Calprotectin (a S100 protein) as a sensitive biomarker for rheumatoid arthritis: new perspectives for an old finding

"...encouraging data showing calprotectin as an independent biomarker of macrophage activation, rheumatoid arthritis disease severity, structural joint damage as well as further progression and potentially therapeutic response are promising."

KEYWORDS: biomarker ■ calprotectin ■ disease activity ■ prognosis ■ rheumatoid arthritis ■ S100 proteins ■ ultrasonography

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Rheumatoid arthritis (RA) is a heterogeneous disease that is characterized with a variable course spanning from monoarticular pattern to polyarthritis, extra-articular manifestations, severe disability and premature mortality. The course of the disease may also be variable with respect to acute-phase response and autoantibody profile that are, however, normal in up to half of patients with recent-onset RA. So far, there are no validated laboratory tests that encompass the complex biological processes of RA. Any marker to be useful in clinical practice should reflect disease activity, joint damage and impairment of physical function [1].

S100 proteins are small calcium-binding cytosolic molecules acting as important regulators of multiple cellular processes, including cell survival, proliferation, differentiation and motility, and have already been associated with diverse inflammatory, autoimmune, degenerative and malignant diseases [2]. One of the first members of the S100 proteins was discovered almost 50 years ago and because of its solubility in 100% ammonium sulfate solution, the protein was termed 'S100'. Calprotectin, a heterodimeric complex of S100A8/A9 (known also as myeloid-related proteins [MRP-8/MRP-14]), is an important member of the S100 family proteins representing ~60% of the soluble cytosol proteins in polymorphonuclear leukocytes, as well as being a major monocyte/macrophage protein [3]. Expression in other cells (e.g., fibroblasts and endothelial cells) has been described.

Calprotectin was recently categorized as an alarmin, an endogenous molecule released mostly by immune cells, that signal tissue and cell damage [3]. There is evidence that calprotectin is secreted via an a tubulin-dependent (nonclassical) pathway and does not require *de novo* protein synthesis. It is activated by microbial molecules, so-called pathogen-associated molecular patterns and other alarmins or cytokines. There is evidence that calprotectin plays an important role in regulating inflammatory and immune processes via several mechanisms. Calprotectin promotes migration of phagocytes to the sites of inflammation and is recognized by Toll-like receptor-4 on monocytes [4]. The interaction between calprotectin and Toll-like receptor-4 triggers an autocrine/paracrine positive feedback loop, which is involved in the amplification of cytokine production through activation of multiple protein kinase-mediated signal transduction pathways [5]. Of note, a crucial role of calprotectin for the development of autoreactive lymphocytes during autoimmunity has been recently proposed [6]. Together these data raise the possibility that calprotectin acts as an important danger signal promoting innate, as well as, adaptive immunity.

In general, calprotectin has been considered to be an important molecule contributing to inflammation. Recent studies demonstrated the association of calprotectin and several human inflammatory diseases, including RA, juvenile idiopathic arthritis, psoriatic arthritis, psoriasis, systemic lupus erythematosus, idiopathic inflammatory myopathies, vasculitis and sepsis or inflammatory bowel diseases (for review see [2]). Increased calprotectin expression has been initially demonstrated in RA synovial membrane [7], which was largely localized to the highly activated macrophages of the lining layer and mostly to those cells that were adjacent to the cartilage–pannus junction, where joint destruction occurs in patients with active disease [5]. In line with these data, Berntzen *et al.* demonstrated significantly elevated synovial fluid, as well as circulating calprotectin levels, in patients with RA compared with control

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individuals with osteoarthritis [8]. Synovial fluid calprotectin levels were significantly higher than that of serum counterparts, suggesting local release of the protein. Importantly, Hammer *et al.* found a positive association among calprotectin, inflammatory and serological markers, as well as with the RA joint disease activity score [9]. Moreover, in a cross-sectional study, they showed independent correlation between calprotectin levels and joint damage in patients with RA [10].

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A critical question was whether calprotectin associates with therapeutic response. In regard to this, several studies have been conducted. It has been shown that TNF-blockade therapy significantly decreased both infiltrations of calprotectin-positive macrophages in RA synovial membrane as well as circulating calprotectin in the blood of RA patients [11]. In the most recent 1-year longitudinal study published by Hammer *et al.,* associations between calprotectin levels and ultrasonographic assessment of the joints during TNF-blockade treatment with adalimumab in 20 RA patients were investigated [12]. Interestingly, the authors found that baseline calprotectin had the highest association with ultrasonographic scores among the traditional inflammatory markers such as ESR, C-reactive protein or serum amyloid A, and this association even increased during the follow-up. This study indicates that calprotectin is associated with overall synovial hypertrophy and the extent of vascularization. In agreement with a previous study [11], calprotectin levels decreased during TNF-blockade treatment and had higher sensitivity to change than the abovementioned inflammatory markers [12]. Supporting this finding, we have demonstrated the normalization of elevated calprotectin in patients with recent-onset RA 3 months after the initiation of conventional treatment [13]. In addition, a decrease in calprotectin, but not C-reactive protein was a significant predictor for improvements in total swollen joint count. Apart from this, it has been shown that calprotectin accelerated the development of atherosclerosis and further development of coronary artery disease [14], which is often seen as a significant comorbidity in RA patients with clinically active disease. Therefore it can be hypothesized that successful therapeutic interventions reducing calprotectin levels could also reduce the progression of cardiovascular complications in RA patients.

As discussed above, calprotectin may represent a good biomarker for RA severity. The 'Holy Grail' of RA is the prediction of disease outcomes and therapeutic responsiveness. There is evidence that fecal calprotectin levels have an important role in diagnosis, follow-up, prediction of relapses and assessment of response to treatment in patients with inflammatory bowel disease [15]. Although calprotectin serum levels are significantly higher in juvenile idiopathic arthritis patients before relapse than during stable remission [16], this has not yet been shown in adult patients with RA. At least in juvenile patients with arthritis, calprotectin can be upregulated even weeks to months before relapse becomes clinically apparent in patients with previously well-controlled disease. Therefore, it could be suggested that calprotectin may help to adjust treatment strategies in this subpopulation of low disease activity subjects. A major challenge is predicting long-term structural damage and functional disability. Recently, calprotectin was established as an independent predictor of clinical and radiographic joint damage over 10 years [17]. To support this critical finding, it has been shown that S100A8 stimulates secretion of several matrix degrading enzymes from chondrocytes and activates osteoclast formation, thus contributing to cartilage and bone destruction during experimental arthritis [18,19]. Therefore, calprotectin may be considered as a potential prognostic biomarker for further structural progression of RA.

> "*...calprotectin was established as an independent predictor of clinical and radiographic joint damage...*"

In conclusion, extensive research to better understand biomarkers that would reflect the complex biological processes of RA is ongoing. Until now, however, individual biomarkers have not been validated to capture the heterogeneity of RA. As there are more efficient and expensive therapies [20], it is necessary to search for better biomarkers to predict outcomes and therapeutic responsiveness. In regard to this, encouraging data showing calprotectin as an independent biomarker of macrophage activation, RA disease severity and structural joint damage, as well as further progression and potentially therapeutic response are promising. In addition, S100 proteins may become promising novel

targets for therapeutic intervention in chronic inflammatory diseases.

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