Testing of the Preliminary OMERACT Validation Criteria for a Biomarker to Be Regarded as Reflecting Structural Damage Endpoints in Rheumatoid Arthritis Clinical Trials: The Example of C-Reactive Protein

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ABSTRACT. Objective. A list of 14 criteria for guiding the validation of a soluble biomarker as reflecting structural damage endpoints in rheumatoid arthritis (RA) clinical trials was drafted by an international working group after a Delphi consensus exercise. C-reactive protein (CRP), a soluble biomarker extensively studied in RA, was then used to test these criteria. Our objectives were: (1) To assess the strength of evidence in support of CRP as a soluble biomarker reflecting structural damage in RA according to the draft validation criteria. (2) To assess the strength of recommendation for inclusion of individual criteria in the draft set.

Methods. A systematic literature review was conducted to elicit evidence in support of each specific criterion composing the 14-criteria draft set. A summary of the key literature findings per criterion was presented to both the working group and to participants in a special interest soluble biomarker group at OMERACT 8. Participants at OMERACT 8 were asked to rate the strength of evidence and the strength of the recommendation in support of each individual criterion on a 0–10 numerical rating scale. Working group members not present at OMERACT voted by a Web-based survey.

Results. Minimal data were extracted from the literature pertaining to those criteria listed under the category of truth. Ratings for strength of evidence were moderate to low (< 7) for CRP as a biomarker reflecting structural damage in RA; this was true for all criteria except those listed under the category of feasibility and 2 listed under the category of discrimination pertaining to assay reproducibility and evidence regarding sources of variability. Ratings for strength of recommendation for inclusion of each of the 14 criteria in the draft set were high (> 7) except for those criteria listed under the category of truth.

Conclusion. The draft criteria serve as a useful template in the evaluation of the strength of evidence in support of a particular soluble biomarker as reflecting structural damage in RA. (J Rheumatol 2007;34:623–33)

Key Indexing Terms: SOLUBLE BIOMARKER C-REACTIVE PROTEIN OMERACT RHEUMATOID ARTHRITIS VALIDATION STRUCTURAL DAMAGE
An OMERACT special interest group in soluble biomarkers was assembled, consisting of individuals with a special interest in clinical and radiographic structural damage outcomes and soluble biomarkers in inflammatory arthritis. The goal of the group was to develop validation criteria for soluble biomarkers considered to be valid markers of structural damage in rheumatoid arthritis (RA) as defined by plain radiography, using a Delphi consensus exercise. A list of 14 criteria was generated and structured according to the key requirements of the OMERACT filter for validation of an outcome measure though focusing on issues of truth and discrimination. It was further decided to examine the performance of the proposed criteria using the example of C-reactive protein (CRP) as a biomarker reflecting structural damage in RA, as extensive literature is available on it.

The first evidence to support a potential association between CRP and structural damage in RA appeared in the literature several decades ago. In addition to being used routinely in clinical practice and clinical trials, CRP is one of the variables within the Disease Activity Score and the American College of Rheumatology 20% response criterion. Further, improvements in the performance of the test and standardization between laboratories make this an excellent candidate biomarker to test the draft OMERACT soluble biomarker validation criteria.

MATERIALS AND METHODS
Systematic literature review. The first step in testing the proposed validation criteria was a review of the literature, focusing on examination of the criteria generated by the consensus exercise. A systematic review was performed using the MeSH terms CRP, C-reactive protein, arthritis, and rheumatoid arthritis, in Medline, Embase, and PubMed and references of articles obtained from the electronic databases. Selected studies focused on measurement of CRP in animal models of inflammatory arthritis; correlations to other markers of structural damage; localization of CRP in joint tissues; cross-sectional comparisons of CRP in patients with RA and age/sex-matched healthy controls; longitudinal studies of patients with RA where structural damage was measured as an endpoint using validated plain radiographic scoring methods for RA; randomized controlled trials that included measurements of CRP and structural damage endpoints; analytical studies that evaluated the reproducibility, sensitivity and specificity of the CRP assay; and studies that analyzed the effects of potential sources of variability in healthy people and patients with RA such as age, sex, menopause, circadian rhythms, physical activity, body mass index, renal/hepatic function, and nonsteroidal antiinflammatory drugs (NSAID). The primary objective of this exercise was to critically evaluate the criteria for a soluble biomarker to be regarded as a valid biomarker reflecting structural damage endpoints in RA. CRP was used to test this process. The focus of this review was therefore to examine the strength of the evidence that CRP behaves as a biomarker reflecting structural damage in RA, from direct histopathological data in animals to circumstantial data in human studies (correlations with other biomarkers reflecting structural damage, e.g., magnetic resonance imaging (MRI)), to direct associations with plain radiographic endpoints in randomized controlled trials.

Rating the strength of evidence supporting CRP as reflecting structural damage in RA and strength of recommendation for inclusion of individual criteria in the draft set. A summary of key findings from the literature pertaining to each criterion was prepared by 2 investigators (SOK, WPM) and presented to both the working group and to the special interest group participants at OMERACT 8. They were first asked to rate the strength of evidence in support of each individual criterion on a 0–10 numerical rating scale (0 = no supporting evidence at all, 10 = unequivocal evidence to support this criterion) according to the following question:

Question 1. “Please rate to what degree you consider the available data from the literature as supporting CRP as a valid surrogate for structural damage in RA according to this specific criterion.”

The wording of this question was developed prior to the OMERACT conference, where it was decided that the term “surrogate” should be restricted to only those variables that have been sufficiently validated as reflecting patient-centered outcomes, i.e., how a patient “feels, functions, and survives.” The purpose of the question was clarified with OMERACT participants prior to the vote, so it was clear that evidence was being evaluated in support of CRP as a biomarker reflecting structural damage in RA. They were then asked to vote on the following question on a 0–10 numerical rating scale in order to determine the strength of the recommendation in support of including each individual criterion in the draft criteria:

Question 2. “Please rate to what degree you consider that this particular criterion should be included in the draft criteria.”

This exercise would then form the basis for further discussion about the requirement for modifications to the draft criteria.

Voting according to Question 1 (strength of evidence) was not conducted for those individual criteria that were not germane to the specific consideration of CRP as reflecting structural damage. All scores are provided as a mean (standard error).

RESULTS
Literature review
The findings from the systematic review of data pertinent to the 14 items of the draft validation criteria have been organized here according to the main subject heading requirements of the OMERACT filter. MeSH terms used in the literature search for each criterion and detailed findings of the search are reported in the Appendix.

A. Truth. The literature review revealed minimal evidence to support the validity of CRP as reflecting structural damage in RA when the search was specifically focused on the 5 criteria listed under the category of truth. Circumstantial evidence included several cross-sectional and prospective studies demonstrating associations with other biomarkers implicated in structural damage, e.g., serum metalloproteinase (MMP-3). The most compelling evidence came from 2 prospective studies in early RA that showed associations between baseline CRP and subsequent development of MRI erosions.

B. Discrimination. The literature review focusing on the 7 criteria listed under the category of discrimination revealed that several large population-based studies have clarified the influence of potential causes of variability on CRP levels. Increased age and body mass index are associated with increased CRP levels, while physical activity is associated with decreased CRP levels. Female sex is also associated with increased levels, although the effect is not consistently

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observed. The effects of circadian rhythms, menopause, renal and hepatic disease, and NSAID have also been studied, and these factors do not appear to contribute to variability. Involvement of larger joints in RA is associated with higher CRP levels. The assay has been well established, with very good reproducibility between assays (coefficient of variation < 10%). Metabolism has been studied in healthy persons and in those with RA. Inflammation has little effect on clearance of CRP. Six prospective observational studies were identified that examined the validity of CRP in reflecting structural damage in RA (Table 1). These were consistent in showing an association with structural damage, although only one study employed a regression analysis that took into account the role of potential confounders. Data from 4 randomized trials of biologics (2 infliximab, 2 etanercept) consistently showed that CRP is associated with radiological progression, although this was evident only in the methotrexate (MTX)/placebo comparator arms and not in patients taking MTX/biologic (Table 2).

C. Feasibility. Evidence to support the 2 criteria listed under the category of feasibility was largely obtained from the manufacturers of the CRP assay. The methods used in clinical practice are internationally standardized with available reference standards. CRP is a relatively stable biomarker both at room temperature and in frozen specimens.

Voting (Table 3)
The strength of evidence in support of CRP as a valid biomarker reflecting structural damage in RA was not rated highly for most of the draft criteria by either OMERACT participants or working group members unable to attend OMERACT. Only criteria related to feasibility attained scores ≥ 8. Criteria 2 (immunohistochemical localization to joints — truth), 3 (sensitivity and specificity for target of joint tissue origin — truth), and 6 (assay reproducibility — discrimination) were not considered germane to the objectives of the voting exercise, while no data were available in the literature to address criteria 4 (relationship of biomarker to synthesis, degradation, turnover of joint tissues — truth) or 12 (reflecting structural damage in preradiographic disease — discrimination). The strength of the recommendation for inclusion in the draft criteria was rated highly for almost all of those criteria categorized under discrimination and feasibility, but not those categorized under truth. Discrepancies in scores between OMERACT participants and working group members voting by Web-based survey were observed primarily for criteria 1, 3, and 12.

DISCUSSION
This exercise demonstrated that a systematic literature review addressing each of the individual criteria of the draft set did not provide strong evidence to support the use of CRP as a valid biomarker reflecting structural damage in RA. More importantly, it suggested that some of the criteria, particularly those itemized under the category of truth, may be considered less useful in this process of validation.

The observation that the criteria categorized under truth were considered less important is not entirely surprising. Evaluation of biomarkers in animal models of arthritis (criterion 1) may be of questionable relevance to human disease due to obvious dissimilarities in pathogenesis and evolution of disease, and differences in the biochemistry and metabolism of tissues among several of the most important variables. Testing the criteria using CRP serves as a good example in questioning the importance of immunohistochemical localization to joint tissues and demonstration of biomarker specificity for target of joint tissue origin (criteria 2 and 3, respectively). Although these criteria appear intuitively important, their lack of relevance in the case of CRP illustrates how immunopathological events occurring in the joint may have proportionate systemic consequences that might lead to the identification of useful biomarkers of non-joint tissue origin that only indirectly reflect pathogenetic events in joint tissues.

Table 1. Prospective observational studies that analyzed CRP as a predictor of structural damage in RA.

<table>
<thead>
<tr>
<th>Study</th>
<th>No. Patients</th>
<th>Disease Duration</th>
<th>Study Duration</th>
<th>Radiographic Scoring System</th>
<th>Main Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matsuda69</td>
<td>118</td>
<td>&lt; 1 yr</td>
<td>2 yrs</td>
<td>Larsen</td>
<td>CRP at 6 mo correlated with change in Larsen score at 6, 12, and 24 mo (r = 0.455, 0.447, 0.384, respectively)</td>
</tr>
<tr>
<td>Van Leeuwen70</td>
<td>149</td>
<td>&lt; 1 yr</td>
<td>3 yrs</td>
<td>Modified Sharp</td>
<td>Correlation coefficients between CRP AUC and radiological progression over 1st 3 yrs were 0.656 for 149 patients, 0.651 for 54 patients with 6 yrs followup</td>
</tr>
<tr>
<td>Plant71</td>
<td>359</td>
<td>2 yrs</td>
<td>5 yrs</td>
<td>Larsen</td>
<td>Correlation between time-integrated CRP and Larsen score change of 0.50</td>
</tr>
<tr>
<td>Dawes72</td>
<td>150</td>
<td>4 yrs</td>
<td>1 yr</td>
<td>Larsen</td>
<td>Reduction in CRP associated with less radiographic progression</td>
</tr>
<tr>
<td>Jansen73</td>
<td>130</td>
<td>3 mo</td>
<td>1 yr</td>
<td>Modified Sharp</td>
<td>CRP was independent predictor of 1-yr radiographic progression</td>
</tr>
<tr>
<td>Listing74</td>
<td>139</td>
<td>&lt; 2 yrs</td>
<td>3 yrs</td>
<td>Ratingen Score (RS)</td>
<td>Elevated CRP was predictive of erosive RA at 4 yrs (78.4% probability of erosive RA with CRP &gt; 15 mg/l)</td>
</tr>
</tbody>
</table>

AUC: area under the curve.
The same reasoning could apply to the perceived limitations in the importance of criterion 4, which stipulates that the relation of the biomarker to synthesis, degradation, and turnover of joint tissue components has been characterized. The higher rating for criterion 5 under truth might reflect increasing confidence in the evidence that changes in other biomarkers, especially MRI, may reflect structural damage in RA. In particular, there is increasing evidence from prospective studies of early RA that support the development of MRI erosions as indicative of eventual structural damage on plain radiography. It is likely premature, however, to consider discarding any of the proposed criteria under the category of truth.

Table 2. Randomized controlled trials that analyzed CRP as a predictor of structural damage.

<table>
<thead>
<tr>
<th>Study</th>
<th>No. Patients</th>
<th>Disease Duration</th>
<th>Study Duration</th>
<th>Treatment Groups</th>
<th>Scoring System</th>
<th>Main Study Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smolen75</td>
<td>1004</td>
<td>0.9 yrs</td>
<td>54 wks</td>
<td>MTX/placebo</td>
<td>Modified</td>
<td>High baseline and time-averaged CRP correlated with greater radiographic progression in MTX/placebo group only, independent of clinical prognostic variables</td>
</tr>
<tr>
<td>Smolen76</td>
<td>428</td>
<td>10 yrs</td>
<td>54 wks</td>
<td>MTX/placebo</td>
<td>Modified</td>
<td>Association between % change in CRP from baseline and radiographic progression = 0.54 in the MTX/placebo group only</td>
</tr>
<tr>
<td>Bathon77</td>
<td>632</td>
<td>11–12 mo</td>
<td>1 yr</td>
<td>MTX/placebo</td>
<td>Modified</td>
<td>Decreases in CRP correlated with lack of radiographic progression in etanercept group (r = 0.45)</td>
</tr>
<tr>
<td>Garnero14</td>
<td>116</td>
<td>12 mo</td>
<td>1 yr</td>
<td>Etanercept/placebo</td>
<td>Modified</td>
<td>Baseline CRP correlated with radiologic progression (r = 0.21)</td>
</tr>
<tr>
<td>Landewe79</td>
<td>682</td>
<td>6–6.5 yrs</td>
<td>1 yr</td>
<td>MTX/placebo</td>
<td>Modified</td>
<td>Time-averaged CRP is associated with radiologic progression in the MTX group only</td>
</tr>
<tr>
<td>Sharp80</td>
<td>US301: 482</td>
<td>6.5–7.0</td>
<td>1 yr</td>
<td>MTX/LEF MTX</td>
<td>Sharp</td>
<td>Weak correlation between time-averaged CRP and radiographic progression (US 301: r = 0.17; MN 302: r = 0.15)</td>
</tr>
<tr>
<td></td>
<td>MN301: 358</td>
<td>5.7–7.6</td>
<td>6 mo</td>
<td>LEF SSPN LEF</td>
<td>Sharp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MN302: 999</td>
<td>3.7–3.8</td>
<td>1 yr</td>
<td>Placebo</td>
<td>Sharp</td>
<td></td>
</tr>
</tbody>
</table>

MTX: methotrexate, LEF: leflunomide, SSPN: salazopyrine

Table 3. Rating (0–10 numerical rating scale) of A. Strength of evidence (SOE) in support of CRP as a surrogate for structural damage in RA according to individual criteria comprising the 14 draft validation criteria; and B. Strength of recommendation (SOR) for inclusion of each criterion in the draft criteria.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>OMERACT Participants</th>
<th>Web Survey Participants (n = 6)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Question A (SOE)</td>
<td>Question B (SOR)</td>
</tr>
<tr>
<td></td>
<td>(n = 16)</td>
<td>(n = 18)</td>
</tr>
<tr>
<td>1</td>
<td>0.47 (0.19)</td>
<td>4.17 (0.49)</td>
</tr>
<tr>
<td>2</td>
<td>NA</td>
<td>4.24 (0.73)</td>
</tr>
<tr>
<td>3</td>
<td>NA</td>
<td>5.77 (0.82)</td>
</tr>
<tr>
<td>4</td>
<td>NA</td>
<td>5.44 (0.61)</td>
</tr>
<tr>
<td>5</td>
<td>5.25 (0.51)</td>
<td>7.35 (0.67)</td>
</tr>
<tr>
<td>6</td>
<td>NA</td>
<td>9.06 (0.22)</td>
</tr>
<tr>
<td>7</td>
<td>7.33 (0.55)</td>
<td>8.67 (0.26)</td>
</tr>
<tr>
<td>8</td>
<td>7.54 (0.54)</td>
<td>8.39 (0.33)</td>
</tr>
<tr>
<td>9</td>
<td>5.46 (0.78)</td>
<td>8.77 (0.28)</td>
</tr>
<tr>
<td>10</td>
<td>5.69 (0.73)</td>
<td>9.18 (0.23)</td>
</tr>
<tr>
<td>11</td>
<td>4.69 (0.69)</td>
<td>9.06 (0.28)</td>
</tr>
<tr>
<td>12</td>
<td>NA</td>
<td>9.00 (0.24)</td>
</tr>
<tr>
<td>13</td>
<td>8.50 (0.48)</td>
<td>9.28 (0.21)</td>
</tr>
<tr>
<td>14</td>
<td>6.86 (0.80)</td>
<td>7.97 (0.49)</td>
</tr>
</tbody>
</table>

* Working group members unable to attend OMERACT. NA: not germane to the specific criterion or no data available from the literature (criteria 4 and 12).
merely because they were not considered useful in the setting where the primary focus of the literature search was CRP. It is possible that other soluble biomarkers that may prove to be superior at revealing structural damage will display characteristics consistent with the proposed criteria, such as localization to joint tissues, and clear correlations with the metabolism of matrix components. Examples might include those biomarkers that reflect cartilage turnover and/or degradation such as urinary C-terminal telopeptide of type II collagen (CTX-II) and serum cartilage oligomeric matrix protein (COMP). This testing exercise therefore needs to be repeated with other candidate soluble biomarkers before any further conclusions can be drawn.

The highest strength of recommendation ratings for inclusion of specific criteria went to the criteria that highlighted assay feasibility with respect to assay simplicity and standardization and biomarker stability at room temperature and in frozen specimens. This reflects the longstanding experience with the biomarker together with its widespread use in both clinical practice and research. In addition, criteria that also received the highest ratings stipulated demonstration of an association between change in the biomarker and change in structural damage scored by plain radiography in well designed prospective cohort studies (criterion 10) and randomized controlled trials (criterion 11). It is therefore somewhat surprising that despite several such studies showing associations between CRP and radiographic progression (summarized in Tables 1 and 2), the strength of evidence was rated as only moderate (range of mean scores 4.69–7.00). For prospective studies (criterion 10), limitations in study design and analysis appeared to be the most likely basis for the low score according to the discussions that took place at OMERACTION. It was noted that only 2 studies analyzed the predictive validity of CRP assessed at a single baseline timepoint.

This may be useful in addressing CRP as a prognostic marker, but does not necessarily indicate that it can substitute for radiography in scoring structural damage in clinical trials. This requires demonstration of an independent association between change in biomarker levels and change in radiological progression. Although some studies measured the association between time-integrated CRP and radiographic change, only one study addressed known predictors of radiographic change as confounders using regression analysis. None used generalized estimating equations with sequential clinical, laboratory, and radiographic assessments to address the effects of changes in disease activity and changes in disease-modifying therapy that might be anticipated during followup. In addition to the failure to adequately address confounders, it was noted that it is difficult to address the strength of an association when the results are presented primarily as correlation analyses. There was also little uniformity in the use of radiological outcome instruments. Only 2 studies assessed the van der Heijde-modified Sharp score, which is the most responsive tool currently available and is the current benchmark for clinical trials, 3 used the Larsen score, and one used the Ratingen score. A major objective of the working group for the next OMERACTION meeting will be to achieve consensus on the optimal approach to the design and analysis of longitudinal studies.

The analysis of CRP as a biomarker reflecting structural damage in the randomized controlled trials with anti-tumor necrosis factor (TNF) agents highlights an important deficiency in the draft criteria. Although CRP is predictive of joint damage in patients who received MTX placebo, it was not predictive in those patients who received MTX plus an anti-TNF agent. In the MTX-only group, significant radiographic progression was likely to occur in patients who had even moderately elevated time-averaged CRP levels or erythrocyte sedimentation rate (ESR). In contrast, the use of MTX in combination with infliximab during the trial effectively slowed radiographic progression, despite elevated time-averaged CRP or ESR or elevated time-averaged swollen joint counts. This would be consistent with a scenario in which CRP is not in the causal pathway of structural damage, but is an indirect byproduct of both TNF- and non-TNF-driven pathophysiological processes driving chronic inflammation but where TNF-driven inflammation is the dominant factor leading to structural joint damage. MTX does not appear to interfere with TNF production, and so high CRP reflecting high TNF production leads to structural damage that may not be ameliorated by MTX alone. This would account for the correlation between time-averaged CRP and structural damage in this treatment group. On the other hand, if anti-TNF agents reduce structural damage, even in the presence of elevated CRP related to non-TNF-driven inflammation, this would account for the lack of correlation between time-averaged CRP and structural damage in those taking combination MTX/anti-TNF therapy. This example emphasizes the importance of criteria proposed a decade ago by Prentice for the validation of surrogate endpoints in phase IIIB clinical trials. In particular, he proposed that it is insufficient to demonstrate that the surrogate endpoint correlates with the clinical endpoint, but it must also be shown that the surrogate fully captures the net effect of treatment on clinical outcomes. This is most likely to be observed if the biomarker is in the only causal pathway of the disease process, and the intervention’s entire effect on the true clinical outcome is mediated through its effect on the surrogate. Even then, biomarker data may be misleading if the therapeutic intervention effect is of insufficient size or duration to alter the true clinical outcome in a meaningful way. Several examples from other fields of medicine also highlight the importance of demonstrating a consistent association between change in the biomarker and change in target outcome in randomized trials in the same drug class as well as other drug classes. For example, several trials have shown that calcium channel blockers are less efficacious than thiazides or angiotensin-converting enzyme inhibitors in preventing hard clinical endpoints despite exert-
ing similar degrees of blood pressure reduction\textsuperscript{84-87}. These considerations were addressed in the development of levels of evidence for validation of a generic biomarker in the surrogate “superworkshop,”\textsuperscript{88} but it may be more appropriate to incorporate these requirements as additional criteria in the draft validation set. This will be reexamined in preparation for OMERACT 9.

In conclusion, this first testing exercise using CRP has shown that the draft validation criteria for soluble biomarkers generated by a Delphi consensus exercise of an OMERACT working group are useful to direct literature search strategies to evaluate the strength of evidence supporting a particular soluble biomarker as revealing structural damage in RA. In addition, further consensus was achieved on the inclusion of specific criteria in the 14-criteria draft set. Deficiencies in the criteria were identified and further testing exercises with additional biomarkers were recommended before modifications to the draft criteria can be proposed.

**APPENDIX**

Truth Criteria

1. Evidence that the biomarker reflects tissue remodeling in established animal models of disease (e.g., collagen arthritis for RA)

MeSH terms used for animal models included: CRP, C-reactive protein, animal models of arthritis, experimental arthritis, collagen-induced arthritis, antigen-induced arthritis, adjuvant arthritis. The published data were organized according to 3 categories reflecting increasing levels of evidence:

i. **Associations of CRP with other biomarkers reflecting tissue remodeling** — lowest level of evidence. There are very limited data that have specifically addressed associations between CRP and other biomarkers in animal models of arthritis. One study of collagen-induced arthritis in non-human primates has shown that CRP levels are significantly associated with urinary excretion of collagen cross-links hydroxylysylpyridinoline and lysylpyridinoline during active periods of inflammation\textsuperscript{8}.

ii. **Association between CRP and plain radiographic damage** — higher level of evidence. Several studies have demonstrated elevated levels of CRP commensurate with the development of inflammation in animal models of arthritis, although none directly examined correlations between CRP levels and radiographic damage scores\textsuperscript{9,10}.

iii. **Association between CRP and histopathological damage scores** — highest level of evidence. Few studies have directly examined the correlation between CRP levels and histopathological scores for damage. Hunneyball, et al\textsuperscript{11} demonstrated that in antigen-induced monoarticular arthritis in rabbits, CRP levels did not correlate with the post-mortem joint assessment for erosive synovitis. Hart, et al\textsuperscript{12} assessed severity of arthritis in primates by a semiquantitative scoring system measuring degree of inflammation and deformity of affected joints.

Primates with the highest CRP values had the most severe disease, but direct correlations between CRP and histological specimens were not analyzed.

2. The biomarker has been immunohistochemically localized to joint tissues

This criterion is not applicable to CRP because interleukin 6 is largely responsible for stimulating the production of CRP by hepatocytes\textsuperscript{13}.

3. The biomarker demonstrates sensitivity and specificity for target of joint tissue origin

This criterion is not applicable to CRP, which is produced by hepatocytes.

4. Relation of biomarker to synthesis, degradation, turnover of joint tissue components has been characterized

This has not been directly studied with the CRP.

5. Levels of the biomarker correlate with scores for other surrogates that have been established as possessing predictive validity for structural damage (e.g., MRI for erosive RA)

MeSH terms used for item 5 include the following: CRP, C-reactive protein, rheumatoid arthritis, COMP, urinary CTX-2, urine NTx, MMP-3, matrix metalloproteinase 3, stromelysin, MRI, ultrasound.

These data were organized to reflect the strength of evidence according to study design (cross-sectional vs prospective cohort vs randomized controlled trial) for soluble biomarkers and imaging parameters.

ii. **Soluble biomarkers. Serum MMP-3.** This marker has been shown to be an independent predictor of structural damage progression in RA\textsuperscript{14}. Small cross-sectional studies have reported associations between CRP and MMP-3 based on correlation coefficient rather than regression analysis\textsuperscript{15-21}. Several prospective longitudinal studies reported associations between MMP-3 and CRP at different timepoints\textsuperscript{22-24} or between time-integrated CRP and MMP-3 values\textsuperscript{25-27}. Parallel reductions in MMP-3 and CRP were noted in patients receiving etanercept over 12 weeks\textsuperscript{28}. One randomized placebo-controlled trial of infliximab in RA showed parallel reductions in CRP and MMP-3 in patients on active therapy\textsuperscript{29}.

**Urinary N-terminal telopeptide of type I collagen (NTx).** One cross-sectional study of this marker of bone resorption showed no correlation with CRP in 184 RA patients\textsuperscript{30}.

**Urinary C-terminal telopeptide of type II collagen (CTX-II).** It has been shown that this marker of cartilage degradation is a significant independent predictor of structural damage in RA\textsuperscript{14,31}. Baseline levels of urinary CTX-II and CRP were significantly associated in a prospective cohort of 116 patients with early RA (< 1 yr) that demonstrated the predictive validity of baseline urinary CTX-II for structural damage, although baseline CRP was not itself predictive\textsuperscript{14}. 
Using the Synchron LX® immunoturbidimetric method as an example, the intraassay coefficient of variation is 5% and the interassay coefficient of variation is 7.5%, based on the manufacturer’s studies.

The effects of the following sources of variability on levels of the biomarker in normal individuals are known: age, sex, menopause, circadian rhythms, body mass index, physical activity, NSAID use, renal and hepatic disease, contribution of different affected joints

MeSH terms used for this criterion included the following: CRP, C-reactive protein, age, sex, menopause, circadian rhythms, body mass index, obesity, physical activity, NSAID, nonsteroidal antiinflammatories, renal dysfunction, renal disease, hepatic dysfunction, hepatic disease, “contribution of different affected joints,” arthritis, joint distribution.

i. Age. The effect of age on levels of CRP has been extensively reviewed in the cardiovascular and to a lesser extent the rheumatologic literature. Presently, unlike ESR, corrections of CRP for age and age-appropriate reference limits have not been implemented, and many laboratories do not have separate reference ranges accounting for differences in sex and age. Cross-sectional cohort comparisons of elderly versus younger controls have consistently shown higher CRP values in elderly individuals, although it is not clear to what extent this represents a true effect of age as opposed to an effect of age-related comorbidities that elevate CRP, including obesity and arthritis. Wener, et al31 looked at more than 22,000 individuals in the United States (part of the Third National Health and Nutrition Evaluation Survey, NHANES III) and confirmed that CRP increases with age, and suggested that age should be among the demographic factors used to adjust the upper reference limit for CRP.35-37, although it is not clear to what extent this represents a true effect of age as opposed to an effect of age-related comorbidities that elevate CRP, including obesity and arthritis. Wener, et al38 looked at more than 22,000 individuals in the United States (part of the Third National Health and Nutrition Evaluation Survey, NHANES III) and confirmed that CRP increases with age, and suggested that age should be among the demographic factors used to adjust the upper reference limit for CRP.38. This conclusion is generally supported by the cardiovascular literature38-41.

ii. Sex. Wener, et al38 found that at ages as young as 20 to 39 years, women had higher 95th centile CRP values compared with men. This is supported by other large population-based studies from the USA, Mexico, and Europe, even after adjustment for body mass index and other confounders (smoking, estrogen use, activity)42-45, although the converse was observed in 2 studies from Asia, suggesting an ethnic component46,47.

CRP levels are higher in women taking oral hormone replacement therapy, which may provide another hormonal explanation for the discrepancy in CRP between men and some women48,49.

iii. Menopause. CRP levels do not appear to be definitively influenced by menopause, although CRP levels appear to be higher in women taking hormone replacement therapy48,49.

iv. Circadian rhythms. Circadian rhythms do not appear to affect the levels of CRP in healthy individuals50,51.

v. Body mass index. Higher BMI is associated with increased CRP levels based on multiple large and small studies52-54, and weight loss resulted in proportional reductions in plasma CRP levels55. BMI in both men and women is therefore considered a confounder in CRP-related studies.

vi. Physical activity. In the MacArthur Studies of Successful Aging, the Cardiovascular Health Study, the Physicians Health Study, and the Health, Aging, and Body Composition Study, CRP levels were lower in physically active elderly individuals, with a significant inverse association between exercise frequency and CRP56-59.

vii. NSAID. Most studies have found that NSAID do not reduce acute-phase reactants including CRP50,61, although several have reported a delayed reduction in a subset of patients with particularly active disease62,63.

viii. Renal disease. CRP is not metabolized by the kidney and has not been associated with change in glomerular filtration rate64.

ix. Hepatic disease. CRP is produced primarily by hepatocytes. Although the literature is limited, hepatic disease appears to reduce but does not abolish the ability to increase CRP expression in the face of infection. Park, et al65 evaluated patients with E. coli bacteremia both with and without liver cirrhosis, and found only a 58% reduction in CRP production in the cirrhotic liver in response to infection.

x. The contribution of different joints. Larger inflamed joints such as the knee or shoulder appear to contribute more to CRP production than small joints such as the fingers66.

8. The metabolism, clearance, and half-life of the biomarker have been characterized in normal individuals and in patients with arthritis

MeSH terms used for this criterion included the following: CRP, C-reactive protein, arthritis, healthy controls, metabolism, half-life, clearance.

In both normal individuals and patients with arthritis, CRP is synthesized after tissue injury or inflammation and is detected at 4 to 6 h after injury, with a peak at 24 to 72 h67. CRP synthesis depends on stimulation by interleukin 6, while...
CRP clearance is via hepatocyte catabolism, which is minimally influenced by inflammation so that half-life varies little with arthritis.68

9. The biomarker demonstrates high sensitivity and specificity in comparisons of the disease population with age- and sex-matched healthy controls

CRP is clearly a nonspecific biomarker reflecting tissue injury from diverse sources, although levels are markedly higher in patients with RA.

10. The biomarker demonstrates independent association with the structural damage endpoint (van der Heijde modification of Sharp score for RA, modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) for AS, joint space narrowing score for OA) at the level of both absolute and relative change in a clinically well defined prospective cohort of adequate sample size and followed for a sufficient duration to detect change in radiographic damage score

MeSH terms used for the following criterion included: CRP, C-reactive protein, radiological damage, van der Heijde modification of Sharp score, Sharp score, rheumatoid arthritis, prospective study.

Multiple prospective observational studies have shown a significant association between CRP and radiographic progression (see Table 1)69-74. Various methods for scoring structural damage were used including the Larsen and the modified Sharp scores.67 These studies had at least 1 year of followup and mostly analyzed the predictive validity of baseline CRP and time-integrated CRP values. One study used a regression analysis in which relevant predictors (initial Sharp/van der Heijde score, age, sex, duration of complaints, DAS28 score, number of tender and swollen joints (28 joint count), HAQ score, IgM rheumatoid factor positivity) were included in the model.73

11. The biomarker demonstrates independent association with the structural damage endpoint (van der Heijde modification of Sharp score for RA, mSASSS for AS, joint space narrowing score for OA) at the level of both absolute and relative change in a randomized controlled trial of adequate sample size and of sufficient duration to detect change in structural damage score

MeSH terms used for the following included: CRP, C-reactive protein, rheumatoid arthritis, van der Heijde modification of Sharp score, Sharp score, Larsen score, randomized controlled trial.

While the majority of data supporting the predictive validity of CRP for structural damage come from prospective, observational cohorts, data from several randomized controlled trials have also shown an independent association of CRP with structural damage (Table 2). In the Active-Controlled Study of Patients Receiving Infliximab for the Treatment of Rheumatoid Arthritis of Early Onset (ASPIRE), high CRP levels at baseline were associated with greater radiographic progression at 1 year in the MTX/placebo but not the MTX/infliximab treatment arms.75 Multivariate logistic regression showed that this was independent of clinical characteristics (swollen and tender joint count, age, sex, rheumatoid factor). In the tertiles with the highest baseline serum CRP, 67% of patients in the MTX-only group showed an increase in radiographic score. In the MTX-only group, significant radiographic progression was likely to occur in patients who had even moderately elevated time-averaged CRP levels during the trial. These data were consistent with substudy findings from the Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy (ATTRACT) trial, which found that patients taking MTX/placebo with normal time-integrated CRP levels had less radiographic progression than those with elevated time-integrated CRP.76 This was not observed in the MTX/infliximab treatment arms.

In the trial of etanercept and MTX in patients with early rheumatoid arthritis (ERA), 632 patients with early RA received either etanercept or weekly oral MTX for 12 months.77 The strongest correlate of the absence of progression was decreased serum CRP concentrations in the group assigned to receive etanercept 25 mg (r = 0.45). In a substudy analysis of 116 patients randomly chosen from the original ERA cohort, baseline CRP was weakly associated with radiological progression over 1 year in the overall cohort (r = 0.21), but correlation coefficient data were not available for individual treatment groups.14 In the Trial of Etanercept and Methotrexate with Radiographic Patient Outcomes (TEMPO), 682 patients were randomized to either MTX or etanercept monotherapy or combination MTX/etanercept.78 Generalized mixed linear modeling analysis to adjust for within-patient correlation showed that high time-averaged CRP over 1 year was associated with radiologic progression only in the MTX/placebo group, as noted in the infliximab trials cited above.79 Much weaker correlations between time-averaged CRP values and radiographic progression have been noted with conventional disease modifying antirheumatic drugs but this has been less well studied than for biologics.80

12. The biomarker demonstrates predictive validity for the structural damage endpoint (van der Heijde modification of Sharp score for RA, mSASSS for AS, joint space narrowing score for OA) in a clinically well defined prospective cohort of patients with preradiographic disease of adequate sample size and followed for a sufficient duration to detect structural damage

No study was found that addressed this criterion.

13. The assay for measurement of the biomarker has been well characterized, is internationally standardized (availability of reference standards), and is methodologically simple

Rate nephelometry and immunoturbidimetry are the most
widely used measures of CRP in laboratories, and correlate well with a more recently developed solid-phase fluorescence immunoassay. These methods are internationally standardized with available reference standards. The turbidimetric method is simple because most sites now use an automated analyzer that automatically proportions the appropriate sample and reagent volume into a cuvette and monitors the change in absorbance at 340 nm.

14. Stability of the biomarker at room temperature and in frozen specimen has been documented.

Separated serum or plasma can stay at room temperature for up to 8 h, after which time they must be stored at 2°–8° C. If the assay has not been performed by 48 h, or the sample must be stored for longer, the sample should be frozen at −15° to −20° C. While this information comes from the Synchron immunoturbidimetric assay, it still reflects stability of CRP at these temperatures.

REFERENCES


Keeling, et al: Testing biomarker validation criteria


