaard S, Youssef PP, Smith MD, Tak PP.
oligo- and polyarticular, resembles more spondyloarthropathy than rheumatoid arthritis. Arthritis Res. Ther. (in press)


Figure 1: Manual counting of a synovial membrane section using a graticule

Figure 2: Semiquantitative scoring of synovial tissue

Figure 3: Digital Image Analysis of synovial tissue: DIA equipment (A), RA synovial tissue stained for CD3 using the immunoperoxidase method with AEC (red) as chromogen x200 (B) and x400 (C), Image shown in (B) captured by DIA software and processed for nuclear staining with red mask applied (D), CD3 staining with red mask applied (E) and both nuclear staining (outlined) and CD3 staining (red mask) (F). Software will automatically detect nuclear staining and immunoperoxidase staining and calculate areas of each but the observer can over-ride the program if necessary.
Table 1: Application of the OMERACT filter to the three methods of quantitation of synovial membrane immunohistochemical labelling currently in use.

<table>
<thead>
<tr>
<th>Quantitation Method</th>
<th>Validity</th>
<th>Reliability</th>
<th>Feasability</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual Counting (MC)</td>
<td>Valid measure when the parameter being measured is localised to a cell. Less certainty about the validity of this method when measuring parameters which are not cell associated or diffuse in their distribution eg cytokines</td>
<td>Reliability demonstrated between observers in the same research laboratory but not tested between different research centres. Reliability not tested for a wide range of biological parameters. Field selection bias could be a problem for RCTs.</td>
<td>Requires minimal equipment which should be readily available in any research centre in the world. Laborious and time consuming method which is not well suited to large scale studies.</td>
<td>26, 27, 30, 37, 38, 40</td>
</tr>
<tr>
<td>Semiquantitative Analysis (SQA)</td>
<td>Most subjective of methods but does attempt to evaluate the tissue as a whole, removing issues of field selection bias. Validated in the measurement of a wide range of biological parameters in synovial tissue</td>
<td>Intra- and inter-observer reliability demonstrated but requires the use of more than one observer with training and standardisation. Use of a atlas of gradings would improve reliability between centres.</td>
<td>Most feasible of methods as it requires no special equipment. Only feasibility issues relate to the training and standardisation of observers between centres. This would be facilitated by the development of an atlas of grades for a range of biological parameters in the synovial membrane</td>
<td>22, 35, 37, 40</td>
</tr>
<tr>
<td>Digital Image Analysis (DIA)</td>
<td>Demonstrated to be valid for the widest range of biological parameters in the synovial membrane including cytokines and metalloproteinases.</td>
<td>Intra- and inter-observer reliability demonstrated for a wide range of biological parameters in the synovial membrane. Automatisation of field acquisition and threshold selection would reduce any remaining sources of bias in this method</td>
<td>This method is the most feasible method of quantitation of synovial tissue staining in RCTs because of the minimisation of bias, the speed of the method and the ability to automate many of the steps involved. The significant “learning curve” and the initial set up costs are a major deterrent to wider application of this method outside RCTs and specialised synovial tissue research centres.</td>
<td>33, 38, 39, 40</td>
</tr>
</tbody>
</table>
**Table 2:** Comparison of advantages/disadvantages of manual counting, semi-quantitative and digital image analysis techniques.

<table>
<thead>
<tr>
<th></th>
<th>Manual Counting</th>
<th>Semi-Quantitative</th>
<th>Digital Image Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observer Bias</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Field Selection Bias</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sensitivity to Change</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Time Intensive</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Cost</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>
Table 3: Published studies on the effect of treatment on the cellular infiltrate and other biological parameters in the synovial membrane

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Cellular Infiltrate</th>
<th>Cytokines</th>
<th>Cell Adhesion Molecules</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td>8, 13, 14, 45</td>
<td>9, 13, 14, 42, 45</td>
<td>9, 13, 14, 45</td>
<td>MMPs: 14, 45 Myeloid Related Proteins: 43</td>
</tr>
<tr>
<td>Leflunomide</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>MMPs: 14</td>
</tr>
<tr>
<td>IM Gold</td>
<td>2, 4</td>
<td>4, 5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>7, 19</td>
<td>4, 7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>TNF Blockers</td>
<td>10, 11, 17, 47, 52</td>
<td>11, 12</td>
<td>10, 52</td>
<td>MMPs: 50</td>
</tr>
<tr>
<td>IL-1 receptor antagonist</td>
<td>16</td>
<td></td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD 38</td>
<td>CD 22</td>
<td>CD 68</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td><img src="image" alt="CD 38 Image" /></td>
<td><img src="image" alt="CD 22 Image" /></td>
<td><img src="image" alt="CD 68 Image" /></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><img src="image" alt="CD 38 Image" /></td>
<td><img src="image" alt="CD 22 Image" /></td>
<td><img src="image" alt="CD 68 Image" /></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="CD 38 Image" /></td>
<td><img src="image" alt="CD 22 Image" /></td>
<td><img src="image" alt="CD 68 Image" /></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="CD 38 Image" /></td>
<td><img src="image" alt="CD 22 Image" /></td>
<td><img src="image" alt="CD 68 Image" /></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="CD 38 Image" /></td>
<td><img src="image" alt="CD 22 Image" /></td>
<td><img src="image" alt="CD 68 Image" /></td>
<td></td>
</tr>
</tbody>
</table>