Serum calprotectin: emerging marker of rheumatoid arthritis

Maurizio Benucci

Rheumatology Unit, S. Giovanni di Dio Hospital, Azienda USL-Toscana Centro Florence, Italy

Rheumatoid arthritis (RA) is a chronic inflammatory disease that mainly affects the synovial membrane of the diarthrodial joints, generating joint destruction at the cartilage and bone level. The main target of the disease is the synovial membrane, despite having numerous systemic manifestations, with evidence that underlines an alteration of immunological tolerance. The natural history of this disease involves serious inconveniences in daily life. Therefore, it is essential to be able to recognize the disease early and slow down its evolution. An early diagnosis, together with the estimation of the degree of aggressiveness of the disease, allows to promptly prescribe the most adequate drug therapy, halt joint damage and relieve painful clinical symptoms. Early diagnosis and disease monitoring are important and necessary, in order to ensure that the patient receives the most appropriate treatment depending on the stage and severity of the disease. The laboratory plays a leading role in RA management and represents the first step in the management of a patient with suspected or known RA. For this reason, laboratory research over the years has focused on the identification of new markers capable of offering high sensitivity and specificity, such as serum calprotectin. This molecule, which was initially called leukocyte protein L1,[1] is a 36 kD protein made up of two 14 kD heavy chains and an 8 kD light chain, belonging to the S100 family of proteins. Calprotectin, also known as MRP8/14 or S100A8/A9, is a heterodimer composed of two calcium-binding S100 proteins: myeloid-related protein 8 (MRP-8 or S100A8) and MRP-14 (or S100A9). Calprotectin is an important proinflammatory element of innate immunity acting as an endogenous ligand of the toll-like receptor (TLR). Originally identified in neutrophil granulocytes, of which it represents 5% of total proteins and 60% of the soluble fraction of the cytosol. Later it was also shown in monocytes, macrophages and many other cells, tissues and body fluids. The increase of this protein has been documented in numerous clinical conditions on an acute inflammatory basis. The serum concentration of calprotectin was correlated with the increase in the number of neutrophil granulocytes and other markers of inflammation, such as C reactive protein (CRP) and erythrocyte sedimentation rate (ESR).[2] This led to evaluate the possibility of using the calprotectin assay not only on serum/plasma, but also on biological samples, such as feces, saliva, urine, CSF, synovial fluid, biopsy material. Calprotectin measured in the stool represents a well-known and reliable diagnostic marker in the context of inflammatory or malignant diseases of the entire gastrointestinal tract. However, the discovery of the correlation of serum calprotectin with disease activity and with the response to treatment in rheumatoid arthritis (RA)[3] has given a new impetus to protein studies. Although the main rheumatological works on this protein concern RA, it is important to know that today the association with disease activity in Still's disease, in ankylosing spondylitis, in psoriatic arthritis, in juvenile idiopathic arthritis, and in systemic lupus erythematosus[4] is also known. In the gastroenterological field, elevated levels of serum calprotectin are found in inflammatory bowel diseases both at the time of diagnosis and during disease monitoring.

Correspondence: Maurizio Benucci, Rheumatology Unit, Azienda Sanitaria USL-Toscana Centro, Hospital S. Giovanni di Dio, via Torregalli 3, 50143 Florence, Italy.
Tel.: +39.055.6932636 - Fax: +39.055.6932099.
E-mail: maubenucci@tiscali.it ; maurizio.benucci@uslcentro.toscana.it

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Serum calprotectin in the diagnosis of rheumatoid arthritis

Numerous clinical studies report that the serum levels of calprotectin are elevated in RA in the active phase,[5-8] especially in patients with rheumatoid factor positive (RF), and then normalize after a specific treatment.[9] The dosing of calprotectin should reflect the degree of local inflammation, in relation to the joints affected by the inflammatory process. In a cross-sectional observational study involving 145 patients with RA, the serum concentration of calprotectin showed a significant correlation ($P<0.001$) with traditional inflammatory indices (CRP and ESR) and with the clinimetric index DAS28.[9] The correlation of calprotectin with traditional markers of inflammation suggests that it has a similar behavior to acute phase proteins. Numerous studies, on the other hand, report that calprotectin levels are correlated with levels of RF[9,10] and that patients with positive RF show elevated levels of calprotectin compared to patients with negative RF. Patients with positive anti-citrullinated peptide (ACPA) and RF IgA and IgM antibodies have elevated baseline calprotectin levels during follow-up compared to patients negative for these serological markers ($P<0.001$).[11] Currently biological agents, such as anti-TNF-α, are available for the treatment of RA in non-responders or patients who have achieved a partial response to background drugs both in combination with MTX and in monotherapy.[12] However, a significant proportion of patients do not respond to treatment with biologics, so the use of predictive markers of treatment response guides the choice of a spe-

cific and targeted treatment and helps to reduce side effects and costs. The study by Choi *et al.* in 2013[13] demonstrated that calprotectin serum levels are related to the clinical picture and symptoms. This study also highlights that calprotectin levels measured at baseline may have a prognostic role in predicting response to treatment, regardless of the mechanism of action.

Numerous studies have also evaluated calprotectin as a predictor of structural damage and treatment response.[2,14] These studies also underline the prognostic role of this marker; in fact, high basal levels of calprotectin are predictive of a rapid and progressive course with early and important erosive damage. The study by Hammer *et al.*[10] in 2010 evaluated calprotectin in discriminating patients with progressive radiographic evidence of structural damage versus patients in the quiescent phase.

On ultrasound examination, the presence of the power doppler signal reflects active inflammation in the synovium and is associated with radiographic progression in early RA.[15,16] Serum calprotectin is therefore associated with the ultrasound assessment of disease activity and high levels of the protein may indicate persistent inflammation in patients in remission or with low disease activity.

Future perspectives and conclusions

Numerous biochemical markers, both traditional and more recently introduced in the clinical practice, have been identified and are useful in the management of the many aspects of RA, thus representing a valuable aid from the diagnosis up to any complications. However, research is directed towards the introduction of new markers, which can be capable of increasing the diagnostic potential and minimizing the need to resort to more expensive diagnostic imaging techniques. Serum calprotectin represents a good candidate in this respect, since elevated levels of this protein have been found in patients with RA especially in the severe state. This protein correlates with disease activity and also shows to have a prognostic role in predicting response to treatment in RA. Furthermore, if used together with other markers, it can greatly optimize the therapeutic choice. The great stability of calprotectin in the sample, unlike cytokines such as IL-6, TNF-α, il-1β, makes it a good candidate for inflammatory diseases and could play an even more important role if it were to be included in future diagnostic or classification criteria for these diseases.

However, the data reported in the literature highlight the poor standardization of the test from the laboratory point of view which is linked to the preanalytical variability (the plasma matrix is more stable than serum),[17,18] to the analytical variability (inter-method), and post - reporting analytics (for an unclear definition of the cut-off).[19]

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