

# Synovial Tissue Sublining CD68 Expression Is a Biomarker of Therapeutic Response in Rheumatoid Arthritis Clinical Trials: Consistency Across Centers

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**ABSTRACT. Objective.** To determine whether the correlation between the mean change in disease activity and the mean change in synovial sublining (sl) CD68 expression could be demonstrated across different academic centers.

**Methods.** Synovial biopsies obtained at arthroscopy from patients with rheumatoid arthritis before and 160 days after rituximab therapy were selected and coded. Paired sections were processed independently at Amsterdam Medical Center (AMC) and at St. Vincent's University Hospital (SVUH), Dublin. Digital image analysis (DIA) was employed at both centers to quantify sublining CD68 expression.

**Results.** After analysis of CD68sl expression at centers in 2 different countries, high levels of intra-center and intercenter agreement were observed. For the pooled sections stained at AMC, the correlation between 2 investigators was  $R = 0.942$ ,  $p = 0.000$ , and for sections stained at SVUH,  $R = 0.899$ ,  $p = 0.001$ . Similarly, the intracenter correlations for  $\Delta$ CD68sl expression after treatment were  $R = 0.998$ ,  $p = 0.000$ , for sections stained at AMC and  $R = 0.880$ ,  $p = 0.000$ , for sections stained at SVUH. The intercenter correlation for the pooled scores of sections stained at AMC was  $R = 0.85$ ,  $p = 0.000$ , and for the sections stained at SVUH,  $R = 0.62$ ,  $p = 0.001$ . The consistent correlation between  $\Delta$ DAS (Disease Activity Score) and  $\Delta$ CD68sl expression across different studies (Pearson correlation = 0.895,  $p < 0.001$ ) was confirmed. The standardized response mean values for  $\Delta$ CD68sl, calculated from analyses at both AMC and SVUH, were consistently 0.5 or greater, indicating a moderate to high potential to detect change.

**Conclusion.** The correlation between mean  $\Delta$ DAS and mean  $\Delta$ CD68sl expression was confirmed across 2 centers. Examination of serial biopsy samples can be used reliably to screen for interesting biological effects at the site of inflammation at an early stage of drug development. (J Rheumatol 2009;36:1800–2; doi:10.3899/jrheum.090348)

## Key Indexing Terms:

SYNOVIUM      MACROPHAGES      RHEUMATOID ARTHRITIS      BIOMARKERS

An OMERACT special interest group consisting of researchers with expertise in synovial tissue analysis was established in 2004. A considerable evidence base has accu-

mulated at one academic center that supports the hypothesis that synovial tissue sublining (sl) CD68 expression may be a biomarker of clinical response to therapeutic intervention

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in patients with rheumatoid arthritis (RA), an observation of potential utility in early phase randomized clinical trials (RCT)<sup>1,2</sup>. The available data were presented at the OMER-ACT meeting in 2006<sup>3</sup> and were comprehensively reviewed more recently<sup>4</sup>. Employing a range of effective therapeutic agents, including prednisolone, methotrexate, leflunomide, and infliximab, a consistent correlation between the mean change in disease activity and the mean change in synovial sublining CD68 ( $\Delta$ CD68sl) expression was observed. Moreover, patient cohorts that received either placebo, stable doses of a disease modifying antirheumatic drug (DMARD), or an ineffective compound demonstrated no change in CD68sl expression. Thus, in relatively small patient cohorts, effective therapies were readily distinguished from ineffective compounds by an analysis of synovial tissue, the primary target of disturbed immunomodulatory pathways in RA. More recent published studies have supported these earlier findings<sup>5-7</sup>.

Following OMERACT 2006, the primary research objective proposed for presentation at OMERACT 2008 was to determine whether the correlation between the mean change in disease activity and the mean  $\Delta$ CD68sl expression could be demonstrated across different academic centers.

## MATERIALS AND METHODS

For the purposes of our study, synovial biopsies obtained at arthroscopy from the same joints before and 160 days after 2 standard infusions of rituximab at the Academic Medical Center (AMC), Amsterdam, were selected<sup>8,9</sup>. All patients had active seropositive RA<sup>11</sup>. The Disease Activity Score 28 (DAS28)<sup>11</sup> was determined at each of the 2 timepoints. Paired sections selected from each biopsy were coded and processed independently at AMC and at St. Vincent's University Hospital (SVUH), Dublin, as described<sup>8,9,12</sup>. There were some methodological differences in tissue preparation and staining techniques. The reagent kits and antibodies were sourced from the same company (Dako) at both centers. Optimum primary antibody dilutions were determined locally. Incubation times were similar in both centers.

Digital image analysis (DIA) was employed at both centers to quantify CD68sl expression. The methodology has been described<sup>12,13</sup>. At AMC, CD68sl scores were derived from an analysis of 18 images acquired from each biopsy section and were expressed as the mean number of positively stained cells per square millimeter<sup>13</sup>. At SVUH, the scores were derived from an analysis of 6 images from each biopsy section and expressed as the mean area of tissue occupied by positively stained cells<sup>12</sup>. When quantifying CD68sl expression, the investigators at each center were unaware of patients' identities, therapeutic responses, or time sequence of each stained section.

In order to evaluate intracenter variation, 2 investigators (RT and MV) from AMC independently quantified CD68sl expression, and the results were compared. In order to evaluate intercenter variation, an investigator from SVUH (EP) independently quantified CD68sl expression in all sections, and results were compared to those derived by RT. Thus, after decoding, intercenter and intracenter variations in quantification of CD68sl expression,  $\Delta$ CD68sl, and the relationships between  $\Delta$ CD68sl and  $\Delta$ DAS were determined.

Determination of the sensitivity of biomarker expression to detect change was based on the standardized response mean (SRM), which is calculated by dividing the mean change by the standard deviation of the mean change. SRM > 0.8 indicates a high potential to detect change, and SRM of 0.5 indicates moderate potential.

## RESULTS

The intracenter correlations for quantification of CD68sl expression were excellent. For the pooled sections stained at AMC, the correlation between 2 investigators was R = 0.942, p = 0.000, and for sections stained at SVUH, R = 0.899, p = 0.001. Similarly, the intracenter correlations for  $\Delta$ CD68sl expression after treatment were R = 0.998 with a P = 0.000 for sections stained at AMC and R = 0.880 with a P = 0.000 for sections stained at SVUH.

The intercenter correlation for the pooled scores of sections stained at AMC was R = 0.85, p = 0.000, and for the sections stained at SVUH, R = 0.62, p = 0.001. The intercenter correlation for  $\Delta$ CD68sl scores derived from sections stained at AMC was R = 0.67, p = 0.013, but only R = 0.22 (not statistically significant) for the  $\Delta$ CD68sl scores derived from sections stained at SVUH.

This discrepancy could be explained by fading of the staining intensity in some sections during an unavoidable delay in acquiring images and quantifying CD68sl expression in sections transported from Dublin to Amsterdam due to an unforeseen illness of one of the investigators. Thus, some of the sections that were stained and images recorded at SVUH demonstrated prominent CD68sl expression, but minimal expression when the images were acquired at AMC some weeks after staining. This phenomenon resulted in inconsistent determination of  $\Delta$ CD68sl values for some sections stained at SVUH and quantified at AMC. This observation further underlines the known importance of analyzing sections stained with amino ethylcarbazole within a few weeks when this dye is used for immunohistochemistry. Further analysis of this issue is in progress and will be reported separately.

The mean DAS28 at baseline was 6.7. The mean value 160 days after rituximab was 5.0 (mean  $\Delta$ DAS28 = 1.7). The relationships between the mean  $\Delta$ CD68sl and the mean  $\Delta$ DAS28 from the 4 completed analyses undertaken in this study (RT, paired AMC-stained sections, n = 19; RT, paired SVUH-stained sections, n = 18; MV, paired AMC-stained sections, n = 19; MV, paired SVUH-stained sections, n = 18) were added to the previously described study cohorts<sup>5</sup>. The consistent correlation between  $\Delta$ DAS and  $\Delta$ CD68sl expression (Pearson correlation = 0.895, p < 0.001) was confirmed. In this regard, it was not feasible to compare the correlation between the  $\Delta$ CD68sl values calculated at SVUH and  $\Delta$ DAS because different units of CD68sl expression were employed.

The SRM values for  $\Delta$ CD68sl, calculated from the analyses at AMC, in addition to the analyses completed at SVUH (EP, paired AMC-stained sections, n = 16; EP, paired SVUH sections, n = 16), were consistently 0.5 or greater, indicating a moderate to high potential to detect change.

## DISCUSSION

Our study was designed to determine if the correlation

between the mean  $\Delta$ DAS and the mean  $\Delta$ CD68sl, consistently observed in several RCT at one academic center, could be confirmed across other centers. Paired biopsy sections obtained before and after rituximab treatment were independently processed, and CD68sl expression before and after treatment was quantified at academic centers in 2 different countries. The stained sections from each center were then exchanged, and CD68sl expression and  $\Delta$ CD68sl were recalculated. After decoding, excellent intracenter and inter-center agreements were observed for the pooled CD68sl expression scores. Excellent intracenter agreement was also observed for  $\Delta$ CD68sl after treatment. Measurement of CD68sl expression SRM values confirmed a moderate to high potential to detect change. Finally, a consistent correlation between the mean  $\Delta$ CD68sl values, calculated by different investigators from sections that were processed in the different centers, and  $\Delta$ DAS28 was confirmed. In a ballot at the concluding OMERACT general assembly, 55% of the delegates agreed with this statement while 19% disagreed. Moreover, 59% of the delegates agreed that CD68 expression in synovial tissue is less susceptible to a placebo effect and expectation bias than clinical evaluation, compared to 13% who disagreed.

The use of arthroscopic synovial biopsy in proof-of-concept clinical trials is feasible and safe. It allows macroscopic evaluation of the synovium. Narrow-diameter arthroscopes provide access to small joints, including the ankle, wrist, metacarpophalangeal, and proximal interphalangeal joints. The arthroscopic procedure is well tolerated, with a low complication rate. In a series of more than 2000 arthroscopic biopsy procedures at the AMC, the complication rate was  $< 0.3\%$ <sup>14</sup>. When voting, 72% of the participants at OMERACT agreed that arthroscopic synovial biopsy was safe and well tolerated, while only 8% disagreed.

In conclusion, examination of serial biopsy samples can be used reliably to screen on the group level for interesting biological effects at the site of inflammation in an early stage of drug development. In addition to immunohistochemistry, other techniques like quantitative polymerase chain reaction<sup>15</sup>, microarray analysis<sup>16</sup>, and proteomics may provide supportive data, although they need further validation as markers of therapeutic response. It can be anticipated that future development will include the use of more extensive markers of joint degradation as well as the use of panels of biomarkers in synovial tissue samples.

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